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(54) **COMPOSITIONS AND METHODS FOR INHIBITING SQUAMOUS CELL CARCINOMA**

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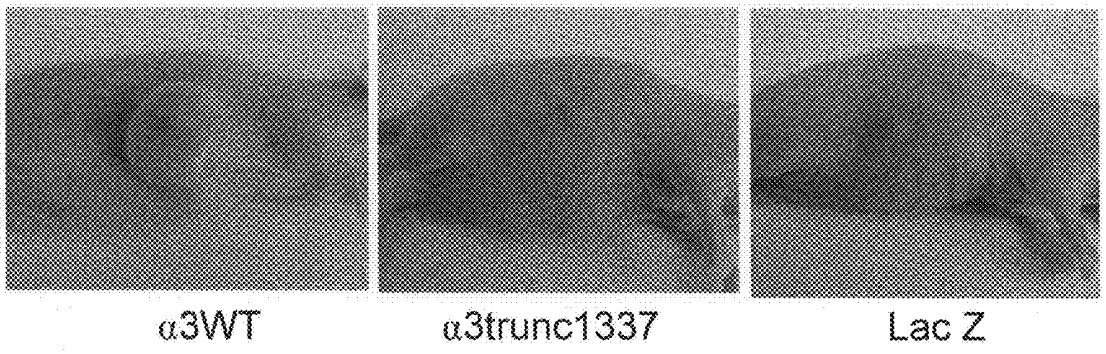
(57) **ABSTRACT**

The present invention relates to compositions and methods for detecting and inhibiting squamous cell carcinoma using agents that target the laminin 5 alpha 3 G4-G5 domain.



