(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 February 2011 (24.02.2011)

PCT

(10) International Publication Number
WO 2011/021386 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01) *C12N 15/113* (2010.01)
A61P 35/00 (2006.01) *G01N 33/50* (2006.01)
C07K 16/30 (2006.01) *G01N 33/53* (2006.01)
C12N 15/09 (2006.01)

(21) International Application Number:
PCT/JP2010/005095

(22) International Filing Date:
18 August 2010 (18.08.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/274,800 21 August 2009 (21.08.2009) US

(71) Applicant *(for all designated States except US)*: **ONCOTHERAPY SCIENCE, INC.** [JP/JP]; 2-1, Sakado 3-chome, Takatsu-ku, Kawasaki-shi, Kanagawa, 2130012 (JP).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **DAIGO, Yataro** [JP/JP]; c/o THE UNIVERSITY OF TOKYO, 3-1, Hongo 7-chome, Bunkyo-ku, Tokyo, 1138654 (JP). **TSUNODA, Takuya** [JP/JP]; c/o ONCOTHERAPY SCIENCE, INC., 2-1, Sakado 3-chome, Takatsu-ku, Kawasaki-shi, Kanagawa, 2130012 (JP). **NAKAMURA, Yusuke** [JP/JP]; c/o THE UNIVERSITY OF TOKYO, 3-1, Hongo 7-chome, Bunkyo-ku, Tokyo, 1138654 (JP).

(74) Agents: **SHIMIZU, Hatsushi** et al.; Kantetsu Tsukuba Bldg. 6F, 1-1-1, Oroshi-machi, Tsuchiura-shi, Ibaraki, 3000847 (JP).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2011/021386 A1

(54) Title: CSTF2 FOR TARGET GENES OF LUNG CANCER THERAPY AND DIAGNOSIS

(57) Abstract: The present invention relates to the roles played by a CSTF2 gene in cancer carcinogenesis and features a method for treating or preventing cancer by administering a double-stranded molecule against the CSTF2 gene or a composition, vector or cell containing such a double stranded molecule. The present invention also features methods for diagnosing cancer or assessing/determining the prognosis of a subject with lung cancer, using the over-expressed CSTF2 gene. To that end, CSTF2 may serve as a novel prognosis biomarker for cancer, particularly lung cancer. Also, disclosed are methods of screening candidate substances for treating and preventing cancer, using as an index their effect on the expression or biological activity of CSTF2.

Description

Title of Invention: CSTF2 FOR TARGET GENES OF LUNG CANCER THERAPY AND DIAGNOSIS

Technical Field

[0001] The present invention relates to the field of biological science, more specifically to the field of cancer research, cancer diagnosis and cancer therapy. In particular, the present invention relates to methods for detecting and diagnosing lung cancer as well as methods for treating and preventing or assessing/determining the prognosis of a subject with lung cancer. Moreover, the present invention relates to methods for screening a candidate substance for treating and/or preventing lung cancer.

[0002] Priority

The present application claims the benefit of U.S. Provisional Application No. 61/274,800, filed on August 21, 2009, the entire contents of which is incorporated by reference herein.

Background Art

[0003] Primary lung cancer is the leading cause of cancer deaths in most countries, and non-small lung cancer (NSCLC) accounts for about 80% of those cases (NPL 1). Detailed molecular mechanism of lung carcinogenesis remains unclear, although various genetic alterations in lung cancer were reported (NPL 2). The patient with an advanced lung cancer often suffers from the fatal disease progress in spite of the improvement in a surgical technique and the chemoradiotherapy (NPL 1). Therefore, it is considered extremely important to understand the biology of lung cancer and to introduce more effective treatments to improve the survival of patients (NPL 3). Within the last two decades, some newly developed cytotoxic agents such as paclitaxel, docetaxel, gemcitabine, and vinorelbine have appeared to offer multiple choices for treatment of patients with advanced NSCLC, however, those regimens shows only a modest survival benefit compared with conventional therapies based cisplatine (NPLs 4, 5).

[0004] At present, the concept of a specific molecular targeting has been applied to the development of a reformative cancer treatment strategy, and the main two approaches are used by treatment: therapeutic monoclonal antibody and small molecular agent (NPL 6). To date, a lot of molecular targeting therapies have been investigated in phase II and phase III trials for the chemotherapy of advanced lung cancers, including tyrosine kinase inhibitors of epidermal growth factor such as gefitinib and erlotinib, tyrosine kinase inhibitors of vascular endothelial growth factor such as vandetanib, sorafenib, sunitinib, and monoclonal antibodies of epidermal growth factor or vascular endothelial growth factor such as bevacizumab and cetuximab (NPLs 6-10). However,

only a limited numbers of patients can choose these treatment regimens due to the problem of toxicity, and the ratio of patients who show the good response is limited even if all kind of treatment is applied (NPLs 6-10).

Citation List

Non Patent Literature

[0005] [NPL 1] Ahmedin J, Rebecca S, Elizabeth W, et al. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43-66

[NPL 2] Sozzi G. Molecular biology of lung cancer. Eur J Cancer 2001;37 Suppl 7:S63-73

[NPL 3] Daigo Y, Nakamura Y. Gen Thorac Cardiovasc Surg 2008;56:43-53

[NPL 4] Kelly K, Crowley J, Bunn PA et al. J Clin Oncol 2001;19:3210-18

[NPL 5] Schiller JH, Harrington D, Belani CP et al. N Engl J Med 2002;346:92-8

[NPL 6] Thatcher Lung Cancer 2007;57 Suppl 2:S18-23

[NPL 7] Sandler A, Gray R, Perry MC, et al. N Engl J Med 2006;355:2542-50

[NPL 8] Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. N Engl J Med 2005;353:123-32

[NPL 9] Thatcher N, Chang A, Parikh P, et al. Lancet 2005;366:1527-37

[NPL 10] Cesare G, Paolo M, Filomena G, et al. Oncologist 2007;12:191-200

Summary of Invention

[0006] Systematic analysis of expression levels of thousands of genes using a cDNA microarray technology is an effective approach to identify target molecules associated with carcinogenic pathways that can be candidates for the development of novel therapeutics and diagnostics. To isolate potential molecular targets for diagnosis and/or treatment of lung cancers, the present inventors previously analyzed genome-wide gene expression profiles of 101 lung cancer tissue samples by means of a cDNA microarray consisting of 27,648 genes or expressed sequence tags (ESTs), using tumor-cell populations purified by laser microdissection (Daigo Y, Nakamura Y. Gen Thorac Cardiovasc Surg 2008;56:43-53, Kikuchi T, Daigo Y, Katagiri T, et al. Oncogene 2003;22:2192-205, Kakiuchi S, Daigo Y, Tsunoda T, Yano S, Sone S, Nakamura Y. Mol Cancer Res 2003;1:485-99, Kakiuchi S, Daigo Y, Ishikawa N, et al. Hum Mol Genet 2004;13:3029-43, Kikuchi T, Daigo Y, Ishikawa N, et al. Int J Oncol 2006; 28:799-805, Taniwaki M, Daigo Y, Ishikawa N, et al. Int J Oncol 2006;29:567-75). For purpose of verifying the biological and clinicopathologic significance of the respective gene products, the present inventors have established a screening system by a combination of the tumor tissue microarray analysis of clinical lung cancer materials with RNA interference technique (Suzuki C, Daigo Y, Kikuchi T, Katagiri T, Nakamura Y. Cancer Res 2003;63:7038-41, Ishikawa N, Daigo Y, Yasui W, et al. Clin

Cancer Res 2004;10:8363-70, Kato T, Daigo Y, Hayama S, et al. Cancer Res 2005;65:5638-46, Furukawa C, Daigo Y, Ishikawa N, et al. Cancer Res 2005;65:7102-10, Ishikawa N, Daigo Y, Takano A, et al. Cancer Res 2005;65:9176-84, Suzuki C, Daigo Y, Ishikawa N, et al. Cancer Res 2005;65:11314-25, Ishikawa N, Daigo Y, Takano A, et al. Cancer Sci 2006;97:737-45, Takahashi K, Furukawa C, Takano A, et al. Cancer Res 2006;66:9408-19, Hayama S, Daigo Y, Kato T, et al. Cancer Res 2006;66:10339-48, Kato T, Hayama S, Yamabuki Y, et al. Clin Cancer Res 2007;13:434-42, Suzuki C, Takahashi K, Hayama S, et al. Mol Cancer Ther 2007;6:542-51, Yamabuki T, Takano A, Hayama S, et al. Cancer Res 2007;67:2517-25, Hayama S, Daigo Y, Yamabuki T, et al. Cancer Res 2007; 67:4113-22, Taniwaki M, Takano A, Ishikawa N, et al. Clin Cancer Res 2007;13:6624-31, Ishikawa N, Takano A, Yasui W, et al. Cancer Res 2007;67:11601-11, Mano Y, Takahashi, K, Ishikawa N, et al. Cancer Sci 2007;98:1902-13, Kato T, Sato N, Hayama S, et al. Cancer Res 2007; 67:8544-53, Kato T, Sato N, Takano A, et al. Clin Cancer Res 2008;14:2363-70, Dunleavy EM, Roche D, Tagami H, et al. Cell 2009;137:485-97, Hirata D, Yamabuki T, Ito T, et al. Clin Cancer Res 2009,15:256-66, Suda T, Tsunoda T, Daigo Y, Nakamura Y, Tahara H. Cancer Sci 2007;98:1803-8, Mizukami Y, Kono K, Daigo Y, et al. Cancer Sci 2008;99:1448-54, Harao M, Hirata S, Irie A, et al. Int J Cancer 2008;123:2616-25).

[0007] This systematic approach revealed that cleavage stimulation factor, 3' pre-RNA, subunit 2, 64 kDa (CSTF2) was frequently overexpressed in the majority of primary lung cancers. CSTF2 encodes a nuclear protein that contains a ribonucleoprotein (RNP)-type RNA binding domain in the N-terminal region. The protein is a member of cleavage stimulation factor complex (CSTF), which plays a role in polyadenylation of mRNA with other 2 members of cleavage stimulation factors (Takagaki Y, MacDonald CC, Shenk T, Manley JL. Proc. Nat. Acad. Sci 1992; 89: 1403-1407). CSTF2 binds to GU-rich elements within the 3'-untranslated region of mRNAs (Colgan DF, Manley JL. Genes Dev 1997;11:2755-2766, MacDonald CC, Wilusz J, Shenk T. Mol Cell Biol 1994;14:6647-6654, Takagaki Y, Manley JL. Mol Cell Biol 1997;17:3907-3914, Deka P, Rajan PK, Perez-Canadillas JM, J Mol Biol 2005;347:719-33). The amount of CSTF2 increases during the G0 to S phase transition in mouse 3T6 fibroblast (Martincic K, Campbell R, Edwalds-Gilbert G, Souan L, Lotze MT, Milcarek C. 1998; 95:11095-100). Strong CSTF2 expression was reported in male germ cells of mice and rats and human cancer cells, or CSTF2 was expressed in mice tissues ubiquitously (Dass B, Tardif S, Park JY, Tian B, Weitlauf HM, Hess RA, Carnes K, Griswold MD, Small CL, Macdonald CC. Proc Natl Acad Sci U S A. 2007;104:20374-9, Huber Z, Monarez RR, Dass B, MacDonald CC. Ann N Y Acad Sci. 2005;1061:163-72, Wallace AM, Denison TL, Attaya EN, MacDonald CC. Biol Reprod 2004;70:1080-7,

Dass B, Attaya EN, Michelle Wallace A, MacDonald CC. Biol Reprod 2001;64:1722-9, Chennathukuzhi VM, Lefrancois S, Morales CR, Syed V, Hecht NB. Mol Reprod Dev 2001;58:460-9, Dass B, McMahon KW, Jenkins NA, Gilbert DJ, Copeland NG, MacDonald CC. J Biol Chem 2001;276:8044-50, Wallace AM, Dass B, Ravnik SE, Tonk V, Jenkins NA, Gilbert DJ, Copeland NG, MacDonald CC. Proc Natl Acad Sci U S A 1999;96:6763-8, Shankarling GS, Coates PW, Dass B, Macdonald CC. BMC Mol Biol 2009 Mar;10:22). In spite of the evidence of CSTF2 function as a member of CSTF in vitro, the significance of activation of CSTF2 in human cancer progression and its clinical potential as a therapeutic target were not described.

[0008] As noted above, the present invention relates to CSTF2, and the roles it plays in lung cancer carcinogenesis. As such, the present invention relates to novel compositions and methods for detecting, diagnosing, treating and/or preventing lung cancer as well as methods for screening for useful substances.

In particular, the present invention arises from the discovery that CSTF2 gene is over-expressed in cancer, but not in normal tissues, and double-stranded molecules composed of specific sequences (in particular, SEQ ID NOs: 9 and 10), targeting CSTF2 gene, are effective for inhibiting cellular growth of lung cancer cells. Specifically, small interfering RNAs (siRNAs) targeting a CSTF2 gene are provided by the present invention. These double-stranded molecules may be utilized in an isolated state or encoded in vectors and expressed from the vectors. Accordingly, it is an object of the present invention to provide such double-stranded molecules as well as vectors and host cells expressing them.

[0009] In one aspect, the present invention provides methods for inhibiting cancer cell growth or treating cancer, including lung cancer, by administering the double-stranded molecules or vectors of the present invention to a subject in need thereof. Such methods encompass administering to a subject a composition containing one or more of the double-stranded molecules or vectors.

In another aspect, the present invention provides compositions for treating lung cancer containing at least one of the double-stranded molecules or vectors of the present invention.

[0010] In yet another aspect, the present invention provides a method of diagnosing or determining a predisposition to cancer in a subject by determining an expression level of CSTF2 gene in a subject-derived biological sample. An increase in the expression level of the gene as compared to a normal control level of the gene indicates that the subject suffers from or is at risk of developing cancer, including lung cancer. In a preferred embodiment, the expression level of the CSTF2 gene can be determined by detecting mRNA of the CSTF2 gene by appropriate probes or primers, or the CSTF2 protein by anti-CSTF2 antibody.

[0011] Moreover, the present invention relates to the discovery that a high expression level of CSTF2 correlates to poor survival rate. Therefore, the present invention provides a method for assessing or determining the prognosis of a patient with lung cancer, which method includes the steps of detecting the expression level of CSTF2 gene, comparing it to a pre-determined reference expression level and determining the prognosis of the patient from the difference therebetween.

[0012] In a further aspect, the present invention provides a method of screening for a candidate substance for treating and/or preventing cancer. Such substances would bind with CSTF2 protein, reduce the biological activity of the CSTF2 protein, reduce the expression of CSTF2 gene or reduce the expression or activity of reporter gene surrogating the CSTF2 gene.

[0013] It will be understood by those skilled in the art that one or more aspects of this invention can meet certain objectives, while one or more other aspects can meet certain other objectives. Each objective may not apply equally, in all its respects, to every aspect of this invention. As such, the preceding objects can be viewed in the alternative with respect to any one aspect of this invention. These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the invention and the following detailed description are of a preferred embodiment, and not restrictive of the invention or other alternate embodiments of the invention.

Brief Description of Drawings

[0014] Various aspects and applications of the present invention will become apparent to the skilled artisan upon consideration of the brief description of the figures and the detailed description of the present invention and its preferred embodiments that follows:

[0015] [fig.1ab]Fig. 1 depicts the expression of CSTF2 in lung tumors: A, expression of CSTF2 in 15 clinical lung cancers [lung adenocarcinoma (ADC), lung squamous cell carcinoma (SCLC), and small-cell lung cancer (SCC)] and 15 lung cancer cell lines, examined by semiquantitative RT-PCR. Expression of beta-actin (ACTB) gene was served as a quantity control. B, expression of CSTF2 protein in lung cancer cell lines by Western blot analysis. Expression of ACTB protein was served as a quantity control. IB, immunoblotting.

[0016] [fig.1c]Fig. 1 depicts the expression of CSTF2 in lung tumors: C, subcellular localization of the CSTF2 proteins examined by confocal microscopy.

[0017] [fig.2ab]Fig. 2 depicts the expression of CSTF2 in normal tissues, and association of CSTF2 overexpression with poor prognosis for NSCLC patients: A, expression of

CSTF2 in normal human tissues, detected by Northern blot analysis. B, expression of CSTF2 in five normal human tissues as well as lung adenocarcinoma cell, detected by immunohistochemical staining using the rabbit polyclonal anti-CSTF2 antibody; counterstaining with hematoxylin (x200).

[0018] [fig.2cd]Fig. 2 depicts the expression of CSTF2 in normal tissues, and association of CSTF2 overexpression with poor prognosis for NSCLC patients: C, representative examples for strong, weak, and absent CSTF2 expression in lung ADC tissues and a normal lung tissue (original magnification, x100). D, Kaplan-Meier analysis of survival of patients with NSCLC (P = 0.0079, log-rank test).

[0019] [fig.3]Fig. 3 depicts inhibition of growth of NSCLC cells by siRNAs against CSTF2: A, expression of CSTF2 in response to siRNA treatment for CSTF2 (si-CSTF2-#1or si-CSTF2-#2) or control siRNAs [si-enhanced green fluorescent protein (si-EGFP) or si-luciferase (si-LUC)] in A549 and LC319 cells, analyzed by semiquantitative RT-PCR (top). B, C, MTT and colony formation assays of the tumor cells transfected with si-CFTF2s or control siRNAs.

[0020] [fig.4]Fig. 4 depicts enhancement of cellular growth by CSTF2 introduction into mammalian cells: A, transient expression of CSTF2 in COS-7 cells, detected by Western blot analysis. The cells were introduced with pcDNA3.1-myc-His-CSTF2 or mock vector. B and C, the cell viability and colony numbers were evaluated by the MTT and colony-formation assays. Assays were done thrice and in triplicate wells.

Description of Embodiments

[0021] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. However, before the present materials and methods are described, it is to be understood that the present invention is not limited to the particular sizes, shapes, dimensions, materials, methodologies, protocols, etc. described herein, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0022] The disclosure of each publication, patent or patent application mentioned in this specification is specifically incorporated by reference herein in its entirety. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0023] In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to

be limiting.

[0024] Definition

The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

[0025] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that similarly functions to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those modified after translation in cells (e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine). The phrase "amino acid analog" refers to compounds that have the same basic chemical structure (an alpha carbon bound to a hydrogen, a carboxy group, an amino group, and an R group) as a naturally occurring amino acid but have a modified R group or modified backbones (e.g., homoserine, norleucine, methionine, sulfoxide, methionine methyl sulfonium). The phrase "amino acid mimetic" refers to chemical compounds that have different structures but similar functions to general amino acids.

[0026] Amino acids may be referred to herein by their commonly known three letter symbols or the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0027] As used herein, the term "biological sample" refers to a whole organism or a subset of its tissues, cells or component parts (e.g., body fluids, including but not limited to blood, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). "Biological sample" further refers to a homogenate, lysate, extract, cell culture or tissue culture prepared from a whole organism or a subset of its cells, tissues or component parts, or a fraction or portion thereof. Lastly, "biological sample" refers to a medium, such as a nutrient broth or gel in which an organism has been propagated, which contains cellular components, such as proteins or polynucleotides.

[0028] The term "polynucleotide", "oligonucleotide", "nucleic acid", and "nucleic acid molecule" are used interchangeably herein to refer to a polymer of nucleic acid residues and, unless otherwise specifically indicated are referred to by their commonly accepted single-letter codes. The terms apply to nucleic acid (nucleotide) polymers in which one or more nucleic acids are linked by ester bonding. The nucleic acid polymers may be composed of DNA, RNA or a combination thereof and encompass both naturally-occurring and non-naturally occurring nucleic acid polymers.

[0029] The terms "polypeptide", "peptide", and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is a modified residue, or a non-naturally occurring residue, such as an artificial chemical mimetic of a corresponding naturally

occurring amino acid, as well as to naturally occurring amino acid polymers.

[0030] The terms "cell proliferative activity", "cell proliferation enhancing activity", cell-proliferating activity" and "cell proliferation promoting activity" are used interchangeably herein to refer to an activity of a polypeptide, which promote or enhance cell proliferation, when the polypeptide is contacted with the cell or the gene encoding the polypeptide is introduced into the cell.

[0031] The terms "isolated" and "purified" used in relation with a substance (e.g., polypeptide, antibody, polynucleotide, etc.) indicates that the substance is substantially free from at least one substance that can be included in the natural source. Thus, an isolated or purified antibody refers to antibodies that are substantially free of cellular material for example, carbohydrate, lipid, or other contaminating proteins from the cell or tissue source from which the protein (antibody) is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The term "substantially free of cellular material" includes preparations of a polypeptide in which the polypeptide is separated from cellular components of the cells from which it is isolated or recombinantly produced.

[0032] Thus, a polypeptide that is substantially free of cellular material includes preparations of polypeptide having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the polypeptide is recombinantly produced, in some embodiments it is also substantially free of culture medium, which includes preparations of polypeptide with culture medium less than about 20%, 10%, or 5% of the volume of the protein preparation. When the polypeptide is produced by chemical synthesis, in some embodiments it is substantially free of chemical precursors or other chemicals, which includes preparations of polypeptide with chemical precursors or other chemicals involved in the synthesis of the protein less than about 30%, 20%, 10%, 5% (by dry weight) of the volume of the protein preparation. That a particular protein preparation contains an isolated or purified polypeptide can be shown, for example, by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining or the like of the gel. In one embodiment, proteins including antibodies of the present invention are isolated or purified.

[0033] An "isolated" or "purified" nucleic acid molecule, for example, a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In one embodiment, nucleic acid molecules encoding proteins of the present invention are isolated or purified.

[0034] Unless otherwise defined, the terms "cancer" refers to cancers over-expressing the

CSTF2 gene, such as lung cancer, including adenocarcinoma (ADC), squamous-cell carcinoma (SCC), large-cell carcinoma (LCC), and small-cell lung cancer (SCLC).

[0035] CSTF2 gene or CSTF2 protein

The nucleic acid and polypeptide sequence of the human CSTF2 gene is shown in SEQ ID NO: 1 and SEQ ID NO: 2 respectively, but not limited to. Furthermore, the above sequence data is also available via the GenBank accession No. NM_001325.

[0036] According to an aspect of the present invention, functional equivalents are also considered to be above "polypeptides". Herein, a "functional equivalent" of a protein is a polypeptide that has a biological activity equivalent to the protein. Namely, any polypeptide that retains the biological ability may be used as such a functional equivalent in the present invention. For example, CSTF2 polypeptide is known to have cell proliferation enhancing activity, RNA binding activity, RNA cleavage activity, RNA polyadenylation activity and such. Polypeptides that retain at least one of these activities are considered as functional equivalents of CSTF2 polypeptide in the present invention. Such functional equivalents include those wherein one or more amino acids are substituted, deleted, added, or inserted to the natural occurring amino acid sequence of the protein. Alternatively, the polypeptide may be composed of an amino acid sequence having at least about 80% homology (also referred to as sequence identity) to the sequence of the respective protein, more preferably at least about 90% to 95% homology, further more preferably 96%, 97%, 98% or 99% homology. In other embodiments, the polypeptide can be encoded by a polynucleotide that hybridizes under stringent conditions to the natural occurring nucleotide sequence of the gene.

[0037] A polypeptide of the present invention may have variations in amino acid sequence, molecular weight, isoelectric point, the presence or absence of sugar chains, or form, depending on the cell or host used to produce it or the purification method utilized. Nevertheless, so long as it has a function equivalent to that of the human protein, it is within the scope of the present invention.

[0038] The phrase "stringent (hybridization) conditions" refers to conditions under which a nucleic acid molecule will hybridize to its target sequence, typically in a complex mixture of nucleic acids, but not detectably to other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10 degrees C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes

complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times of background, preferably 10 times of background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42 degrees C, or, 5x SSC, 1% SDS, incubating at 65 degrees C, with wash in 0.2x SSC, and 0.1% SDS at 50 degrees C.

[0039] In the context of the present invention, a condition of hybridization for isolating a DNA encoding a polypeptide functionally equivalent to the above human protein can be routinely selected by a person skilled in the art. For example, hybridization may be performed by conducting pre-hybridization at 68 degrees C for 30 min or longer using "Rapid-hyb buffer" (Amersham LIFE SCIENCE), adding a labeled probe, and warming at 68 degrees C for 1 hour or longer. The following washing step can be conducted, for example, in a low stringent condition. An exemplary low stringent condition may include 42 degrees C, 2x SSC, 0.1% SDS, preferably 50 degrees C, 2x SSC, 0.1% SDS. High stringency conditions are often preferably used. An exemplary high stringency condition may include washing 3 times in 2x SSC, 0.01% SDS at room temperature for 20 min, then washing 3 times in 1x SSC, 0.1% SDS at 37 degrees C for 20 min, and washing twice in 1x SSC, 0.1% SDS at 50 degrees C for 20 min. However, several factors, such as temperature and salt concentration, can influence the stringency of hybridization and one skilled in the art can suitably select the factors to achieve the requisite stringency.

[0040] Generally, it is known that modifications of one or more amino acid in a protein do not influence the function of the protein. In fact, mutated or modified proteins, proteins having amino acid sequences modified by substituting, deleting, inserting, and/or adding one or more amino acid residues of a certain amino acid sequence, have been known to retain the original biological activity (Mark et al., Proc Natl Acad Sci USA 81: 5662-6 (1984); Zoller and Smith, Nucleic Acids Res 10:6487-500 (1982); Dalbadie-McFarland et al., Proc Natl Acad Sci USA 79: 6409-13 (1982)). Accordingly, one of skill in the art will recognize that individual additions, deletions, insertions, or substitutions to an amino acid sequence which alter a single amino acid or a small percentage of amino acids or those considered to be a "conservative modifications", wherein the alteration of a protein results in a protein with similar functions, are acceptable in the context of the instant invention.

[0041] So long as the activity the protein is maintained, the number of amino acid mutations is not particularly limited. However, it is generally preferred to alter 5% or less of the amino acid sequence. Accordingly, in a preferred embodiment, the number of amino

acids to be mutated in such a mutant is generally 30 amino acids or less, preferably 20 amino acids or less, more preferably 10 amino acids or less, more preferably 6 amino acids or less, and even more preferably 3 amino acids or less.

[0042] An amino acid residue to be mutated is preferably mutated into a different amino acid in which the properties of the amino acid side-chain are conserved (a process known as conservative amino acid substitution). Examples of properties of amino acid side chains are hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). Conservative substitution tables providing functionally similar amino acids are well known in the art. For example, the following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Aspargine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins 1984).

[0043] Such conservatively modified polypeptides are included in the present protein. However, the present invention is not restricted thereto and the protein includes non-conservative modifications, so long as at least one biological activity of the protein is retained. Furthermore, the modified proteins do not exclude polymorphic variants, interspecies homologues, and those encoded by alleles of these proteins.

[0044] Moreover, the CSTF2 gene encompasses polynucleotides that encode such functional equivalents of the protein. In addition to hybridization, a gene amplification method, for example, the polymerase chain reaction (PCR) method, can be utilized to isolate a polynucleotide encoding a polypeptide functionally equivalent to the protein, using a primer synthesized based on the sequence above information. Polynucleotides and polypeptides that are functionally equivalent to the human gene and protein, respectively, normally have a high homology to the originating nucleotide or amino acid sequence of. "High homology" typically refers to a homology of 40% or higher, preferably 60% or higher, more preferably 80% or higher, even more preferably 90% to 95% or higher, even more preferably 96%, 97%, 98%, 99% or higher. The

homology of a particular polynucleotide or polypeptide can be determined by following the algorithm in "Wilbur and Lipman, Proc Natl Acad Sci USA 80: 726-30 (1983)".

[0045] Antibody

The terms "antibody" as used herein is intended to include immunoglobulins and fragments thereof which are specifically reactive to the designated protein or peptide thereof. An antibody can include human antibodies, primatized antibodies, chimeric antibodies, bispecific antibodies, humanized antibodies, antibodies fused to other proteins or radiolabels, and antibody fragments. Furthermore, an antibody herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. An "antibody" indicates all classes (e.g., IgA, IgD, IgE, IgG and IgM).

[0046] The present invention uses antibodies against CSTF2. These antibodies will be provided by known methods.

[0047] Exemplary techniques for the production of the antibodies used in accordance with the present invention are described.

[0048] (i) Polyclonal antibodies:

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOC12, or R'N=C=NR, where R and R are different alkyl groups.

[0049] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 or 5 micrograms of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking substance.

[0050] Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[0051] (ii) Monoclonal antibodies:

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies including the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

[0052] For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler G & Milstein C. Nature. 1975 Aug 7;256(5517):495-7, or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0053] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)).

[0054] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0055] Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Manassas, Virginia, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor D, et al., J Immunol. 1984 Dec;133(6):3001-5; Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0056] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-

linked immunoabsorbent assay (ELISA).