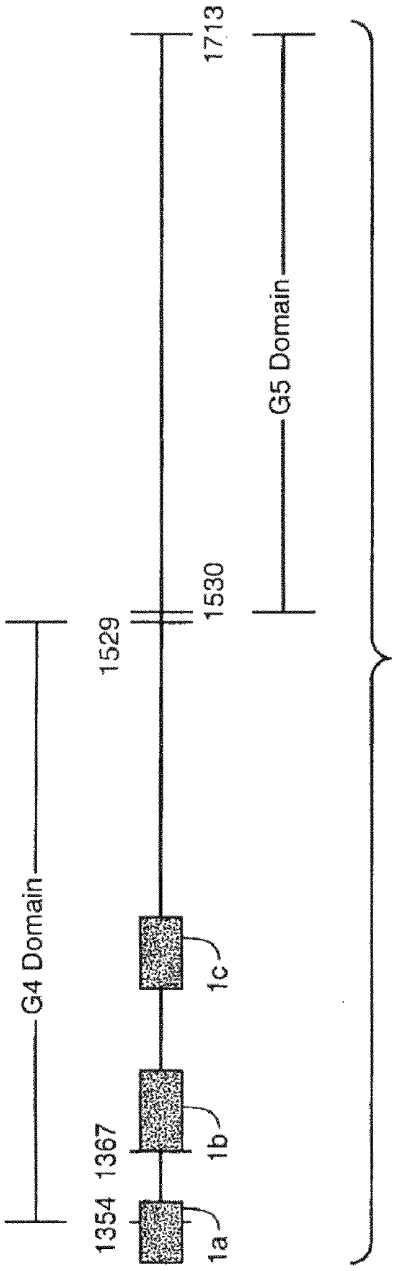

G4 Domain
1354
1367
1529
1530
1713
1a
1b
1c
G5 Domain

FIG._1A

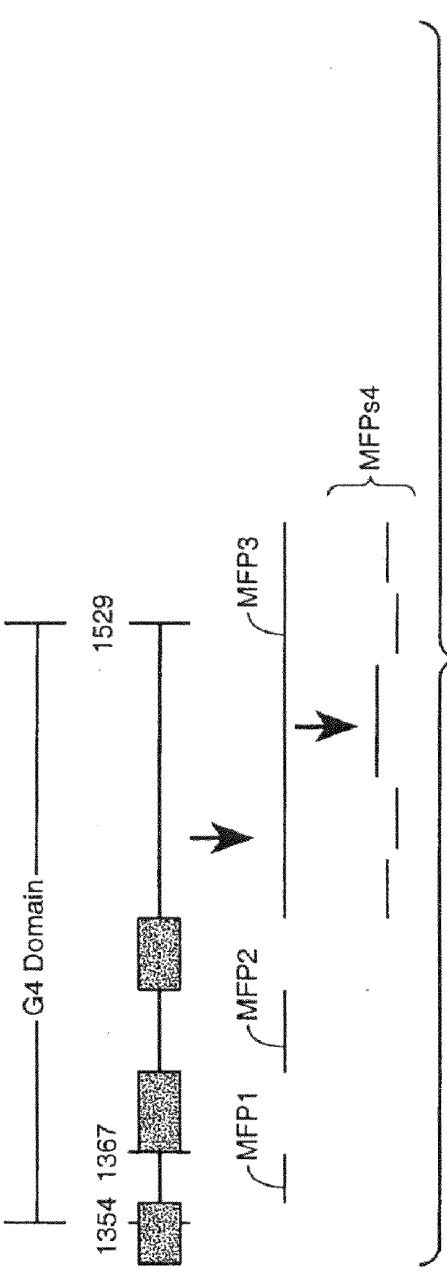

G4 Domain
1354
1367
1529
MFP1
MFP2
MFP3
MFPs4

FIG._1B

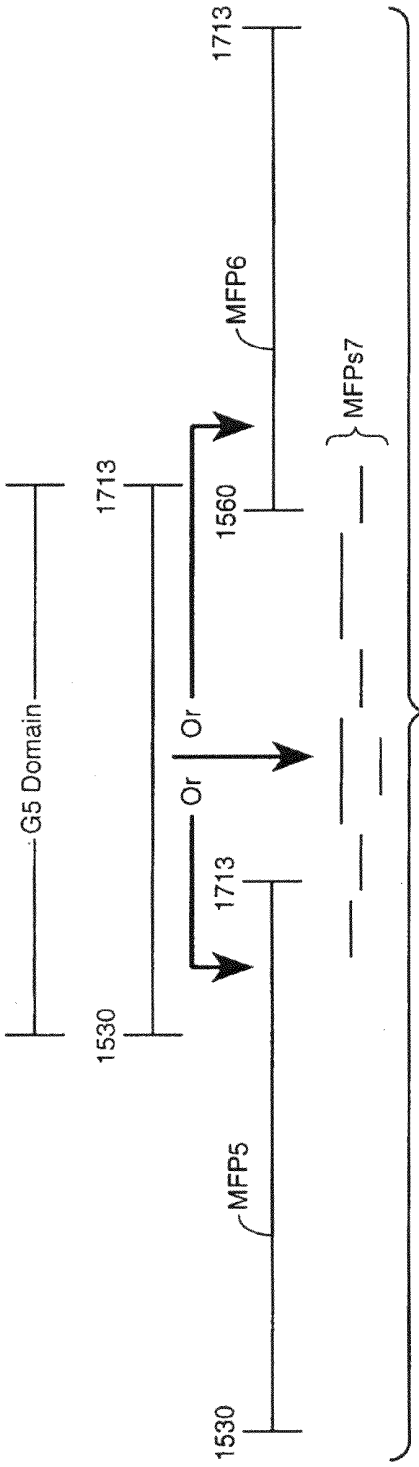

G5 Domain
1530
1713
Or
1713
MFP5
1530
Or
1560
1713
MFP6
MFPs7

FIG._1C

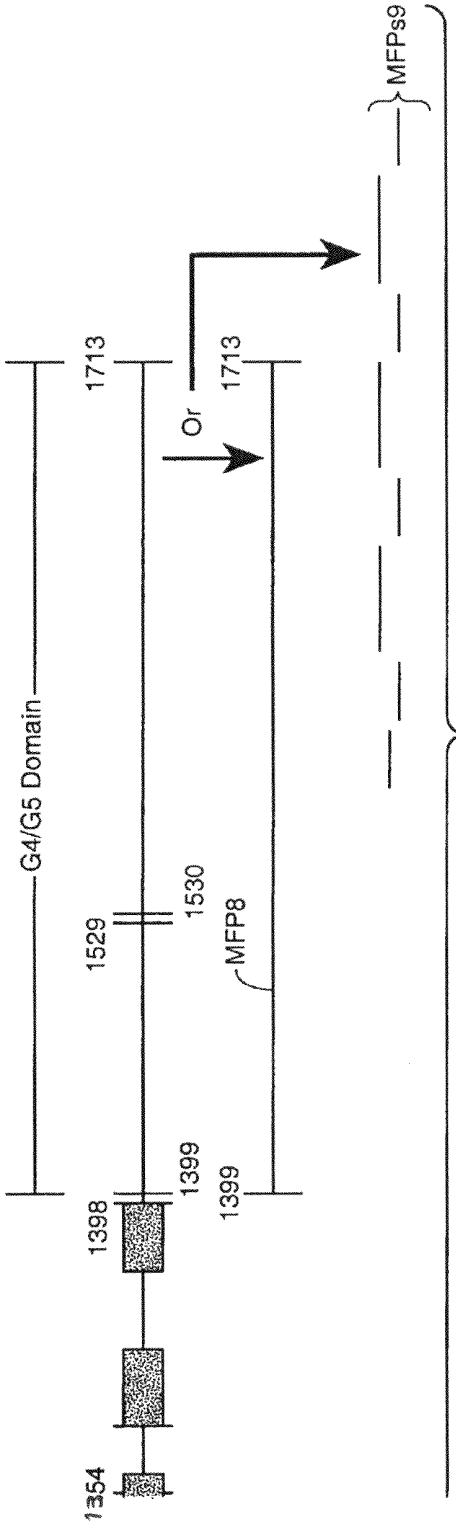

G4/G5 Domain
1354
1398
1399
1529
1530
1713
MFP8
1399
Or
1713
MFPs9

FIG._1D

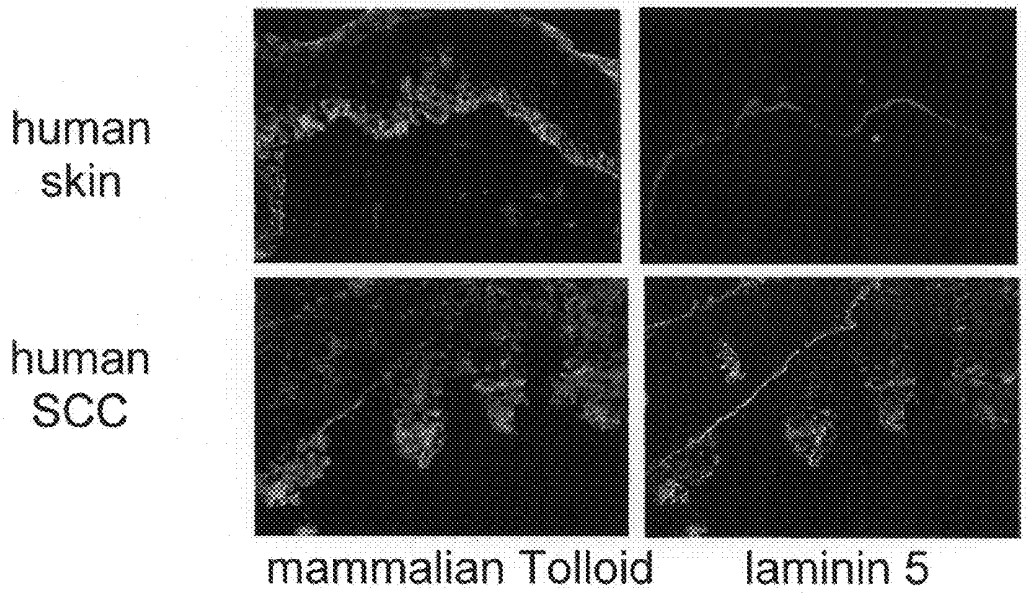


FIG._2

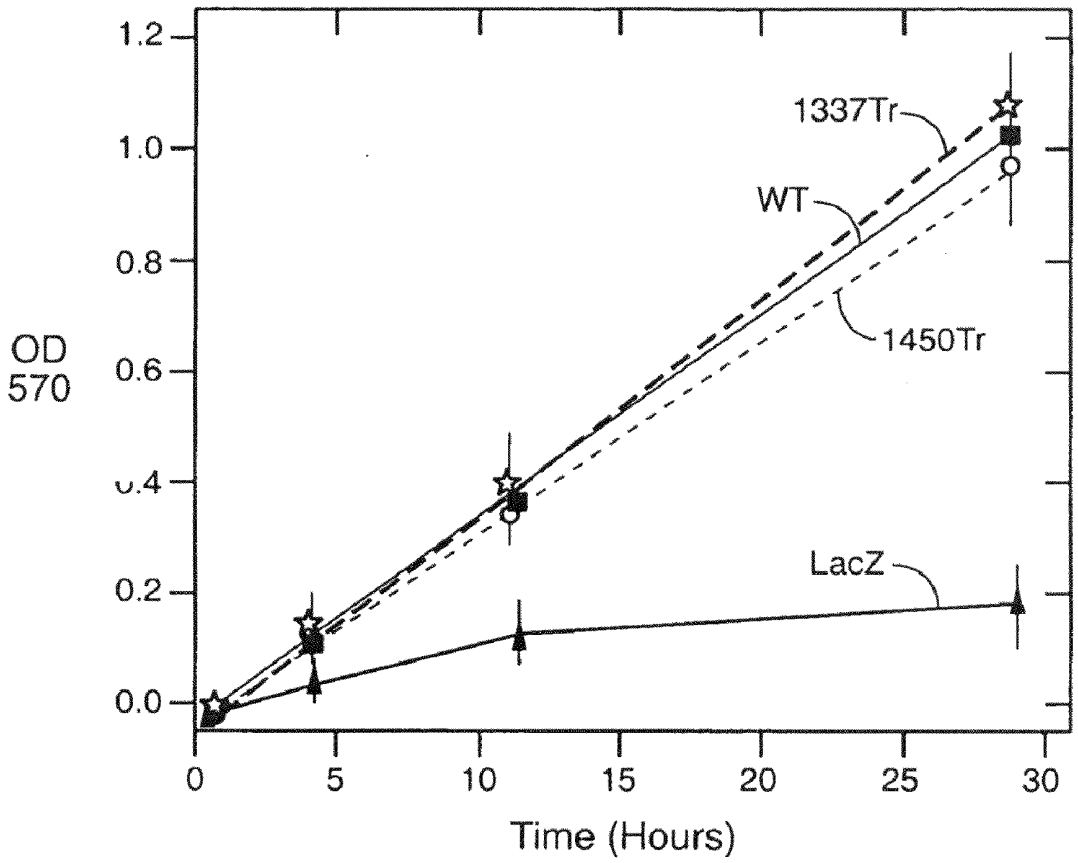

1.2
1.0
0.8
0.6
0.4
0.2
0.0
OD
570
0
5
10
15
20
25
30
Time (Hours)
1337Tr
WT
1450Tr
LacZ

FIG._3

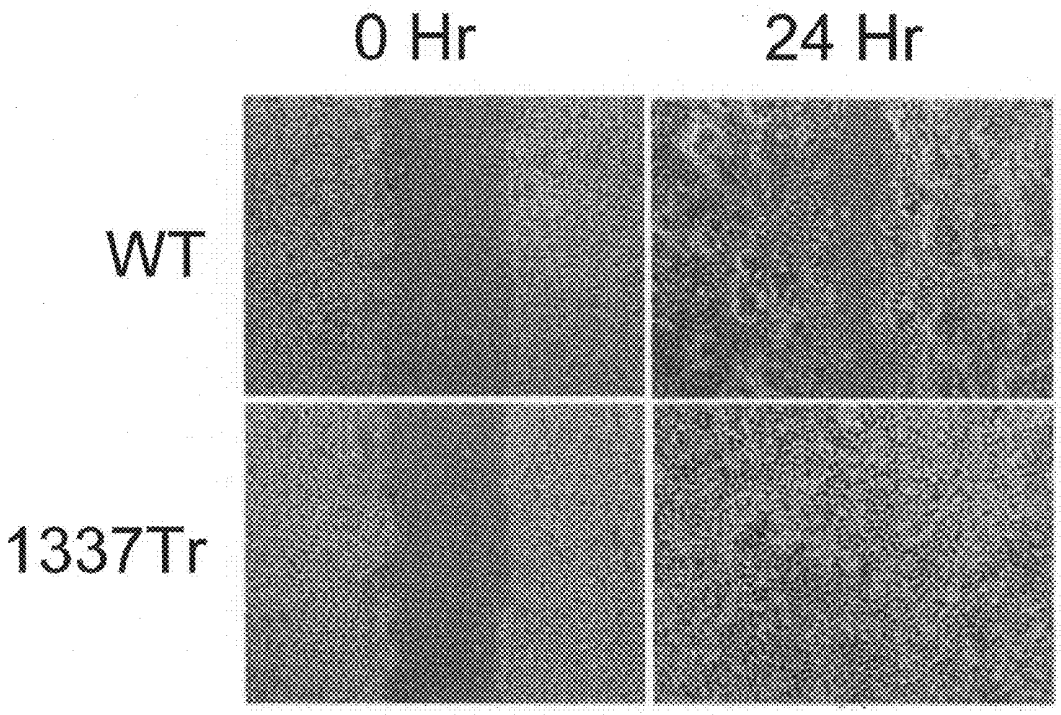


FIG._4

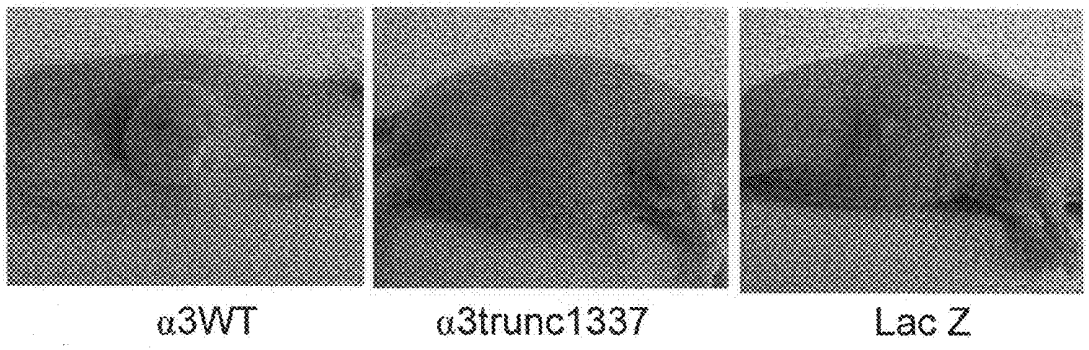


FIG._5

FIGURE 6

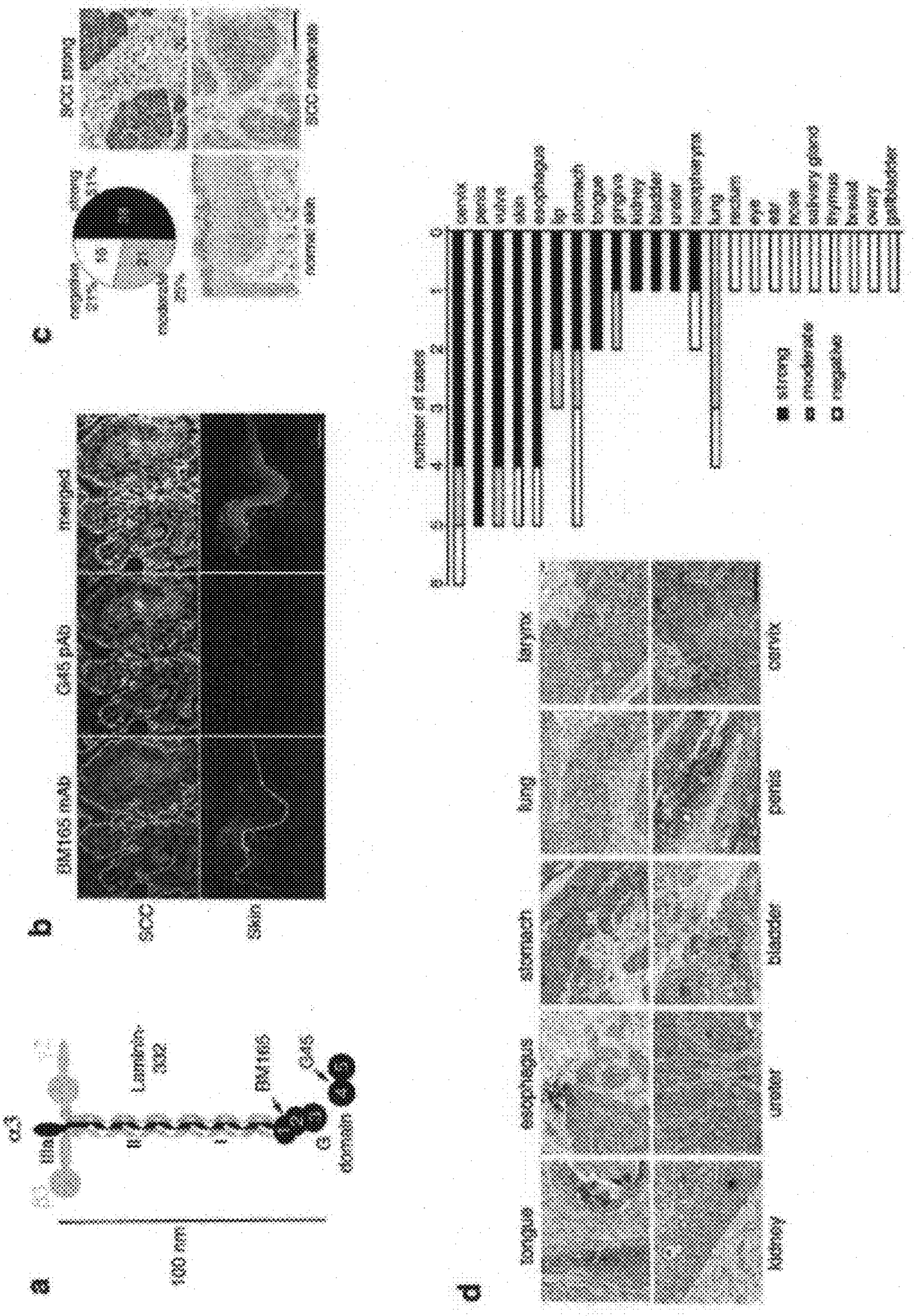
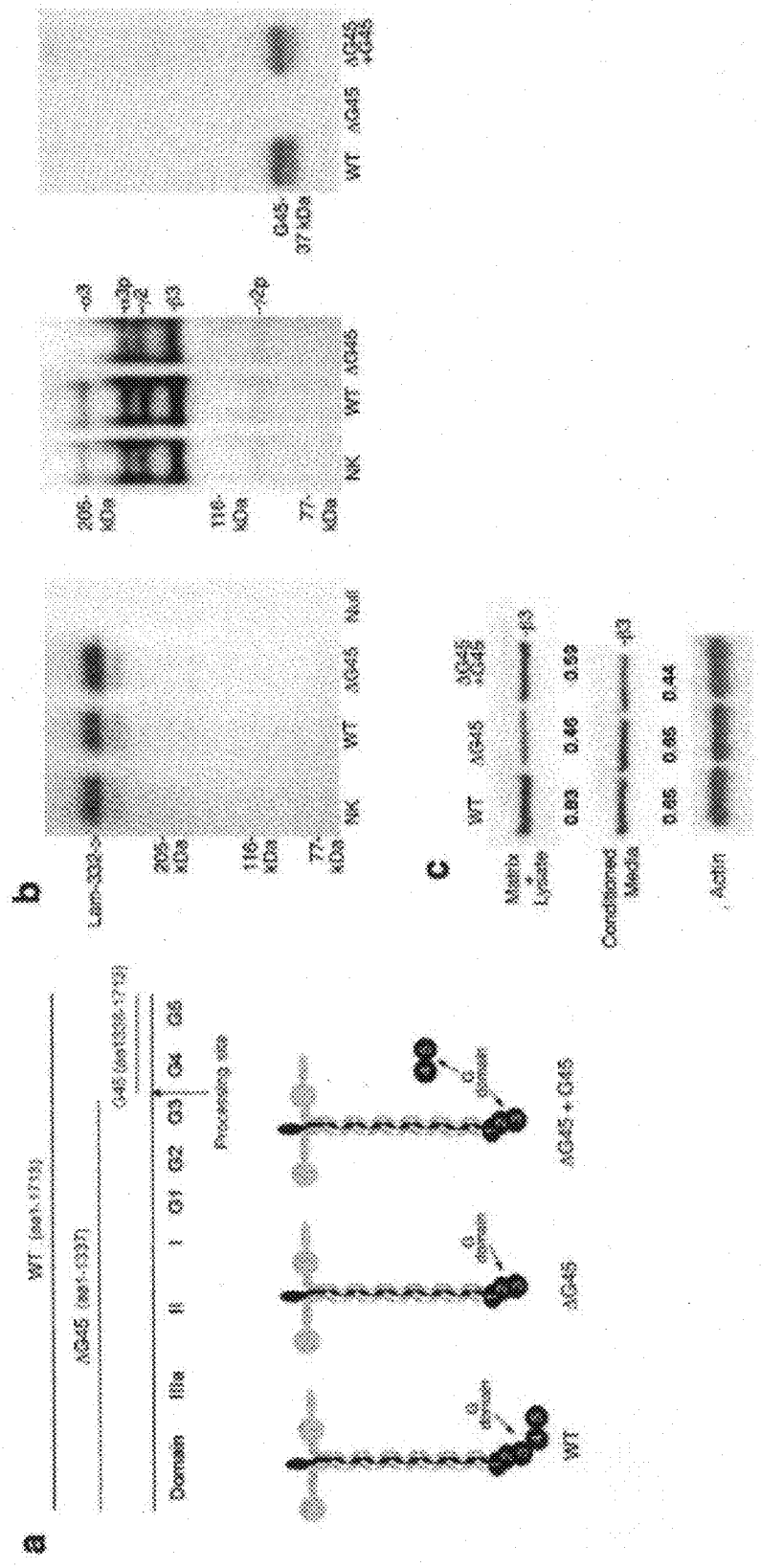

a
100 nm
Laminin-332
BM165
G45
b
SCC
Skin
BM165 mAb
G45 pAb
merged
c
d
number of cases
strong
moderate
negative
tongue
esophagus
stomach
lung
larynx
kidney
ureter
bladder
penis
cervix