[0057] The binding affinity of the monoclonal antibody can, for example, be determined by the 30 Scatchard analysis of Munson PJ & Rodbard D. Anal Biochem. 1980 Sep 1;107(1):220-39.

[0058] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPML-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

[0059] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0060] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra A. Curr Opin Immunol. 1993 Apr;5(2):256-62 and Pluckthun A. Immunol Rev. 1992 Dec;130:151-88.

[0061] Another method of generating specific antibodies, or antibody fragments, reactive against CSTF2 is to screen expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with CSTF2. For example, complete Fab fragments, VH regions and Fv regions can be expressed in bacteria using phage expression libraries. See for example, Ward ES, et al., Nature. 1989 Oct 12;341(6242):544-6; Huse WD, et al., Science. 1989 Dec 8;246(4935):1275-81; and McCafferty J, et al., Nature. 1990 Dec 6;348(6301):552-4. Screening such libraries with a CSTF2 peptide, can identify immunoglobulin fragments reactive with CSTF2. Alternatively, the SCID-hu-mouse (available from Genpharm) can be used to produce antibodies or fragments thereof.

[0062] In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty J, et al., Nature. 1990 Dec 6;348(6301):552-4; Clarkson T, et al., Nature. 1991 Aug

15;352(6336):624-8; and Marks JD, et al., J MoL BioL, 222: 581-597 (1991) J Mol Biol. 1991 Dec 5;222(3):581-97 describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks JD, et al., Biotechnology (N Y). 1992 Jul;10(7):779-83), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse P, et al., Nucleic Acids Res. 1993 May 11;21(9):2265-6). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0063] The DNA also may be modified, for example, by substituting the coding sequence for human heavy-and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison SL, et al., Proc Natl Acad Sci U S A. 1984 Nov;81(21):6851-5), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[0064] Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody including one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[0065] (iii) Humanized antibodies:

Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones PT, et al., Nature. 1986 May 29-Jun 4;321(6069):522-5; Riechmann L, et al., Nature. 1988 Mar 24;332(6162):323-7; Verhoeyen M, et al., Science. 1988 Mar 25;239(4847):1534-6), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US Pat No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0066] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The

human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims MJ, et al., J Immunol. 1993 Aug 15;151(4):2296-308; Chothia C & Lesk AM. J Mol Biol. 1987 Aug 20;196(4):901-17). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter P, et al., Proc Natl Acad Sci U S A. 1992 May 15;89(10):4285-9; Presta LG, et al., J Immunol. 1993 Sep 1;151(5):2623-32).

[0067] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs, which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences, are available. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen, is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0068] (iv) Human antibodies:

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits A, et al., Proc Natl Acad Sci U S A. 1993 Mar 15;90(6):2551-5; Nature. 1993 Mar 18;362(6417):255-8; Bruggemann M, et al., Year Immunol. 1993;7:33-40; and U.S. Patent Nos. 5,591,669; 5,589,369 and 5,545,807.

[0069] Alternatively, phage display technology (McCafferty J, et al., Nature. 1990 Dec 6;348(6301):552-4) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from

unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson KS & Chiswell DJ. Curr Opin Struct Biol. 1993 ;3:564-71. Several sources of V-gene segments can be used for phage display.

[0070] Clackson T, et al., Nature. 1991 Aug 15;352(6336):624-8 isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self antigens) can be isolated essentially following the techniques described by Marks JD, et al., J Mol Biol. 1991 Dec 5;222(3):581-97, or Griffiths AD, et al., EMBO J. 1993 Feb;12(2):725-34. See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

[0071] Human antibodies may also be generated by in vitro activated B cells (see U.S. Patent Nos. 20 5,567,610 and 5,229,275). A preferred means of generating human antibodies using SCID mice is disclosed in commonly-owned, co-pending applications.

[0072] (v) Antibody fragments:

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto K & Inouye K. J Biochem Biophys Methods. 1992 Mar;24(1-2):107-17; Brennan M, et al., Science. 1985 Jul 5;229(4708):81-3). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from E. coli and chemically coupled to form F (ab') 2 fragments (Carter P, et al., Biotechnology (N Y). 1992 Feb;10(2):163-7). According to another approach, F (ab') 2 fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; US Pat Nos. 5,571,894 and 5,587,458. The antibody fragment may also be a "linear antibody", e.g., as described in US Pat No.5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

[0073] (vi) Non-antibody binding protein:

The terms "non-antibody binding protein" or "non-antibody ligand" or "antigen binding protein" interchangeably refer to antibody mimics that use non-immunoglobulin protein scaffolds, including adnectins, avimers, single chain polypeptide binding molecules, and antibody-like binding peptidomimetics, as discussed in more detail below.

[0074] Other substances have been developed that target and bind to targets in a manner similar to antibodies. Certain of these "antibody mimics" use non-immunoglobulin protein scaffolds as alternative protein frameworks for the variable regions of antibodies.

[0075] For example, Ladner et al. (US Pat No. 5,260,203) describe single polypeptide chain binding molecules with binding specificity similar to that of the aggregated, but molecularly separate, light and heavy chain variable region of antibodies. The single-chain binding molecule contains the antigen binding sites of both the heavy and light variable regions of an antibody connected by a peptide linker and will fold into a structure similar to that of the two peptide antibody. The single-chain binding molecule displays several advantages over conventional antibodies, including, smaller size, greater stability and are more easily modified.

[0076] Ku et al. (Proc Natl Acad Sci USA 92(14):6552-6556 (1995)) describe an alternative to antibodies based on cytochrome b562. Ku et al. (1995) generated a library in which two of the loops of cytochrome b562 were randomized and selected for binding against bovine serum albumin. The individual mutants were found to bind selectively with BSA similarly with anti-BSA antibodies.

[0077] Lipovsek et al. (US Pat Nos. 6,818,418 and 7,115,396) describe an antibody mimic featuring a fibronectin or fibronectin-like protein scaffold and at least one variable loop. Known as Adnectins, these fibronectin-based antibody mimics exhibit many of the same characteristics of natural or engineered antibodies, including high affinity and specificity for any targeted ligand. Any technique for evolving new or improved binding proteins can be used with these antibody mimics.

[0078] The structure of these fibronectin-based antibody mimics is similar to the structure of the variable region of the IgG heavy chain. Therefore, these mimics display antigen binding properties similar in nature and affinity to those of native antibodies. Further, these fibronectin-based antibody mimics exhibit certain benefits over antibodies and antibody fragments. For example, these antibody mimics do not rely on disulfide bonds for native fold stability, and are, therefore, stable under conditions which would normally break down antibodies. In addition, since the structure of these fibronectin-based antibody mimics is similar to that of the IgG heavy chain, the process for loop randomization and shuffling, that is similar to the process of affinity maturation of antibodies in vivo, can be employed in vitro.

[0079] Beste et al. (Proc Natl Acad Sci USA 96(5):1898-1903 (1999)) describe an antibody mimic based on a lipocalin scaffold (Anticalin(registered trademark)). Lipocalins are composed of a beta-barrel with four hypervariable loops at the terminus of the protein. Beste (1999) subjected the loops to random mutagenesis and selected for binding with, for example, fluorescein. Three variants exhibited specific binding with fluorescein, with one variant showing binding similar to that of an anti-fluorescein antibody. Further analysis revealed that all of the randomized positions are variable, indicating that Anticalin(registered trademark) would be suitable to be used as an alternative to antibodies.

[0080] Anticalins(registered trademark) are small, single chain peptides, typically between 160 and 180 residues, which provide several advantages over antibodies, including decreased cost of production, increased stability in storage and decreased immunological reaction.

[0081] Hamilton et al. (US Pat No. 5,770,380) describe a synthetic antibody mimic using the rigid, non-peptide organic scaffold of calixarene, attached with multiple variable peptide loops used as binding sites. The peptide loops all project from the same side geometrically from the calixarene, with respect to each other. Because of this geometric confirmation, all of the loops are available for binding, increasing the binding affinity to a ligand. However, in comparison to other antibody mimics, the calixarene-based antibody mimic does not consist exclusively of a peptide, and therefore it is less vulnerable to attack by protease enzymes. Neither does the scaffold consist purely of a peptide, DNA or RNA, meaning this antibody mimic is relatively stable in extreme environmental conditions and has a long life span. Further, since the calixarene-based antibody mimic is relatively small, it is less likely to produce an immunogenic response.

[0082] Murali et al. (Cell Mol Biol. 49(2):209-216 (2003)) describe a methodology for reducing antibodies into smaller peptidomimetics, they term "antibody like binding peptidomimetics" (ABiP) which can also be useful as an alternative to antibodies.

[0083] Silverman et al. (Nat Biotechnol. 23: 1556-1561 (2005)) describe fusion proteins that are single-chain polypeptides including multiple domains termed "avimers." Developed from human extracellular receptor domains by in vitro exon shuffling and phage display, the avimers are a class of binding proteins somewhat similar to antibodies in their affinities and specificities for various target molecules. The resulting multidomain proteins can include multiple independent binding domains that can exhibit improved affinity (in some cases sub-nanomolar) and specificity compared with single-epitope binding proteins. Additional details concerning methods of construction and use of avimers are disclosed, for example, in US Pat. App. Pub. Nos. 20040175756, 20050048512, 20050053973, 20050089932 and 20050221384.

[0084] In addition to non-immunoglobulin protein frameworks, antibody properties have also been mimicked in substances including, but not limited to, RNA molecules and unnatural oligomers (e.g., protease inhibitors, benzodiazepines, purine derivatives and beta-turn mimics), all of which are suitable for use with the present invention.

[0085] (vii) Pharmaceutical formulations:

Therapeutic formulations of present antibodies used in accordance with the present invention may be prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol ; cyclohexanol; 3-pentanol; and m-cresol) ; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

[0086] Lyophilized formulations adapted for subcutaneous administration are described in W097/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the mammal to be treated herein.

[0087] The formulation herein may also contain more than one active substances as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide a chemotherapeutic agent, cytokine or immunosuppressive agent. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disease or disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

[0088] The active ingredients may also be entrapped in microcapsules prepared, for

example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly- (methyl methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, micro-emulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0089] Sustained-release preparations may be prepared. Suitable examples of sustained release preparations include semipermeable matrices of solid hydrophobic polymers containing the agent, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)), polylactides (U. S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0090] (x)Treatment with an antibody:

A composition including present antibodies may be formulated, dosed, and administered in a fashion consistent with good medical practice. Preferably, the present antibody will be a human, chimeric or humanized antibody scFv, or antibody fragment. Factors for consideration in this context include the particular lung cancer being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disease or disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The therapeutically effective amount of the antibody to be administered will be governed by such considerations.

[0091] As a general proposition, the therapeutically effective amount of the antibody administered parenterally per dose will be in the range of about 0.1 to 20 mg/kg of patient body weight per day, with the typical initial range of antibody used being in the range of about 2 to 10 mg/kg.

[0092] As noted above, however, these suggested amounts of antibody are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above.

[0093] For example, relatively higher doses may be needed initially for the treatment of ongoing and acute diseases. To obtain the most efficacious results, depending on the disease or disorder, the antibody may be administered as close to the first sign, diagnosis, appearance, or occurrence of the disease or disorder as possible or during re-

missions of the disease or disorder.

[0094] The antibody may be administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local immunosuppressive treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration.

[0095] In addition, the antibody may suitably be administered by pulse infusion, e.g., with declining doses of the antibody. Preferably the dosing is given by injections, most preferably intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

[0096] One additionally may administer other substances, such as cytotoxic agents, chemotherapeutic agents, immunosuppressive agents and/or cytokines with the antibody herein. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

[0097] Aside from administration of the antibody to the patient, the present invention contemplates administration of the antibody by gene therapy. Such administration of a nucleic acid encoding an antibody is encompassed by the expression "administering a therapeutically effective amount of an antibody". See, for example, W096/07321 published March 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

[0098] There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; in vivo and ex vivo. For in vivo delivery the nucleic acid is injected directly into the patient, usually at the site where the antibody is required. For ex vivo treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsulated within porous membranes which are implanted into the patient (see, e.g., U. S. Patent Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for ex vivo delivery of the gene is a retrovirus.

[0099] The currently preferred in vivo nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated

virus) and lipid-based systems (useful lipids for lipid mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. When liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g., capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu et al., J. Biol. Chem. 262: 4429-4432 (1987); and Wagner et al., Proc. Nad. Acad. Sci. USA 87: 3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols, see Anderson et al., Science 256: 808-813 (1992). See also WO 93/25673 and the references cited therein.

[0100] In another embodiment, the present invention also provides the use of an antibody against CSTF2 of the present invention in manufacturing a pharmaceutical composition for treating a cancer expressing the CSTF2 gene.

[0101] Alternatively, the present invention further provides an antibody against CSTF2 of the present invention for use in treating a cancer expressing the CSTF2 gene.

[0102] Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition for treating a cancer expressing the CSTF2 gene, wherein the method or process comprises step for formulating a pharmaceutically or physiologically acceptable carrier with an antibody against CSTF2 as active ingredients.

[0103] In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition for treating a cancer expressing the CSTF2 gene, wherein the method or process comprises step for admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is an antibody against CSTF2.

[0104] Double-stranded molecule

As used herein, the term "isolated double-stranded molecule" refers to a nucleic acid molecule that inhibits expression of a target gene and includes, for example, short interfering RNA (siRNA; e.g., double-stranded ribonucleic acid (dsRNA) or small hairpin RNA (shRNA)) and short interfering DNA/RNA (siD/R-NA; e.g., double-stranded chimera of DNA and RNA (dsD/R-NA) or small hairpin chimera of DNA and RNA (shD/R-NA)). Herein, "double-stranded molecule" is also referred to as "double-stranded nucleic acid ", " double-stranded nucleic acid molecule", "double-stranded polynucleotide", "double-stranded polynucleotide molecule", "double-stranded

oligonucleotide" and "double-stranded oligonucleotide molecule".

[0105] As used herein, the term "target sequence" refers to a nucleotide sequence within mRNA or cDNA sequence of a target gene, which will result in suppress of translation of the whole mRNA of the target gene if a double-stranded molecule targeting the sequence is introduced into a cell expressing the target gene. A nucleotide sequence within mRNA or cDNA sequence of a gene can be determined to be a target sequence when a double-stranded molecule comprising a sequence corresponding to the target sequence inhibits expression of the gene in a cell expressing the gene. The double stranded polynucleotide which suppresses the gene expression may consists of the target sequence and 3'overhang having 2 to 5 nucleotides in length (e.g., uu).

[0106] When a target sequence is shown by cDNA sequence, a sense strand sequence of a double-stranded cDNA, i.e., a sequence that mRNA sequence is converted into DNA sequence, is used for defining a target sequence. A double-stranded molecule is composed of a sense strand that has a sequence corresponding to a target sequence and an antisense strand that has a complementary sequence to the target sequence, and the antisense strand hybridizes with the sense strand at the complementary sequence to form a double-stranded molecule.

[0107] Herein, the phrase "corresponding to" means converting a target sequence according to the kind of nucleic acid that constitutes a sense strand of a double-stranded molecule. For example, when a target sequence is shown in DNA sequence and a sense strand of a double-stranded molecule has an RNA region, base "t"s within the RNA region is replaced with base "u"s. On the other hand, when a target sequence is shown in RNA sequence and a sense strand of a double-stranded molecule has a DNA region, base "u"s within the DNA region is replaced with "t"s.

[0108] For example, when a target sequence is shown in the RNA sequence of SEQ ID NO: 10 and the sense strand of the double-stranded molecule has the 3' side half region composed of DNA, "a sequence corresponding to a target sequence" is "5'- CACUU-UACUUTCTGTAACT-3'".

[0109] Also, a complementary sequence to a target sequence for an antisense strand of a double-stranded molecule can be defined according to the kind of nucleic acid that constitutes the antisense strand. For example, when a target sequence is shown in the RNA sequence of SEQ ID NO: 10 and the antisense strand of the double-stranded molecule has the 5' side half region composed of DNA, "a complementary sequence to a target sequence" is "3'- GUGAAAUGAAAGACATTGA -5'".

On the other hand, when a double-stranded molecule is composed of RNA, the sequence corresponding to a target sequence shown in SEQ ID NO: 10 is the RNA sequence of SEQ ID NO: 10, and the complementary sequence corresponding to a target sequence shown in SEQ ID NO: 10 is the RNA sequence of "3'- GUGAAAU-

GAAAGACAUUGA -5'".

[0110] A double-stranded molecule may has one or two 3' overhangs having 2 to 5 nucleotides in length (e.g., uu) and/or a loop sequence that links a sense strand and an antisense strand to form hairpin structure, in addition to a sequence corresponding to a target sequence and complementary sequence thereto.

[0111] As used herein, the term "siRNA" refers to a double-stranded RNA molecule which prevents translation of a target mRNA. Standard techniques of introducing siRNA into the cell are used, including those in which DNA is a template from which RNA is transcribed. The siRNA includes a sense nucleic acid sequence (also referred to as "sense strand"), a antisense nucleic acid sequence (also referred to as "antisense strand") or both. The siRNA may be constructed such that a single transcript has both the sense and complementary antisense nucleic acid sequences of the target gene, e.g., a hairpin. The siRNA may either be a dsRNA or shRNA.

[0112] As used herein, the term "dsRNA" refers to a construct of two RNA molecules composed of complementary sequences to one another and that have annealed together via the complementary sequences to form a double-stranded RNA molecule. The nucleotide sequence of two strands may include not only the "sense" or "antisense" RNAs selected from a protein coding sequence of target gene sequence, but also RNA molecule having a nucleotide sequence selected from non-coding region of the target gene.

[0113] The term "shRNA", as used herein, refers to an siRNA having a stem-loop structure, composed of the first and second regions complementary to one another, i.e., sense and antisense strands. The degree of complementarity and orientation of the regions is sufficient such that base pairing occurs between the regions, the first and second regions is joined by a loop region, the loop results from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The loop region of an shRNA is a single-stranded region intervening between the sense and antisense strands and may also be referred to as "intervening single-strand".

[0114] As used herein, the term "siD/R-NA" refers to a double-stranded polynucleotide molecule which is composed of both RNA and DNA, and includes hybrids and chimeras of RNA and DNA and prevents translation of a target mRNA. Herein, a hybrid indicates a molecule wherein a polynucleotide composed of DNA and a polynucleotide composed of RNA hybridize to each other to form the double-stranded molecule; whereas a chimera indicates that one or both of the strands composing the double stranded molecule may contain RNA and DNA. Standard techniques of introducing siD/R-NA into the cell are used. The siD/R-NA includes a CSTF2 sense nucleic acid sequence (also referred to as "sense strand"), a CSTF2 antisense nucleic acid sequence (also referred to as "antisense strand") or both. The siD/R-NA may be

constructed such that a single transcript has both the sense and complementary antisense nucleic acid sequences from the target gene, e.g., a hairpin. The siD/R-NA may either be a dsD/R-NA or shD/R-NA.

[0115] As used herein, the term "dsD/R-NA" refers to a construct of two molecules composed of complementary sequences to one another and that have annealed together via the complementary sequences to form a double-stranded polynucleotide molecule. The nucleotide sequence of two strands may include not only the "sense" or "antisense" polynucleotides sequence selected from a protein coding sequence of target gene sequence, but also polynucleotide having a nucleotide sequence selected from non-coding region of the target gene. One or both of the two molecules constructing the dsD/R-NA are composed of both RNA and DNA (chimeric molecule), or alternatively, one of the molecules is composed of RNA and the other is composed of DNA (hybrid double-strand).

[0116] The term "shD/R-NA", as used herein, refers to an siD/R-NA having a stem-loop structure, composed of the first and second regions complementary to one another, i.e., sense and antisense strands. The degree of complementarity and orientation of the regions is sufficient such that base pairing occurs between the regions, the first and second regions are joined by a loop region, the loop results from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The loop region of an shD/R-NA is a single-stranded region intervening between the sense and antisense strands and may also be referred to as "intervening single-strand".

[0117] As used herein, an "isolated nucleic acid" is a nucleic acid removed from its original environment (e.g., the natural environment if naturally occurring) and thus, synthetically altered from its natural state. In the present invention, examples of isolated nucleic acid include DNA, RNA, and derivatives thereof.

[0118] A double-stranded molecule against CSTF2 gene, which hybridizes to target mRNA, decreases or inhibits production of a CSTF2 protein encoded by the CSTF2 gene by associating with the normally single-stranded mRNA transcript of the gene, thereby interfering with translation and thus, inhibiting expression of the protein.

[0119] The expression of CSTF2 gene in lung cancer cell lines can be inhibited by dsRNA against CSTF2 gene. Therefore the present invention provides isolated double-stranded molecules that are capable of inhibiting the expression of the CSTF2 gene when introduced into a cell expressing the gene. The target sequence of double-stranded molecule may be designed by an siRNA design algorithm such as that mentioned below.

[0120] CSTF2 target sequence includes, for example, nucleotide sequence of SEQ ID NOs: 9 and 10. In other words, the present invention also provides double-stranded molecules whose target sequence comprises or consisting of SEQ ID NO: 9 or 10.

[0121] Specifically, the present invention provides the following double-stranded molecules [1] to [19]:

[1] An isolated double-stranded molecule that, when introduced into a cell, inhibits in vivo expression of a CSTF2 gene and cell proliferation, wherein the double-stranded molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule;

[2] The double-stranded molecule of [1], wherein the double-stranded molecule acts on mRNA, matching a target sequence selected from among SEQ ID NO: 9, and SEQ ID NO: 10;

[3] The double-stranded molecule of [1], wherein the sense strand contains a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10;

[4] The double-stranded molecule of any one of [1] to [3], wherein the sense strand hybridizes with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 100 nucleotides;

[5] The double-stranded molecule of [4], wherein the sense strand hybridize with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 75 nucleotides;

[6] The double-stranded molecule of [5], wherein the sense strand hybridize with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 50 nucleotides;

[7] The double-stranded molecule of [6] wherein the sense strand hybridize with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 25 nucleotides;

[8] The double-stranded molecule of [7], wherein the sense strand hybridize with antisense strand at the target sequence to form the double-stranded molecule having a length of between about 19 and about 25 nucleotides;

[9] The double-stranded molecule of any one of [1] to [8], composed of a single polynucleotide having both the sense and antisense strands linked by an intervening single-strand;

[10] The double-stranded molecule of [9], having the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10, [B] is the intervening single-strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to the target sequence selected in [A];

[11] The double-stranded molecule of any one of [1] to [10], composed of RNA;

[12] The double-stranded molecule of any one of [1] to [10], composed of both DNA and RNA;

[13] The double-stranded molecule of [12], wherein the molecule is a hybrid of a DNA polynucleotide and an RNA polynucleotide;

[14] The double-stranded molecule of [13] wherein the sense and the antisense strands are composed of DNA and RNA, respectively;

[15] The double-stranded molecule of [12], wherein the molecule is a chimera of DNA and RNA;

[16] The double-stranded molecule of [15], wherein a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are RNA;

[17] The double-stranded molecule of [16], wherein the flanking region is composed of 9 to 13 nucleotides; and

[18] The double-stranded molecule of any one of [1] to [17], wherein the molecule contains one or two 3' overhang(s); and

[19] The double-stranded molecule of [3], wherein the sense strand hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between 19 and 25 nucleotide pair in length.

[0122] The double-stranded molecule of the present invention will be described in more detail below.

Methods for designing double-stranded molecules having the ability to inhibit target gene expression in cells are known (See, for example, US Patent No. 6,506,559, herein incorporated by reference in its entirety). For example, a computer program for designing siRNAs is available from the Ambion website (on the worldwide web at ambion.com/techlib/misc/siRNA_finder.html).

The computer program selects target nucleotide sequences for double-stranded molecules based on the following protocol.

[0123] Selection of Target Sites:

1. Beginning with the AUG start codon of the transcript, scan downstream for AA di-nucleotide sequences. Record the occurrence of each AA and the 3' adjacent 19 nucleotides as potential siRNA target sites. Tuschl et al. recommend to avoid designing siRNA to the 5' and 3' untranslated regions (UTRs) and regions near the start codon (within 75 bases) as these may be richer in regulatory protein binding sites, and UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex.

2. Compare the potential target sites to the appropriate genome database (human, mouse, rat, etc.) and eliminate from consideration any target sequences with significant homology to other coding sequences. Basically, BLAST, which can be found on the NCBI server at: www.ncbi.nlm.nih.gov/BLAST/, is used (Altschul SF et al., Nucleic Acids Res 1997 Sep 1, 25(17): 3389-402).

3. Select qualifying target sequences for synthesis. Selecting several target sequences along the length of the gene to evaluate is typical.

[0124] Using the above protocol, the target sequences of the double-stranded molecules against CSTF2 gene were designed as SEQ ID NOs: 9 and 10.

[0125] Double-stranded molecules targeting the above-mentioned target sequences were respectively examined for their ability to suppress the growth of cells expressing the target gene. Therefore, the present invention provides double-stranded molecules targeting the sequences selected from the group consisting of SEQ ID NO: 9 and 10.

[0126] Examples of double-stranded molecules that target the above-mentioned target sequence of the CSTF2 gene include isolated polynucleotides that contain the nucleic acid sequences corresponding to target sequences and/or complementary sequences to the target sequences. Preferred examples of polynucleotides targeting the CSTF2 gene include those containing the sequence corresponding to SEQ ID NO: 9 or 10 and/or complementary sequences to these sequences. In an embodiment, a double-stranded molecule is composed of two polynucleotides, one polynucleotide has a sequence corresponding to a target sequence, i.e., sense strand, and another polypeptide has a complementary sequence to the target sequence, i.e., antisense strand. The sense strand polynucleotide and the antisense strand polynucleotide hybridize to each other to form double-stranded molecule. Examples of such double-stranded molecules include dsRNA and dsD/R-NA.

[0127] In an another embodiment, a double-stranded molecule is composed of a polynucleotide that has both a sequence corresponding to a target sequence, i.e., sense strand, and a complementary sequence to the target sequence, i.e., antisense strand. Generally, the sense strand and the antisense strand are linked by a intervening strand, and hybridize to each other to form a hairpin loop structure. Examples of such double-stranded molecule include shRNA and shD/R-NA.

[0128] In other words, a double-stranded molecule of the present invention comprises a sense strand polynucleotide having a nucleotide sequence of the target sequence and anti-sense strand polynucleotide having a nucleotide sequence complementary to the target sequence, and both of polynucleotides hybridize to each other to form the double-stranded molecule. In the double-stranded molecule comprising the polynucleotides, a part of the polynucleotide of either or both of the strands may be RNA, and when the target sequence is defined with a DNA sequence, the nucleotide "t" within the target sequence and complementary sequence thereto is replaced with "u".

[0129] In one embodiment of the present invention, such a double-stranded molecule of the present invention comprises a stem-loop structure, composed of the sense and antisense strands. The sense and antisense strands may be joined by a loop. Accordingly, the present invention also provides the double-stranded molecule

comprising a single polynucleotide containing both the sense strand and the antisense strand linked or flanked by an intervening single-strand.

[0130] In the present invention, double-stranded molecules targeting the CSTF2 gene may have a sequence selected from among SEQ ID NOs: 9 and 10 as a target sequence. Accordingly, preferable examples of the double-stranded molecule of the present invention include polynucleotides that hybridize to each other at a sequence corresponding to SEQ ID NO: 9 or 10 and a complementary sequence thereto, and a polynucleotide that has a sequence corresponding to SEQ ID NO: 9 or 10 and a complementary sequence thereto.

[0131] The double-stranded molecule of the present invention may be directed to a single target CSTF2 gene sequence or may be directed to a plurality of target CSTF2 gene sequences.

[0132] A double-stranded molecule of the present invention targeting the above-mentioned targeting sequence of a CSTF2 gene include isolated polynucleotides that contain any of the nucleic acid sequences of target sequences and/or complementary sequences to the target sequences. Examples of polynucleotides targeting a CSTF2 gene include those containing the sequence of SEQ ID NO: 9 or 10 and/or complementary sequences to these nucleotides. However, the present invention is not limited to these examples, and minor modifications in the aforementioned nucleic acid sequences are acceptable so long as the modified molecule retains the ability to suppress the expression of a CSTF2 gene. Herein, the phrase "minor modification" as used in connection with a nucleic acid sequence indicates one, two or several substitution, deletion, addition or insertion of nucleic acids to the sequence.

[0133] In the context of the present invention, the term "several" as applies to nucleic acid substitutions, deletions, additions and/or insertions may mean 3-7, preferably 3-5, more preferably 3-4, even more preferably 3 nucleic acid residues.