FIGURE 7


a
WT (aa1-1715)
Δ345 (aa1-1331)
Δ345 (aa1338-1715)
Domain
IIIa
II
I
G1
G2
G3
G4
G5
Processing site
WT
Δ345
Δ345 + Δ45
b
Lam-332-γ
265 kDa
116 kDa
72 kDa
NK
WT
Δ345
Null
-β3
-γ2
-β3
-γ2
-β3
NK
WT
Δ345
Δ45 37 kDa
WT
Δ345
Δ345 +Δ45
c
WT
Δ345
Δ345 +Δ45
-β3
Matrix + Lysate
0.83
0.40
0.59
Conditioned Media
0.66
0.65
0.44
Actin

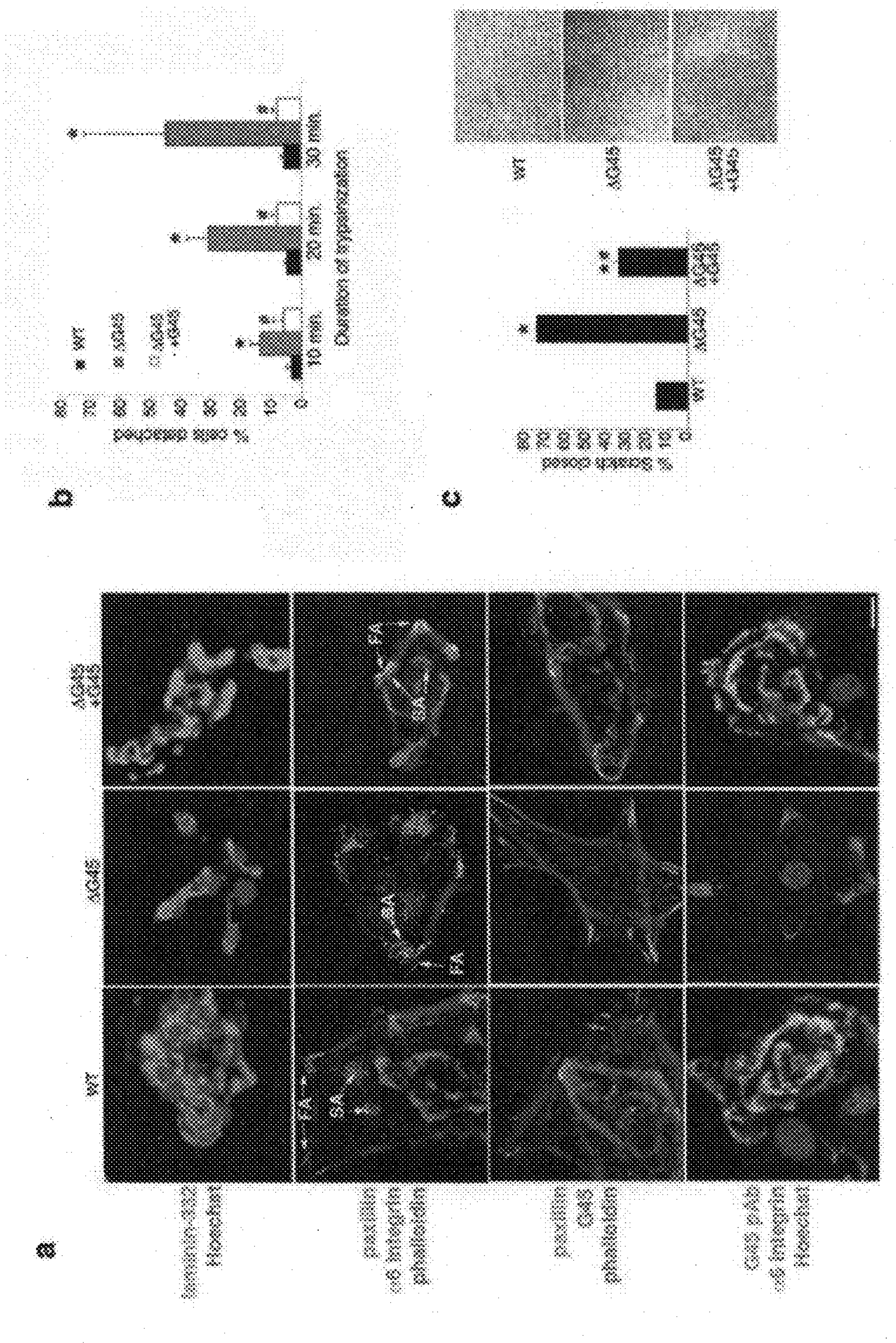


FIGURE 8

FIGURE 9

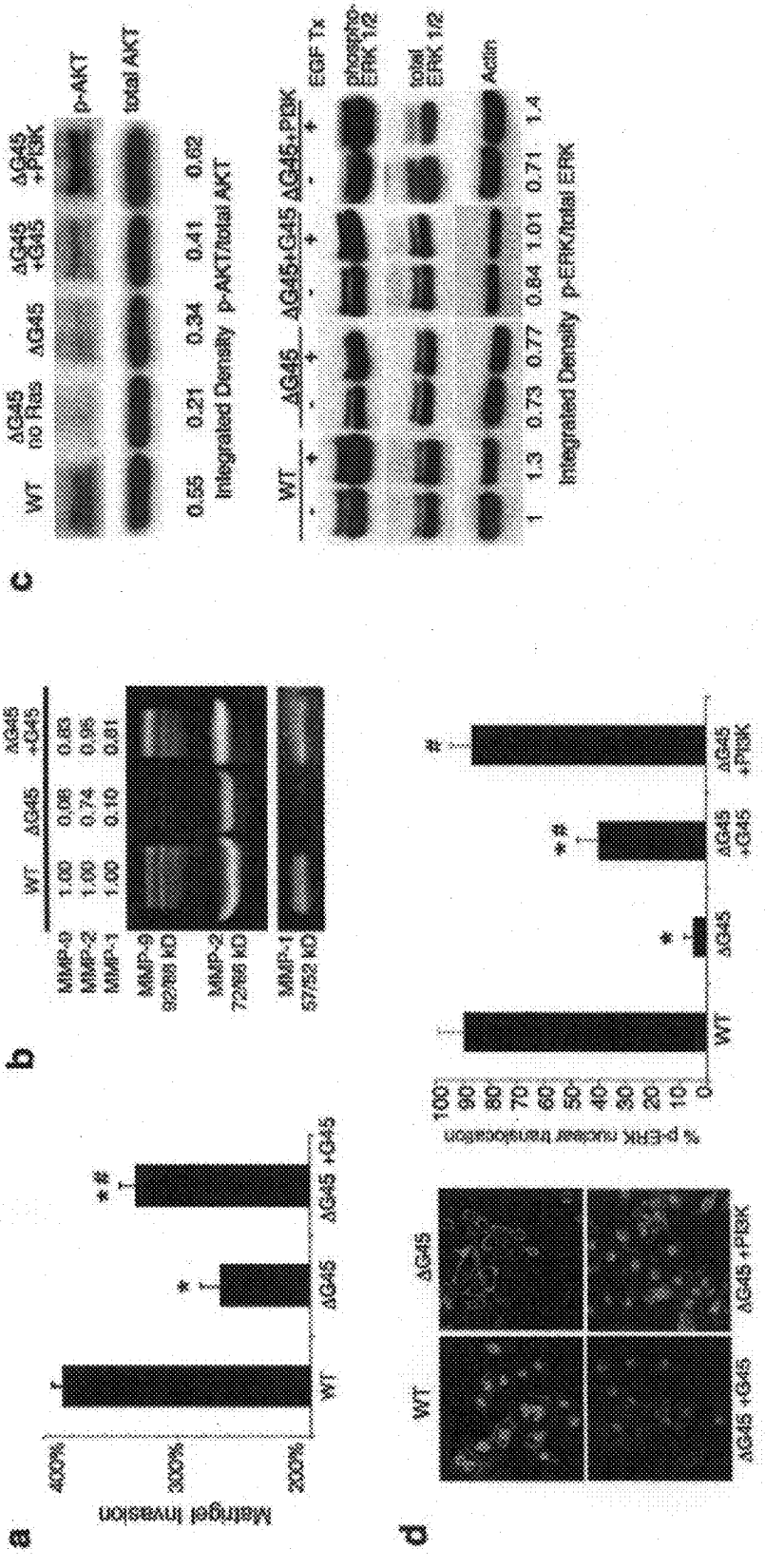

a
Matrigel Invasion
400%
300%
200%
WT
ΔG45
ΔG45 +G45
b
WT
ΔG45
ΔG45 +G45
MMP-9 1.00 0.98 0.83
MMP-2 1.00 0.74 0.95
MMP-1 1.00 0.10 0.81
MMP-9 92/86 kD
MMP-2 72/66 kD
MMP-1 57/52 kD
c
WT
ΔG45 no Ras
ΔG45
ΔG45 +G45
ΔG45 +PBK
p-AKT
total AKT
0.55 0.21 0.34 0.41 0.82
Integrated Density p-AKT/total AKT
WT
ΔG45
ΔG45+G45
ΔG45+PBK
EGF Tx
phospho-ERK 1/2
total ERK 1/2
Actin
1 1.3 0.73 0.77 0.84 1.01 0.71 1.4
Integrated Density p-ERK/total ERK
d
WT
ΔG45
ΔG45 +G45
ΔG45 +PBK
% p-ERK nuclear translocation
100
90
80
70
60
50
40
30
20
10
0
WT
ΔG45
ΔG45 +G45
ΔG45 +PBK

FIGURE 10

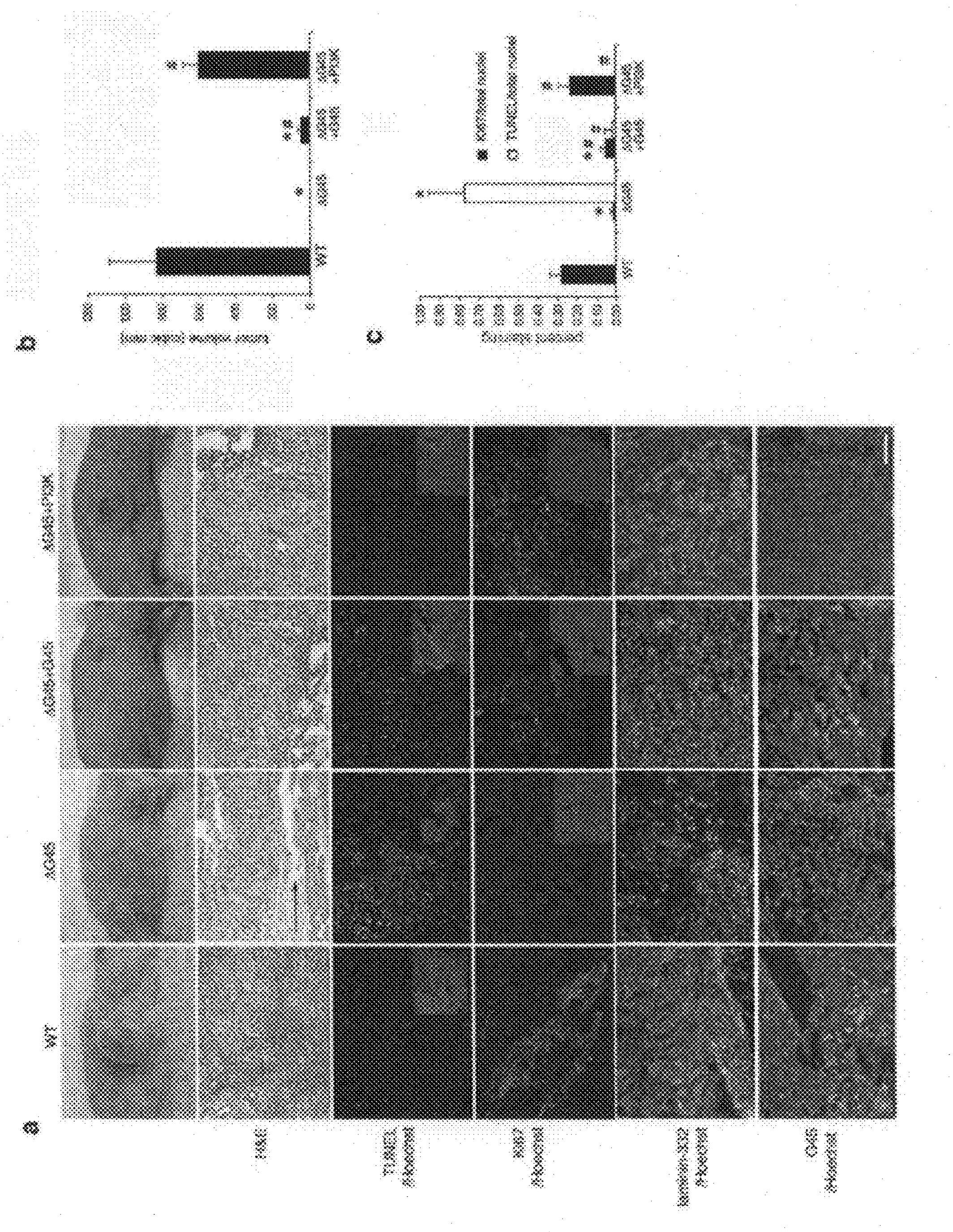
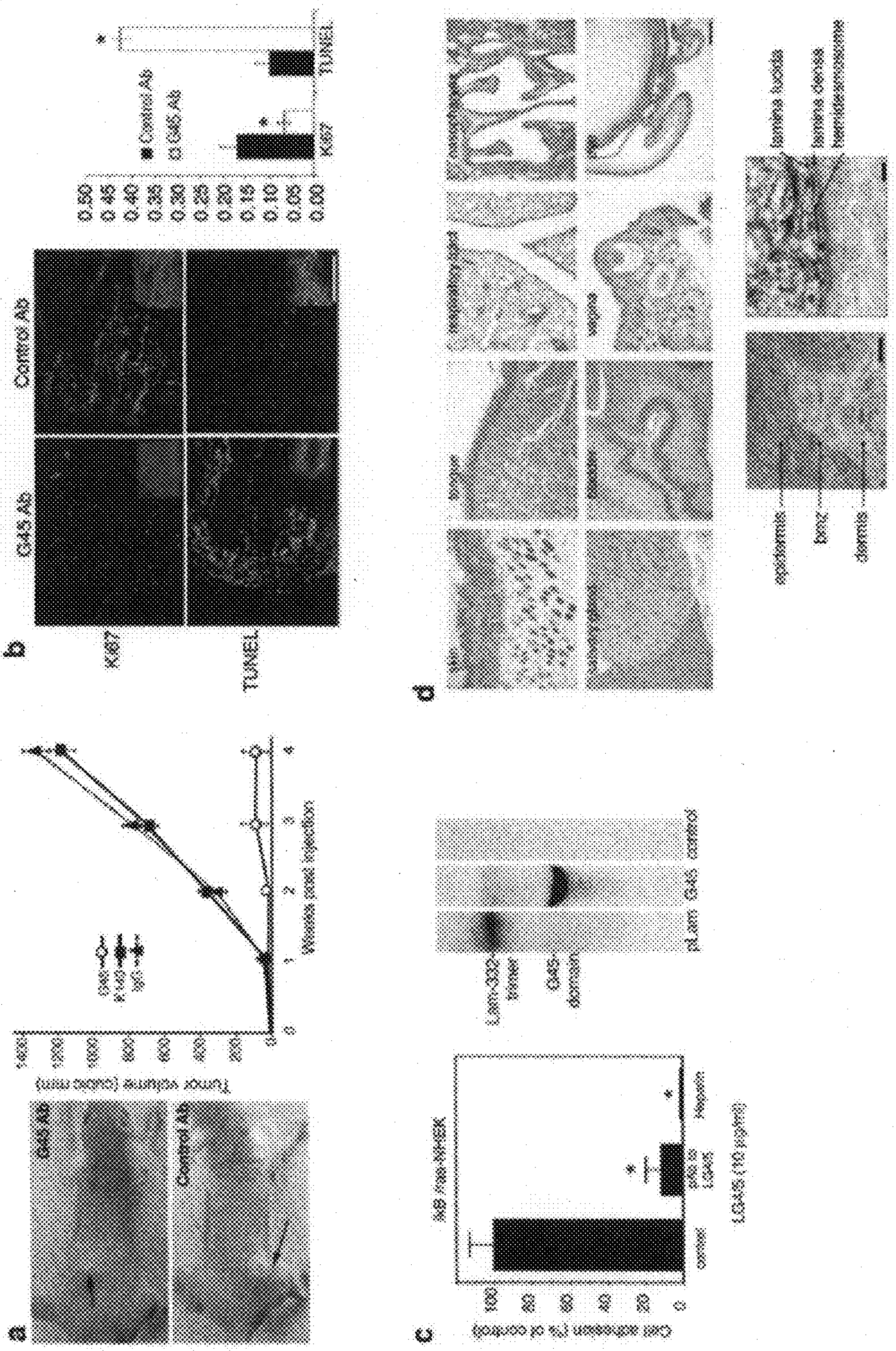


FIGURE 11


a
G45 Ab
Control Ab
Tumor volume (cubic mm)
Weeks post injection
b
G45 Ab
Control Ab
Ki67
TUNEL
Control Ab
G45 Ab
c
Cell adhesion (% of control)
control
Heparin
Lam-332 trimer
G45 domain
pLam
G45
control
d
epidermis
bmz
dermis
lamina lucida
lamina densa
hemidesmosome

COMPOSITIONS AND METHODS FOR INHIBITING SQUAMOUS CELL CARCINOMA

[0001] The present invention relates to compositions and methods for detecting and inhibiting squamous cell carcinoma using agents that target the laminin 5 alpha 3 G4-G5 domain.

INTRODUCTION

[0002] Squamous cell carcinoma (SCC) is common form of cancer and is the second most common form of skin cancer in the United States. SCCs are highly invasive and metastatic. SCCs are associated with a comparatively high risk of recurrence, resulting in significant mortality. SCC can be diagnosed by biopsy. However, SCCs are typically not as distinct as basal cell carcinomas or melanomas, making detection and diagnosis difficult. Current methods of treatment, i.e. surgery, radiotherapy, and chemotherapy, require continued monitoring due to the metastatic nature of the disease. The development of alternative methods of detection and treatment is therefore desirable.

[0003] The compositions and methods described herein are directed towards identifying agents that can detect and inhibit proteins associated with SCC tumorigenesis. Of particular interest, are agents that interact with the laminin-5 alpha 3 chain G4 and/or G5 domains.

SUMMARY

[0004] Provided herein are compositions and methods useful for detecting and treating squamous cell carcinoma (SCC). The compositions generally comprise antibodies capable of binding a migration facilitating protein (MFP) of a laminin 5 alpha 3 chain G4 and/or G5 domain or subdomain. MFPs typically comprise 8, 9, 10 or more amino acids present in the laminin 5 alpha 3 G4 and/or 5 domains that do not comprise a recognized cleavage site for bone morphogenetic protein-1 (BMP-1) and BMP-1 related proteins. For example, MFPs can be generated comprising: (1) the G5 subdomain; (2) the G4 subdomain lying between amino acid 1358 and amino acid 1366; (3) the G4 subdomain lying between amino acid 1375 and amino acid 1390; (4) the G4 subdomain lying between amino acid 1399 and 1530; and, (5) the G4-5 subdomain lying between amino acid 1399 and amino acid 1713. As will be appreciated by a person of skill in the art, MFPs encoding other subdomains within the laminin 5 alpha 3 G4 and/or 5 domains can also be generated and used in the methods of the present invention. The compositions can include additional components, such as, detectable labels and a pharmaceutically acceptable carrier.

[0005] The methods generally involve administering a therapeutically effective amount of a composition comprising one or more antibodies capable of inhibiting SCC tumorigenesis to a patient diagnosed with SCC. Treatment of a patient diagnosed with SCC with the compositions described herein can be combined with other medical means for treating SCC, such as surgery, radiotherapy, and chemotherapy. The SCC can be selected from the group consisting of skin cancer, lung cancer, head cancer, gastric cancer, colorectal cancer, throat cancer, cancer of the urinary tract, cancer of the reproductive tract, esophageal cancer, and bronchiogenic carcinoma.

[0006] Also provided are methods that utilize the MFPs described above. In some embodiments, a method is provided for detecting the binding activity of a candidate agent in a sample that comprises the steps of: (a) contacting the sample with an. MFP under conditions effective to permit binding between the MFP and the candidate agent (if present); and, (b) detecting the binding of the candidate agent.

[0007] A number of different assays can be used to detect binding of the candidate agent. For example, in some embodiments, the candidate agent is labeled and binding determined directly. In other embodiments, the binding of the candidate agent is determined through the use of competitive binding assays in which the competitor is a binding moiety known to bind the MFP, i.e., an antibody. Displacement of the competitor by the candidate agent is an indication that the candidate agent is capable of binding the MFP.

[0008] Also provided herein are methods for screening for candidate agents that inhibit SCC tumorigenesis. In some embodiments, a method is provided for screening for candidate agents that inhibit SCC tumor development comprising the steps of: a) subcutaneously injecting nude mice with a suspension comprising: i) Ras/IKB transformed epithelial cells; ii) a migration facilitating protein (MFP) of a laminin G4 and/or G5 domain or subdomain; iii) one or more candidate agents; and b) determining the presence or absence of a tumor.

[0009] In some embodiments, a method is provided to evaluate the effect of a candidate SCC drug comprising administering the drug to a patient diagnosed with SCC and removing a cell sample from the patient. A number of different assays can be used to evaluate the effect of the candidate drug. For example, the expression profile of the cell sample can be determined and compared with an expression profile of a healthy individual. In some embodiments, the cell sample can be analyzed for the presence or absence of an MFP associated with SCC development before and after treatment with a candidate drug. In yet other embodiments, the size of the tumor before and after treatment with a candidate drug can be analyzed to determine if the drug is effective in inhibiting the invasion of nearby normal cells.

[0010] Also provided herein is a method for diagnosing SCC comprising removing a cell sample from an individual and analyzing the cell with one or more MFPs determined to be involved in SCC proliferation and/or metastasis.

[0011] These and other features of the compositions and methods described herein will become more apparent from the detailed description below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0013] Aspects of the invention, can be more fully understood with respect to the following drawings. In the drawings, similar elements are referenced with like numbers.

[0014] FIG. **1**A provides a cartoon illustrating the G4 (1) and G5 (2) domains of the alpha 3 chain of laminin 5, including the cleavage recognition sites (1a, 1b, 1c) for bone morphogenetic protein-1 (BMP-1); FIG. **1**B illustrates exemplary embodiments of migration facilitating proteins (MFPs) that can be generated from the G4 domain; FIG. **1**C illustrates exemplary embodiments of migration facilitating proteins

(MFPs) that can be generated from the G5 domain; FIG. **1**D illustrates exemplary embodiments of migration facilitating proteins (MFPs) that can be generated comprising amino acids present in both the G4 and G5 domains.