[0134] According to the present invention, a double-stranded molecule of the present invention can be tested for its ability using the methods utilized in the Examples. In the Examples herein below, double-stranded molecules composed of sense strands of various portions of mRNA of the CSTF2 gene or antisense strands complementary thereto were tested in vitro for their ability to decrease production of a CSTF2 gene product in lung cancer cell lines (e.g., using A549 and SBC-5) according to standard methods. Furthermore, for example, reduction in the CSTF2 gene product in cells contacted with the candidate double-stranded molecule compared to cells cultured in the absence of the candidate molecule can be detected by, e.g., RT-PCR using primers for a CSTF2 mRNA mentioned under Example: "Semi-quantitative RT-PCR". Sequences which decrease the production of the CSTF2 gene product in vitro cell-based assays can then be tested for there inhibitory effects on cell growth. Sequences

which inhibit cell growth in vitro cell-based assay can then be tested for their in vivo ability using animals with cancer, e.g., nude mouse xenograft models, to confirm decreased production of the CSTF2 gene product and decreased cancer cell growth.

[0135] When the isolated polynucleotide is RNA or derivatives thereof, base "t" should be replaced with "u" in the nucleotide sequences. As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a polynucleotide, and the term "binding" means the physical or chemical interaction between two polynucleotides. When the polynucleotide includes modified nucleotides and/or non-phosphodiester linkages, these polynucleotides may also bind each other as same manner. Generally, complementary polynucleotide sequences hybridize under appropriate conditions to form stable duplexes containing few or no mismatches. Furthermore, the sense strand and antisense strand of the isolated polynucleotide of the present invention can form double-stranded molecule or hairpin loop structure by the hybridization. In a preferred embodiment, such duplexes contain no more than 1 mismatch for every 10 matches. In an especially preferred embodiment, where the strands of the duplex are fully complementary, such duplexes contain no mismatches.

[0136] The polynucleotide is preferably less than 1009 nucleotides in length for CSTF2. For example, the polynucleotide is less than 500, 200, 100, 75, 50, or 25 nucleotides in length for the gene. The isolated polynucleotides of the present invention are useful for forming double-stranded molecules against the CSTF2 gene or preparing template DNAs encoding the double-stranded molecules. When the polynucleotides are used for forming double-stranded molecules, the polynucleotide may be longer than 19 nucleotides, preferably longer than 21 nucleotides, and more preferably has a length of between about 19 and 25 nucleotides. Accordingly, the present invention provides the double-stranded molecules comprising a sense strand and an antisense strand, wherein the sense strand comprises a nucleotide sequence corresponding to a target sequence. In preferable embodiments, the sense strand hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between 19 and 25 nucleotide pair in length.

[0137] The double-stranded molecule serves as a guide for identifying homologous sequences in mRNA for the RNA-induced silencing complex (RISC), when the double-stranded molecule is introduced into cells. The identified target RNA is cleaved and degraded by the nuclease activity of Dicer, through which the double-stranded molecule eventually decreases or inhibits production (expression) of the polypeptide encoded by the RNA. Thus, a double-stranded molecule of the invention can be defined by its ability to generate a single-strand that specifically hybridizes to the mRNA of the CSTF2 gene under stringent conditions. Herein, the portion of the

mRNA that hybridizes with the single-strand generated from the double-stranded molecule is referred to as "target sequence" or "target nucleic acid" or "target nucleotide". In the present invention, nucleotide sequence of the "target sequence" can be shown using not only the RNA sequence of the mRNA, but also the DNA sequence of cDNA synthesized from the mRNA.

[0138] The double-stranded molecules of the invention may contain one or more modified nucleotides and/or non-phosphodiester linkages. Chemical modifications well known in the art are capable of increasing stability, availability, and/or cell uptake of the double-stranded molecule. The skilled person will be aware of other types of chemical modification which may be incorporated into the present molecules (WO03/070744; WO2005/045037). In one embodiment, modifications can be used to provide improved resistance to degradation or improved uptake. Examples of such modifications include, but are not limited to, phosphorothioate linkages, 2'-O-methyl ribonucleotides (especially on the sense strand of a double-stranded molecule), 2'-deoxy-fluoro ribonucleotides, 2'-deoxy ribonucleotides, "universal base" nucleotides, 5'-C- methyl nucleotides, and inverted deoxybasic residue incorporation (US20060122137).

[0139] In another embodiment, modifications can be used to enhance the stability or to increase targeting efficiency of the double-stranded molecule. Examples of such modifications include, but are not limited to, chemical cross linking between the two complementary strands of a double-stranded molecule, chemical modification of a 3' or 5' terminus of a strand of a double-stranded molecule, sugar modifications, nucleobase modifications and/or backbone modifications, 2-fluoro modified ribonucleotides and 2'-deoxy ribonucleotides (WO2004/029212). In another embodiment, modifications can be used to increased or decreased affinity for the complementary nucleotides in the target mRNA and/or in the complementary double-stranded molecule strand (WO2005/044976). For example, an unmodified pyrimidine nucleotide can be substituted for a 2-thio, 5-alkynyl, 5-methyl, or 5-propynyl pyrimidine. Additionally, an unmodified purine can be substituted with a 7-deaza, 7-alkyl, or 7-alkenyl purine. In another embodiment, when the double-stranded molecule is a double-stranded molecule with a 3' overhang, the 3'- terminal nucleotide overhanging nucleotides may be replaced by deoxyribonucleotides (Elbashir SM et al., Genes Dev 2001 Jan 15, 15(2): 188-200). For further details, published documents such as US20060234970 are available. The present invention is not limited to these examples and any known chemical modifications may be employed for the double-stranded molecules of the present invention so long as the resulting molecule retains the ability to inhibit the expression of the target gene.

[0140] Furthermore, the double-stranded molecules of the invention may include both DNA and RNA, e.g., dsD/R-NA or shD/R-NA. Specifically, a hybrid polynucleotide of a

DNA strand and an RNA strand or a DNA-RNA chimera polynucleotide shows increased stability. Mixing of DNA and RNA, i.e., a hybrid type double-stranded molecule composed of a DNA strand (polynucleotide) and an RNA strand (polynucleotide), a chimera type double-stranded molecule containing both DNA and RNA on any or both of the single strands (polynucleotides), or the like may be formed for enhancing stability of the double-stranded molecule.

[0141] The hybrid of a DNA strand and an RNA strand may have the structures either where the sense strand is DNA and the antisense strand is RNA, or the opposite so long as it can inhibit expression of the target gene when introduced into a cell expressing the gene. Preferably, the sense strand polynucleotide is DNA and the antisense strand polynucleotide is RNA. Also, the chimera type double-stranded molecule may have the structures either where both of the sense and antisense strands are composed of DNA and RNA, or where any one of the sense and antisense strands is composed of DNA and RNA so long as it has an activity to inhibit expression of the target gene when introduced into a cell expressing the gene. In order to enhance stability of the double-stranded molecule, the molecule preferably contains as much DNA as possible, whereas to induce inhibition of the target gene expression, the molecule is required to be RNA within a range to induce sufficient inhibition of the expression.

[0142] As a preferred example of the chimera type double-stranded molecule, an upstream partial region (i.e., a region flanking to the target sequence or complementary sequence thereof within the sense or antisense strands) of the double-stranded molecule is RNA. Preferably, the upstream partial region indicates the 5' side (5'-end) of the sense strand and the 3' side (3'-end) of the antisense strand. Alternatively, regions flanking to 5'-end of sense strand and/or 3'-end of antisense strand are referred to upstream partial region. That is, in preferable embodiments, a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are composed of RNA. For instance, the chimera or hybrid type double-stranded molecule of the present invention include following combinations.

sense strand:
5'-[---DNA---]-3'
3'-(RNA)-[DNA]-5'
:antisense strand,
sense strand:
5'-(RNA)-[DNA]-3'
3'-(RNA)-[DNA]-5'
:antisense strand, and
sense strand:

5'-(RNA)-[DNA]-3'
3'-(---RNA---)-5'
:antisense strand.

[0143] The upstream partial region preferably is a domain composed of 9 to 13 nucleotides counted from the terminus of the target sequence or complementary sequence thereto within the sense or antisense strands of the double-stranded molecules. Moreover, preferred examples of such chimera type double-stranded molecules include those having a strand length of 19 to 21 nucleotides in which at least the upstream half region (5' side region for the sense strand and 3' side region for the antisense strand) of the polynucleotide is RNA and the other half is DNA. In such a chimera type double-stranded molecule, the effect to inhibit expression of the target gene is much higher when the entire antisense strand is RNA (US20050004064).

[0144] In the present invention, the double-stranded molecule may form a hairpin, such as a short hairpin RNA (shRNA) and short hairpin consisting of DNA and RNA (shD/R-NA). The shRNA or shD/R-NA is a sequence of RNA or mixture of RNA and DNA making a tight hairpin turn that can be used to silence gene expression via RNA interference. The shRNA or shD/R-NA includes the sense target sequence and the antisense target sequence on a single strand wherein the sequences are separated by a loop sequence. Generally, the hairpin structure is cleaved by the cellular machinery into dsRNA or dsD/R-NA, which is then bound to RISC. This complex binds to and cleaves mRNAs which match the target sequence of the dsRNA or dsD/R-NA.

[0145] A loop sequence composed of an arbitrary nucleotide sequence can be located between the sense and antisense sequence in order to form the hairpin loop structure. Thus, the present invention also provides a double-stranded molecule having the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence, [B] is an intervening single-strand and [A'] is the antisense strand containing a complementary sequence to the target sequence. The target sequence may be selected from among, for example, nucleotide sequence of SEQ ID NOs: 9 and 10.

[0146] The present invention is not limited to these examples, and the target sequence in [A] may be modified sequences from these examples so long as the double-stranded molecule retains the ability to suppress the expression of the targeted CSTF2 gene. The region [A] hybridizes to [A'] to form a loop composed of the region [B]. The intervening single-stranded portion [B], i.e., loop sequence may be preferably 3 to 23 nucleotides in length. The loop sequence, for example, can be selected from among the following sequences (http://www.ambion.com/techlib/tb/tb_506.html). Furthermore, loop sequence consisting of 23 nucleotides also provides active siRNA (Jacque JM et al., Nature 2002 Jul 25, 418(6896): 435-8, Epub 2002 Jun 26):

CCC, CCACC, or CCACACC: Jacque JM et al., Nature 2002 Jul 25, 418(6896): 435-8, Epub 2002 Jun 26;
UUCG: Lee NS et al., Nat Biotechnol 2002 May, 20(5): 500-5; Fruscoloni P et al., Proc Natl Acad Sci USA 2003 Feb 18, 100(4): 1639-44, Epub 2003 Feb 10; and
UUCAAGAGA: Dykxhoorn DM et al., Nat Rev Mol Cell Biol 2003 Jun, 4(6): 457-67.

[0147] Examples of preferred double-stranded molecules of the present invention having hairpin loop structure are shown below. In the following structure, the loop sequence can be selected from among AUG, CCC, UUCG, CCACC, CTCGAG, AAGCUU, CCACACC, and UUCAAGAGA; however, the present invention is not limited thereto:

GGCUUUAGUCCCGGGCAGA-[B]-UCUGCCCGGGACUAAAGCC
(for target sequence SEQ ID NO: 9);
CACUUUACUUUCUGUAACU-[B]- AGUUACAGAAAGUAAAGUG
(for target sequence SEQ ID NO: 10);

[0148] Furthermore, in order to enhance the inhibition activity of the double-stranded molecules, several nucleotides can be added to 3 'end of the sense strand and/or antisense strand of the target sequence, as 3' overhangs. The number of nucleotides to be added is at least 2, generally 2 to 10, preferably 2 to 5. The added nucleotides form single strand at the 3 'end of the antisense strand of the double-stranded molecule. The nucleotides for 3' overhang are preferably "u" or "t", but are not limited to. When the double-stranded molecule has a harpin loop structure, a 3' overhang is added to the 3' end of the antisense strand.

[0149] The method for preparing the double-stranded molecule is not particularly limited though it is preferable to use a chemical synthetic method known in the art. According to the chemical synthesis method, sense and antisense single-stranded polynucleotides are separately synthesized and then annealed together via an appropriate method to obtain a double-stranded molecule. Specific example for the annealing includes wherein the synthesized single-stranded polynucleotides are mixed in a molar ratio of preferably at least about 3:7, more preferably about 4:6, and most preferably substantially equimolar amount (i.e., a molar ratio of about 5:5). Next, the mixture is heated to a temperature at which double-stranded molecules dissociate and then is gradually cooled down. The annealed double-stranded polynucleotide can be purified by usually employed methods known in the art. Example of purification methods include methods utilizing agarose gel electrophoresis or wherein remaining single-stranded polynucleotides are optionally removed by, e.g., degradation with appropriate enzyme.

[0150] The regulatory sequences flanking CSTF2 sequences may be identical or different, such that their expression can be modulated independently, or in a temporal or spatial

manner. The double-stranded molecules can be transcribed intracellularly by cloning CSTF2 gene templates into a vector containing, e.g., a RNA pol III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter.

[0151] Alternatively, the double-stranded molecules may be transcribed intracellularly by cloning its coding sequence into a vector containing a regulatory sequence that directs the expression of the double-stranded molecule in an adequate cell (e.g., a RNA poly III transcription unit from the small nuclear RNA (snRNA) U6 or the human H1 RNA promoter) adjacent to the coding sequence. The regulatory sequences flanking the coding sequences of double-stranded molecule may be identical or different, such that their expression can be modulated independently, or in a temporal or spatial manner. Details of vectors which are capable of producing the double-stranded molecules will be described below.

[0152] Vectors encoding a double-stranded molecule of the present invention:

Also included in the present invention are vectors encoding one or more of the double-stranded molecules described herein, and a cell containing such a vector.

[0153] Specifically, the present invention provides the following vector of [1] to [10].

[1] A vector, encoding a double-stranded molecule that, when introduced into a cell, inhibits in vivo expression of CSTF2 gene and cell proliferation, wherein the double-stranded molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule.

[2] The vector of [1], encoding the double-stranded molecule acts on mRNA, matching a target sequence selected from among SEQ ID NO: 9 and SEQ ID NO: 10;

[3] The vector of [1], wherein the sense strand contains a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10;

[4] The vector of any one of [1] to [3], encoding the double-stranded molecule, wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 100 nucleotide pair in length;

[5] The vector of [4], encoding the double-stranded molecule, wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 75 nucleotide pair in length;

[6] The vector of [5], encoding the double-stranded molecule, wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having a length of less than about 50 nucleotide pair in length;

[7] The vector of [6] encoding the double-stranded molecule having, wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the

target sequence to form the double-stranded molecule a length of less than about 25 nucleotide pair in length;

[8] The vector of [7], encoding the double-stranded molecule, wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having a length of between about 19 and about 25 nucleotide pair in length;

[9] The vector of any one of [1] to [8], wherein the double-stranded molecule is composed of a single polynucleotide having both the sense and antisense strands linked by an intervening single-strand;

[10] The vector of [9], encoding the double-stranded molecule having the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10, [B] is the intervening single-strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to the target sequence selected in [A].

[0154] A vector of the present invention preferably encodes a double-stranded molecule of the present invention in an expressible form. Herein, the phrase "in an expressible form" indicates that the vector, when introduced into a cell, will express the molecule. In a preferred embodiment, the vector includes regulatory elements necessary for expression of the double-stranded molecule. Accordingly, in one embodiment, the expression vector encodes the nucleic acid sequences of the present invention and is adapted for expression of said nucleic acid sequences. Such vectors of the present invention may be used for producing the present double-stranded molecules, or directly as an active ingredient for treating cancer.

[0155] Vectors of the present invention can be produced, for example, by cloning the sequences encoding a sense and antisense strand of the double-stranded molecule against CSTF2 gene into an expression vector so that regulatory sequences are operatively-linked to the sequences encoding the strands in a manner to allow expression (by transcription of the DNA molecule) of both strands (Lee NS et al., Nat Biotechnol 2002 May, 20(5): 500-5). For example, RNA molecule that is the antisense to mRNA is transcribed by a first promoter (e.g., a promoter sequence flanking to the 3' end of the cloned DNA) and RNA molecule that is the sense strand to the mRNA is transcribed by a second promoter (e.g., a promoter sequence flanking to the 5' end of the cloned DNA). The sense and antisense strands hybridize in vivo to generate a double-stranded molecule constructs for silencing of the gene. Alternatively, two vectors constructs respectively encoding the sense and antisense strands of the double-stranded molecule are utilized to respectively express the sense and anti-sense strands and then forming a double-stranded molecule construct. Furthermore, the cloned sequence may encode a construct having a secondary structure (e.g., hairpin); namely,

a single transcript of a vector contains both the sense and complementary antisense sequences of the target gene.

[0156] The vectors of the present invention may also be equipped to achieve stable insertion into the genome of the target cell (see, e.g., Thomas KR & Capecchi MR, Cell 1987, 51: 503-12 for a description of homologous recombination cassette vectors). See, e.g., Wolff et al., Science 1990, 247: 1465-8; US Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., US Patent No. 5,922,687).

[0157] The vectors of the present invention include, for example, viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox (see, e.g., US Patent No. 4,722,848). This approach involves the use of vaccinia virus, e.g., as a vector to express nucleotide sequences that encode the double-stranded molecule. Upon introduction into a cell expressing the target gene, the recombinant vaccinia virus expresses the molecule and thereby suppresses the proliferation of the cell. Another example of useable vector includes Bacille Calmette Guerin (BCG). BCG vectors are described in Stover et al., Nature 1991, 351: 456-60. A wide variety of other vectors are useful for therapeutic administration and production of the double-stranded molecules; examples include adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like. See, e.g., Shata et al., Mol Med Today 2000, 6: 66-71; Shedlock et al., J Leukoc Biol 2000, 68: 793-806; and Hipp et al., In Vivo 2000, 14: 571-85.

[0158] Methods of inhibiting or reducing growth of a cancer cell or treating cancer using a double-stranded molecule of the present invention:

The present invention provides methods for inhibiting cancer cell growth, e.g., lung cancer cell growth, by inducing dysfunction of a CSTF2 gene via inhibiting the expression of CSTF2 gene. The CSTF2 gene expression can be inhibited by any of the aforementioned double-stranded molecules of the present invention which specifically target the CSTF2 gene or the vectors of the present invention that can express at lease one of the double-stranded molecules.

[0159] Such ability of the present double-stranded molecules and vectors to inhibit cell growth of cancerous cell indicates that they can be used for methods for treating cancer. Thus, the present invention provides methods to treat patients with lung cancer by administering a double-stranded molecule against a CSTF2 gene or a vector expressing the molecule without adverse effect because that the gene were hardly detected in normal organs.

[0160] Specifically, the present invention provides the following methods [1] to [36]:

[1] A method of treating or preventing cancer in a subject, comprising administering to a subject a pharmaceutically effective amount of a double-stranded molecule against a CSTF2 gene or a vector encoding the double-stranded molecule, wherein the double-stranded molecule, when introduced into a cell, inhibits an expression of the CSTF2 gene;

[2] The method of [1], wherein the double-stranded molecule acts on mRNA which matches a target sequence selected from among SEQ ID NO: 9 and SEQ ID NO: 10;

[3] The method of [1], wherein the sense strand contains a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10;

[4] The method of any one of [1] to [3], wherein plural kinds of the double-stranded molecules are administered;

[5] The method of any one of [1] to [4], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 100 nucleotides pairs in length;

[6] The method of [5], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 75 nucleotide pairs in length;

[7] The method of [6], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 50 nucleotide pairs in length;

[8] The method of [7], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 25 nucleotide pairs in length;

[9] The method of [9], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between about 19 and about 25 nucleotide pairs in length;

[10] The method of any one of [1] to [9], wherein the double-stranded molecule is composed of a single polynucleotide containing both the sense strand and the antisense strand linked by an intervening single-strand;

[11] The method of [10], wherein the double-stranded molecule has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10, [B] is the intervening single strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to the target sequence selected in [A];

[12] The method of any one of [1] to [11], wherein the double-stranded molecule is an RNA;

[13] The method of any one of [1] to [11], wherein the double-stranded molecule

contains both DNA and RNA;

[14] The method of [13], wherein the double-stranded molecule is a hybrid of a DNA polynucleotide and an RNA polynucleotide;

[15] The method of [14], wherein the sense and antisense strand polynucleotides are composed of DNA and RNA, respectively;

[16] The method of [13], wherein the double-stranded molecule is a chimera of DNA and RNA;

[17] The method of [16], wherein a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are composed of RNA;

[18] The method of [17], wherein the flanking region is composed of 9 to 13 nucleotides;

[19] The method of any one of [1] to [18], wherein the double-stranded molecule contains one or more 3' overhang(s);

[20] The method of any one of [1] to [19], wherein the double-stranded molecule is contained in a composition which includes, in addition to the molecule, a transfection-enhancing agent and pharmaceutically acceptable carrier.

[21] The method of any one of [1] to [20], wherein the double-stranded molecule is encoded by a vector;

[22] The method of [21], wherein the double-stranded molecule encoded by the vector acts on mRNA which matches a target sequence selected from among SEQ ID NO: 9 and SEQ ID NO: 10.

[23] The method of [21], wherein the sense strand of the double-stranded molecule encoded by the vector contains the sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10.

[24] The method of any one of [21] to [23], wherein plural kinds of the double-stranded molecules are administered;

[25] The method of any one of [21] to [24], wherein the sense strand of the double-stranded molecule encoded by the vector has a length of less than about 100 nucleotides pair in lengths;

[26] The method of [25], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 75 nucleotides pair in length;

[27] The method of [26], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 50 nucleotides pair in length;

[28] The method of [27], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded

molecule having has a length of less than about 25 nucleotides pair in length;
[29] The method of [28], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between about 19 and about 25 nucleotides pair in length;
[30] The method of any one of [21] to [29], wherein the double-stranded molecule encoded by the vector is composed of a single polynucleotide containing both the sense strand and the antisense strand linked by an intervening single-strand;
[31] The method of [30], wherein the double-stranded molecule encoded by the vector has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10, [B] is a intervening single-strand is composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to the target sequence selected in [A];
[34] The method of any one of [21] to [31], wherein the double-stranded molecule encoded by the vector is contained in a composition which includes, in addition to the molecule, a transfection-enhancing agent and pharmaceutically acceptable carrier; and
[35] The method of any one of [1] to [34], wherein the cancer is lung cancer.
[36] The method of [35], wherein the lung cancer is adenocarcinoma, squamous cell carcinoma, large cell carcinoma or small cell lung carcinoma.

[0161] The method of the present invention will be described in more detail below.

The growth of cells expressing a CSTF2 gene may be inhibited by contacting the cells with a double-stranded molecule against the CSTF2 gene, a vector expressing the molecule or a composition containing the same. The cell may be further contacted with a transfection agent. Suitable transfection agents are known in the art. The phrase "inhibition of cell growth" indicates that the cell proliferates at a lower rate or has decreased viability as compared to a cell not exposed to the molecule. Cell growth may be measured by methods known in the art, e.g., using the MTT cell proliferation assay.

[0162] The growth of any kind of cell may be suppressed according to the present method so long as the cell expresses or over-expresses the target gene of the double-stranded molecule of the present invention. Exemplary cells include lung cancer cells such as NSCLC and SCLC.

[0163] Thus, patients suffering from or at risk of developing disease related to CSTF2, e.g., cancer may be treated by administering at least one of the present double-stranded molecules, at least one vector expressing at least one of the molecules or at least one composition containing at least one of the molecules. For example, patients with lung cancer may be treated according to the present methods. The type of cancer may be identified by standard methods according to the particular type of tumor to be diagnosed. Lung cancer may be diagnosed, for example, with Carcinoembryonic

antigen (CEA), CYFRA, pro-GRP and so on, as lung cancer marker, or with Chest X-Ray and/or Sputum Cytology. More preferably, patients treated by the methods of the present invention are selected by detecting the expression of CSTF2 gene in a biopsy specimen obtained from the patient by methods known in the art, for example, RT-PCR or immunoassay. Preferably, before the treatment of the present invention, the biopsy specimen from the subject is confirmed for a CSTF2 gene over-expression by methods known in the art, for example, immunohistochemical analysis or RT-PCR.

[0164] According to the present method to inhibit cell growth and thereby treating cancer, when administering plural kinds of the double-stranded molecules (or vectors expressing or compositions containing the same), each of the molecules may have different structures but acts on mRNA which matches the same target sequence. Alternatively plural kinds of the double-stranded molecules may acts on mRNA which matches different target sequence of same gene or acts on mRNA which matches different target sequence of different gene. For example, the method may utilize double-stranded molecules directed to different target sequences of CSTF2 gene. Alternatively, for example, the method may utilize double-stranded molecules directed to one, two or more target sequence of CSTF2 gene and other genes.

[0165] For inhibiting cell growth, a double-stranded molecule of present invention may be directly introduced into the cells in a form to achieve binding of the molecule with corresponding mRNA transcripts. Alternatively, as described above, a DNA encoding the double-stranded molecule may be introduced into cells as a vector. For introducing the double-stranded molecules and vectors into the cells, transfection-enhancing agent, such as FuGENE (Roche diagnostics), Lipofectamine 2000 (Invitrogen), Oligofectamine (Invitrogen), and Nucleofector (Wako pure Chemical), may be employed.

[0166] A treatment is deemed "efficacious" if it leads to clinical benefit such as, reduction in expression of a CSTF2 gene, or a decrease in size, prevalence, or metastatic potential of the cancer in the subject. When the treatment is applied prophylactically, "efficacious" means that it retards or prevents cancers from forming or prevents or alleviates a clinical symptom of cancer. Efficaciousness is determined in association with any known method for diagnosing or treating the particular tumor type.

[0167] To the extent that the methods and compositions of the present invention find utility in the context of "prevention" and "prophylaxis", such terms are interchangeably used herein to refer to any activity that reduces the burden of mortality or morbidity from disease. Prevention and prophylaxis can occur "at primary, secondary and tertiary prevention levels." While primary prevention and prophylaxis avoid the development of a disease, secondary and tertiary levels of prevention and prophylaxis encompass activities aimed at the prevention and prophylaxis of the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already es-

tablished disease by restoring function and reducing disease-related complications. Alternatively, prevention and prophylaxis can include a wide range of prophylactic therapies aimed at alleviating the severity of the particular disorder, e.g. reducing the proliferation and metastasis of tumors.

[0168] The treatment and/or prophylaxis of cancer and/or the prevention of postoperative recurrence thereof include any of the following steps, such as the surgical removal of cancer cells, the inhibition of the growth of cancerous cells, the involution or regression of a tumor, the induction of remission and suppression of occurrence of cancer, the tumor regression, and the reduction or inhibition of metastasis. Effectively treating and/or the prophylaxis of cancer decreases mortality and improves the prognosis of individuals having cancer, decreases the levels of tumor markers in the blood, and alleviates detectable symptoms accompanying cancer. For example, reduction or improvement of symptoms constitutes effectively treating and/or the prophylaxis include 10%, 20%, 30% or more reduction, or stable disease.

[0169] It is understood that the double-stranded molecule of the invention degrades the CSTF2 mRNA in substoichiometric amounts. Without wishing to be bound by any theory, it is believed that the double-stranded molecule of the invention causes degradation of the target mRNA in a catalytic manner. Thus, compared to standard cancer therapies, significantly less a double-stranded molecule needs to be delivered at or near the site of cancer to exert therapeutic effect.

[0170] One skilled in the art can readily determine an effective amount of the double-stranded molecule of the invention to be administered to a given subject, by taking into account factors such as body weight, age, sex, type of disease, symptoms and other conditions of the subject; the route of administration; and whether the administration is regional or systemic. Generally, an effective amount of the double-stranded molecule of the invention is an intercellular concentration at or near the cancer site of from about 1 nanomolar (nM) to about 100 nM, preferably from about 2 nM to about 50 nM, more preferably from about 2.5 nM to about 10 nM. It is contemplated that greater or smaller amounts of the double-stranded molecule can be administered. The precise dosage required for a particular circumstance may be readily and routinely determined by one of skill in the art.

[0171] The present methods can be used to inhibit the growth or metastasis of cancer expressing CSTF2 gene; for example lung cancer, including NSCLC and SCLC. In particular, a double-stranded molecule containing a target sequence of SEQ ID NO: 9 or 10 is particularly preferred for the treatment of lung cancer.

[0172] For treating cancer, the double-stranded molecule of the invention can also be administered to a subject in combination with a pharmaceutical agent different from the double-stranded molecule. Alternatively, the double-stranded molecule of the

invention can be administered to a subject in combination with another therapeutic method designed to treat cancer. For example, the double-stranded molecule of the invention can be administered in combination with therapeutic methods currently employed for treating cancer or preventing cancer metastasis (e.g., radiation therapy, surgery and treatment using chemotherapeutic agents, such as cisplatin, carboplatin, cyclophosphamide, 5-fluorouracil, adriamycin, daunorubicin or tamoxifen).

[0173] In the present methods, the double-stranded molecule can be administered to the subject either as a naked double-stranded molecule, in conjunction with a delivery substance, or as a recombinant plasmid or viral vector which expresses the double-stranded molecule.