[0015] FIG. **2** depicts normal human skin epithelia cells and SCC epithelial derived tumor cells.

[0016] FIG. 3 illustrates the results from a migration assay comparing wild-type cells, and keratinocytes transformed with truncated versions of the laminin-5 alpha 3 chain.

[0017] FIG. **4** illustrates a scratch assay in which cells lacking the G4 and G5 domain (i.e. 1337TR) migrate more efficiently than cells expressing wild-type laminin-5 alpha 3 chain.

[0018] FIG. **5** illustrates a mouse model of human SCC. The left panel depicts tumor formation in nude mice transformed with RAS/IKB keratinocytes transformed with wild-type laminin-5 alpha 3 chain. The middle and right panels illustrate that SCC tumors are not formed in nude mice transformed with RAS/IKB laminin-5 negative keratinocytes (right panel) or with a laminin-5 construct lacking the G4 and G5 domains (middle panel).

[0019] FIGS. **6**A-**6**D. Laminin-332 G45 is present in human SCC tumors but absent in normal skin. (a) Schematic diagram of laminin-332's α3, β3 and γ2 chains, highlighting α3 chain domain structure (black), proteolytic cleavage and epitopes for BM165 mAb and G45 pAb. (b) Immunofluorescence microscopic analysis of frozen sections of human SCC (upper panels) and neonatal skin (lower panels) using BM165 mAb (green) and G45 pAb (red). Merged images with nuclear Hoechst staining (blue) are shown in the panels to the right. Images are representative of four frozen SCC and skin samples tested. Scale bar=50 μm. (c) Results of analysis of 75 cases of paraffin embedded human cutaneous SCC using G45 pAb by immunohistochemical analysis. Upper left diagram shows number and percentage of samples which showed negative, moderate or strong expression. Bottom and right panels show representative examples of moderate/strong expression, as well as negative skin control. Scale bar=50 μm. (d) Left: Representative samples of 56 cases of paraffin embedded human extra-cutaneous SCC from various tissues using G45 pAb by immunohistochemical analysis showing moderate to strong expression. Scale bar=50 μm. Right: results of extra-cutaneous SCC tissue survey, showing tissues of origin, and intensity of staining for G45 pAb antibody.

[0020] FIGS. **7**A-**7**C. Laminin-332 G45 expression and function in human keratinocytes. (a) Schematic of the laminin α3 cDNA constructs used in this study. WT=wild type full length laminin α3 construct. ΔG45=laminin α3 chain truncated at amino acid 1337 (major processing site) (20). G45=portion of the laminin α3 chain G domain removed during processing. (b) Retroviral expression of laminin α3 cDNA constructs in JEB^{null} keratinocytes Left panel, nonreduced immunoblot of conditioned keratinocyte medium using laminin-332 pAb, position of laminin-332 trimer and molecular weight markers (kD) shown to the left. NK=normal human keratinocytes. Center panel, reduced immunoblot of extracted keratinocyte matrix using laminin-332 pAb, positions of molecular weight markers (kD) are shown to the left and positions of individual laminin-332 chains are shown to the right. Right panel, nonreduced immunoblot analysis of conditioned keratinocyte medium using G45 pAb, position of G45 shown on left. (c) Quantification of cell layer (Matrix+Lysate) and conditioned media fractions from laminin α3 null keratinocytes expressing the indicated laminin α3 constructs by immunoblot using anti-laminin β3 antibody K140, or anti-actin antibody followed by densitometric analysis showed as integrated density of the ratio of laminin β3 to actin bands. Position of laminin β3 band shown on right.

[0021] FIGS. **8**A-**8**C. Laminin-332 G45 influences matrix receptor organization and function. (a) Effects of G45 on keratinocyte matrix deposition and adhesion complex formation. Keratinocytes expressing the indicated α3 chain constructs were analyzed by triple-label confocal microscopy. Color of secondary antibodies and staining are designated by the color of the text listing the primary antibody. FA, focal adhesion; SA, stable adhesion. Scale bar=10 μm. (b) G45 promotes resistance to trypsin dissociation. Established keratinocyte cultures expressing the indicated laminin α3 constructs were subjected to dilute trypsin at the indicated intervals and % of cells dissociated was quantified. Data represents the results of triplicate experiments, error bars=±SD, *=p-value<0.05 compared to WT cells, #=p-value<0.05 compared to ΔG45 cells (c) G45 modulates keratinocyte migration during in vitro wound healing assay. Confluent monolayers of keratinocytes expressing indicated laminin constructs were tested for their ability to migrate into 1 mm scratches over the course of 24 hours and % closure of scratch was quantified as shown. Data represents the results of triplicate experiments, error bars=±SD, *=p-value<0.05 compared to WT cells, #=p-value<0.01 compared to ΔG45 cells.

[0022] FIGS. **9**A-**9**D. Laminin-332 G45 drives human SCC tumorigenesis and invasion by increasing activation of MMPs, ERK and PI3-K pathways, promoting proliferation and inhibiting apoptosis. Ras-IκBα transformed JEB^{null} keratinocytes expressing the laminin constructs indicated in FIG. **2**, (and in some instances activated p110 PI3-K subunit) were analyzed under the following conditions: (a) Keratinocytes expressing G45 showed increased cellular Matrigel invasion. Data represents triplicate independent experiments ±SD and are quantified as a percentage of basal invasion by JEB^{null} cells alone, *=p-value<0.05 compared to WT cells, #=p-value<0.05 compared to ΔG45 cells (b) G45 expression promotes MMP activity. Conditioned medium was analyzed by gel zymography, and intensity of digestion of bands by indicated metalloproteinases was quantified by densitometry as shown above the zymogram (c) G45 expression promotes PI3-K pathway and ERK activation. Top: cell lysates were analyzed by immunoblot using phospho-AKT (p-AKT) and total AKT antibodies. Ratio of phospho-AKT to total AKT staining was quantified using densitometry. All cell studied were Ras/IKBα transformed except where indicated (no Ras). Bottom: lysates from growth factor starved cells were analyzed by immunoblot before or after EGF treatment, using antibodies to phospho-ERK, total ERK and actin. Densitometric analysis is shown at the bottom as phosphorylated ERK1/2 normalized to untreated WT controls and total ERK1/2 expression. (d) G45 promotes phospho-ERK nuclear translocation. Left: Representative images from JEB^{null} keratinocytes expressing, the indicated laminin α3 constructs were visualized by immunofluorescence microscopy using phospho-ERK antibody. Graph on right shows quantification of the effects of G45 on phospho-ERK nuclear translocation. Data represents triplicate independent experiments ±SD and are quantified as a percentage of phopho-ERK staining nuclei over total nuclei, *=p-value<0.001 compared to WT cells, #=p-value<0.01 compared to ΔG45 cells.

[0023] FIGS. **10**A-**10**C. G45 promotes human SCC tumorigenesis in vivo. (a) Representative photos in the top panel show a lack of tumor growth in cells lacking G45 expression (ΔG45, top panel), but a partial restoration when G45 was separately expressed (ΔG45+G45), and a near-complete restoration of tumorigenesis when activated p110 (ΔG45+PI3K) Histologic invasion of underlying muscle (second panel) was absent in tumors lacking G45 expression (arrow). Apoptosis was greatly increased in tumors lacking G45 (third panel) as shown by TUNEL assay of frozen tumor sections, inset: Hoechst nuclear stain. Proliferation was greatly reduced in tumors lacking G45 (fourth panel) shown by Ki67 antibody analysis of frozen tumor sections, inset: Hoechst nuclear stain. Laminin-332 deposition (fifth panel) was greatly reduced in the absence of G45 subunit, but was partially rescued by expression of G45 and fully rescued by activated p110 (PI3-K) expression as shown by immunofluorescence microscopy of frozen tumor sections using laminin-332 polyclonal antibody. G45 construct produced levels of G45 expression in tumor sections equivalent to WT construct as assessed by immunofluorescence microscopy of frozen tumor sections using G45 pAb (bottom panel). Scale bar=100 μm. (b) G45 promotes human SCC tumorigenesis. Tumor growth was measured 4 weeks after subcutaneous transfer of indicated cells to immunodeficient mice. Error bars, ±SD. *=p-value<0.05 compared to WT cells, #=p-value<0.05 compared to ΔG45 cells. (c) G45 promotes proliferation and protection from apoptosis. TUNEL assay and Ki67 staining as assessed by immunofluorescence microscopy of frozen tumor sections was quantified as a percentage of staining of total nuclei. Error bars, ±SD. *=p-value<0.05 compared to WT cells, #=p-value<0.05 compared to ΔG45 cells.

[0024] FIGS. **11**A-**11**D. Laminin α3 G45 pAb inhibited human SCC tumorigenesis without disrupting normal epithelial adhesion. (a) G45 pAb blocked human tumorigenesis. Left: representative photos of Ras/IκBα-transformed SCC tumors in immunodeficient mice after 4 weeks of treatment with G45 pAb or control IgG. Right: quantification of tumor growth during weekly tumor volume measurements in-mice treated with G45 pAb, laminin β3 mAb K140 or control IgG. (b) Following 4 weeks of G45 pAb antibody injection, proliferation was reduced in tumors as shown by analysis of frozen tumor sections using immunofluorescence microscopy and Ki67 antibody. Left: proliferation was significantly reduced and apoptosis was greatly increased with G45 pAb treatment, as shown by Ki67 staining and TUNEL assay of frozen tumor sections; insets: Hoechst nuclear stain. Scale bar=100 μm. Right: quantification expressed as number of cells with Ki67 or TUNEL staining, respectively, as a percentage of total nuclei. All error bars=±SD, *=p-value<0.05 compared to control Ab (c) Left: Ras/IKBα transformed normal human epidermal keratinocytes (NHEK) were plated on dishes coated with recombinant laminin α3 G45 domain in the presence of 20 μg/ml control or affinity purified G45 IgG or heparin for one hour, then attached cells were analyzed by colorimetery and quantified as percentage of control. Right: laminin α3 G45 pAb reacted with native mouse laminin-332 as shown by nonreduced immunoblot of conditioned mouse keratinocyte medium using laminin-332 pAb (pLam), G45 pAb (G45) or nonimmune rabbit IgG (control). *=p-value<0.01 compared to control (d) Upper photos: Representative micrographs of G45 antibody treated mouse tissues known to express laminin-332 show no evidence of epithelial detachment or other histologic defects scale bar=200 μm. Lower photos: transmission electron microscopy of antibody treated mouse skin revealed no vesiculation or disruption of BMZ ultrastructure in representative samples. Left bar=5 μm, right bar=100 nm.

DETAILED DESCRIPTION

[0025] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the inventions described herein. In this application, the use of the singular includes the plural unless specifically state otherwise. Also, the use of "or" means "and/or" unless state otherwise. Similarly, "comprise," "comprises," "comprising," "include," "includes" and "including" are not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which the invention belongs.

DEFINITIONS

[0026] As used herein, the following terms and phrases are intended to have the following meanings:

[0027] "Antibody" has its standard meaning and is intended to refer to intact molecules as to fragments thereof, such as Fab, F(ab')2, and Fv fragments, that are capable of binding an epitope.

[0028] "Cancer" has its standard meaning and is intended to refer to any malignant tumor of potentially unlimited growth that expands locally by proliferation and systemically by metastasis.

[0029] "Neoplasm" has its standard meaning and is intended to refer to the abnormal growth of a tissue, such as a tumor.

[0030] "Nucleobase" means those naturally occurring and those synthetic nitrogenous, aromatic moieties commonly found in the nucleic acid arts. Examples of nucleobases include purines and pyrimidines, genetically encoding nucleobases, analogs of genetically encoding nucleobases, and purely synthetic nucleobases. Specific examples of genetically encoding nucleobases include adenine, cytosine, guanine, thymine, and uracil. Specific examples of analogs of genetically encoding nucleobases and synthetic nucleobases include 5-methylcytosine, pseudoisocytosine, 2-thiouracil and 2-thiothymine, 2-aminopurine, N9-(2-amino-6-chloropurine-), N9-(2,6-diaminopurine), hypoxanthine, N9-(7-deaza-guanine), N9-(7-deaza-8-aza-guanine) and N8-(7-deaza-8-aza-adenine). 5-propynyl-uracil, 2-thio-5-propynyl-uracil. Other non-limiting examples of suitable nucleobases include those nucleobases illustrated in FIGS. **2**(A) and **2**(B) of U.S. Pat. No. 6,357,163, incorporated herein by reference in its entirety.

[0031] "Nucleoside" refers to a nucleobase linked to a pentose sugar. Pentose sugars include ribose, 2'-deoxyribose, 3'-deoxyribose, and 2',3'-dideoxyribose.

[0032] "Nucleoside analog" refers to a nucleobase linked to a sugar, other than a pentose sugar. For example, a nucleobase linked to hexose.

[0033] "Nucleotide" refers to compound comprising a nucleobase, a pentose sugar and a phosphate. Thus, as used herein a nucleotide refers to a phosphate ester of a nucleoside, e.g., a triphosphate.

[0034] "Nucleobase Polymer or Oligomer" refers to two or more nucleobases that are connected by linkages that permit

the resultant nucleobase polymer or oligomer to hybridize to a polynucleotide having at least a partially complementary nucleobase sequence. Nucleobase polymers or oligomers include, but are not limited to, poly- and oligonucleotides (e.g., DNA and RNA polymers and oligomers), poly- and oligonucleotide analogs and poly- and oligonucleotide mimics, such as polyamide nucleic acids or peptide nucleic acids. Polyamide nucleic acids and peptide nucleic acids are two different phrases used in the literature to describe the same molecule, abbreviated herein as PNA. Nucleobase polymers or oligomers can vary in size from a few nucleobases, for example, from 2 to 40 nucleobases, to several hundred nucleobases, to several thousand nucleobases, or more.

[0035] "Polynucleotides or Oligonucleotides" refer to nucleobase polymers or oligomers in which the nucleobases are linked by sugar phosphate linkages (sugar-phosphate backbone). Exemplary poly- and oligonucleotides include polymers of 2'-deoxyribonucleotides (DNA) and polymers of ribonucleotides (RNA). A polynucleotide may be composed entirely of ribonucleotides, entirely of 2'-deoxyribonucleotides or combinations thereof.

[0036] "Polynucleotide or Oligonucleotide Analog" refers to nucleobase polymers or oligomers in which the nucleobases are linked by a phosphate backbone comprising one or more sugar analogs or phosphate analogs. Typical oligonucleotide or polynucleotide analogs include, but are not limited to, sugar alkylphosphonates, sugar phosphoramidites, sugar alkyl- or substituted alkylphosphotriesters, sugar phosphorothioates, sugar phosphorodithioates, sugar phosphates and sugar phosphate analogs in which the sugar is other than 2'-deoxyribose or ribose, nucleobase polymers having positively charged sugar-guanidyl interlinkages such as those described in U.S. Pat. No. 6,013,785 and U.S. Pat. No. 5,696,253 (see also, Dagani 1995, Chem. & Eng. News 4-5:1153; Dempey et al., 1995, J. Am. Chem. Soc. 117:6140-6141). Such positively charged analogues in which the sugar is 2'-deoxyribose are referred to as "DNGs," whereas those in which the sugar is ribose are referred to as "RNGs." Specifically included within the definition of poly- and oligonucleotide analogs are locked nucleic acids (LNAs; see, e.g. Elayadi et al., 2002, Biochemistry 41:9973-9981; Koshkin et al., 1998, J. Am. Chem. Soc. 120:13252-3; Koshkin et al., 1998, Tetrahedron Letters, 39:4381-4384; Jumar et al., 1998, Bioorganic & Medicinal Chemistry Letters 8:2219-2222; Singh and Wengel, 1998, Chem. Commun., 12:1247-1248; WO 00/56746; WO 02/28875; and, WO 01/48190; all of which are incorporated herein by reference in their entireties).

[0037] "Polynucleotide or oligonucleotide mimic" refers to nucleobase polymers or oligomers in which the nucleobases are connected by a linkage other than a sugar-phosphate linkage or a sugar-phosphate analog linkage. Mimics with a specific linkage include peptide nucleic acids (PNAs) as described in any one or more of U.S. Pat. Nos. 5,539,082, 5,527,675, 5,623,049, 5,714,331, 5,718,262, 5,736,336, 5,773,571, 5,766,855, 5,786,461, 5,837,459, 5,891,625, 5,972,610, 5,986,053, 6,107,470, 6,451,968, 6,441,130, 6,414,112 and 6,403,763; all of which are incorporated herein by reference. Other types of mimics are described in the following publications: Lagriffoul et al., 1994, Bioorganic & Medicinal Chemistry Letters, 4: 1081-1082; Petersen et al., 1996, Bioorganic & Medicinal Chemistry Letters, 6: 793-796; Diderichsen et al, 1996, Tett. Lett. 37: 475-478; Fujii et al., 1997, Bioorg. Med. Chem. Lett. 7: 637-627; Jordan et al., 1997, Bioorg. Med. Chem. Lett. 7: 687-690; Krotz et al., 1995, Tett. Lett. 36: 6941-6944; Lagriffoul et al, 1994, Bioorg. Med. Chem. Lett. 4: 1081-1082; Diederichsen, U., 1997, Bioorganic & Medicinal Chemistry 25 Letters, 7: 1743-1746; Lowe et al., 1997, J. Chem. Soc. Perkin Trans. 1, 1: 539-546; Lowe et al., 1997, J. Chem. Soc. Perkin Trans. 11: 547-554; Lowe et al., 1997, 1. Chem. Soc. Perkin Trans. 1 1:5 55-560; Howarth et al., 1997, 1. Org. Chem. 62: 5441-5450; Altmann, K-H et al., 1997, Bioorganic & Medicinal Chemistry Letters, 7: 1119-1122; Diederichsen, U., 1998, Bioorganic & Med. Chem. Lett., 8:165-168; Diederichsen et al., 1998, Angew. Chem. mt. Ed., 37: 302-305; Cantin et al., 1997, Tett. Lett., 38: 4211-4214; Ciapetti et al., 1997, Tetrahedron, 53: 1167-1176; Lagriffoule et al., 1997, Chem. Eur. 1. 3: 912-919; Kumar et al., 2001, Organic Letters 3(9): 1269-1272; and the Peptide-Based Nucleic Acid Mimics (PENAMs) of Shah et al. as disclosed in WO 96/04000. All of which are incorporated herein by reference.

[0038] The oligonucleotides may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand ("Watson") also defines the sequence of the other strand ("Crick"); thus the sequences described herein also includes the complement of the sequence.

[0039] "Protein" has its standard meaning and is intended to refer to at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e., "analogs" such as peptoids [see Simon et al., Proc. Natl. Acad. Sci. U.S.A. 89(20:9367-71 (1992)], generally depending on the method of synthesis. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline, and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. In addition, any amino acid representing a component of the variant proteins of the present invention can be replaced by the same amino acid but of the opposite chirality. Thus, any amino acid naturally occurring in the L-configuration (which may also be referred to as the R or S, depending upon the structure of the chemical entity) may be replaced with an amino acid of the same chemical structural type, but of the opposite chirality, generally referred to as the D-amino acid but which can additionally be referred to as the R— or the S—, depending upon its composition and chemical configuration. Such derivatives generally have the property of greatly increased stability, and therefore are advantageous in the formulation of compounds which may have longer in vivo half lives, when administered by oral, intravenous, intramuscular, intraperitoneal, topical, rectal, intraocular, or other routes.

[0040] In some embodiments, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradations. Proteins including non-naturally occurring amino acids may be synthesized or in some cases, made recombinantly; see van Hest et al., FEBS Lett 428:(1-2) 68-70 May 22, 1998 and Tang et al., Abstr. Pap Am. Chem. S218: U138 Part 2 Aug. 22, 1999, both of which are expressly incorporated by reference herein.

[0041] "Squamous cell carcinoma" has its standard meaning and is intended to refer to any neoplasm or tumor of epithelial cells.

[0042] "Tumorigenesis" has its standard meaning and is intended to refer to the basic developmental processes that produce tumors. These basic properties include the ability to proliferate or invade nearby normal cells and the ability to migrate from the site where the tumor initiated, i.e. metastasis.

[0043] The invention finds use in the prevention, treatment, detection or research of squamous cell carcinomas. Carcinomas are malignancies that originate in the epithelial tissues. Epithelial cells cover the external surface of the body, line the internal cavities, and form the lining of glandular tissues. In adults, carcinomas are the most common forms of cancer.

[0044] "Diagnosis" as used herein generally includes determination of a subject's susceptibility to a disease or disorder, determination as to whether a subject is presently affected by a disease or disorder, prognosis of a subject affected by a disease or disorder (e.g., identification of pre-metastatic or metastatic cancerous states, stages of cancer, or responsiveness of cancer to therapy), and use of therametrics (e.g., monitoring a subject's condition to provide information as to the effect or efficacy of therapy).

[0045] The term "biological sample" encompasses a variety of sample types obtained from an organism and can be used in a diagnostic or monitoring assay. The term encompasses blood and other liquid samples of biological origin, solid tissue samples, such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The term encompasses samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components. The term encompasses a clinical sample, and also includes cells in cell culture, cell supernatants, cell lysates, serum, plasma, biological fluids, and tissue samples.

[0046] The terms "treatment", "treating", "treat" and the like are used herein to generally refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete stabilization or cure for a disease and/or adverse effect attributable to the disease. "Treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease or symptom from occurring in a subject which may be predisposed to the disease or symptom but has not yet been diagnosed as having it; (b) inhibiting the disease symptom, i.e., arresting its development; or (c) relieving the disease symptom, i.e., causing regression of the disease or symptom.

[0047] The terms "individual," "subject," "host," and "patient," used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. Other subjects may include cattle, dogs, cats, guinea pigs, rabbits, rats, mice, horses, and the like.

Exemplary Embodiments

[0048] Provided herein are: (1) migration facilitating proteins (MFPs) derived from the laminin-5 α3 chain G4 and/or G5 domains; (2) antibodies which bind to MFPs, thereby inhibiting tumorigenesis of neoplastic epithelial cells; (3) methods for screening for agents, such as antibodies, small molecules, etc., that specifically bind one or more of the MFPs described herein; (4) methods for screening for agents that inhibit squamous cell carcinoma (SCC) tumor development using MFPs, (5) methods for diagnosing SCC; and, (6) methods for determining the efficacy of candidate agents used to treat SCC. All of these inventions rely upon MFPs, nucleic acids that encode MFPs and other molecules, such as antibodies, that bind MFPs.

[0049] Laminin-5 (formerly called kalinin, nicein, or BM6000) is a heterotrimeric extracellular matrix protein that is initially synthesized and secreted in an unprocessed form with an α3 chain of 200 kDa, a β3 chain of 140 kDA, and a γ2 chain of 155 kDA. (Marinkovich et al., 1992, J. Biol. Chem., 267: 17900-17906). Laminin-5 is a component of the basal lamina, the structure that provides tissue integrity, as well as the foundation for migration, growth and differentiation of cells. It is therefore not surprising that processes that interfere with wild type functions of laminin-5 produce diseases in humans and other mammals.

[0050] Large deposits of laminin-5 are found at the leading edges of squamous cell carcinomas (SCCs). This deposition of laminin-5 is believed to serve as a substrate for tumor invasion (see, e.g., Pyke et al., 1995, Canc. Res. 55: 4132-4139; Berndt et al., 1997, Invasion and Metastasis, 17: 251-258). Increased laminin-5 immunoreactivity is indicative of a poor prognosis in patients with squamous cell carcinoma (SCC). Laminin-5 is also preferentially expressed by invading malignant cells of many human carcinomas in additions to SCCs, colon and mammary carcinomas (Pyke, et al., 1994, Am. J. Pathol. 145(4):782-791) and malignant gliomas (Fukushima et al., 1998, Int. J. Cancer, 76: 63-72).

[0051] Processing of extracellular matrix proteins by proteases is emerging as a key mechanisms in processes such as wound healing and tumor metastasis. Several proteases have been implicated in laminin-5-processing (see, e.g., Veitch et al., 2003, J. Biol. Chem., 278: 15661-15668; and U.S. patent Pub. No. 2002/0076736). In fully formed tissues, laminin-5 is completely processed and is devoid of the G4 and G5 domains (Marinkovich et al., 1992, J. Biol. Chem., 267: 17900-17906). Without being bound by theory, it appears that specific proteolytic processing can convert laminin-5 from a pro-migratory signal required for cell migration during tumor invasion and tissue remodeling to an adhesive substrate devoid of the G4 and G5 domains.