[0174] Suitable delivery substances for administration in conjunction with the present double-stranded molecule include the Mirus Transit TKO lipophilic substance; lipofectin; lipofectamine; cellfectin; or polycations (e.g., polylysine), or liposomes. A preferred delivery substance is a liposome.

[0175] Liposomes can aid in the delivery of the double-stranded molecule to a particular tissue, such as lung tumor tissue, and can also increase the blood half-life of the double-stranded molecule. Liposomes suitable for use in the invention are formed from standard vesicle-forming lipids, which generally include neutral or negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of factors such as the desired liposome size and half-life of the liposomes in the blood stream. A variety of methods are known for preparing liposomes, for example as described in Szoka et al., Ann Rev Biophys Bioeng 1980, 9: 467; and US Pat. Nos. 4,235,871; 4,501,728; 4,837,028; and 5,019,369, the entire disclosures of which are herein incorporated by reference.

[0176] Preferably, the liposomes encapsulating the present double-stranded molecule includes a ligand molecule that can deliver the liposome to the cancer site. Ligands which bind to receptors prevalent in tumor or vascular endothelial cells, such as monoclonal antibodies that bind to tumor antigens or endothelial cell surface antigens, are preferred.

[0177] Particularly preferably, the liposomes encapsulating the present double-stranded molecule are modified so as to avoid clearance by the mononuclear macrophage and reticuloendothelial systems, for example, by having opsonization-inhibition moieties bound to the surface of the structure. In one embodiment, a liposome of the invention can include both opsonization-inhibition moieties and a ligand.

[0178] Opsonization-inhibiting moieties for use in preparing the liposomes of the invention are typically large hydrophilic polymers that are bound to the liposome membrane. As used herein, an opsonization inhibiting moiety is "bound" to a liposome membrane when it is chemically or physically attached to the membrane, e.g., by the intercalation

of a lipid-soluble anchor into the membrane itself, or by binding directly to active groups of membrane lipids. These opsonization-inhibiting hydrophilic polymers form a protective surface layer which significantly decreases the uptake of the liposomes by the macrophage-monocyte system ("MMS") and reticuloendothelial system ("RES"); e.g., as described in US Pat. No. 4,920,016, the entire disclosure of which is herein incorporated by reference. Liposomes modified with opsonization-inhibition moieties thus remain in the circulation much longer than unmodified liposomes. For this reason, such liposomes are sometimes called "stealth" liposomes.

[0179] Stealth liposomes are known to accumulate in tissues fed by porous or "leaky" microvasculature. Thus, target tissue characterized by such microvasculature defects, for example, solid tumors, will efficiently accumulate these liposomes; see Gabizon et al., Proc Natl Acad Sci USA 1988, 18: 6949-53. In addition, the reduced uptake by the RES lowers the toxicity of stealth liposomes by preventing significant accumulation in liver and spleen. Thus, liposomes of the invention that are modified with opsonization-inhibition moieties can deliver the present double-stranded molecule to tumor cells.

[0180] Opsonization inhibiting moieties suitable for modifying liposomes are preferably water-soluble polymers with a molecular weight from about 500 to about 40,000 daltons, and more preferably from about 2,000 to about 20,000 daltons. Such polymers include polyethylene glycol (PEG) or polypropylene glycol (PPG) derivatives; e.g., methoxy PEG or PPG, and PEG or PPG stearate; synthetic polymers such as polyacrylamide or poly N-vinyl pyrrolidone; linear, branched, or dendrimeric polyamidoamines; polyacrylic acids; polyalcohols, e.g., polyvinylalcohol and polyxylitol to which carboxylic or amino groups are chemically linked, as well as gangliosides, such as ganglioside GM1. Copolymers of PEG, methoxy PEG, or methoxy PPG, or derivatives thereof, are also suitable. In addition, the opsonization inhibiting polymer can be a block copolymer of PEG and either a polyamino acid, polysaccharide, polyamidoamine, polyethyleneamine, or polynucleotide. The opsonization inhibiting polymers can also be natural polysaccharides containing amino acids or carboxylic acids, e.g., galacturonic acid, glucuronic acid, mannuronic acid, hyaluronic acid, pectic acid, neuraminic acid, alginic acid, carrageenan; aminated polysaccharides or oligosaccharides (linear or branched); or carboxylated polysaccharides or oligosaccharides, e.g., reacted with derivatives of carbonic acids with resultant linking of carboxylic groups.

[0181] Preferably, the opsonization-inhibiting moiety is a PEG, PPG, or derivatives thereof. Liposomes modified with PEG or PEG-derivatives are sometimes called "PEGylated liposomes".

[0182] The opsonization inhibiting moiety can be bound to the liposome membrane by any one of numerous well-known techniques. For example, an N-hydroxysuccinimide ester

of PEG can be bound to a phosphatidyl-ethanolamine lipid-soluble anchor, and then bound to a membrane. Similarly, a dextran polymer can be derivatized with a stearylamine lipid-soluble anchor via reductive amination using Na(CN)BH3 and a solvent mixture such as tetrahydrofuran and water in a 30:12 ratio at 60 degrees C.

[0183] Vectors expressing a double-stranded molecule of the invention are discussed above. Such vectors expressing at least one double-stranded molecule of the invention can also be administered directly or in conjunction with a suitable delivery substance, including the Mirus Transit LT1 lipophilic substance; lipofectin; lipofectamine; cellfectin; polycations (e.g., polylysine) or liposomes. Methods for delivering recombinant viral vectors, which express a double-stranded molecule of the invention, to an area of cancer in a patient are within the skill of the art.

[0184] The double-stranded molecule of the invention can be administered to the subject by any means suitable for delivering the double-stranded molecule into cancer sites. For example, the double-stranded molecule can be administered by gene gun, electroporation, or by other suitable parenteral or enteral administration routes.

[0185] Suitable enteral administration routes include oral, rectal, or intranasal delivery.

Suitable parenteral administration routes include intravascular administration (e.g., intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature); peri- and intra-tissue injection (e.g., peri-tumoral and intra-tumoral injection); subcutaneous injection or deposition including subcutaneous infusion (such as by osmotic pumps); direct application to the area at or near the site of cancer, for example by a catheter or other placement device (e.g., a suppository or an implant including a porous, non-porous, or gelatinous material); and inhalation. It is preferred that injections or infusions of the double-stranded molecule or vector be given at or near the site of cancer.

[0186] The double-stranded molecule of the invention can be administered in a single dose or in multiple doses. When the administration of the double-stranded molecule of the invention is by infusion, the infusion can be a single sustained dose or can be delivered by multiple infusions. Injection of the agent directly into the tissue at or near the site of cancer is preferred. Multiple injections of the agent into the tissue at or near the site of cancer are particularly preferred.

[0187] One skilled in the art can also readily determine an appropriate dosage regimen for administering the double-stranded molecule of the invention to a given subject. For example, the double-stranded molecule can be administered to the subject once, for example, as a single injection or deposition at or near the cancer site. Alternatively, the double-stranded molecule can be administered once or twice daily to a subject for a period of from about three to about twenty-eight days, more preferably from about seven to about ten days. In a preferred dosage regimen, the double-stranded molecule

is injected at or near the site of cancer once a day for seven days. When a dosage regimen includes multiple administrations, it is understood that the effective amount of a double-stranded molecule administered to the subject can include the total amount of a double-stranded molecule administered over the entire dosage regimen.

[0188] In the present invention, cancer overexpressing CSTF2 can be treated with at least one active ingredient selected from the group consisting of:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding thereof, and

(c) a vector encoding thereof.

[0189] The cancer includes, but is not limited to, lung cancer. Accordingly, prior to the administration of double-stranded molecule of the present invention as an active ingredient, it is preferable to confirm whether the expression level of CSTF2 in the cancer cells or tissues to be treated is enhanced as compared with normal cells of the same organ. Thus, in one embodiment, the present invention provides a method for treating a cancer (over)expressing CSTF2, which method may include the steps of:

i) determining the expression level of CSTF2 in cancer cells or tissue(s) obtained from a subject with the cancer to be treated;

ii) comparing the expression level of CSTF2 with normal control; and

iii) administrating at least one component selected from the group consisting of;

(a) a double-stranded molecule of the present invention,

(b) DNA encoding thereof, and

(c) a vector encoding thereof,

to a subject with a cancer overexpressing CSTF2 as compared with normal control. Alternatively, the present invention also provides a pharmaceutical composition comprising at least one component selected from the group consisting of:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding thereof, and

(c) a vector encoding thereof,

for use in administrating to a subject having a cancer overexpressing CSTF2. In other words, the present invention further provides a method for identifying a subject to be treated with:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding thereof, or

(c) a vector encoding thereof,

which method may include the step of determining an expression level of CSTF2 in subject-derived cancer cells or tissue(s), wherein an increase of the level compared to a normal control level of the gene indicates that the subject has cancer which may be treated with:

(a) a double-stranded molecule of the present invention,
(b) DNA encoding thereof, or
(c) a vector encoding thereof.

[0190] The method of treating a cancer of the present invention will be described in more detail below.

A subject to be treated by the present method is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

[0191] According to the present invention, the expression level of CSTF2 in cancer cells or tissues obtained from a subject is determined. The expression level can be determined at the transcription (nucleic acid) product level, using methods known in the art. For example, the mRNA of CSTF2 may be quantified using probes by hybridization methods (e.g., Northern hybridization). The detection may be carried out on a chip or an array. The use of an array is preferable for detecting the expression level of CSTF2. Those skilled in the art can prepare such probes utilizing the sequence information of CSTF2. For example, the cDNA of CSTF2 may be used as the probes. If necessary, the probes may be labeled with a suitable label, such as dyes, fluorescent substances and isotopes, and the expression level of the gene may be detected as the intensity of the hybridized labels.

[0192] Furthermore, the transcription product of CSTF2 (e.g., SEQ ID NO: 1) may be quantified using primers by amplification-based detection methods (e.g., RT-PCR). Such primers may be prepared based on the available sequence information of the gene.

[0193] Specifically, a probe or primer used for the present method hybridizes under stringent, moderately stringent, or low stringent conditions to the mRNA of CSTF2. As used herein, the phrase "stringent (hybridization) conditions" refers to conditions under which a probe or primer will hybridize to its target sequence, but not to other sequences. Stringent conditions are sequence-dependent and will be different under different circumstances. Specific hybridization of longer sequences is observed at higher temperatures than shorter sequences. Generally, the temperature of a stringent condition is selected to be about 5 degree Centigrade lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature (under a defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to their target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the

temperature is at least about 30 degree Centigrade for short probes or primers (e.g., 10 to 50 nucleotides) and at least about 60 degree Centigrade for longer probes or primers. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

[0194] Alternatively, the translation product may be detected for the diagnosis of the present invention. For example, the quantity of CSTF2 protein (SEQ ID NO: 2) may be determined. Methods for determining the quantity of the protein as the translation product include immunoassay methods that use an antibody specifically recognizing the protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, F(ab')2, Fv, etc.) of the antibody may be used for the detection, so long as the fragment or modified antibody retains the binding ability to the CSTF2 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof.

[0195] As another method to detect the expression level of CSTF2 gene based on its translation product, the intensity of staining may be measured via immunohistochemical analysis using an antibody against the CSTF2 protein. Namely, in this measurement, strong staining indicates increased presence/level of the protein and, at the same time, high expression level of CSTF2 gene.

[0196] The expression level of a target gene, e.g., the CSTF2 gene, in cancer cells can be determined to be increased if the level increases from the control level (e.g., the level in normal cells) of the target gene by, for example, 10%, 25%, or 50%; or increases to more than 1.1 fold, more than 1.5 fold, more than 2.0 fold, more than 5.0 fold, more than 10.0 fold, or more.

[0197] The control level may be determined at the same time with the cancer cells by using a sample(s) previously collected and stored from a subject/subjects whose disease state(s) (cancerous or non-cancerous) is/are known. In addition, normal cells obtained from non-cancerous regions of an organ that has the cancer to be treated may be used as normal control. Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing previously determined expression level(s) of CSTF2 gene in samples from subjects whose disease states are known. Furthermore, the control level can be derived from a database of expression patterns from previously tested cells. Moreover, according to an aspect of the present invention, the expression level of CSTF2 gene in a biological sample may be compared to multiple control levels, which are determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the subject-derived biological sample. Moreover, it is preferred to use the standard value of the expression levels of CSTF2 gene in a population with a

known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean +/- 2 S.D. or mean +/- 3 S.D. may be used as the standard value.

[0198] In the context of the present invention, a control level determined from a biological sample that is known to be non-cancerous is referred to as a "normal control level". On the other hand, if the control level is determined from a cancerous biological sample, it is referred to as a "cancerous control level".

[0199] When the expression level of CSTF2 gene is increased as compared to the normal control level, or is similar/equivalent to the cancerous control level, the subject may be diagnosed with cancer to be treated.

[0200] Compositions containing a double-stranded molecule of the present invention:

In addition to the above, the present invention also provides pharmaceutical compositions that include at least one of the present double-stranded molecules or the vectors coding for the molecules. Specifically, the present invention provides the following compositions [1] to [34]:

[1] A composition for inhibiting a growth of cancer cell and/or treating or preventing cancer, wherein the composition includes at least one isolated double-stranded molecule against CSTF2 gene, or vector encoding the double-stranded molecule, wherein the double-stranded molecule, when introduced into a cell, inhibits the expression of CSTF2 and the cell proliferation, wherein the double-stranded molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded molecule, and a pharmaceutically acceptable carrier;

[2] The composition of [1], wherein the double-stranded molecule acts on mRNA which matches a target sequence selected from among SEQ ID NO: 9 and SEQ ID NO:10;

[3] The composition of [1], wherein the sense strand of the double-stranded molecule contains a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10;

[4] The composition of [1], wherein the composition contains plural kinds of the double-stranded molecules;

[5] The composition of any one of [1] to [3], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 100 nucleotide pairs in length;

[6] The composition of [5], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 75 nucleotide pairs in length;

[7] The composition of [6], wherein the sense strand of the double-stranded molecule

hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 50 nucleotide pairs in length;
[8] The composition of [7], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 25 nucleotide pairs in length;
[9] The composition of [8], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between about 19 and about 25 nucleotide pairs in length;
[10] The composition of any one of [1] to [9], wherein the double-stranded molecule is composed of a single polynucleotide containing the sense strand and the antisense strand linked by an intervening single-strand;
[11] The composition of [10], wherein the double-stranded molecule has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand sequence contains a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10, [B] is the intervening single-strand consisting of 3 to 23 nucleotides, and [A'] is the antisense strand contains a sequence complementary to the target sequence selected in[A];
[12] The composition of any one of [1] to [11], wherein the double-stranded molecule is an RNA;
[13] The composition of any one of [1] to [11], wherein the double-stranded molecule is DNA and/or RNA;
[14] The composition of [13], wherein the double-stranded molecule is a hybrid of a DNA polynucleotide and an RNA polynucleotide;
[15] The composition of [14], wherein the sense and antisense strand polynucleotides are composed of DNA and RNA, respectively;
[16] The composition of [13], wherein the double-stranded molecule is a chimera of DNA and RNA;
[17] The composition of [16], wherein a region flanking to the 3'-end of the antisense strand, or both of a region flanking to the 5'-end of sense strand and a region flanking to the 3'-end of antisense strand are composed of RNA;
[18] The composition of [17], wherein the flanking region is composed of 9 to 13 nucleotides;
[19] The composition of any one of [1] to [18], wherein the double-stranded molecule contains one of two 3' overhang(s);
[20] The composition of any one of [1] to [19], wherein the composition further includes a transfection-enhancing agent;
[21] The composition of any one of [1] to [20], wherein the double-stranded molecule is encoded by a vector and contained in the composition;

[22] The composition of [21], wherein the double-stranded molecule encoded by the vector acts on mRNA which matches a target sequence selected from among SEQ ID NO: 9 and SEQ ID NO: 10;

[23] The composition of [21], wherein the sense strand of the double-stranded molecule encoded by the vector contains the sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10;

[24] The composition of any one of [21] to [23], wherein plural kinds of the double-stranded molecules are administered;

[25] The composition of any one of [21] to [24], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 100 nucleotide pairs in length;

[26] The composition of [25], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 75 nucleotide pairs in length;

[27] The composition of [26], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 50 nucleotide pairs in length;

[28] The composition of [27], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having less than about 25 nucleotide pairs in length;

[29] The composition of [28], wherein the sense strand of the double-stranded molecule hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between about 19 and about 25 nucleotide pairs in length;

[30] The composition of any one of [21] to [29], wherein the double-stranded molecule encoded by the vector is composed of a single polynucleotide containing both the sense strand and the antisense strand linked by an intervening single-strand;

[31] The composition of [30], wherein the double-stranded molecule has the general formula 5'-[A]-[B]-[A']-3', wherein [A] is the sense strand containing a sequence corresponding to a target sequence selected from among SEQ ID NOs: 9 and 10, [B] is a intervening single-strand composed of 3 to 23 nucleotides, and [A'] is the antisense strand containing a sequence complementary to the target sequence selected in [A];

[32] The composition of any one of [21] to [31], wherein the composition further includes a transfection-enhancing agent; and

[33] The composition of any one of [1] to [32], wherein the cancer is lung cancer.

[34] The composition of [33], wherein the lung cancer is adenocarcinoma, squamous cell carcinoma, large cell carcinoma or small cell lung carcinoma.

[0201] Suitable compositions of the present invention are described in additional detail

below.

The double-stranded molecules of the invention are preferably formulated as pharmaceutical compositions prior to administering to a subject, according to techniques known in the art. Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. As used herein, "pharmaceutical formulations" include formulations for human and veterinary use. Methods for preparing pharmaceutical compositions of the invention are within the skill in the art, for example as described in Remington's Pharmaceutical Science, 17th ed., Mack Publishing Company, Easton, Pa. (1985), the entire disclosure of which is herein incorporated by reference.

[0202] The present pharmaceutical formulations contain at least one of the double-stranded molecules or vectors encoding them of the present invention (e.g., 0.1 to 90% by weight), or a physiologically acceptable salt of the molecule, mixed with a physiologically acceptable carrier medium. Preferred physiologically acceptable carrier media are water, buffered water, normal saline, 0.4% saline, 0.3% glycine, hyaluronic acid and the like.

[0203] According to the present invention, the composition may contain plural kinds of the double-stranded molecules, each of the molecules may be directed to the different target sequences of the same gene, or different target sequences of different genes. For example, the composition may contain double-stranded molecules directed to target sequences of CSTF2 gene. Alternatively, for example, the composition may contain double-stranded molecules directed to one, two or more target sequences of CSTF2 gene and other genes.

[0204] Furthermore, the present composition may contain a vector coding for one or plural double-stranded molecules. For example, the vector may encode one, two or several kinds of the present double-stranded molecules. Alternatively, the present composition may contain plural kinds of vectors, each of the vectors coding for a different double-stranded molecule.

[0205] Moreover, the present double-stranded molecules may be contained as liposomes in the present composition. See under the item of "Methods of inhibiting or reducing growth of a cancer cell or treating cancer using a double-stranded molecule of the present invention" for details of liposomes.

[0206] Compositions of the present invention may be pharmaceutical compositions. Pharmaceutical compositions of the invention can also include conventional pharmaceutical excipients and/or additives. Suitable pharmaceutical excipients include stabilizers, antioxidants, osmolality adjusting agents, buffers, and pH adjusting agents. Suitable additives include physiologically biocompatible buffers (e.g., tromethamine hydrochloride), additions of chelants (such as, for example, DTPA or DTPA-bisamide) or

calcium chelate complexes (for example calcium DTPA, CaNaDTPA-bisamide), or, optionally, additions of calcium or sodium salts (for example, calcium chloride, calcium ascorbate, calcium gluconate or calcium lactate). Pharmaceutical compositions of the invention can be packaged for use in liquid form, or can be lyophilized.

[0207] For solid compositions, conventional nontoxic solid carriers can be used; for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

[0208] For example, a solid pharmaceutical composition for oral administration can include any of the carriers and excipients listed above and 10-95%, preferably 25-75%, of one or more double-stranded molecule of the invention. A pharmaceutical composition for aerosol (inhalational) administration can include 0.01-20% by weight, preferably 1-10% by weight, of one or more double-stranded molecule of the invention encapsulated in a liposome as described above, and propellant. A carrier can also be included as desired; e.g., lecithin for intranasal delivery.

[0209] In addition to the above, the present composition may contain other pharmaceutical active ingredients so long as they do not inhibit the in vivo function of the present double-stranded molecules. For example, the composition may contain chemotherapeutic agents conventionally used for treating cancers.

[0210] In another embodiment, the present invention also provides the use of the double-stranded nucleic acid molecules of the present invention in manufacturing a pharmaceutical composition for treating a lung cancer characterized by the expression of CSTF2. For example, the present invention relates to a use of double-stranded nucleic acid molecule inhibiting the expression of a CSTF2 gene in a cell, which molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded nucleic acid molecule and targets to a sequence selected from among SEQ ID NOs: 9 and 10, for manufacturing a pharmaceutical composition for treating lung cancer expressing CSTF2.

[0211] Alternatively, the present invention further provides the double-stranded nucleic acid molecules of the present invention for use in treating a cancer expressing the CSTF2 gene.

Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition for treating a lung cancer characterized by the expression of CSTF2, wherein the method or process includes a step for formulating a pharmaceutically or physiologically acceptable carrier with a double-stranded nucleic acid molecule inhibiting the expression of CSTF2 in a cell, which over-expresses the gene, wherein the molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded nucleic acid

molecule and targets to a sequence selected from among SEQ ID NOs: 9 and 10 as active ingredients.

[0212] In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition for treating a lung cancer characterized by the expression of CSTF2, wherein the method or process includes a step for admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is a double-stranded nucleic acid molecule inhibiting the expression of CSTF2 in a cell, which over-expresses the gene, wherein the molecule includes a sense strand and an antisense strand complementary thereto, hybridized to each other to form the double-stranded nucleic acid molecule and targets to a sequence selected from among SEQ ID NOs: 9 and 10.

[0213] Method of detecting or diagnosing lung cancer

The expression of CSTF2 gene was found to be specifically elevated in lung cancer cells (Figs. 1A-1C). Therefore, the gene identified herein as well as their transcription and translation products find diagnostic utility as markers for lung cancer, and by measuring the expression of CSTF2 gene in a cell sample, lung cancer can be diagnosed. Specifically, the present invention provides a method for diagnosing lung cancer by determining the expression level of CSTF2 in a subject. Lung cancers that can be diagnosed by the present method include NSCLC and SCLC. Furthermore, NSCLC, including lung adenocarcinoma (ADC), lung squamous cell carcinoma (SCC) and large cell carcinoma (LCC), can also be diagnosed or detected by the present invention.

[0214] According to the present invention, an intermediate result for examining the condition of a subject may be provided. Such intermediate result may be combined with additional information to assist a doctor, nurse, or other practitioner to diagnose that a subject suffers from the disease. That is, the present invention provides a diagnostic marker CSTF2 for examining cancer. Alternatively, the present invention provides a method for detecting or identifying cancer cells in a subject-derived lung tissue sample, said method including the step of determining the expression level of the CSTF2 gene in a subject-derived biological sample, wherein an increase in said expression level as compared to a normal control level of said gene indicates the presence or suspicion of cancer cells in the tissue. Such result may be combined with additional information to assist a doctor, nurse, or other healthcare practitioner in diagnosing a subject as afflicted with the disease. In other words, the present invention may provide a doctor with useful information to diagnose a subject as afflicted with the disease. For example, according to the present invention, when there is doubt regarding the presence of cancer cells in the tissue obtained from a subject, clinical decisions can be reached by considering the expression level of the CSTF2 gene, plus a different aspect

of the disease including tissue pathology, levels of known tumor marker(s) in blood, and clinical course of the subject, etc. For example, some well-known diagnostic lung tumor markers in blood are IAP, ACT, BFP, CA19-9, CA50, CA72-4, CA130, CEA, KMO-1, NSE, SCC, SP1, Span-1, TPA, CSLEX, SLX, STN and CYFRA. Namely, in this particular embodiment of the present invention, the outcome of the gene expression analysis serves as an intermediate result for further diagnosis of a subject's disease state.

[0215] Specifically, the present invention provides the following methods [1] to [11]:

[1] A method for diagnosing lung cancer or a predisposition for developing lung cancer in a subject, the method including the steps of:

(a) detecting the expression level of the CSTF2 gene in a subject-derived biological sample; and

(b) correlating an increase in the expression level detected as compared to a normal control level of the gene to the presence of disease in the subject;

[2] The method of [1], wherein the expression level is at least 10% greater than the normal control level;

[3] The method of [1] or [2], wherein the expression level is detected by a method selected from among:

(a) detecting an mRNA encoded by a CSTF2 gene,

(b) detecting a protein encoded by a CSTF2 gene, and

(c) detecting a biological activity of a protein encoded by a CSTF2 gene;

[4] The method of [3], wherein the expression level is determined by detecting hybridization of a probe to the mRNA encoded by the CSTF2 gene;

[5] The method of [3], wherein the expression level is determined by detecting the binding of an antibody against the protein encoded by the CSTF2 gene to the protein encoded by the CSTF2 gene;

[6] The method of any one of [1] to [5], wherein the biological sample includes biopsy sample, sputum or blood;

[7] The method of any one of [1] to [6], wherein the subject-derived biological sample includes an epithelial cell;

[8] The method of any one of [1] to [7], wherein the subject-derived biological sample includes a cancerous cell;

[9] The method of [8], wherein the subject-derived biological sample includes a cancerous epithelial cell.

[10] The method of any one of [1] to [9], wherein the subject-derived biological sample includes a lung tissue or lung cells.

[11] The method of any one of [1] to [10], wherein the lung cancer is NSCLC or SCLC;

[0216] The method of diagnosing lung cancer will be described in more detail below.

A subject to be diagnosed by the present method is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

[0217] It is preferred to collect a biological sample from a subject to be diagnosed to perform the diagnosis. Any biological material can be used as a biological sample for the determination so long as it includes the objective transcription or translation product of CSTF2 gene. The biological samples include, but are not limited to, bodily tissues which are desired for diagnosing or are suspected of suffering from cancer, and fluids, such as blood, sputum, pleural effusion and urine. Preferably, the biological sample contains a cell population including an epithelial cell, more preferably a cancerous epithelial cell or an epithelial cell derived from tissue suspected to be cancerous. Further, if necessary, the cell may be purified from the obtained bodily tissues and fluids, and then used as the biological sample. In a preferred embodiment, a biological sample contains a lung tissue or lung cells. Such biological sample can be obtained by collecting a lung tissue or lung cells from a region suspected cancerous in a subject's lung, for example, by biopsy.

[0218] According to the present invention, the expression level of CSTF2 gene in a subject-derived biological sample is determined. The expression level can be determined at the transcription (mRNA) product level, using methods known in the art. For example, the mRNA of CSTF2 gene may be quantified using probes by hybridization methods (e.g., Northern hybridization). The detection may be carried out on a chip or an array. The use of an array is preferable for detecting the expression level of a plurality of genes (e.g., various cancer specific genes) including CSTF2 gene. Those skilled in the art can prepare such probes utilizing the sequence information of the CSTF2 gene (SEQ ID NO: 1; GenBank accession number: NM_001325). For example, the cDNA of CSTF2 gene may be used as the probes. If necessary, the probe may be labeled with a suitable label, such as dyes, fluorescent and isotopes, and the expression level of the gene may be detected as the intensity of the hybridized labels.

[0219] Furthermore, the transcription product of CSTF2 gene may be quantified using primers by amplification-based detection methods (e.g., RT-PCR). Such primers can also be prepared based on the available sequence information of the gene. For example, the primers or probes (SEQ ID NOs: 3, 4, 7 and 8) used in the Example may be employed for the detection by RT-PCR or Northern blot, but the present invention is not restricted thereto.

[0220] Specifically, a probe or primer used for the present method hybridizes under stringent, moderately stringent, or low stringent conditions to the mRNA of CSTF2 gene. As also defined above, the phrase "stringent (hybridization) conditions" refers to

conditions under which a probe or primer will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different under different circumstances. Specific hybridization of longer sequences is observed at higher temperatures than shorter sequences. Generally, the temperature of a stringent condition is selected to be about 5 degrees Centigrade lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 degrees Centigrade for short probes or primers (e.g., 10 to 50 nucleotides) and at least about 60 degrees Centigrade for longer probes or primers. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

[0221] Alternatively, the translation product may be detected for the diagnosis of the present invention. For example, the quantity of a CSTF2 protein may be determined. A method for determining the quantity of the protein as the translation product includes immunoassay methods that use an antibody specifically recognizing the protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, F(ab')2, Fv, etc.) of the antibody may be used for the detection, so long as the fragment retains the binding ability to a CSTF2 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof.

[0222] As another method to detect the expression level of a CSTF2 gene based on its translation product, the intensity of staining may be observed via immunohistochemical analysis using an antibody against a CSTF2 protein. Namely, the observation of strong staining indicates increased presence of the protein and at the same time high expression level of a CSTF2 gene.

[0223] Moreover, in addition to the expression level of a CSTF2 gene, the expression level of other cancer-associated genes, for example, genes known to be differentially expressed in lung cancer may also be determined to improve the accuracy of the diagnosis.

[0224] The expression level of cancer marker gene including a CSTF2 gene in a biological sample can be considered to be increased if it increases from the control level of the corresponding cancer marker gene by, for example, 10%, 25%, or 50%; or increases to

more than 1.1 fold, more than 1.5 fold, more than 2.0 fold, more than 5.0 fold, more than 10.0 fold, or more.

[0225] The control level may be determined at the same time with the test biological sample by using a sample(s) previously collected and stored from a subject/subjects whose disease state (cancerous or non-cancerous) is/are known. Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing previously determined expression level(s) of a CSTF2 gene in samples from subjects whose disease state are known. Furthermore, the control level can be a database of expression patterns from previously tested cells. Moreover, according to an aspect of the present invention, the expression level of the CSTF2 gene in a biological sample may be compared to multiple control levels, which control levels are determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the patient-derived biological sample. Moreover, it is preferred, to use the standard value of the expression levels of the CSTF2 gene in a population with a known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean +/- 2 S.D. or mean +/- 3 S.D. may be used as standard value.

[0226] In the context of the present invention, a control level determined from a biological sample that is known not to be cancerous is referred to as a "normal control level". On the other hand, if the control level is determined from a cancerous biological sample, it is referred to as a "cancerous control level".

[0227] When the expression level of a CSTF2 gene is increased as compared to the normal control level or is similar to the cancerous control level, the subject may be diagnosed to be suffering from or at a risk of developing cancer. Furthermore, in the case where the expression levels of multiple cancer-related genes are compared, a similarity in the gene expression pattern between the sample and the reference which is cancerous indicates that the subject is suffering from or at a risk of developing cancer.

[0228] Difference between the expression levels of a test biological sample and the control level can be normalized to the expression level of control nucleic acids, e.g., housekeeping genes, whose expression levels are known not to differ depending on the cancerous or non-cancerous state of the cell. Exemplary control genes include, but are not limited to, beta-actin, glyceraldehyde 3 phosphate dehydrogenase, and ribosomal protein P1.

[0229] Alternatively, the present invention provides use of a reagent for preparing a diagnostic reagent for diagnosing cancer. In some embodiments, the reagent can be selected from the group consisting of:

(a) a reagent for detecting mRNA of the CSTF2 gene;

(b) a reagent for detecting the CSTF2 protein; and

(c) a reagent for detecting the biological activity of the CSTF2 protein.

[0230] Specifically, such reagent is an oligonucleotide that hybridizes to the CSTF2 polynucleotide, or an antibody that binds to the CSTF2 polypeptide.

[0231] In the present invention, it is revealed that CSTF2 is not only a useful diagnostic marker, but also suitable target for cancer therapy. Therefore, cancer treatment targeting CSTF2 can be achieved by the present invention. In the present invention, the cancer treatment targeting CSTF2 refers to suppression or inhibition of CSTF2 activity and/or expression in the cancer cells. Any anti-CSTF2 agents may be used for the cancer treatment targeting CSTF2. In the present invention, the anti-CSTF2 agents include following substance as active ingredient:

(a) a double-stranded molecule of the present invention,

(b) DNA encoding thereof, or

(c) a vector encoding thereof.

Accordingly, in a preferred embodiment, the present invention provides a method of (i) diagnosing whether a subject has the cancer to be treated with anti-CSTF2 agent, and/or (ii) selecting a subject for cancer treatment targeting CSTF2, which method includes the steps of:

(a) determining the expression level of CSTF2 gene in cancer cells or tissue(s) obtained from a subject who is suspected to have the cancer to be treated;

(b) comparing the expression level of CSTF2 gene with a normal control level;

(c) diagnosing the subject as having the cancer to be treated, if the expression level of CSTF2 is increased as compared to the normal control level; and

(d) selecting the subject for cancer treatment, if the subject is diagnosed as having the cancer to be treated, in step (c).

Alternatively, such a method includes the steps of:

(a) determining the expression level of CSTF2 gene in cancer cells or tissue(s) obtained from a subject who is suspected to have the cancer to be treated;

(b) comparing the expression level of CSTF2 gene with a cancerous control level;

(c) diagnosing the subject as having the cancer to be treated, if the expression level of CSTF2 gene is similar or equivalent to the cancerous control level; and

(d) selecting the subject for cancer treatment, if the subject is diagnosed as having the cancer to be treated, in step (c).

[0232] Method for assessing the prognosis of cancer

The present invention relates to the novel discovery that CSTF2 expression is significantly associated with poorer prognosis of patients. Thus, the present invention provides a method for determining or assessing the prognosis of a patient with cancer, in particular, lung cancer, by detecting the expression level of CSTF2 in a biological sample of the patient; comparing the detected expression level to a control level; and

determining an increased expression level to the control level as indicative of poor prognosis (poor survival). Specifically, the present invention provides a method for assessing or determining prognosis of a subject with lung cancer, which method includes steps of:

(a) detecting an expression level of a CSTF2 gene in a subject-derived biological sample;

(b) comparing the detected expression level to a control level; and

(c) determining prognosis of the subject based on the comparison of (b).

[0233] Herein, the term "prognosis" refers to a forecast as to the probable outcome of the disease as well as the prospect of recovery from the disease as indicated by the nature and symptoms of the case. Accordingly, a less favorable, negative, poor prognosis is defined by a lower post-treatment survival term or survival rate. Conversely, a positive, favorable, or good prognosis is defined by an elevated post-treatment survival term or survival rate.

[0234] The terms "assessing the prognosis" refer to the ability of predicting, forecasting or correlating a given detection or measurement with a future outcome of cancer of the patient (e.g., malignancy, likelihood of curing cancer, survival, and the like). For example, a determination of the expression level of CSTF2 gene over time enables a predicting of an outcome for the patient (e.g., increase or decrease in malignancy, increase or decrease in grade of a cancer, likelihood of curing cancer, survival, and the like).

[0235] In the context of the present invention, the phrase "assessing (or determining) the prognosis" is intended to encompass predictions and likelihood analysis of cancer, progression, particularly cancer recurrence, metastatic spread and disease relapse. The present method for assessing prognosis is intended to be used clinically in making decisions concerning treatment modalities, including therapeutic intervention, diagnostic criteria such as disease staging, and disease monitoring and surveillance for metastasis or recurrence of neoplastic disease.

[0236] The patient-derived biological sample used for the method may be any sample derived from the subject to be assessed so long as the CSTF2 gene can be detected in the sample. Preferably, the biological sample is a lung cell (a cell obtained from the lung). Furthermore, the biological sample may include bodily fluids such as sputum, blood, serum, or plasma. Moreover, the sample may be cells purified from a tissue. The biological samples may be obtained from a patient at various time points, including before, during, and/or after a treatment. For example, a lung cancer cell(s) obtained from a subject to be assessed is a preferable biological sample.

[0237] According to the present invention, it was shown that the higher the expression level of the CSTF2 gene measured in the patient-derived biological sample, the poorer the

prognosis for post-treatment remission, recovery, and/or survival and the higher the likelihood of poor clinical outcome. Thus, according to the present method, the "control level" used for comparison may be, for example, the expression level of the CSTF2 gene detected before any kind of treatment in an individual or a population of individuals who showed good or positive prognosis of cancer, after the treatment, which herein will be referred to as "good prognosis control level". Alternatively, the "control level" may be the expression level of the CSTF2 gene detected before any kind of treatment in an individual or a population of individuals who showed poor or negative prognosis of cancer, after the treatment, which herein will be referred to as "poor prognosis control level". The "control level" is a single expression pattern derived from a single reference population or from a plurality of expression patterns. Thus, the control level may be determined based on the expression level of the CSTF2 gene detected before any kind of treatment in a patient of cancer, or a population of the patients whose disease state (good or poor prognosis) is known. In the context of the present invention, cancer is lung cancer. It is preferred, to use the standard value of the expression levels of the CSTF2 gene in a patient group with a known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean +/- 2 S.D. or mean +/- 3 S.D. may be used as standard value.

[0238] The control level may be determined at the same time with the test biological sample by using a sample(s) previously collected and stored before any kind of treatment from cancer patient(s) (control or control group) whose disease state (good prognosis or poor prognosis) are known.

[0239] Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing the expression level of the CSTF2 gene in samples previously collected and stored from a control group. Furthermore, the control level can be a database of expression patterns from previously tested cells.

[0240] Moreover, according to an aspect of the present invention, the expression level of the CSTF2 gene in a biological sample may be compared to multiple control levels, which control levels are determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the patient-derived biological sample.

[0241] According to the present invention, a similarity in the expression level of the CSTF2 gene to a good prognosis control level indicates a more favorable prognosis of the patient and an increase in the expression level to the good prognosis control level indicates less favorable, poorer prognosis for post-treatment remission, recovery, survival, and/or clinical outcome. On the other hand, a decrease in the expression level of CSTF2 to the poor prognosis control level indicates a more favorable prognosis of the patient and a similarity in the expression level to the poor prognosis control level

indicates less favorable, poorer prognosis for post-treatment remission, recovery, survival, and/or clinical outcome. For example, a lung cancer cell(s) obtained from a subject who showed good, or poor prognosis of cancer after treatment is a preferable biological sample for good, or poor prognosis control level, respectively.

[0242] The expression level of the CSTF2 gene in a biological sample can be considered altered when the expression level differs from the control level by more than 1.0, 1.5, 2.0, 5.0, 10.0, or more fold.

[0243] The difference in the expression level between the test biological sample and the control level can be normalized to a control, e.g., housekeeping gene. For example, polynucleotides whose expression levels are known not to differ between the cancerous and non-cancerous cells, including those coding for beta-actin, glyceraldehyde 3-phosphate dehydrogenase, and ribosomal protein P1, may be used to normalize the expression level of the CSTF2 gene.

[0244] The expression level may be determined by detecting the gene transcript in the patient-derived biological sample using techniques well known in the art. The gene transcripts detected by the present method include both the transcription and translation products, such as mRNA and protein.

[0245] For instance, the transcription product of the CSTF2 gene can be detected by hybridization, e.g., Northern blot hybridization analyses, that use a CSTF2 gene probe to the gene transcript. The detection may be carried out on a chip or an array. The use of an array is preferable for detecting the expression level of the CSTF2 gene. As another example, amplification-based detection methods, such as reverse-transcription based polymerase chain reaction (RT-PCR) which use primers specific to the CSTF2 gene may be employed for the detection (see Example). The CSTF2 gene-specific probe or primers may be designed and prepared using conventional techniques by referring to the whole sequence of the CSTF2 gene (SEQ ID NO: 1). For example, the primers (SEQ ID NOs: 3 and 4) used in the Example may be employed for the detection by RT-PCR, but the present invention is not restricted thereto.

[0246] Specifically, a probe or primer used for the present method hybridizes under stringent, moderately stringent, or low stringent conditions to the mRNA of the CSTF2 gene.

[0247] Alternatively, the translation product may be detected for the assessment of the present invention. For example, the quantity of the CSTF2 protein may be determined. A method for determining the quantity of the protein as the translation product includes immunoassay methods that use an antibody specifically recognizing the CSTF2 protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, F(ab')2, Fv, etc.) of the antibody may be used for the detection, so long as the fragment retains the binding

ability to the CSTF2 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof.

[0248] As another method to detect the expression level of the CSTF2 gene based on its translation product, the intensity of staining may be observed via immunohistochemical analysis using an antibody against a CSTF2 protein. Namely, the observation of strong staining indicates increased presence of the CSTF2 protein and at the same time high expression level of the CSTF2 gene.

[0249] Furthermore, the CSTF2 protein is known to have a cell proliferating activity. Therefore, the expression level of the CSTF2 gene can be determined using such cell proliferating activity as an index. For example, cells which express CSTF2 are prepared and cultured in the presence of a biological sample, and then by detecting the speed of proliferation, or by measuring the cell cycle or the colony forming ability the cell proliferating activity of the biological sample can be determined.

[0250] Moreover, in addition to the expression level of the CSTF2 gene, the expression level of other lung cancer-associated genes, for example, genes known to be differentially expressed in lung cancer may also be determined to improve the accuracy of the assessment. Examples of such other lung cell-associated genes include those described in WO 2004/031413 and WO 2005/090603, the contents of which are incorporated by reference herein.

[0251] Alternatively, according to the present invention, an intermediate result may also be provided in addition to other test results for assessing the prognosis of a subject. Such intermediate result may assist a doctor, nurse, or other practitioner to assess, determine, or estimate the prognosis of a subject. Additional information that may be considered, in combination with the intermediate result obtained by the present invention, to assess prognosis includes clinical symptoms and physical conditions of a subject.

[0252] In other words, the expression level of the CSTF2 gene is useful prognostic marker for assessing, predicting or determining the prognosis of a subject suffering from lung cancer (e.g. NSCLC). Therefore, the present invention also provides a method for detecting prognostic marker for assessing, predicting or determining the prognosis of a subject suffering from lung cancer including NSCLC, which comprises steps of

a) detecting or determining an expression level of a CSTF2 gene in a subject-derived biological sample, and

b) correlating the expression level detected or determined in step a) with the prognosis of the subject.

In particular, according to the present invention, an increased expression level to the control level is indicative of potential or suspicion of poor prognosis (poor survival).

[0253] The patient to be assessed for the prognosis of cancer according to the method is

preferably a mammal and includes human, non-human primate, mouse, rat, dog, cat, horse, and cow.

[0254] Alternatively, the present invention provides use of a reagent for preparing a reagent for assessing prognosis of cancer. In some embodiments, the reagent is selected from the group consisting of:

(a) a reagent for detecting mRNA of the CSTF2 gene;

(b) a reagent for detecting the CSTF2; and

(c) a reagent for detecting the biological activity of the CSTF2 protein.

Specifically, such reagent is an oligonucleotide that hybridizes to the CSTF2 polynucleotide, or an antibody that binds to the CSTF2 polypeptide.

[0255] A kit for diagnosing cancer or assessing the prognosis of cancer:

The present invention provides a kit for diagnosing cancer or assessing the prognosis of cancer. Alternatively, the present invention also provides a kit for determining a subject suffering from cancer that can be treated with the double-stranded molecule of the present invention or vector encoding thereof, which may also be useful in assessing and/or monitoring the efficacy of a cancer treatment. In a preferred embodiment, the cancer is lung cancer. Specifically, the kit includes at least one substance or reagent for detecting the expression of the CSTF2 gene in a patient-derived biological sample, which substance may be selected from the group of:

(a) a substance or reagent for detecting mRNA of the CSTF2 gene;

(b) a substance or reagent for detecting the CSTF2 protein; and

(c) a substance or reagent for detecting the biological activity of the CSTF2 protein.

Suitable substances or reagents for detecting mRNA of the CSTF2 gene include nucleic acids that specifically bind to or identify the CSTF2 mRNA, such as oligonucleotides which have a complementary sequence to a part of the CSTF2 mRNA. These kinds of oligonucleotides are exemplified by primers and probes that are specific to the CSTF2 mRNA. These kinds of oligonucleotides may be prepared based on methods well known in the art. If needed, the substance or reagent for detecting the CSTF2 mRNA may be immobilized on a solid matrix. Moreover, more than one substance or reagent for detecting the CSTF2 mRNA may be included in the kit.

[0256] The probes or primers may be of specific sizes. The sizes are selected from the group consisting of at least 10 nucleotides, at least 12 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides and the probes and primers may range in size from 5-10 nucleotides, 10-15 nucleotides, 15-20 nucleotides, 20-25 nucleotides and 25-30 nucleotides.

[0257] On the other hand, suitable substances or reagents for detecting the CSTF2 protein include antibodies to the CSTF2 protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv,

Fab, F(ab')2, Fv, etc.) of the antibody may be used as the substance or reagent, so long as the fragment retains the binding ability to the CSTF2 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof. Furthermore, the antibody may be labeled with signal generating molecules via direct linkage or an indirect labeling technique. Labels and methods for labeling antibodies and detecting the binding of antibodies to their targets are well known in the art and any labels and methods may be employed for the present invention. Moreover, more than one substance or reagent for detecting the CSTF2 protein may be included in the kit.

[0258] Furthermore, the biological activity can be determined by, for example, measuring the cell proliferating activity due to the expressed CSTF2 protein in the biological sample. For example, the cell is cultured in the presence of a patient-derived biological sample, and then by detecting the speed of proliferation, or by measuring the cell cycle or the colony forming ability the cell proliferating activity of the biological sample can be determined. If needed, the substance or reagent for detecting the CSTF2 mRNA may be immobilized on a solid matrix. Moreover, more than one substance for detecting the biological activity of the CSTF2 protein may be included in the kit.

[0259] The kit may contain more than one of the aforementioned substances or reagents. Furthermore, the kit may include a solid matrix and substance for binding a probe against the CSTF2 gene or antibody against the CSTF2 protein, a medium and container for culturing cells, positive and negative control substances or reagents, and a secondary antibody for detecting an antibody against the CSTF2 protein. For example, tissue samples obtained from patient with good prognosis or poor prognosis may serve as useful control substances or reagents. A kit of the present invention may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts (e.g., written, tape, CD-ROM, etc.) with instructions for use. These substances or reagents and such may be included in a container with a label. Suitable containers include bottles, vials, and test tubes. The containers may be formed from a variety of materials, such as glass or plastic.

[0260] As an embodiment of the present invention, when the substance or reagent is a probe against the CSTF2 mRNA, the substance or reagent may be immobilized on a solid matrix, such as a porous strip, to form at least one detection site. The measurement or detection region of the porous strip may include a plurality of sites, each containing a nucleic acid (probe). A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites may be located on a strip separated from the test strip. Optionally, the different detection sites may contain different amounts of im-

mobilized nucleic acids, i.e., a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of CSTF2 mRNA present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a test strip.

[0261] The kit of the present invention may further include a positive control sample or CSTF2 standard sample. The positive control sample of the present invention may be prepared by collecting CSTF2 positive blood samples and then those CSTF2 level are assayed. Alternatively, a purified CSTF2 protein or polynucleotide may be added to CSTF2 free serum to form the positive sample or the CSTF2 standard.

[0262] Screening for an anti-lung cancer substance

In the context of the present invention, substances to be identified through the present screening methods may be any substance or composition including several substances. Furthermore, the test substance exposed to a cell or protein according to the screening methods of the present invention may be a single substance or a combination of substances. When a combination of substances is used in the methods, the substances may be contacted sequentially or simultaneously.

[0263] Any test substance, for example, cell extracts, cell culture supernatant, products of fermenting microorganism, extracts from marine organism, plant extracts, purified or crude proteins, peptides, non-peptide substances, synthetic micromolecular substances (including nucleic acid constructs, such as antisense RNA, siRNA, Ribozymes, and aptamer etc.) and natural substances can be used in the screening methods of the present invention. The test substance of the present invention can be also obtained using any of the numerous approaches in combinatorial library methods known in the art, including (1) biological libraries, (2) spatially addressable parallel solid phase or solution phase libraries, (3) synthetic library methods requiring deconvolution, (4) the "one-bead one-substance" library method and (5) synthetic library methods using affinity chromatography selection. The biological library methods using affinity chromatography selection is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of substances (Lam, Anticancer Drug Des 1997, 12: 145-67). Examples of methods for the synthesis of molecular libraries can be found in the art (DeWitt et al., Proc Natl Acad Sci USA 1993, 90: 6909-13; Erb et al., Proc Natl Acad Sci USA 1994, 91: 11422-6; Zuckermann et al., J Med Chem 37: 2678-85, 1994; Cho et al., Science 1993, 261: 1303-5; Carell et al., Angew Chem Int Ed Engl 1994, 33: 2059; Carell et al., Angew Chem Int Ed Engl 1994, 33: 2061; Gallop et al., J Med Chem 1994, 37: 1233-51). Libraries of substances may be presented in solution (see Houghten, Bio/Techniques

1992, 13: 412-21) or on beads (Lam, Nature 1991, 354: 82-4), chips (Fodor, Nature 1993, 364: 555-6), bacteria (US Pat. No. 5,223,409), spores (US Pat. No. 5,571,698; 5,403,484, and 5,223,409), plasmids (Cull et al., Proc Natl Acad Sci USA 1992, 89: 1865-9) or phage (Scott and Smith, Science 1990, 249: 386-90; Devlin, Science 1990, 249: 404-6; Cwirla et al., Proc Natl Acad Sci USA 1990, 87: 6378-82; Felici, J Mol Biol 1991, 222: 301-10; US Pat. Application 2002103360).

[0264] A substance in which a part of the structure of the substance screened by any of the present screening methods is converted by addition, deletion and/or replacement, is included in the substances obtained by the screening methods of the present invention.

[0265] Furthermore, when the screened test substance is a protein, for obtaining a DNA encoding the protein, either the whole amino acid sequence of the protein may be determined to deduce the nucleic acid sequence coding for the protein, or partial amino acid sequence of the obtained protein may be analyzed to prepare an oligo DNA as a probe based on the sequence, and screen cDNA libraries with the probe to obtain a DNA encoding the protein. The obtained DNA is confirmed its usefulness in preparing the test substance which is a candidate for treating or preventing cancer.

[0266] Test agents useful in the screenings described herein can also be antibodies that specifically bind to a CSTF2 protein or partial peptides thereof that lack the biological activity of the original proteins in vivo.

[0267] Although the construction of test agent libraries is well known in the art, herein below, additional guidance in identifying test substances and construction libraries of such substances for the present screening methods are provided.

[0268] In the present invention, it is revealed that suppression of the expression level and/or biological activity of CSTF2 lead to suppression of the growth of cancer cells. Therefore, when a substance suppresses the expression and/or activity of CSTF2, the suppression is indicative of a potential therapeutic effect in a subject. In the present invention, a potential therapeutic effect refers to a clinical benefit with a reasonable expectation. In the present invention, such clinical benefit includes;

(a) reduction in expression of the CSTF2 gene,

(b) a decrease in size, prevalence, or metastatic potential of the cancer in the subject,

(c) preventing cancers from forming, or

(d) preventing or alleviating a clinical symptom of cancer.

[0269] (i) Molecular modeling:

Construction of test agent libraries is facilitated by knowledge of the molecular structure of substances known to have the properties sought, and/or the molecular structure of CSTF2. One approach to preliminary screening of test agents suitable for further evaluation is computer modeling of the interaction between the test agent and its target.

[0270] Computer modeling technology allows the visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new substances that will interact with the molecule. The three-dimensional construct typically depends on data from X-ray crystallographic analysis or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new substance will link to the target molecule and allow experimental manipulation of the structures of the substance and target molecule to perfect binding specificity. Prediction of what the molecule-substance interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

[0271] An example of the molecular modeling system described generally above includes the CHARMm and QUANTA programs, Polygen Corporation, Waltham, Mass. CHARMm performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

[0272] A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen et al. Acta Pharmaceutica Fennica 1988, 97: 159-66; Ripka, New Scientist 1988, 54-8; McKinlay & Rossmann, Annu Rev Pharmacol Toxiciol 1989, 29: 111-22; Perry & Davies, Prog Clin Biol Res 1989, 291: 189-93; Lewis & Dean, Proc R Soc Lond 1989, 236: 125-40, 141-62; and, with respect to a model receptor for nucleic acid components, Askew et al., J Am Chem Soc 1989, 111: 1082-90.

[0273] Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, Calif., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. See, e.g., DesJarlais et al., J Med Chem 1988, 31: 722-9; Meng et al., J Computer Chem 1992, 13: 505-24; Meng et al., Proteins 1993, 17: 266-78; Shoichet et al., Science 1993, 259: 1445-50.

[0274] Once a putative inhibitor has been identified, combinatorial chemistry techniques can be employed to construct any number of variants based on the chemical structure of the identified putative inhibitor, as detailed below. The resulting library of putative inhibitors, or "test substances" may be screened using the methods of the present invention to identify test agents treating or preventing the lung cancer.

[0275] (ii) Combinatorial chemical synthesis:

Combinatorial libraries of test substances may be produced as part of a rational drug design program involving knowledge of core structures existing in known inhibitors. This approach allows the library to be maintained at a reasonable size, facilitating high

throughput screening. Alternatively, simple, particularly short, polymeric molecular libraries may be constructed by simply synthesizing all permutations of the molecular family making up the library. An example of this latter approach would be a library of all peptides six amino acids in length. Such a peptide library could include every 6 amino acid sequence permutation. This type of library is termed a linear combinatorial chemical library.

[0276] Preparation of combinatorial chemical libraries is well known to those of skill in the art, and may be generated by either chemical or biological synthesis. Combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., US Patent 5,010,175; Furka, Int J Pept Prot Res 1991, 37: 487-93; Houghten et al., Nature 1991, 354: 84-6). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptides (e.g., PCT Publication No. WO 91/19735), encoded peptides (e.g., WO 93/20242), random bio-oligomers (e.g., WO 92/00091), benzodiazepines (e.g., US Patent 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (DeWitt et al., Proc Natl Acad Sci USA 1993, 90:6909-13), vinylogous polypeptides (Hagihara et al., J Amer Chem Soc 1992, 114: 6568), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., J Amer Chem Soc 1992, 114: 9217-8), analogous organic syntheses of small compound libraries (Chen et al., J. Amer Chem Soc 1994, 116: 2661), oligocarbamates (Cho et al., Science 1993, 261: 1303), and/or peptidylphosphonates (Campbell et al., J Org Chem 1994, 59: 658), nucleic acid libraries (see Ausubel, Current Protocols in Molecular Biology 1995 supplement; Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989, Cold Spring Harbor Laboratory, New York, USA), peptide nucleic acid libraries (see, e.g., US Patent 5,539,083), antibody libraries (see, e.g., Vaughan et al., Nature Biotechnology 1996, 14(3):309-14 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al., Science 1996, 274: 1520-22; US Patent 5,593,853), and small organic molecule libraries (see, e.g., benzodiazepines, Gordon EM. Curr Opin Biotechnol. 1995 Dec 1;6(6):624-31.; isoprenoids, US Patent 5,569,588; thiazolidinones and metathiazanones, US Patent 5,549,974; pyrrolidines, US Patents 5,525,735 and 5,519,134; morpholino compounds, US Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

[0277] Devices for the preparation of combinatorial libraries are commercially available (see, e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (see, e.g., ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, MO, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

[0278] (iii) Other candidates:

Another approach uses recombinant bacteriophage to produce libraries. Using the "phage method" (Scott & Smith, Science 1990, 249: 386-90; Cwirla et al., Proc Natl Acad Sci USA 1990, 87: 6378-82; Devlin et al., Science 1990, 249: 404-6), very large libraries can be constructed (e.g., 106 -108 chemical entities). A second approach uses primarily chemical methods, of which the Geysen method (Geysen et al., Molecular Immunology 1986, 23: 709-15; Geysen et al., J Immunologic Method 1987, 102: 259-74); and the method of Fodor et al. (Science 1991, 251: 767-73) are examples. Furka et al. (14th International Congress of Biochemistry 1988, Volume #5, Abstract FR:013; Furka, Int J Peptide Protein Res 1991, 37: 487-93), Houghten (US Patent 4,631,211) and Rutter et al. (US Patent 5,010,175) describe methods to produce a mixture of peptides that can be tested as agonists or antagonists.

[0279] Aptamers are macromolecules composed of nucleic acid that bind tightly to a specific molecular target. Tuerk and Gold (Science. 249:505-510 (1990)) discloses SELEX (Systematic Evolution of Ligands by Exponential Enrichment) method for selection of aptamers. In the SELEX method, a large library of nucleic acid molecules (e.g., 1015 different molecules) can be used for screening.

[0280] Screening for a CSTF2 binding substance

In present invention, over-expression of CSTF2 gene was detected in lung cancer, in spite of no expression in normal organs (Figs. 1 and 2). Furthermore, suppression of the expression of CSTF2 gene by an siRNA against CSTF2 gene induced suppression of cancer cell growth (Fig. 3). These results indicate that CSTF2 gene plays a crucial role in cancer cells. Therefore, using the CSTF2 gene, proteins encoded by the gene, the present invention provides a method of screening for a substance that binds to CSTF2 polypeptide. Due to the expression of CSTF2 gene in lung cancer, a substance binds to CSTF2 polypeptide is expected to suppress the proliferation of lung cancer cells, and thus be useful for treating or preventing lung cancer. Therefore, the present invention also provides a method for screening a candidate substance that suppresses the proliferation of lung cancer cells, and a method for screening a candidate substance for treating or preventing lung cancer using the CSTF2 polypeptide. Specially, in an embodiment of the present method of screening for a candidate substance for treating or preventing cancer, or inhibiting cancer cell growth, the method includes the steps of:

(a) contacting a test substance with a CSTF2 polypeptide or a fragment thereof;

(b) detecting the binding activity between the polypeptide or fragment thereof and the test substance; and

(c) selecting the test substance that binds to the polypeptide or a fragment as a candidate substance for treating or preventing cancer.