Migration Facilitating Sequences

[0052] Accordingly, provided herein are polynucleotide and amino acid sequences associated with SCC, herein termed "migration facilitating sequences" or "MFSs". The proteins having the various amino acid sequences are referred to herein as "migration facilitating proteins" or "MFPs". Association in this context means that the amino acid and polynucleotide sequences are either differentially expressed or altered in SCCs or neoplastic epithelial cells as compared to normal epithelial tissue. "SCC" refers herein to any malignant neoplasm or tumor of epithelial cells. Specific examples of epithelial cells include squamous cells, squamous carcinoma cells, keratinocytes, mucosal epithelial cells, such as oral mucosal cells, gastrointestinal epithelial cells, corneal epithelium of the eye, and epithelial cells of the urinary and reproductive tract. Specific examples of SCC carcinomas arising from neoplastic epithelial cells include skin, lung, head, neck, oral, gastric, colorectal, throat, urinary tract, reproductive tract, esophageal, etc.

[0053] SCC is commonly sun-induced, i.e., actinically derived SCC. SCC can also result from transplant or invasive surgery, or follow other immunosuppressive situations. Chronic inflammation can lead to development of SCC at the site of inflammation, e.g., a burn or scar, Majolin's ulcer, etc. SCC can be virally induced, for example, SCC can result from human papillomavirus-induced (HPV) infection. SCC can include adenoid (acantholytic) SCC, spindle cell SCC, verrucous carcinoma (VC), keratoacanthoma (KA), nodular SCC periungual SCC, and other epithelial carcinomas.

[0054] MFSs can include both polynucleotide and amino acid sequences. In some embodiments, the MFSs are recombinant polynucleotides. By the term "recombinant polynucleotide" herein is meant polynucleotides, originally formed in vitro, in general, by the manipulation of the polynucleotide by polymerases and endonucleases, in a form not normally found in nature. Thus, an isolated polynucleotide, in a linear form, or an expression vector formed in vitro by ligating polynucleotide molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant polynucleotide is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, i.e. using the in vivo cellular machinery of the host cell rather than in vitro manipulations; however, such polynucleotides, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention.

[0055] As will be appreciated by those in the art, and more fully outlined below, MFSs comprising polynucleotides are useful in a variety of applications, including diagnostic applications, where they can be used as hybridization probes to detect MFSs in SCCs, as well as in therapeutic applications, such as the development of antisense sequences that can be used to affect the expression and activity of MFPs in SCCs.

[0056] MFSs include those that are up-regulated, (e.g., expressed at a higher level), as well as those that are down-regulated, (e.g., expressed at a lower level) in SCCs. MFSs also include sequences that have been altered (i.e. truncated sequences or sequences with a one or more mutations, such as point mutations, deletions, insertions, etc.) and show either the same expression profile or an altered profile. In some embodiments, the MFSs are from humans. However, as will be appreciated by a person of skill in the art, MFSs from other organism may be useful in animal models of disease and drug evaluation. Thus, other MFSs are provided. For example, MFSs can be obtained from vertebrates, including mammals, such as rodents (rats, mice, hamsters, guinea pigs, etc.), primates, farm animals (including sheep, goats, pigs, cows, horses, etc), as well invertebrates, such as Drosophila. MFSs from other organisms may be obtained using the techniques outlined below.

[0057] In some embodiments, MFSs are those that are altered but show either the same expression profile or an altered profile as compared to normal epithelial tissue of the same differentiation stage. "Altered MFSs" as used herein refers to sequences which are truncated, contain insertions or contain point mutations.

[0058] An MFS can be initially identified by substantial nucleic acid and/or amino acid sequence homology to the MFS's outlined herein. Such homology can be based upon the overall oligonucleotide or amino acid sequence, and is generally determined, using either homology programs or hybridization conditions. As is known in the art, a number of different programs are available for determining polynucleotide or amino acid sequence homology including sequence based alignment programs, sequence homology based alignment programs, structural alignment programs etc. Non-limiting examples of sequence-based alignment programs include Smith-Waterman searches (Smith & Waterman, Adv. Appl. Math. 2:482 (1981)), Needleman-Wunsch (Needleman & Wunsch, J. Mol. Biol. 48:443 (1970)), Double Affine Smith-Waterman, frame search, Gribskov/GCG profile search, Gribskov/GCG profile scan, profile frame search, Bucher generalized profiles, Hidden Markov models, Hframe, Double Frame, Blast, Psi-Blast, Clustal, and GeneWise. Sequence homology based alignment methods are described in Altschul et al. (Altschul et al., J. Mol. Biol. 215(3):403 (1990)). Examples of structural alignment programs include VAST from the NCBI; SSAP (Orengo and Taylor, Methods Enzymol 266(617-635 (1996)) SARF2 (Alexandrov, Protein Eng 9(9):727-732. (1996)) CE (Shindyalov and Boume, Protein Eng 11(9):739-747. (1998)); (Orengo et al., Structure 5(8):1093-108 (1997); Dali (Holm et al., Nucleic Acid Res. 26(1):316-9 (1998), Computerized implementations of some of the above described algorithms are also available (e.g., BLASTx, BLAST, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.); the Best Fit sequence program described by Devereux et al., Nucl. Acid Res. 12:387-395 (1984).

[0059] Polynucleotide homology can also be determined through hybridization studies; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference. Generally, stringent conditions are selected, although less stringent hybridization conditions can be used. Typically, stringent conditions are selected to be about 5-10/C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993).

[0060] In some embodiments, MFSs are polynucleotides. Polynucleotides comprising MFSs can be generated from either a full length genomic and/or cDNA polynucleotide encoding a laminin-5 α3 chain. In some embodiments, MFSs are generated from the human α3 chain of laminin-5 (Ryan et al., 1994, J. Biol. Chem., 269: 22779-22787; Gen Bank Accession No. NM.sub.--000227). MFSs of various lengths spanning the G4 and/or G5 domains can be generated. For example, an polynucleotide spanning a subdomain of the G4 domain of the human α3 chain of laminin-5 can be generated by starting at nucleotide position 4196 and ending at nucleotide 4588. An oligonucleotide spanning the G5 domain of the human α3 chain of laminin-5 can be generated by starting at nucleotide position 4590 and ending at nucleotide 5140. An oligonucleotide spanning a subdomain of the G4 domain and the entire G5 domain of the human α3 chain of laminin-5 can be generated by starting at nucleotide position 4196 and ending at nucleotide 5140.

[0061] The exact number of nucleotides or nucleotide analogs chosen will vary depending on the sequence of the nucleotides selected and the presence of nucleotides encoding amino acids that comprise antigenic determinants. By "epitope" or "determinant" "or antigenic determinant" herein

is meant a portion of a protein that can generate and/or bind an antibody or T-cell receptor in the context of MHC. For example, the presence of antigenic determinants within the G4 and G5 domains can be identified by searching databases for MHC ligands and peptide motifs (Rammensee, H., et al. (1999) Immunogenetics, 50:213-219). This information can be used to generate MFSs comprising MHC epitopes. Typically, epitopes recognized by MHC class I molecules comprise between 8 and 11 amino acids, thus, an MFS encoding an MHC class I epitope can range between 24 to 33 nucleotides. Viral peptides recognized by MHC class II molecules comprise between 10 to 20 amino acids, thus, an MFS encoding an MHC class II epitope can range between 30 to 60 nucleotides (Fundamental Immunology, 4th edition, W. E. Paul, ed., Lippincott-Raven Publishers, 1999, Chapter 39, pp 1295-1334). In other embodiments, MFSs range between 24 to 1050, or from 60 to 300 nucleotides, or from 60 to 405 nucleotides, or from 60 to 555 nucleotides, or from 60 to 600 nucleotides, or from 60 to 750 nucleotides, or from 60 to 900 nucleotides or from 60 to 1050 nucleotides. In yet other embodiments, MFSs range from 150 to 300 nucleotides, or from 150 to 405 nucleotides, or from 150 to 450 nucleotides, or from 150 to 525 nucleotides, or from 150 to 600 nucleotides, or from 150 to 750 nucleotides, or from 150 to 1050 nucleotides, or from 300 to 600 nucleotides, or from 300 to 900 nucleotides, or from 300 to 1050 nucleotides.

Migration Facilitating Proteins

[0062] In some embodiments, "migration facilitating proteins" or "MFPs" are generated from the amino acid sequence encoding the laminin-5 α3 G4 and/or G5 domains or subdomains thereof. "MFPs" are proteins that are capable of supporting migration of nearby tissue or tissue located at distal points in the body by neoplastic epithelial cells. MFPs also can be recombinant. A "recombinant MFP protein" is a protein made using recombinant techniques, i.e. through the expression of a recombinant oligonucleotide as described above. A recombinant protein is distinguished from a naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated or purified away from some or all of the proteins and compounds with which it is normally associated in its wild type host, and thus may be substantially pure. Generally, an isolated protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of the total protein in a given sample. A substantially pure protein comprises at least about 75% by weight of the total protein, with at least about 80% being preferred, and at least about 90% being particularly preferred. The definition includes the production of an MFP from one organism in a different organism or host cell. Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Alternatively, the protein may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions, as discussed below.

[0063] In some embodiments, MFPs are generated from the G4 and G5 domains of the human α3 chain of laminin-5 (see FIG. **1**A). MFPs of various lengths spanning the G4 and/or G5 domains can be generated. FIG. **1**B illustrates an exemplary embodiment of the generation of MFPs from the G4 domain. As illustrated in FIGS. **1**B-D, a number of MFPs can be generated from the G4 and/or G5 domains comprising varying numbers of amino acids or amino acid analogs. The exact number of amino acids or amino acid analogs chosen will vary depending on the sequence of the amino acids selected, the presence of bone morphogenetic-1 cleavage sites, and the presence of amino acids comprising antigenic determinants.

[0064] As discussed above, the presence of antigenic determinants within the G4 and G5 domains can be identified by searching databases for MHC ligands and peptide motifs (Rammensee, H., et al. (1999) Immunogenetics, 50:213-219). This information can be used to identify MHC epitopes. Typically, epitopes recognized by MHC class I molecules comprise between 8 and 11 amino acids while epitopes recognized by MHC class II molecules comprise between 10 to 20 amino acids (Fundamental Immunology, 4th edition, W. E. Paul, ed., Lippincott-Raven Publishers, 1999, Chapter 39, pp 1295-1334). Thus, in some embodiments, MFPs range between 8 to 11. In other embodiments, MFPs range between 10 to 20 amino acids. In other embodiments, MFPs range from 8 to 350 amino acids. In still other embodiments, MFPs range between 20 to 100 amino acids, or from 20 to 135 amino acids, or from 20 to 185 amino acids, or from 20 to 200 amino acids, or from 20 to 250 amino acids, or from 20 to 300 amino acids or from 20 to 350 amino acids. In yet other embodiments, MFPs range from 50 to 100 amino acids, or from 50 to 135 amino acids, or from 50 to 150 amino acids, or from 50 to 175 amino acids, or from 50 to 200 amino acids, or from 50 to 250 amino acids, or from 50 to 350 amino acids, or from 100 to 200 amino acids, or from 100 to 300 amino acids, or from 100 to 350 amino acids.

[0065] The MFPs may be unprocessed or processed. As used herein "unprocessed" refers to an MFP that is still associated with the laminin-5 α3 chain. By "processed" herein is meant that the MFP is dissociated from the laminin-5 α3 chain.

[0066] FIG. **1**B illustrates an exemplary embodiment of MFPs that can be generated from the G4 domain. FIG. **1**B depicts 3 MFPs: MFP 1, MFP 2, And MFP 3. Known cleavage sites for bone morphogenetic protein-1 (BMP-1) are indicated by the solid boxes labeled 1a, 1b, and 1c (see U.S. patent Pub. No. 2002/0076736). As illustrated in FIG. **1**B, the MFPs described herein do not comprise cleavage sites for bone morphogenetic protein-1 (BMP-1) or related BMP-1 proteins. As will be appreciated by a skilled artisan, other MFPs (MFPs 4) can be generated from the G4 domain, comprising from 8 up to 130 amino acids.

[0067] FIG. **1**C illustrates an exemplary embodiment of MFPs that can be generated from the G5 domain. As illustrated in FIG. **1**C, one MFP can be made, i.e. MFP 5 spanning the entire G5 domain. In other embodiments one MFP can be made, i.e. MFP 6, which spans a subdomain of the G5 domain. Alternatively, a number of MFPs, i.e. MFPs 7, can be made comprising from 8 up to 182 amino acids.