[0281] In another embodiment, the present invention also provides a method of screening for a candidate substance for treating or preventing cancer, or inhibiting cancer cell

growth, using the CSTF2 polypeptide or fragments thereof including the steps as follows:

(a) contacting a test substance with the CSTF2 polypeptide or a functional fragment thereof; and

(b) detecting the binding activity between the polypeptide or fragment thereof and the test substance of step (a), and

(c) correlating the binding activity of (b) with the therapeutic effect of the test substance.

[0282] Alternatively, according to the present invention, the potential therapeutic effect of a test substance or compound on treating or preventing cancer can also be evaluated or estimated. In some embodiments, the present invention provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of CSTF2, the method including steps of:

(a) contacting a test substance with a polypeptide encoded by a polynucleotide of CSTF2;

(b) detecting the binding activity between the polypeptide and the test substance; and

(c) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance binds to the polypeptide.

[0283] In the present invention, the therapeutic effect may be correlated with the binding activity of CSTF2 polypeptide or a functional fragment thereof. For example, when the test substance binds to CSTF2 polypeptide or a functional fragment thereof, the test substance may identified or selected as the candidate substance having the therapeutic effect. Alternatively, when the test substance does not binds to CSTF2 polypeptide or a functional fragment thereof, the test agent or compound may identified as the substance having no significant therapeutic effect.

[0284] The method of the present invention will be described in more detail below.

The CSTF2 polypeptide to be used for screening may be a recombinant polypeptide or a protein derived from the nature or a partial peptide thereof. The polypeptide to be contacted with a test substance can be, for example, a purified polypeptide, a soluble protein, a form bound to a carrier or a fusion protein fused with other polypeptides.

[0285] As a method of screening for proteins, for example, that bind to the CSTF2 polypeptide using the CSTF2 polypeptide, many methods well known by a person skilled in the art can be used. Such a screening can be conducted by, for example, immunoprecipitation method, specifically, in the following manner. The gene encoding the CSTF2 polypeptide is expressed in host (e.g., animal) cells and so on by inserting the gene to an expression vector for foreign genes, such as pSV2neo, pcDNA I, pcDNA3.1, pCAGGS and pCD8.

[0286] The promoter to be used for the expression may be any promoter that can be used commonly and include, for example, the SV40 early promoter (Rigby in Williamson (ed.), Genetic Engineering, vol. 3. Academic Press, London, 83-141 (1982)), the EF-alpha promoter (Kim et al., Gene 91: 217-23 (1990)), the CAG promoter (Niwa et al., Gene 108: 193 (1991)), the RSV LTR promoter (Cullen, Methods in Enzymology 152: 684-704 (1987)) the SR alpha promoter (Takebe et al., Mol Cell Biol 8: 466 (1988)), the CMV immediate early promoter (Seed and Aruffo, Proc Natl Acad Sci USA 84: 3365-9 (1987)), the SV40 late promoter (Gheysen and Fiers, J Mol Appl Genet 1: 385-94 (1982)), the Adenovirus late promoter (Kaufman et al., Mol Cell Biol 9: 946 (1989)), the HSV TK promoter and so on.

[0287] The introduction of the gene into host cells to express a foreign gene can be performed according to any methods, for example, the electroporation method (Chu et al., Nucleic Acids Res 15: 1311-26 (1987)), the calcium phosphate method (Chen and Okayama, Mol Cell Biol 7: 2745-52 (1987)), the DEAE dextran method (Lopata et al., Nucleic Acids Res 12: 5707-17 (1984); Sussman and Milman, Mol Cell Biol 4: 1641-3 (1984)), the Lipofectin method (Derijard B., Cell 76: 1025-37 (1994); Lamb et al., Nature Genetics 5: 22-30 (1993): Rabindran et al., Science 259: 230-4 (1993)) and so on.

[0288] The polypeptide encoded by the CSTF2 gene can be expressed as a fusion protein including a recognition site (epitope) of a monoclonal antibody by introducing the epitope of the monoclonal antibody, whose specificity has been revealed, to the N- or C- terminus of the polypeptide. A commercially available epitope-antibody system can be used (Experimental Medicine 13: 85-90 (1995)). Vectors which can express a fusion protein with, for example, beta-galactosidase, maltose binding protein, glutathione S-transferase, green florescence protein (GFP) and so on by the use of its multiple cloning sites are commercially available. Also, a fusion protein prepared by introducing only small epitopes consisting of several to a dozen amino acids so as not to change the property of the CSTF2 polypeptide by the fusion is also reported. Epitopes, such as polyhistidine (His-tag), influenza aggregate HA, human c-myc, FLAG, Vesicular stomatitis virus glycoprotein (VSV-GP), T7 gene 10 protein (T7-tag), human simple herpes virus glycoprotein (HSV-tag), E-tag (an epitope on monoclonal phage) and such, and monoclonal antibodies recognizing them can be used as the epitope-antibody system for screening proteins binding to the CSTF2 polypeptide (Experimental Medicine 13: 85-90 (1995)).

[0289] In immunoprecipitation, an immune complex is formed by adding these antibodies to cell lysate prepared using an appropriate detergent. The immune complex consists of the CSTF2 polypeptide, a polypeptide including the binding ability with the polypeptide, and an antibody. Immunoprecipitation can be also conducted using an-

tibodies against the CSTF2 polypeptide, besides using antibodies against the above epitopes, which antibodies can be prepared as described above. An immune complex can be precipitated, for example by Protein A sepharose or Protein G sepharose when the antibody is a mouse IgG antibody. If the polypeptide encoded by the CSTF2 gene is prepared as a fusion protein with an epitope, such as GST, an immune complex can be formed in the same manner as in the use of the antibody against the CSTF2 polypeptide, using a substance specifically binding to these epitopes, such as glutathione-Sepharose 4B.

[0290] Immunoprecipitation can be performed by following or according to, for example, the methods in the literature (Harlow and Lane, Antibodies, 511-52, Cold Spring Harbor Laboratory publications, New York (1988)).

[0291] SDS-PAGE is commonly used for analysis of immunoprecipitated proteins and the bound protein can be analyzed by the molecular weight of the protein using gels with an appropriate concentration. Since the protein bound to the CSTF2 polypeptide is difficult to detect by a common staining method, such as Coomassie staining or silver staining, the detection sensitivity for the protein can be improved by culturing cells in culture medium containing radioactive isotope, 35S-methionine or 35S-cysteine, labeling proteins in the cells, and detecting the proteins. The target protein can be purified directly from the SDS-polyacrylamide gel and its sequence can be determined, when the molecular weight of a protein has been revealed.

[0292] As a method of screening for proteins binding to the CSTF2 polypeptide using the polypeptide, for example, West-Western blotting analysis (Skolnik et al., Cell 65: 83-90 (1991)) can be used. Specifically, a protein binding to the CSTF2 polypeptide can be obtained by preparing a cDNA library from cultured cells (e.g., LC176, LC319, A549, NCI-H23, NCI-H226, NCI-H522, PC3, PC9, PC14, SK-LU-1, EBC-1, RERF-LC-AI, SK-MES-1, SW900, and SW1573) expected to express a protein binding to the CSTF2 polypeptide using a phage vector (e.g., ZAP), expressing the protein on LB-agarose, fixing the protein expressed on a filter, reacting the purified and labeled CSTF2 polypeptide with the above filter, and detecting the plaques expressing proteins bound to the CSTF2 polypeptide according to the label. The polypeptide of the invention may be labeled by utilizing the binding between biotin and avidin, or by utilizing an antibody that specifically binds to the CSTF2, or a peptide or polypeptide (for example, GST) that is fused to the CSTF2 polypeptide. Methods using radioisotope or fluorescence and such may be also used.

[0293] Alternatively, in another embodiment of the screening method of the present invention, a two-hybrid system utilizing cells may be used ("MATCHMAKER Two-Hybrid system", "Mammalian MATCHMAKER Two-Hybrid Assay Kit", "MATCHMAKER one-Hybrid system" (Clontech); "HybriZAP Two-Hybrid Vector

System" (Stratagene); the references "Dalton and Treisman, Cell 68: 597-612 (1992)", "Fields and Sternglanz, Trends Genet 10: 286-92 (1994)").

[0294] In the two-hybrid system, the polypeptide of the invention is fused to the SRF-binding region or GAL4-binding region and expressed in yeast cells. A cDNA library is prepared from cells expected to express a protein binding to the polypeptide of the invention, such that the library, when expressed, is fused to the VP16 or GAL4 transcriptional activation region. The cDNA library is then introduced into the above yeast cells and the cDNA derived from the library is isolated from the positive clones detected (when a protein binding to the polypeptide of the invention is expressed in yeast cells, the binding of the two activates a reporter gene, making positive clones detectable). A protein encoded by the cDNA can be prepared by introducing the cDNA isolated above to E. coli and expressing the protein. As a reporter gene, for example, Ade2 gene, lacZ gene, CAT gene, luciferase gene and such can be used in addition to the HIS3 gene.

[0295] A substance binding to the polypeptide encoded by a CSTF2 gene can also be screened using affinity chromatography. For example, the polypeptide of the invention may be immobilized on a carrier of an affinity column, and a test substance, containing a protein capable of binding to the polypeptide of the invention, is applied to the column. A test substance herein may be, for example, cell extracts, cell lysates, etc. After loading the test substance, the column is washed, and substances bound to the polypeptide of the invention can be prepared. When the test substance is a protein, the amino acid sequence of the obtained protein is analyzed, an oligo DNA is synthesized based on the sequence, and cDNA libraries are screened using the oligo DNA as a probe to obtain a DNA encoding the protein.

[0296] A biosensor using the surface plasmon resonance phenomenon may be used as a mean for detecting or quantifying the bound substance in the present invention. When such a biosensor is used, the interaction between the polypeptide of the invention and a test substance can be observed real-time as a surface plasmon resonance signal, using only a minute amount of polypeptide and without labeling (for example, BIAcore, Pharmacia). Therefore, it is possible to evaluate the binding between the polypeptide of the invention and a test substance using a biosensor such as BIAcore.

[0297] The methods of screening for molecules that bind when the immobilized CSTF2 polypeptide is exposed to synthetic chemical substances, or natural substance banks or a random phage peptide display library, and the methods of screening using high-throughput based on combinatorial chemistry techniques (Wrighton et al., Science 273: 458-64 (1996); Verdine, Nature 384: 11-13 (1996); Hogan, Nature 384: 17-9 (1996)) to isolate not only proteins but chemical substances that bind to the CSTF2 protein (including agonist and antagonist) are well known to one skilled in the art.

[0298] In addition to the CSTF2 polypeptide, fragments of the polypeptides may be used for the present screening, so long as it retains at least one biological activity of the natural occurring CSTF2 polypeptide.

[0299] The polypeptides or fragments thereof may be further linked to other substances, so long as the polypeptides and fragments retain at least one of their biological activities. Usable substances include: peptides, lipids, sugar and sugar chains, acetyl groups, natural and synthetic polymers, etc. These kinds of modifications may be performed to confer additional functions or to stabilize the polypeptide and fragments.

[0300] The polypeptides or fragments used for the present method may be obtained from nature as naturally occurring proteins via conventional purification methods or through chemical synthesis based on the selected amino acid sequence. For example, conventional peptide synthesis methods that can be adopted for the synthesis include:

1) Peptide Synthesis, Interscience, New York, 1966;

2) The Proteins, Vol. 2, Academic Press, New York, 1976;

3) Peptide Synthesis (in Japanese), Maruzen Co., 1975;

4) Basics and Experiment of Peptide Synthesis (in Japanese), Maruzen Co., 1985;

5) Development of Pharmaceuticals (second volume) (in Japanese), Vol. 14 (peptide synthesis), Hirokawa, 1991;

6) WO99/67288; and

7) Barany G. & Merrifield R.B., Peptides Vol. 2, "Solid Phase Peptide Synthesis", Academic Press, New York, 1980, 100-118.

[0301] Alternatively, the proteins may be obtained through any known genetic engineering methods for producing polypeptides (e.g., Morrison J., J Bacteriology 1977, 132: 349-51; Clark-Curtiss & Curtiss, Methods in Enzymology (eds. Wu et al.) 1983, 101: 347-62). For example, first, a suitable vector including a polynucleotide encoding the objective protein in an expressible form (e.g., downstream of a regulatory sequence including a promoter) is prepared, transformed into a suitable host cell, and then the host cell is cultured to produce the protein. More specifically, a gene encoding the CSTF2 polypeptide is expressed in host (e.g., animal) cells and such by inserting the gene into a vector for expressing foreign genes, such as pSV2neo, pcDNA I, pcDNA3.1, pCAGGS, or pCD8. A promoter may be used for the expression. Any commonly used promoters may be employed including, for example, the SV40 early promoter (Rigby in Williamson (ed.), Genetic Engineering, vol. 3. Academic Press, London, 1982, 83-141), the EF-alpha promoter (Kim et al., Gene 1990, 91:217-23), the CAG promoter (Niwa et al., Gene 1991, 108:193), the RSV LTR promoter (Cullen, Methods in Enzymology 1987, 152:684-704), the SR alpha promoter (Takebe et al., Mol Cell Biol 1988, 8:466), the CMV immediate early promoter (Seed et al., Proc Natl Acad Sci USA 1987, 84:3365-9), the SV40 late promoter (Gheysen et al., J Mol Appl

Genet 1982, 1:385-94), the Adenovirus late promoter (Kaufman et al., Mol Cell Biol 1989, 9:946), the HSV TK promoter, and such. The introduction of the vector into host cells to express the CSTF2 gene can be performed according to any methods, for example, the electroporation method (Chu et al., Nucleic Acids Res 1987, 15:1311-26), the calcium phosphate method (Chen et al., Mol Cell Biol 1987, 7:2745-52), the DEAE dextran method (Lopata et al., Nucleic Acids Res 1984, 12:5707-17; Sussman et al., Mol Cell Biol 1985, 4:1641-3), the Lipofectin method (Derijard B, Cell 1994, 7:1025-37; Lamb et al., Nature Genetics 1993, 5:22-30; Rabindran et al., Science 1993, 259:230-4), and such.

[0302] The CSTF2 protein may also be produced in vitro adopting an in vitro translation system.

The CSTF2 polypeptide to be contacted with a test substance can be, for example, a purified polypeptide, a soluble protein, or a fusion protein fused with other polypeptides.

In the present invention, it is revealed that suppressing the expression of CSTF2 gene reduces cell growth. Thus, by screening for a candidate substance that binds to polypeptide CSTF2, a candidate substance that has the potential to treat or prevent cancers can be identified. Potential of these candidate substances or agents to treat or prevent cancers may be evaluated by second and/or further screening to identify therapeutic substance for cancers.

[0303] Screening for a substance suppressing the biological activity of CSTF2

The present invention provides a method for screening a substance that suppresses the proliferation of cancer cells, and a method for screening a substance for treating or preventing cancer, including lung cancer. Thus, the present invention provides a method of screening for a substance for treating or preventing cancer, or inhibiting cancer cell growth, using the polypeptide encoded by CSTF2 gene including the steps as follows:

(a) contacting a test substance with a CSTF2 polypeptide;

(b) detecting the biological activity of the polypeptide of step (a); and

(c) selecting the test substance that suppresses the biological activity of the CSTF2 polypeptide as compared to the biological activity of the polypeptide detected in the absence of the test substance.

[0304] In another embodiment, the present invention also provides a method of screening for a substance for treating or preventing cancer, or inhibiting cancer cell growth, using the polypeptide encoded by CSTF2 gene including the steps as follows:

(a) contacting a test substance with the CSTF2 polypeptide; and

(b) detecting the biological activity of the polypeptide of step (a), and

(c) correlating the biological activity of (b) with the therapeutic effect of the test

substance.

[0305] Alternatively, in some embodiments, the present invention provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of CSTF2, the method including steps of:

(a) contacting a test substance with a polypeptide encoded by a polynucleotide of CSTF2 gene;

(b) detecting the biological activity of the polypeptide of step (a); and

(c) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a substance suppresses the biological activity of the polypeptide encoded by the polynucleotide of CSTF2 gene as compared to the biological activity of said polypeptide detected in the absence of the test substance.

[0306] In the present invention, the therapeutic effect may be correlated with the biological activity of CSTF2 polypeptide. For example, when the test substance suppresses or inhibits the biological activity of CSTF2 polypeptide as compared to a level detected in the absence of the substance, the test substance may identified or selected as the candidate substance having the therapeutic effect. Alternatively, when the test substance does not suppress or inhibit the biological activity of CSTF2 polypeptide as compared to a level detected in the absence of the test substance, the test substance may identified as the substance having no significant therapeutic effect.

[0307] The method of the present invention will be described in more detail below.

Any polypeptides can be used for screening so long as they include the biological activity of the CSTF2 protein. Such biological activity includes cell-proliferating activity, RNA binding activity, mRNA cleavage activity and mRNA polyadenylation activity of the CSTF2 protein. For example, CSTF2 protein can be used and polypeptides functionally equivalent to the CSTF2 protein can also be used. Such polypeptides may be expressed endogenously or exogenously by cells.

[0308] The substance isolated by this screening is a candidate for antagonists of the polypeptide encoded by CSTF2 gene. The term "antagonist" refers to molecules that inhibit the function of the polypeptide by binding thereto. The term also refers to molecules that reduce or inhibit expression of the gene encoding CSTF2. Moreover, a substance isolated by this screening is a candidate for substances which inhibit the in vivo interaction of the CSTF2 polypeptide with molecules (including DNAs, RNAs and proteins).

[0309] When the biological activity to be detected in the present method is cell proliferation, it can be detected, for example, by preparing cells which express the CSTF2 polypeptide, culturing the cells in the presence of a test substance, and determining the

speed of cell proliferation, measuring the cell cycle and such, as well as by measuring survival cells or the colony forming activity, for example, shown in Fig. 3 or 4. The substances that reduce the speed of proliferation of the cells expressed CSTF2 are selected as candidate substance for treating or preventing cancer, including lung cancer.

[0310] In the present invention, it is revealed that suppressing the expression of CSTF2 gene reduces cell growth. Thus, by screening for a candidate substance that reduces the biological activity of CSTF2 polypeptide, a candidate substance that has the potential to treat or prevent cancers can be identified. Potential of these candidate substances to treat or prevent cancers may be evaluated by second and/or further screening to identify therapeutic substance for cancers.

[0311] More specifically, the method includes the step of:

(a) contacting a test substance with cells overexpressing CSTF2 gene;

(b) measuring cell-proliferating activity; and

(c) selecting the test substance that reduces the cell-proliferating activity in the comparison with the cell-proliferating activity in the absence of the test substance.

In preferable embodiments, the method of the present invention may further include the steps of:

(d) selecting the test substance that have no effect to the cells no or little expressing CSTF2.

[0312] "Suppress the biological activity" as defined herein are preferably at least 10% suppression of the biological activity of CSTF2 in comparison with in absence of the substance, more preferably at least 25%, 50% or 75% suppression and most preferably at 90% suppression.

[0313] In the preferred embodiments, control cells which do not express CSTF2 polypeptide are used. Accordingly, the present invention also provides a method of screening for a candidate substance for inhibiting the cell growth or a candidate substance for treating or preventing CSTF2 associating disease, using the CSTF2 polypeptide or fragments thereof including the steps as follows:

(a) culturing cells which express a CSTF2 polypeptide or a functional fragment thereof, and control cells that do not express a CSTF2 polypeptide or a functional fragment thereof in the presence of a test substance;

(b) detecting the biological activity of the cells which express the protein and control cells; and

(c) selecting the test substance that inhibits the biological activity in the cells which express the protein as compared to the proliferation detected in the control cells and in the absence of said test substance.

[0314] In some embodiments, RNA binding activity, mRNA cleavage activity or mRNA

polyadenylation activity may be used as a biological activity of the CSTF2 polypeptide to be detected in the screening method of the present invention. Methods for detecting these activities are well-known in the art. When any one of those activities is detected in the screening, polypeptides containing a RNA recognition motif of the CSTF2 polypeptide may be preferably used as functional equivalents of the CSTF2 polypeptide. For example, the RNA recognition motif of the CSTF2 polypeptide having an amino acid sequence of SEQ ID NO: 2 is the region consisting of amino acid 17 to 90 of SEQ ID NO: 2.

[0315] Screening for a substance altering the expression of CSTF2

The present invention provides a method of screening for a substance that inhibits the expression of CSTF2 gene. A substance that inhibits the expression of CSTF2 gene is expected to suppress the proliferation of lung cancer cells, and thus is useful for treating or preventing lung cancer. Therefore, the present invention also provides a method for screening a candidate substance that suppresses the proliferation of lung cancer cells, and a method for screening a candidate substance for treating or preventing lung cancer. In the context of the present invention, such screening may include, for example, the following steps:

(a) contacting a test substance with a cell expressing CSTF2 gene;

(b) detecting the expression level of the CSTF2 gene; and

(c) selecting the test substance that reduces the expression level of CSTF2 gene as compared to the expression level detected in the absence of the test substance.

[0316] In another enbodiment, the present invention also provides a method for screening a candidate substance that suppresses the proliferation of cancer cells, and a method for screening a candidate substance for treating or preventing CSTF2 associating disease.

[0317] In the context of the present invention, such screening may include, for example, the following steps:

(a) contacting a test substance with a cell expressing the CSTF2 gene;

(b) detecting the expression level of the CSTF2 gene; and

(c) correlating the expression level of (b) with the therapeutic effect of the test substance.

[0318] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of CSTF2, the method including steps of:

(a) contacting a test substance with a cell expressing CSTF2; and;

(b) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduces the expression level of CSTF2 as compared to a control.

[0319] In the present invention, the therapeutic effect may be correlated with the expression level of the CSTF2 gene. For example, when the test substance reduces the expression level of the CSTF2 gene as compared to a level detected in the absence of the test substance, the test substance may identified or selected as the candidate substance having the therapeutic effect. Alternatively, when the test substance does not reduce the expression level of the CSTF2 gene as compared to a level detected in the absence of the test substance, the test substance may identified as the substance having no significant therapeutic effect.

[0320] The method of the present invention will be described in more detail below.

Cells expressing the CSTF2 gene include, for example, cell lines established from lung cancer; such cells can be used for the above screening of the present invention (e.g., A427, A549, LC319, PC14, PC3, PC9, NCI-H1373, NCI-H1781, NCI-H358, NCI-H226, NCI-H520, NCI-H1703, NCI-H2170, EBC-1, RERF-LC-AI, LX1, DMS114, DMS273, SBC-3, SBC-5, NCI-H196, NCI-H446, SK-MES-1, LU61). The expression level can be estimated by methods well known to one skilled in the art, for example, RT-PCR, Northern blot assay, Western blot assay, immunostaining and flow cytometry analysis. "Reduce the expression level" as defined herein are preferably at least 10% reduction of expression level of CSTF2 gene in comparison to the expression level in absence of the substance, more preferably at least 25%, 50% or 75% reduced level and most preferably at 95% reduced level. The substance herein includes chemical substance, double-strand nucleotide, and so on. The preparation of the double-strand nucleotide is in aforementioned description. In the method of screening, a substance that reduces the expression level of CSTF2 gene can be selected as candidate substances to be used for the treatment or prevention of lung cancer.

[0321] In the present invention, it is revealed that suppressing the expression of CSTF2 gene reduces cell growth. Thus, by screening for a substance that reduces the expression level of the CSTF2 gene, a candidate substance that has the potential to treat or prevent cancers can be identified. Potential of these candidate substances to treat or prevent cancers may be evaluated by second and/or further screening to identify therapeutic substance for cancers.

[0322] Alternatively, the screening method of the present invention may include the following steps:

(a) contacting a test substance with a cell into which a vector, including the transcriptional regulatory region of CSTF2 gene and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

(b) measuring the expression level or activity of the reporter gene; and

(c) selecting the test substance that reduces the expression level or activity of the reporter gene.

[0323] In another embodiment, the present invention also provides a method for screening a candidate substance that suppresses the proliferation of cancer cells, and a method for screening a candidate substance for treating or preventing CSTF2 associating disease.

[0324] According to another aspect, the present invention provides a method which includes the following steps of:

(a) contacting a test substance with a cell into which a vector, composed of the transcriptional regulatory region of the CSTF2 gene and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

(b) detecting the expression or activity of said reporter gene; and

(c) correlating the expression level of (b) with the therapeutic effect of the test substance.

[0325] Alternatively, in some embodiments, the present invention also provides a method for evaluating or estimating a therapeutic effect of a test substance on treating or preventing cancer or inhibiting cancer associated with over-expression of CSTF2, the method including steps of:

[0326] (a) contacting a test substance with a cell into which a vector, including the transcriptional regulatory region of CSTF2 gene and a reporter gene that is expressed under the control of the transcriptional regulatory region, has been introduced;

(b) measuring the expression or activity of said reporter gene; and

(c) correlating the potential therapeutic effect and the test substance, wherein the potential therapeutic effect is shown, when a test substance reduces the expression or activity of said reporter gene.

[0327] In the present invention, the therapeutic effect may be correlated with the expression level or activity of said reporter gene. For example, when the substance reduces the expression level or activity of said reporter gene as compared to a level detected in the absence of the test substance, the test substance may identified or selected as the candidate substance having the therapeutic effect. Alternatively, when the test substance does not reduce the expression level or activity of said reporter gene as compared to a level detected in the absence of the test substance, the test substance may identified as the substance having no significant therapeutic effect.

[0328] Suitable reporter genes and host cells are well known in the art. For example, reporter genes are luciferase, green florescence protein (GFP), Discosoma sp. Red Fluorescent Protein (DsRed), Chrolamphenicol Acetyltransferase (CAT), lacZ and beta-glucuronidase (GUS), and host cell is COS7, HEK293, HeLa and so on. The reporter construct required for the screening can be prepared by connecting reporter gene sequence to the transcriptional regulatory region of CSTF2 gene. The transcriptional regulatory region of CSTF2 herein is the region from start codon to at least 500 bp upstream, preferably 1,000 bp, more preferably 5000 or 10,000 bp upstream. A nu-

cleotide segment containing the transcriptional regulatory region can be isolated from a genome library or can be propagated by PCR. The reporter construct required for the screening can be prepared by connecting reporter gene sequence to the transcriptional regulatory region of any one of these genes. Methods for identifying a transcriptional regulatory region, and also assay protocol are well known (Molecular Cloning third edition chapter 17, 2001, Cold Springs Harbor Laboratory Press).

[0329] The vector containing the reporter construct is infected to host cells and the expression or activity of the reporter gene is detected by method well known in the art (e.g., using luminometer, absorption spectrometer, flow cytometer and so on). "Reduces the expression or activity" as defined herein are preferably at least 10% reduction of the expression or activity of the reporter gene in comparison with in absence of the substance, more preferably at least 25%, 50% or 75% reduction and most preferably at 95% reduction.

[0330] In the present invention, it is revealed that suppressing the expression of CSTF2 gene reduces cell growth. Thus, by screening for a candidate substance that reduces the expression or activity of the reporter gene, a candidate substance that has the potential to treat or prevent cancers can be identified. Potential of these candidate substances to treat or prevent cancers may be evaluated by second and/or further screening to identify therapeutic substance for cancers.

[0331] Aspects of the present invention are described in the following examples, which are not intended to limit the scope of the invention described in the claims.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Examples

[0332] Materials and Methods

Cell lines and tissue samples.

Fifteen human lung-cancer cell lines used in this study included five adenocarcinomas (NCI-H1781, NCI-H1373, LC319, A549, and PC-14), five squamous cell carcinomas (SK-MES-1, NCI-H520, NCI-H1703, NCI-H2170, and LU61), one large-cell carcinoma (LX1), and four small-cell lung cancers (SBC-3, SBC-5, DMS114, and DMS273). All cells were grown in monolayer in appropriate media supplemented with 10% FCS and were maintained at 37 degrees C in humidified air with 5% CO2.

Human small airway epithelial cells (SAEC) used as a normal control were grown in optimized medium (Cambrex Bioscience, Inc). Primary NSCLC tissue samples as well as their corresponding normal tissues adjacent to resection margins from patients having no anticancer treatment before tumor resection had been obtained earlier with informed consent (Kikuchi T, Daigo Y, Katagiri T, et al. Oncogene 2003; 22:2192-205, Taniwaki M, Daigo Y, Ishikawa N, et al. Int J Oncol 2006; 29: 567-75, Kato T, Daigo Y, Hayama S, et al. Cancer Res 2005; 65:5638-46). All tumors were staged on the basis of the pathologic tumor-node-metastasis classification of the International Union Against Cancer (Table 1; Sobin L, Wittekind CH. TNM classification of malignant tumors. 6th ed. New York: Wiley-Liss; 2002). Formalin-fixed primary lung tumors and adjacent normal lung tissue samples used for immunostaining on tissue microarrays had been obtained from 327 patients (196 adenocarcinomas, 98 squamous cell carcinomas, 23 large-cell carcinomas, and 10 adenosquamous carcinomas; 99 female and 228 male patients; median age of 64.7 y with a range of 29-85 y) undergoing surgery at Saitama Cancer Center. These patients that received resection of their primary cancers did not receive any preoperative treatment, and among them only patients with positive lymph node metastasis were treated with platinum-based adjuvant chemotherapies after their surgery. This study and the use of all clinical materials mentioned were approved by individual institutional ethics committees.

[0333] [Table 1]

Association between CSTF2-positivity in NSCLC tissues and patients' characteristics (n=327)

		Total	CSTF2 strong positive	CSTF2 weak positive	CSTF2 absent	P value strong vs. weak/absent
		n = 327	n = 77	n = 165	n = 85	
Gender						
	Male	228	55	120	53	0.7775
	Female	99	22	45	32	
Age (years)						
	< 65	150	33	77	40	0.6016
	>= 65	177	44	88	45	
Histology						
	ADC	196	45	97	54	0.7910
	Non-ADC	131	32	68	31	
Smoking						
	Smoker	234	51	124	59	0.2497
	Non-smoker	93	26	41	26	
pT factor						
	T1	135	26	68	41	0.1458
	T2+T3	192	51	97	44	
pN factor						
	N0	208	45	107	56	0.2824
	N1+N2	119	32	58	29	

ADC, adenocarcinoma
non-ADC, squamous-cell carcinoma plus large-cell carcinoma and adenosquamous-cell carcinoma
* P < 0.05 (Fisher's exact test)

[0334] Semiquantitative reverse transcription-PCR.

A total of 3 micrograms of mRNA aliquot from each sample were reverse transcribed

to single-stranded cDNAs using random primer (Roche Diagnostics) and Superscript II (Invitrogen). Semiquantitative reverse transcription-PCR (RT-PCR) experiments were carried out with the following sets of synthesized primers specific for human CSTF2 (Gene Accession NO. NM_001325) or with beta-actin (ACTB)-specific primers as an internal control: CSTF2, 5'-GTCATGCAGGGAACAGGAAT-3' (SEQ ID NO: 3) and 5'-TGAGTCATTCAAGGGTTAGGATG-3' (SEQ ID NO: 4); ACTB, 5'-GAGGTGATAGCATTGCTTTCG-3' (SEQ ID NO: 5) and 5'-CAAGTCAGTGTACAGGTAAGC-3' (SEQ ID NO: 6). PCR reactions were optimized for the number of cycles to ensure product intensity to be within the linear phase of amplification.

[0335] Northern blot analysis.

Human multiple tissue blots covering 23 tissues (BD Bioscience) were hybridized with a 32P-labeled 521-bp PCR product of CSTF2 that was prepared as a probe using primers 5'-CGAGGCTTGTTAGGAGATGC-3' (SEQ ID NO: 7) and 5'-CCCCCATGTTAAGGACTG-3' (SEQ ID NO: 8). Prehybridization, hybridization, and washing were done following manufacturer's recommendation. The blots were autoradiographed with intensifying screens at -80 degrees C for 14 days.

[0336] Western blotting.

Tumor cells were lysed in lysis buffer; 50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 0.5% NP40, 0.5% sodium deoxycholate, and Protease Inhibitor Cocktail Set III (Calbiochem). The protein content of each lysate was determined by a Bio-Rad protein assay kit with bovine serum albumin as a standard. Ten micrograms of each lysate were resolved on 7.5% to 12% denaturing polyacrylamide gels (with 3% polyacrylamide stacking gel) and transferred electrophoretically onto a nitrocellulose membrane (GE Healthcare Biosciences). After blocking with 5% nonfat dry milk in TBST (Tris Buffered Saline with Tween 20), the membrane was incubated for 1 hour at room temperature with a rabbit polyclonal antibody. A commercially available rabbit polyclonal anti-CSTF2 antibody was purchased from ATLAS, Inc., and was probed to be specific to human CSTF2, by Western blot analysis using lysates of lung cancer cell lines. Immunoreactive proteins were incubated with horseradish peroxidase-conjugated secondary antibodies (GE Healthcare Bio-sciences) for 1 hour at room temperature. After washing with TBST, the reactants were developed using the enhanced chemiluminescence kit (GE Healthcare Bio-sciences).

[0337] Immunofluorescence analysis.

Cultured cells washed twice with PBS (-), fixed in 4% formaldehyde solution for 60 min at 4 degrees C, and rendered permeable by treatment for 5 min with PBS (-) containing 0.1% Triton X-100. Cells were covered with CAS Block (Zymed) for 10 min to block nonspecific binding before the primary antibody reaction. Then the cells

were incubated with antibody to human CSTF2 or c-myc-tagged protein.

[0338] Immunohistochemistry and tissue microarray.

To investigate clinicopathologic significance of the CSTF2 protein in clinical lung cancer samples that had been formalin fixed and embedded in paraffin blocks, the sections were stained using Envision+ Kit/horseradish peroxidase (DakoCytomation) in the following manner. For antigen retrieval, slides were immersed in Target Retrieval Solution pH 9 (DakoCytomation) and boiled at 108 degrees C for 15 min in an autoclave. A rabbit polyclonal anti-human CSTF2 antibody (0.06 microgram/ml; ATLAS) was added to each slide after blocking of endogenous peroxidase and proteins, and the sections were incubated with horseradish peroxidase-labeled anti-rabbit IgG [Histofine Simple Stain MAX PO (G), Nichirei] as the secondary antibody. Substrate-chromogen was added, and the specimens were counterstained with hematoxylin.

[0339] Tumor tissue microarrays were constructed with 327 formalin-fixed primary NSCLCs which had been obtained by a single institution (please see above) with an identical protocol to collect, fix, and preserve the tissues after resection (Chin S F, Daigo Y, Huang HE, et al. Mol Pathol 2003;56:275-9, Callagy G, Cattaneo E, Daigo Y, et al. Diagn Mol Pathol 2003;12:27-34, Callagy G, Pharoah P, Chin SF, et al. J Pathol 2005;205:388-96). Considering the histologic heterogeneity of individual tumors, tissue area for sampling was selected based on visual alignment with the corresponding H&E-stained section on a slide. Three, four, or five tissue cores (diameter, 0.6 mm; depth, 3-4 mm) taken from a donor tumor block were placed into a recipient paraffin block with a tissue microarrayer (Beecher Instruments). A core of normal tissue was punched from each case, and 5-micrometer sections of the resulting microarray block were used for immunohistochemical analysis. Three independent investigators semiquantitatively assessed CSTF2 positivity without prior knowledge of clinicopathologic data. Because the intensity of staining within each tumor tissue core was mostly homogeneous, the intensity of CSTF2 staining was semiquantitatively evaluated using the following criteria: strong positive (scored as 2+), dark brown staining in >50% of tumor cells completely obscuring nucleus and cytoplasm; weak positive (1+), any lesser degree of brown staining appreciable in tumor cell nucleus and cytoplasm; absent (scored as 0), no appreciable staining in tumor cells. Cases were accepted as strongly positive only if reviewers independently defined them as such.

[0340] Statistical analysis.

Statistical analyses were done using the StatView statistical program (SaS). Strong CSTF2 immunoreactivity was assessed for association with clinicopathologic variables such as age, gender, pathologic tumor-node-metastasis stage, and histologic type using the Fisher exact test. Tumor-specific survival curves were calculated from the date of

surgery to the time of death related to NSCLC, or to the last follow-up observation. Kaplan-Meier curves were calculated for each relevant variable and for CSTF2 expression; differences in survival times among patient subgroups were analyzed using the log-rank test. Univariate and multivariate analyses were done with the Cox proportional hazard regression model to determine associations between clinicopathologic variables and cancer-related mortality. First, associations between death and possible prognostic factors including age, gender, histology, pT classification, and pN classification were analyzed, taking into consideration one factor at a time. Second, multivariate analysis was applied on backward (stepwise) procedures that always forced strong CSTF2 expression into the model, along with any and all variables that satisfied an entry level of $P < 0.05$. As the model continued to add factors, independent factors did not exceed an exit level of $P < 0.05$.

[0341] RNA interference assay.

To evaluate the biological functions of CSTF2 in lung and esophageal cancer cells, small interfering RNA (siRNA) duplexes against the target genes (SIGMA) were used. The target sequences of the synthetic oligonucleotides for RNA interference were as follows: si-CSTF2-#1, 5'-GGCUUUAGUCCCGGGCAGA-3' (SEQ ID NO: 9); si-CSTF2-#2, 5'-CACUUUACUUUCUGUAACU-3' (SEQ ID NO: 10), control 1: (EGFP, enhanced green fluorescence protein [GFP] gene, a mutant of Aequorea gictoria GFP), 5'-GAAGCAGCACGACUUCUUC-3' (SEQ ID NO: 11); control 2 (LUC, luciferase gene from Photinus pyralis), 5'-CGUACGCGGAAUACUUCGA-3' (SEQ ID NO: 12). Lung cancer cell lines, A549 and LC319, were plated onto 10-cm dishes (8.0 x 105 per dish), and transfected with either of the siRNA oligonucleotides (100 nmol/L) using 30 microlitter of Lipofectamine 2000 (Invitrogen) according to the manufacturers' instructions. After 7 days of incubation, these cells were stained by Giemsa solution to assess colony formation, and cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

[0342] Results

Expression of CSTF2 in lung cancers and normal tissues.

To identify novel target molecules for the development of therapeutic agents and/or biomarkers for lung cancer, first, genome-wide gene expression profile analysis was carried out for 101 lung cancers using a cDNA microarray consisting 27,648 genes or ESTs (Kikuchi T, Daigo Y, Katagiri T, et al. Oncogene 2003;22:2192-205, Kakiuchi S, Daigo Y, Tsunoda T, Yano S, Sone S, Nakamura Y. Mol Cancer Res 2003;1:485-99, Kakiuchi S, Daigo Y, Ishikawa N, et al. Hum Mol Genet 2004;13:3029-43, Kikuchi T, Daigo Y, Ishikawa N, et al. Int J Oncol 2006; 28:799-805, Taniwaki M, Daigo Y, Ishikawa N, et al. Int J Oncol 2006;29:567-75). It was identified the CSTF2 transcript to be overexpressed (3-fold or higher) in the majority of lung cancer samples

examined, whereas CSTF2 was scarcely expressed in any of 29 normal tissues except testis. As a result, CSTF2 was considered to be a good candidate gene for novel molecular target. The CSTF2 overexpression was confirmed by semiquantitative RT-PCR experiments in 12 of 15 lung cancer tissues and in 15 of 15 lung-cancer cell lines examined (Fig. 1A). In addition, the expression of CSTF2 proteins was confirmed in 9 lung cancer cell lines by western blot analysis using anti-CSTF2 antibody (Fig. 1B). To determine the subcellular localization of endogenous CSTF2 in lung cancer cells, immunofluorescence analysis was carried out using anti-CSTF2 antibody and its staining in the nucleus of SBC5 cells was found (Fig. 1C).

[0343] Northern blot analysis with a CSTF2 cDNA as a probe identified a 2.6-kb transcript only in testis among 16 normal human tissues examined (Fig. 2A). In addition, the expression of CSTF2 protein in five normal tissues (heart, lung, liver, kidney, and testis) was examined as well as lung cancers using anti-CSTF2 antibody. It was found that CSTF2 positive staining was observed in nucleus of testicular cells, but not in other normal tissues (Fig. 2B).

[0344] Association of CSTF2 overexpression with poor prognosis for NSCLC patients.

To verify the biological and clinicopathological significance of CSTF2 in pulmonary carcinogenesis, immunohistochemical staining was carried out on tissue microarrays containing primary NSCLC tissues from 327 patients who underwent curative surgical resection. CSTF2 positive staining with the anti-CSTF2 polyclonal antibody was observed in nucleus in lung cancer cells, but staining was negative in any of their adjacent normal lung cells or stromal cells surrounding tumor cells. The CSTF2 expression levels on the tissue array were classified with ranging from absent (scored as 0) to weak/strong positive (scored as 1+ to 2+) (Fig. 2C). Of the 327 NSCLCs, CSTF2 was strongly stained in 77 cases (24%; score 2+), weakly stained in 165 cases (50%; score 1+), and not stained in 85 cases (26%: score 0; details are shown in Table 1). Next, a correlation of CSTF2 expression levels (strong positive versus weak positive/absent) with various clinicopathologic variables was examined and it was found that strong CSTF2 expression was associated with poor prognosis for NSCLC patients after the resection of primary tumors ($P = 0.0079$, log-rank test; Fig. 2D), but not associated with any other clinicopathologic variables. In addition, univariate analysis was examined to evaluate associations between patient prognosis and several clinicopathologic factors including age (>=65 versus <65 years), gender (male versus female), histology (non-adenocarcinoma versus adenocarcinoma), smoking history (smoker versus non-smoker), pT stage (tumor size; T2-T3 versus T1), pN stage (lymph node metastasis; N1-N2 versus N0), and CSTF2 expression (score 2+ versus 0, 1+). All those parameters except smoking history were significantly associated with poor prognosis (Table 2). Multivariate analysis using the Cox proportional hazard model

indicated that pT stage, pN stage, age, and strong CSTF2 positivity were independent prognostic factors for NSCLC (Table 2).

[0345] [Table 2]

Cox's proportional hazards model analysis of prognostic factors in patients with NSCLCs

		Hazards ratio	95% CI	Unfavorable/Favorable	*P*-value
Univariate analysis					
	CSTF2	1.617	1.130-2.314	Strong(+) / Weak(+) or (-)	0.0085*
	Age (years)	1.672	1.188-2.353	>= 65 / <65	0.0032*
	Gender	1.512	1.037-2.206	Male / Female	0.0317*
	Histlogy	1.434	1.033-1.989	non ADC / ADC	0.0311*
	pT factor	2.176	1.505-3.146	T2+T3+T4 / T1	<0.0001*
	pN factor	1.965	1.416-2.728	N1+N2 / N0	<0.0001*
	Smoking	1.252	0.858-1.826	Smoker / non smoker	0.2437
Multivariate analysis					
	marker	1.548	0.951-1.875	Strong(+) / Weak(+) or (-)	0.0177*
	Age (years)	1.770	1.385-2.519	>= 65 / <65	0.0006
	Gender	1.346	0.939-1.939	Male / Female	0.2301
	Histlogy	0.959	0.775-1.463	ADC / non ADC	0.9607
	pT factor	1.877	1.697-3.068	T2+T3+T4 / T1	0.0085*
	pN factor	2.244	1.697-3.068	N1+N2 / N0	<0.0001*

ADC, adenocarcinoma

non-ADC, squamous-cell carcinoma plus large-cell carcinoma and adenosquamous-cell carcinoma

[0346] Inhibition of growth of lung cancer cells by siRNA for CSTF2.

To assess whether up-regulation of CSTF2 plays a role in growth or survival of lung-cancer cells, synthetic oligonucleotide siRNAs against CSTF2 (si-CSTF2-#1 and si-CSTF2-#2) along with control siRNAs (si-LUC and si-EGFP) were transfected into A549 and LC319 cells in which CSTF2 was endogenously overexpressed. The mRNA levels of CSTF2 in cells transfected with si-CSTF2-#1 and si-CSTF2-#2 were significantly decreased in comparison with those transfected with either control siRNAs (Fig. 3A). Cell viability and colony numbers investigated by MTT assay and colony-formation assays were reduced significantly in the cells transfected with si-CSTF2-#1 and si-CSTF2-#2 (Figs. 3B and 3C).

[0347] Activation of mammalian cellular proliferation by CSTF2.

To examine a potential role of CSTF2 in tumorigenesis, plasmids designed to express CSTF2 (pcDNA3.1/myc-His-CSTF2) were constructed and transfected into COS-7 cells. Exogenous CSTF2 expression was confirmed by western-blot analysis (Fig. 4A). MTT and colony-formation assays was carried out, and it was found that growth of the COS-7 cells transfected with CSTF2 was significantly enhanced, compared with the COS-7 cells transfected with the mock vector (Figs. 4B and 4C).

[0348] Discussion

To develop molecular-targeting anticancer drugs that are expected to be highly specific to malignant cells, with minimal risk of adverse reactions, a powerful target screening system was established to identify proteins and their interacting proteins that were activated specifically in lung cancer cells. Firstly, a genome-wide expression profile was analyzed for 101 lung cancer samples through the genome-wide cDNA mi-

croarray system containing 27,648 genes coupled with laser microdissection. After verification of very low or absent expression of such genes in normal organs by cDNA microarray analysis and multiple-tissue Northern blot analysis, the protein expression of candidate targets was analyzed among hundreds of clinical samples on tissue microarrays, then loss of function phenotypes was investigated using RNA interference systems, and further biological functions of the proteins were defined. Through these analyses, candidate genes for the development of novel diagnostic biomarkers, therapeutic drugs, and/or immunotherapy, were identified, which were up-regulated in cancer cells but not expressed in normal organs, except testis, placenta, and/or fetus tissues (Daigo Y, Nakamura Y. Gen Thorac Cardiovasc Surg 2008;56:43-53, Kikuchi T, Daigo Y, Katagiri T, et al. Oncogene 2003;22:2192-205, Kakiuchi S, Daigo Y, Tsunoda T, Yano S, Sone S, Nakamura Y. Mol Cancer Res 2003;1:485-99, Kakiuchi S, Daigo Y, Ishikawa N, et al. Hum Mol Genet 2004;13:3029-43, Kikuchi T, Daigo Y, Ishikawa N, et al. Int J Oncol 2006; 28:799-805, Taniwaki M, Daigo Y, Ishikawa N, et al. Int J Oncol 2006;29:567-75, Suzuki C, Daigo Y, Kikuchi T, Katagiri T, Nakamura Y. Cancer Res 2003;63:7038-41, Ishikawa N, Daigo Y, Yasui W, et al. Clin Cancer Res 2004;10:8363-70, Kato T, Daigo Y, Hayama S, et al. Cancer Res 2005;65:5638-46, Furukawa C, Daigo Y, Ishikawa N, et al. Cancer Res 2005;65:7102-10, Ishikawa N, Daigo Y, Takano A, et al. Cancer Res 2005;65:9176-84, Suzuki C, Daigo Y, Ishikawa N, et al. Cancer Res 2005;65:11314-25, Ishikawa N, Daigo Y, Takano A, et al. Cancer Sci 2006;97:737-45, Takahashi K, Furukawa C, Takano A, et al. Cancer Res 2006;66:9408-19, Hayama S, Daigo Y, Kato T, et al. Cancer Res 2006;66:10339-48, Kato T, Hayama S, Yamabuki Y, et al. Clin Cancer Res 2007;13:434-42, Suzuki C, Takahashi K, Hayama S, et al. Mol Cancer Ther 2007;6:542-51, Yamabuki T, Takano A, Hayama S, et al. Cancer Res 2007;67:2517-25, Hayama S, Daigo Y, Yamabuki T, et al. Cancer Res 2007; 67:4113-22, Taniwaki M, Takano A, Ishikawa N, et al. Cancer. Clin Cancer Res 2007;13:6624-31, Ishikawa N, Takano A, Yasui W, et al. Cancer Res 2007;67:11601-11, Mano Y, Takahashi, K, Ishikawa N, et al. Cancer Sci 2007;98:1902-13, Kato T, Sato N, Hayama S, et al. Cancer Res 2007; 67:8544-53, Kato T, Sato N, Takano A, et al. Clin Cancer Res 2008;14:2363-70, Dunleavy EM, Roche D, Tagami H, et al. Cell 2009;137:485-97, Hirata D, Yamabuki T, Ito T, et al. Clin Cancer Res 2009,15:256-66, Suda T, Tsunoda T, Daigo Y, Nakamura Y, Tahara H. Cancer Sci 2007;98:1803-8, Mizukami Y, Kono K, Daigo Y, et al. Cancer Sci 2008;99:1448-54).

[0349] As noted above, CSTF2, encoding a member of cleavage stimulation factor, was overexpressed in lung cancer with high frequency and likely to play an important role in the growth of lung cancers. Knockdown of CSTF2 expression by siRNA suppressed

the growth of lung cancer cells. Moreover, clinicopathologic evidence obtained through our tissue microarray experiments showed that NSCLC patients with CSTF2-strong positive expression had shorter cancer-specific survival periods than those with CSTF2-weak positive/negative expression. The results obtained by in vitro and in vivo assays strongly suggest that CSTF2 is likely to be an important growth factor and be associated with a more malignant phenotype of lung cancer cells.

[0350] In conclusion, CSTF2 gene can play an important role in the growth/progression of lung cancers. CSTF2 overexpression in resected specimens can be a useful indicator of adjuvant therapy to the lung cancer patients who are likely to have poor prognosis.

Industrial Applicability

[0351] The gene-expression analysis of cancers described herein using the genome-wide cDNA microarray has identified specific genes as targets for cancer prevention and therapy. Based on the expression of a differentially expressed gene, CSTF2, the present invention provides molecular diagnostic markers for identifying and detecting cancer, in particular, lung cancer.

[0352] The data provided herein add to a comprehensive understanding of cancers, facilitate development of novel diagnostic strategies, and provide clues for identification of molecular targets for therapeutic drugs and preventative agents. Such information contributes to a more profound understanding of tumorigenesis, and provides indicators for developing novel strategies for diagnosis, treatment, and ultimately prevention of cancers.

[0353] As demonstrated herein, cell growth is suppressed by double-stranded molecules that specifically target the CSTF2 gene. Thus, these novel double-stranded molecules are useful as anti-cancer pharmaceuticals.

[0354] The expression of the CSTF2 gene is markedly elevated in cancer, specifically lung cancer, as compared to normal organs. Accordingly, this gene can be conveniently used as a diagnostic marker for cancer, in particular, lung cancer, and the proteins encoded thereby find utility in diagnostic assays for cancer. Furthermore, cancer patients with high expression level of CSTF2 gene were found to tend to be poor prognosis. Therefore, CSTF2 gene is useful to assess prognosis of cancer patient.

[0355] Moreover, the present invention provides new therapeutic approaches for treating cancer including lung cancer. The CSTF2 gene is a useful target for the development of anti-cancer pharmaceuticals.

All patents, patent applications, and publications cited herein are incorporated by reference in their entirety.

[0356] Furthermore, while the invention has been described in detail and with reference to specific embodiments thereof, it is to be understood that the foregoing description is

exemplary and explanatory in nature and is intended to illustrate the invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the invention. Thus, the invention is intended to be defined not by the above description, but by the following claims and their equivalents.

Claims

[Claim 1] A method for diagnosing cancer or a predisposition for developing cancer in a subject, wherein the method comprises steps of:
(a) determining an expression level of a CSTF2 gene in a subject-derived biological sample by any one of method(s) selected from a group consisting of:
(i) detecting an mRNA of a CSTF2 gene,
(ii) detecting a protein encoded by a CSTF2 gene, and
(iii) detecting a biological activity of a protein encoded by a CSTF2 gene; and
(b) correlating an increase in the expression level determined in step (a) as compared to a normal control level of the CSTF2 gene to a presence of cancer in the subject.

[Claim 2] The method of claim 1, wherein the expression level determined in step (a) is at least 10% greater than the normal control level.

[Claim 3] The method of claim 1, wherein the subject-derived biological sample includes biopsy sample, sputum, blood, pleural effusion or urine.

[Claim 4] A method for assessing or determining prognosis of a subject with cancer, wherein the method comprises steps of:
(a) detecting an expression level of a CSTF2 gene in a subject-derived biological sample;
(b) comparing the detected expression level to a control level; and
(c) determining prognosis of the subject based on the comparison of (b).

[Claim 5] The method of claim 4, wherein the control level is a good prognosis control level and an increase of the expression level compared to the control level indicates poor prognosis.

[Claim 6] The method of claim 5, wherein the increase is at least 10% greater than the control level.

[Claim 7] The method of claim 4, wherein the expression level is determined by any one of method(s) selected from a group consisting of:
(a) detecting an mRNA of a CSTF2 gene;
(b) detecting a protein encoded by a CSTF2 gene; and
(c) detecting a biological activity of a protein encoded by a CSTF2 gene.

[Claim 8] The method of claim 4, wherein the subject-derived biological sample includes biopsy sample, sputum or blood, pleural effusion or urine.

[Claim 9] A kit for diagnosing cancer or assessing or determining the prognosis of a subject with cancer, which comprises a reagent selected from a group consisting of:
(a) a reagent for detecting an mRNA of a CSTF2 gene;
(b) a reagent for detecting a protein encoded by a CSTF2 gene; and
(c) a reagent for detecting a biological activity of a protein encoded by a CSTF2 gene.

[Claim 10] The kit of claim 9, wherein the reagent comprises a probe or primers to a gene transcript of the CSTF2 gene, or an antibody against a translation product of the CSTF2 gene.

[Claim 11] An isolated double-stranded molecule that, when introduced into a cell, inhibits in vivo expression of a CSTF2 gene as well as cell proliferation, wherein the molecule including a sense strand and an antisense strand complementary thereto, wherein the strands hybridized to each other to form the double-stranded molecule.

[Claim 12] The double-stranded molecule of claim 11, wherein the sense strand comprises a sequence corresponding to a target sequence selected from a group consisting of SEQ ID NOs: 9 and 10.

[Claim 13] The double-stranded molecule of claim 11 or 12, wherein the sense strand hybridizes with antisense strand at the target sequence to form the double-stranded molecule having between 19 and 25 nucleotide pair in length.

[Claim 14] The double-stranded molecule of any one of claims 11 to 13, which consists of a single polynucleotide including both the sense and antisense strands linked by an intervening single-strand.

[Claim 15] The double-stranded molecule of claim 14, which has a general formula 5'-[A]-[B]-[A']-3', wherein [A] is a sense strand comprises a sequence corresponding to a target sequence selected from a group consisting of SEQ ID NOs: 9 and 10, [B] is an intervening single-strand consisting of 3 to 23 nucleotides, and [A'] is an antisense strand including a complementary sequence to the target sequence selected in [A].

[Claim 16] A vector encoding the double-stranded molecule of any one of claims 11 to 15.

[Claim 17] A method of treating or preventing cancer in a subject, wherein the method comprises administering to a subject a pharmaceutically effective amount of a double-stranded molecule against a CSTF2 gene or a vector encoding the double-stranded molecule, wherein the double-stranded molecule, when introduced into a cell expressing the CSTF2

gene, inhibits an expression of the CSTF2 gene.

[Claim 18] The method of claim 17, wherein the double-stranded molecule is that of any one of claims 11 to 15.

[Claim 19] The method of claim 17, wherein the vector is that of claim 16.

[Claim 20] A composition for treating cancer expressing a CSTF2 gene, wherein the composition comprises at least one isolated double-stranded molecule against a CSTF2 gene or vector encoding the double-stranded molecule, wherein the double stranded molecule, when introduced into a cell expressing the CSTF2 gene, inhibits an expression of the CSTF2 gene, and a pharmaceutically acceptable carrier.

[Claim 21] The composition of claim 20, wherein the double-stranded molecule is that of any one of claims 11 to 15.

[Claim 22] The composition of claim 20, wherein the vector is that of claim 16.

[Claim 23] A method of screening for a candidate substance for treating or preventing cancer, or inhibiting cancer cell growth, wherein the method comprises steps of:
(a) contacting a test substance with a CSTF2 polypeptide or a fragment thereof;
(b) detecting a binding activity between the polypeptide or fragment and the test substance; and
(c) selecting a test substance that binds to the polypeptide or a fragment as a candidate substance for treating or preventing cancer.

[Claim 24] A method of screening for a candidate substance for treating or preventing cancer, or inhibiting cancer cell growth, wherein the method comprises steps of:
(a) contacting a test substance with a CSTF2 polypeptide or a fragment thereof;
(b) detecting a biological activity of the polypeptide or fragment;
(c) comparing the biological activity of the polypeptide or fragment with the biological activity detected in the absence of the test substance; and
(d) selecting the test substance that suppresses the biological activity of the polypeptide as a candidate substance for treating or preventing cancer.

[Claim 25] The method of claim 24, wherein the biological activity is cell proliferative activity, RNA binding activity, mRNA cleavage activity or mRNA polyadenylation activity.

[Claim 26] A method of screening for a candidate substance for treating or

preventing cancer, or inhibiting cancer cell growth, wherein the method comprises steps of:
(a) contacting a test substance with a cell expressing a CSTF2 gene; and
(b) selecting the test substance that reduces the expression level of a CSTF2 gene in comparison with the expression level detected in the absence of the test substance.

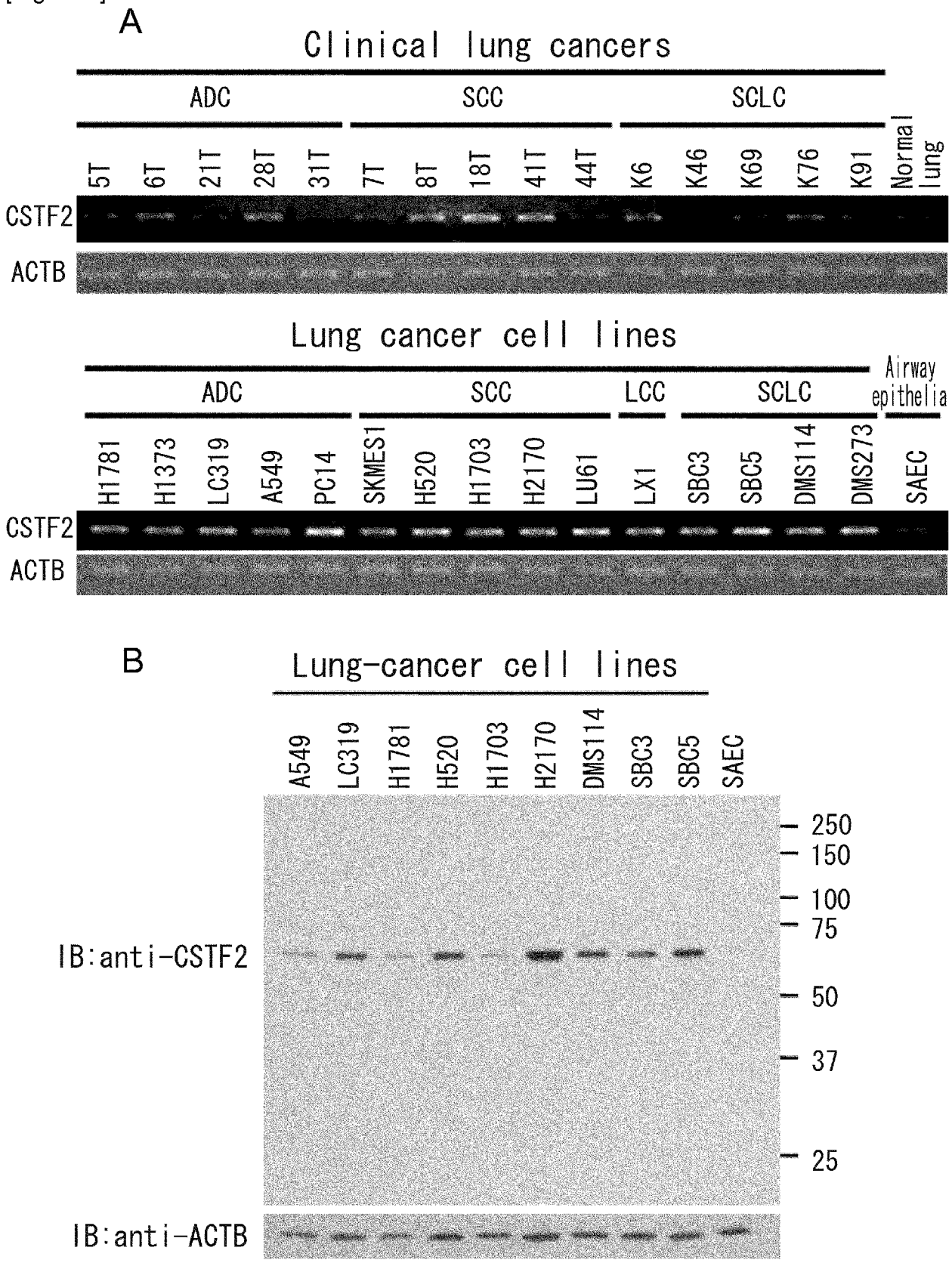
[Claim 27] A method of screening for a candidate substance for treating or preventing cancer, or inhibiting cancer cell growth, wherein the method including steps of:
(a) contacting a test substance with a cell into which a vector, including a transcriptional regulatory region of a CSTF2 gene and a reporter gene that is expressed under a control of the transcriptional regulatory region, has been introduced;
(b) measuring the expression or activity of the reporter gene; and
(c) selecting a test substance that reduces the expression or activity level of the reporter gene as compared to the expression or activity level detected in the absence of the test substance.

[Claim 28] A method for treating or preventing cancer in a subject, comprising administering to the subject an anti-CSTF2 antibody or an immunologically active fragment thereof.

[Claim 29] The method of any one of claims 1 to 8, 17 to 19 and 23 to 28, the kit of claim 9 or 10, or the composition of any one of 20 to 22, wherein the cancer is lung cancer.

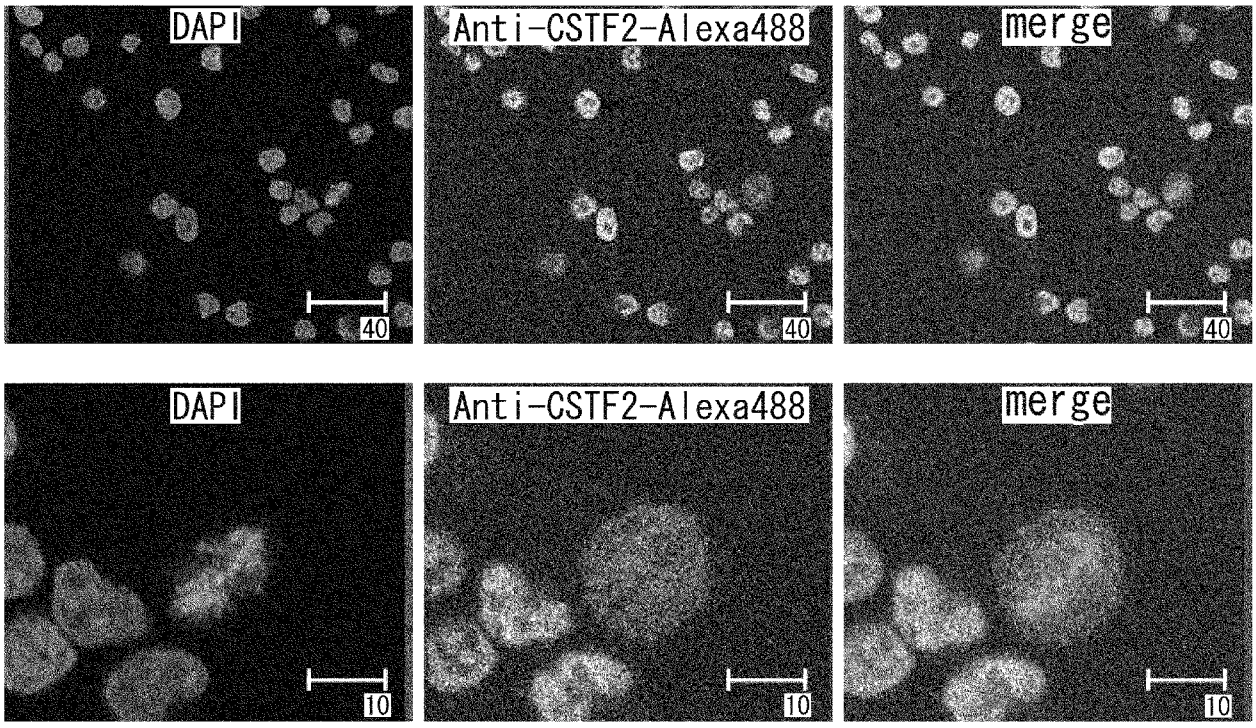
[Claim 30] Vectors comprising each of a combination of polynucleotide comprising a sense strand nucleic acid and an antisense strand nucleic acid, wherein said sense strand nucleic acid comprises nucleotide sequence corresponding to SEQ ID NO: 9 or 10 and said antisense strand nucleic acid consists of a sequence complementary to the sense strand, wherein the transcripts of said sense strand and said antisense strand hybridize to each other to form a double stranded molecule, and wherein said vectors, when introduced into a cell expressing CSTF2 gene, inhibits the cell proliferation.

[Fig. 1ab]

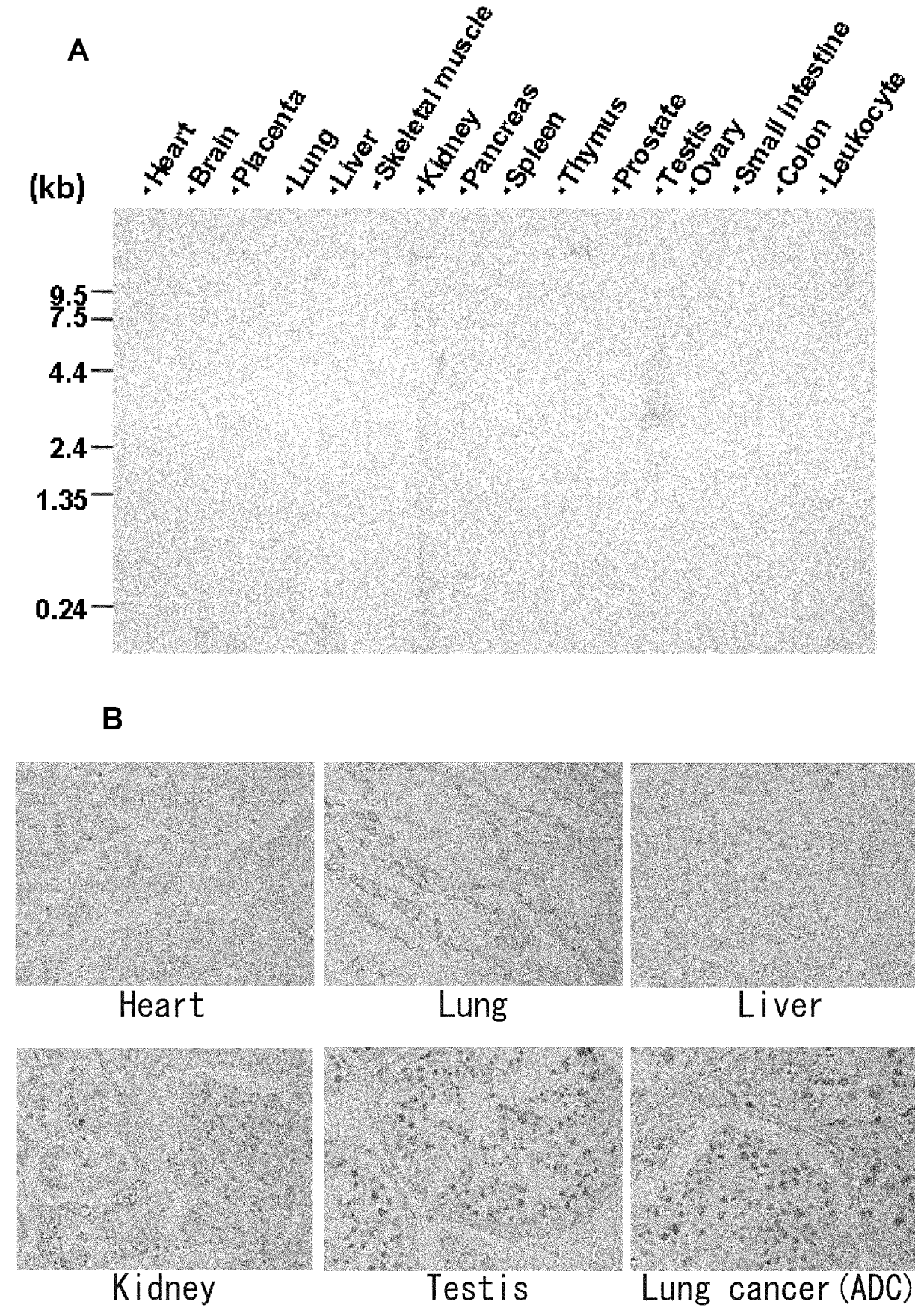

A
Clinical lung cancers
ADC
SCC
SCLC
5T
6T
21T
28T
31T
7T
8T
18T
41T
44T
K6
K46
K69
K76
K91
Normal lung
CSTF2
ACTB
Lung cancer cell lines
ADC
SCC
LCC
SCLC
Airway epithelia
H1781
H1373
LC319
A549
PC14
SKMES1
H520
H1703
H2170
LU61
LX1
SBC3
SBC5
DMS114
DMS273
SAEC
CSTF2
ACTB
B
Lung-cancer cell lines
A549
LC319
H1781
H520
H1703
H2170
DMS114
SBC3
SBC5
SAEC
250
150
100
75
50
37
25
IB:anti-CSTF2
IB:anti-ACTB

[Fig. 1c]

C

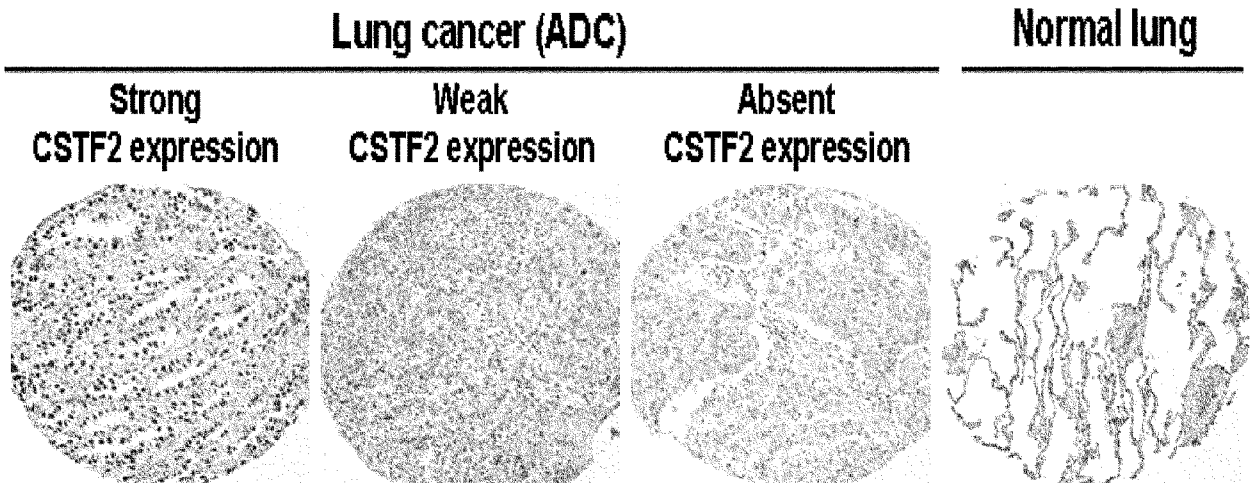

DAPI
Anti-CSTF2-Alexa488
merge
40
40
40
DAPI
Anti-CSTF2-Alexa488
merge
10
10
10

[Fig. 2ab]

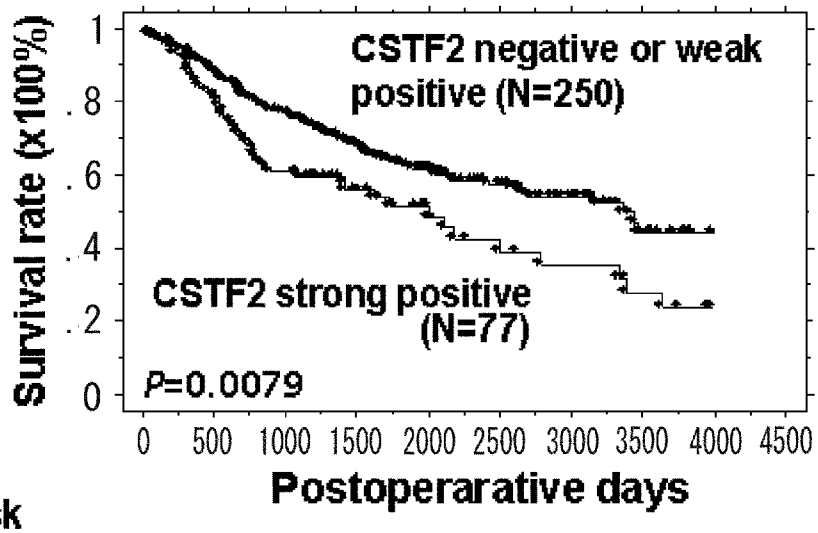

A
(kb)
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Small intestine
Colon
Leukocyte
9.5
7.5
4.4
2.4
1.35
0.24
B
Heart
Lung
Liver
Kidney
Testis
Lung cancer (ADC)

[Fig. 2cd]

C



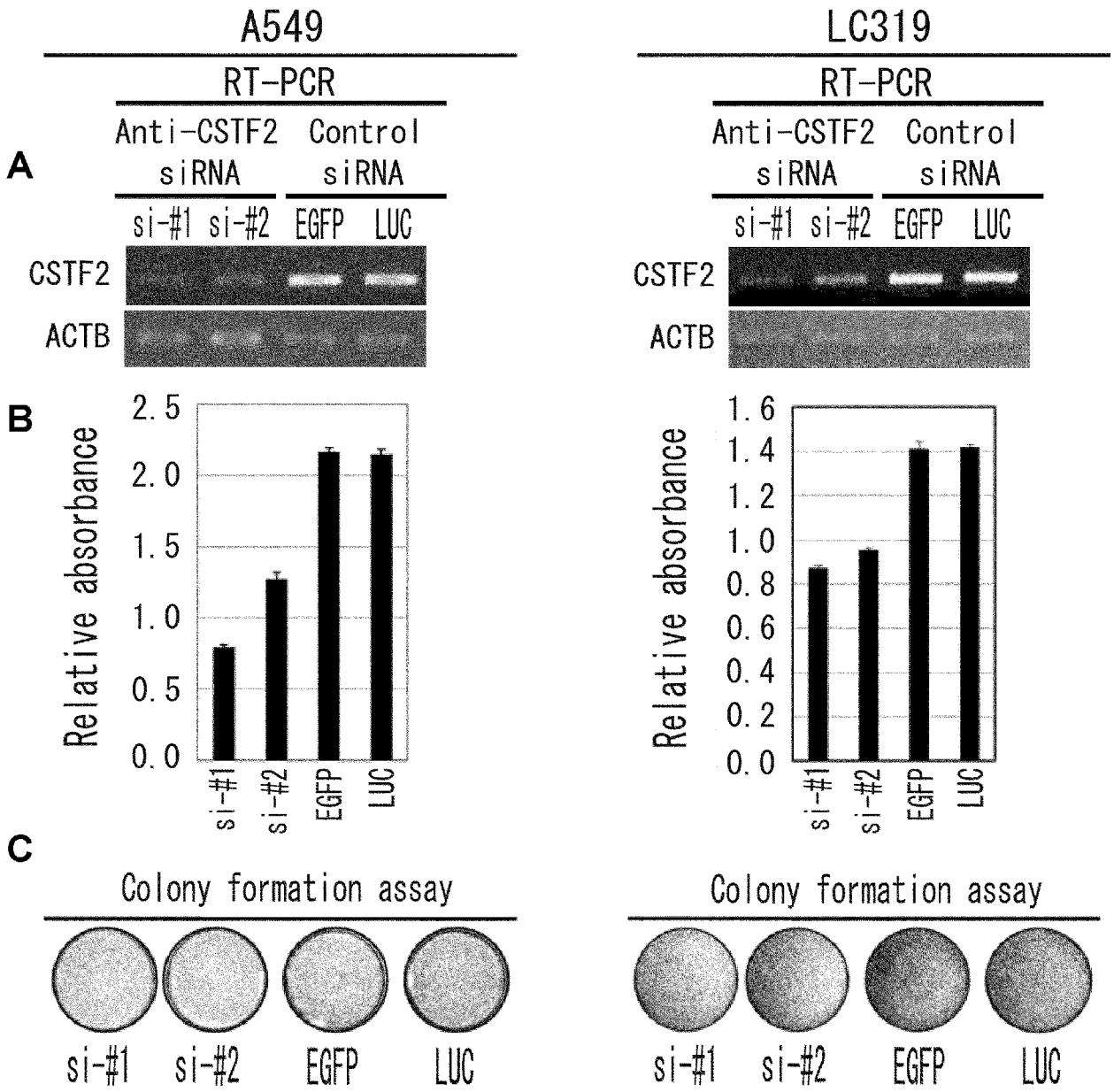
D



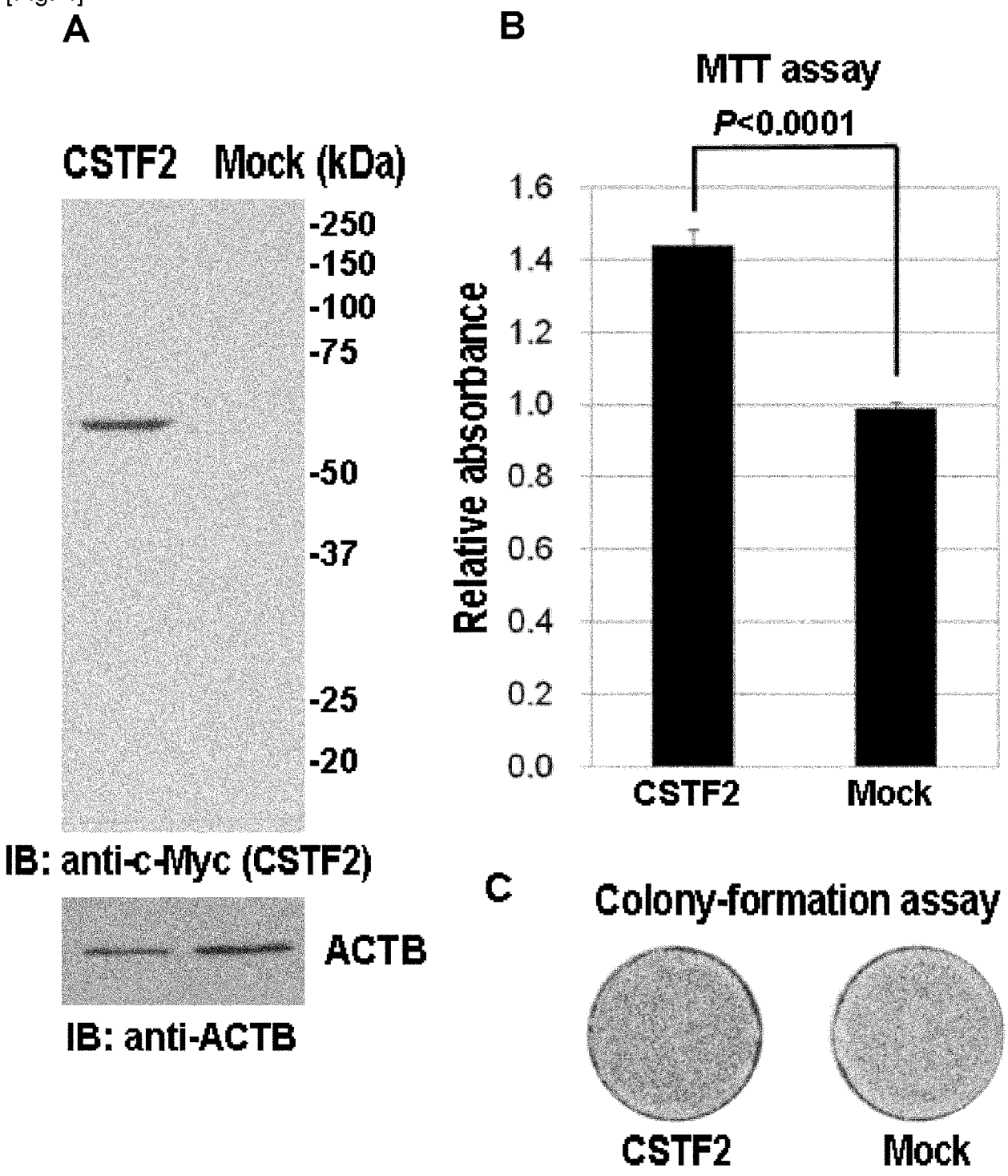
Number at risk

CSTF2 negative or weak positive	250	223	193	135	93	57	33	16
CSTF2 strong positive	77	64	47	28	19	12	10	7

[Fig. 3]


A549
LC319
RT-PCR
RT-PCR
A
Anti-CSTF2 siRNA
Control siRNA
Anti-CSTF2 siRNA
Control siRNA
si-#1 si-#2 EGFP LUC
si-#1 si-#2 EGFP LUC
CSTF2
ACTB
CSTF2
ACTB
B
Relative absorbance
2.5
2.0
1.5
1.0
0.5
0.0
Relative absorbance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
si-#1
si-#2
EGFP
LUC
si-#1
si-#2
EGFP
LUC
C
Colony formation assay
Colony formation assay
si-#1 si-#2 EGFP LUC
si-#1 si-#2 EGFP LUC

[Fig. 4]


A
CSTF2 Mock (kDa)
-250
-150
-100
-75
-50
-37
-25
-20
IB: anti-c-Myc (CSTF2)
ACTB
IB: anti-ACTB
B
MTT assay
P<0.0001
Relative absorbance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
CSTF2
Mock
C
Colony-formation assay
CSTF2
Mock

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2010/005095

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12Q1/68, A61P35/00, C07K16/30, C12N15/09, C12N15/113, G01N33/50, G01N33/53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA/BIOSIS/MEDLINE/WPIDS(STN), JSTPlus/JMEDPlus/JST7580(JDreamII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/030615 A2 (GENENTEC, INC.) 2004.04.15, Claim, Fig.6239, Fig.6240 & JP 2006-516089 A & US 2007/0224201 A1 & EP 1594447 A2	9-16, 20-27, 30
Y	WO 2001/036470 A2 (MATRITECH, INC.) 2001.05.25, Claim & JP 2004-500056 A & US 2006/0160154 A1	9-16, 20-27, 30
Y	WO 2007/013665 A2 (ONCOTHERAPY SCIENCE, INC.) 2007.02.01, Claim & JP 2009-502115 A & US 2009/0317392 A1 & EP 1907581 A2	9-16, 20-27, 30
A	WO 2001/033229 A1 (UNIVERSITEIT VAN AMSTERDAM) 2001.05.10, Claim & JP 2003-513282 A & EP 1226439 A1	9-16, 20-27, 30

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06.09.2010	14.09.2010

Name and mailing address of the ISA/JP
Japan Patent Office
3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan

Authorized officer
Midori TOMINAGA
4N 4434
Telephone No. +81-3-3581-1101 Ext. 3488

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2010/005095

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TAKAGAKI Y. et al., The human 64-kDa polyadenylylation factor contains a ribonucleoprotein-type RNA binding domain and unusual auxiliary motifs, Proc. Natl. Acad. Sci. USA, 1992, Vol.89, pp.1403-1407	9-16, 20-27, 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2010/005095

CLASSIFICATION OF SUBJECT MATTER
C12Q1/68(2006.01)i, A61P35/00(2006.01)i, C07K16/30(2006.01)i,
C12N15/09(2006.01)i, C12N15/113(2010.01)i, G01N33/50(2006.01)i,
G01N33/53(2006.01)i

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2010/005095

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 1-8, 17-19, 28-29
because they relate to subject matter not required to be searched by this Authority, namely:
Methods for treatment of the human body by therapy, and diagnostic methods practiced on the human body.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

专利名称(译)	CSTF2用于肺癌治疗和诊断的靶基因		
公开(公告)号	EP2467499A1	公开(公告)日	2012-06-27
申请号	EP2010809732	申请日	2010-08-18
[标]申请(专利权)人(译)	肿瘤疗法科学股份有限公司		
申请(专利权)人(译)	肿瘤治疗科学，INC.		
当前申请(专利权)人(译)	肿瘤治疗科学，INC.		
[标]发明人	DAIGO YATARO TSUNODA TAKUYA NAKAMURA YUSUKE		
发明人	DAIGO, YATARO TSUNODA, TAKUYA NAKAMURA, YUSUKE		
IPC分类号	C12Q1/68 A61P35/00 C07K16/30 C12N15/09 C12N15/113 G01N33/50 G01N33/53		
CPC分类号	G01N33/5011 C07K16/18 C12N15/113 C12N2310/14 C12Q1/6886 C12Q2600/118 C12Q2600/136 C12Q2600/158 G01N33/57423		
优先权	61/274800 2009-08-21 US		
外部链接	Espacenet		

摘要(译)

本发明涉及CSTF2基因在癌症致癌中所起的作用，并且本发明涉及通过给予针对CSTF2基因的双链分子或含有这种双链分子的组合物，载体或细胞来治疗或预防癌症的方法。本发明的特征还在于使用过表达的CSTF2基因诊断癌症或评估/确定患有肺癌的受试者的预后的方法。为此，CSTF2可以作为癌症，特别是肺癌的新型预后生物标志物。此外，公开了筛选用于治疗和预防癌症的候选物质的方法，使用它们对CSTF2的表达或生物活性的影响作为指标。