[0068] FIG. **1**D illustrates an exemplary embodiment of MFPs that can be generated from the G5 and the G5 domain. As illustrated in FIG. **1**D, one MFP can be made, i.e. MFP 8, spanning the G4-G5 domain. As illustrated in FIG. **1**D, MFP 8 does not contain cleavage sites for BMP-1 or related BMP-1

proteins. Alternatively, a number of MFPs, i.e., MFPs 9, can be made comprising from 8 up to 315 amino acids.

Expression Systems

[0069] MFSs polynucleotides encoding MFPs can be used to make a variety of expression vectors to express MFPs which can then be used in the diagnostic, screening and therapeutic applications described below. The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the oligonucleotide encoding the MFP protein. The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0070] An oligonucleotide is "operably linked" when it is placed into a functional relationship with another oligonucleotide sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. The transcriptional and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the MFP protein; for example, transcriptional and translational regulatory nucleic acid sequences from *Bacillus* are preferably used to express the MFP protein in *Bacillus.* Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

[0071] In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences.

[0072] Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

[0073] In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a procaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

[0074] In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

[0075] MFPs are produced by culturing a host cell transformed with an expression vector containing an oligonucleotide encoding an MFP, under the appropriate conditions to induce or cause expression of the MFP. The conditions appropriate for MFP expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

[0076] Appropriate host cells include yeast, bacteria, archaebacteria, fungi, and insect, plant and animal cells, including mammalian cells. Of particular interest are primary human keratinocytes, although other cells also can be used, i.e. *Drosophila melanogaster* cells, *Saccharomyces cerevisiae* and other yeasts, *E. coli, Bacillus subtilis,* 293 cells, CHO, other human cell and cell lines.

[0077] In some embodiments, the MFPs are expressed in mammalian cells. Mammalian expression systems are also known in the art, and include retroviral systems. A preferred expression vector system is a retroviral vector system such as is generally described in Dajee et al., 2003, Nature, 421: 639-643, which is incorporated herein by reference in its entirety. Of particular use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include, but are not limited to, the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter. Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. Examples of transcription terminator and polyadenlytion signals include those derived form SV40.

[0078] For example, the full length laminin-5 α3 cDNA can be ligated into a pENTR1A.™. vector (Invitrogen). The full length laminin-5 α3 cDNA can be cleaved and the PCR used to obtain a MF oligonucleotide sequence from the G4 and/or G5 domain. The resulting PCR product can be ligated into a pENTR1A™ vector and the cloning product confirmed by sequencing. The cloned product can then be transferred from the pENTR1A™ vector to a Gateway adapted LSRZ retroviral vector through lambda phage recombination. See Dajee et al., 2003, Nature, 421: 639-643.

[0079] In some embodiments, MFPs are expressed in bacterial systems. Bacterial expression systems are well known in the art. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters are also useful; for example, the tac promoter is a hybrid of the trp and lac promoter sequences.

Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable. The expression vector may also include a signal peptide sequence that provides for secretion of the MFP in bacteria. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways. These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for *Bacillus subtilis, E. coli, Streptococcus cremoris,* and *Streptococcus lividans,* among others. The bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

[0080] The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, is well known in the art, and will vary with the host cell used. Techniques include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, viral infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0081] MFS's and MFPs can be identified as described in the examples. For example, in a specific embodiment, various oligonucleotides can be generated from the G4 and G5 domain of the human laminin-5 α3 and subcloned into a retroviral vector. The resulting retroviral vectors can be transduced into cell cultures and the cells analyzed for cell scattering and cell migration (see e.g., Examples and FIGS. **5** and **6**; see also Ryan, et al., 1994, J. Biol. Chem., 269: 22779-22787). Alternatively, laminin-5 negative primary human keratinocytes co-expressing Ras, a stable NF-κB repressor mutant of IκBα (i.e. IKB), and one or more MFS(s) can be retrovirally transduced and used to regenerate human skin on immune deficient mice.(i.e. nude mice). The subsequent development of neoplasms can be monitored and compared to wild type mice (see e.g., Examples, FIG. **7**; and Dajee et al., 2003, Nature, 421:639-643).

[0082] In some embodiments, matrigel, which contains heparin sulfate proteoglycan, is used as a matrix for the suspension of RAS/IKB transformed keratinocytes prior to subcutaneous injection into nude mice. In other embodiments, MFPs can be suspended in matrigel prior to injection of RAS/IKB transformed keratinocytes.

[0083] In some embodiments, MFPs are purified or isolated after expression. MFPs may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing. For example, the MFP may be purified using a standard anti-MFP antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982). The degree of purification necessary will vary depending on the use of the MFP protein. In some instances no purification will be necessary.

Antisense Sequences

[0084] The MFSs and MFPs can be used in a variety of different ways. In some embodiments, MFSs can be used to make antisense therapeutic agents that affect the expression and activity of MFPs. Antisense technology relies on the modulation of expression of a target protein through the specific binding of an antisense sequence to a target sequence encoding the target protein or directing its expression. (See, e.g., Agrawal, S., ed., 1996, Antisense Therapeutics, Humana Press Inc., Totawa N. J.; Alama et al. (1997) Pharmacol Res. 36(3):171-178; Crooke, S. T., 1997, Adv. Pharmacol. 40:1-49; and Lavrosky et al., 1997, Biochem. Mol. Med. 62(1):11-22.). Antisense sequences are nucleic acid sequences capable of specifically hybridizing to at least a portion of a target sequence. Antisense sequences can bind to cellular mRNA or genomic DNA, blocking translation or transcription and thus interfering with expression of a targeted protein product. Antisense sequences can be any nucleic acid material, including DNA, RNA, or any nucleic acid mimics or analogs. (See, e.g., Rossi et al., 1991, Antisense Res. Dev. 1(3):285-288; Pardridge et al., 1995, Proc. Nat. Acad. Sci. 92 (12):5592-5596; Nielsen, P. E. and G. Haaima, 1997, Chem. Soc. Rev. 96:73-78; and Lee et al., 1998, Biochemistry 37 (3):900-1010.). Delivery of antisense sequences can be accomplished in a variety of ways, such as through intracellular delivery using an expression vector. Site-specific delivery of exogenous genes is also contemplated, such as techniques in which cells are first transfected in culture and stable transfectants are subsequently delivered to the target site.

[0085] Typically, antisense oligonucleotides between 15 to 25 nucleobases or nucleobase analogs are capable of producing the desired therapeutic effect, i.e., direct disruption of translation of an MFP. In addition, chemically reactive groups, such as iron-linked ethylenediamine-tetraacet-ic acid (EDTA-Fe), can be attached to antisense oligonucleotides, causing cleavage of the RNA at the site of hybridization. These and other uses of antisense methods to inhibit the in vitro translation of genes are well known in the art (see, e.g., Marcus-Sakura (1988) Anal. Biochem. 172:289).

[0086] In some embodiments, antisense oligonucleotides are designed such that they disrupt the translation of the laminin-5 α3 chain. In other embodiments, antisense oligonucleotides are designed such that they disrupt the translation of an MFP from the G4 domain or subdomain thereof. In still other embodiments, antisense oligonucleotides are designed such that they disrupt the translation of an MFP from the G5 domain or subdomain thereof. In yet other embodiment, antisense oligonucleotides are designed such that they disrupt the translation of an MFP from the G4 and G5 domain or subdomain thereof.

[0087] Delivery of antisense agents can be achieved intracellularly through using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein (see, e.g., Slater et al., 1998, J. Allergy Cli.

Immunol. 102 (3): 469-475). Delivery of antisense sequences can also be achieved through various viral vectors, including retrovirus and adeno-associated virus vectors. (See, e.g., Miller, 1990, Blood, 76: 271; and Uckert and Walther, 1994, Pharacol. Ther., 63(3): 323-347). Suitable viral vectors include, but are not limited to, adenoviruses, herpes viruses, vaccinia, and RNA viruses such as retroviruses.

[0088] Retroviral vectors can be derivatives of murine or avian retrovirus. Retroviral vectors can be made target-specific by inserting, for example, a polynucleotide encoding a protein or proteins such that the desired ligand is expressed on the surface of the viral vector. The ligand can be a glycolipid carbohydrate or protein. Preferred targeting can also be accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the antisense polynucleotide. See, e.g, WO 91/04753.

[0089] Other delivery mechanisms that can be used for delivery of antisense sequences to target cells include colloidal dispersion and liposome-derived systems, artificial viral envelopes, and other systems available to one of skill in the art (see, e.g., Rossi, 1995, Br. Med. Bull. 51 (1): 217-225; Morris et al., 1997, Nucl. Acids Res. 25 (14): 2730-2736; Boado et al., 1998, J. Pharm. Sci. 87 (11): 1308-1315; and WO 90/10448). For example, delivery systems can make use of macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

Antibodies

[0090] In some embodiments, the MFPs are used to generate antibodies that can be used in the screening and therapeutic applications described herein. Preferably, the MFP should comprise at least one epitope or determinant. In some embodiments, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity.

[0091] Within the context of the present invention, antibodies are understood to include monoclonal antibodies and polyclonal antibodies, antibody fragments (e.g., Fab and $F(ab')_2$), chimeric antibodies bifunctional or bispecific antibodies and tetrameric antibody complexes. Antibodies are understood to be reactive against a selected antigen on the surface of a T cell if they bind with an appropriate affinity (association constant), e.g. greater than or equal to 10^7M^{-1}. Additionally, antibodies that may be used in the methods of the present invention may also be described or specified in terms of their binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6}M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-9} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, 10^{-15} M.

[0092] MFPs can be evaluated to determine regions of immunogenicity. As discussed above, methods of analysis and epitope selection are well-known in the art. Analysis and selection can also be accomplished, for example, by various software packages, such as LASERGENE NAVIGATOR software (DNASTAR; Madison, Wis.). The polypeptides or fragments used to induce antibodies should be antigenic, but need not necessarily be biologically active. An antigenic fragment or polypeptide is at least 5 amino acids in length, more preferably, at least 10 amino acids in length, and most preferably, at least 15 amino acids in length. It is preferable that the antibody-inducing fragment or polypeptide is identical to at least a portion of the amino acid sequence of the G4 and/or G5 domain, or subdomains thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor, and antibodies can be produced against the chimeric molecule.

[0093] Methods for the production of antibodies are well-known in the art. For example, various hosts, including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with the MFP or any immunogenic fragment or peptide thereof. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are especially preferable.

[0094] Monoclonal and polycolonal antibodies can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. Techniques for in vivo and in vitro production are well-known in the art (see, e.g., Pound, J. D., 1998, Immunochemical Protocols, Humana Press, Totowa N. J.; Harlow, E. and D. Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, New York). The production of chimeric antibodies is also well-known, as is the production of single-chain antibodies (see, e.g., Morrison et al., 1984, Proc. Natl. Acad. Sci. 81: 6851-6855; Neuberger et al., 1984, Nature, 312: 604-608; Takeda et al., 1985, Nature, 314: 452-454). Antibodies with related specificity, but of distinct idiotypic composition, may be generated, for example, by chain shuffling from random combinatorial immunoglobin libraries (see, e.g., Burton, 1991, Proc. Natl. Acad. Sci. 88: 11120-11123).

[0095] Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents (see, e.g., Orlandi et al., 1989, Proc. NatI. Acad. Sci. 86: 3833-3837; Winter, G. and C. Milstein, 1991, Nature, 349: 293-299). Antibody fragments which contain specific binding sites for the target polypeptide may also be generated. Such antibody fragments include, but are not limited to, F(ab').sub.**2** fragments, which can be produced by pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab').sub.2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (see, e.g., Huse et al., 1989, Science, 254: 1275-1281).

[0096] In some embodiments, the antibodies are bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for a MFP, and the other one is for any other antigen, such as a cell-surface protein or receptor or receptor subunit, preferably one that is tumor specific.

[0097] In some embodiments, the antibodies to MFPs are humanized antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general,.the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework residues (FR) regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986, Nature, 321: 522-525; Riechmann et al., 1988, Nature, 332: 323-329; and Presta, 1992, Curr. Op. Strucf. Biol., 2: 593-596).

[0098] Methods for humanizing non-human antibodies are well known in the art. Generally, 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 et al., 1986, Nature, 321: 522-525; Riechmann et al., 1988, Nature, 332: 323-329; Verhoeyen et al., 1988, Science, 239: 1534-1536), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. 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 CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0099] Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, 1991, J. Mol. Biol., 227: 381; Marks et al., 1991, J. Mol. Biol., 222: 581). The techniques of Cole et al. and Boemer et al. are also available for the preparation of human monoclonal antibodies (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77, and Boemer et al., 1991, J. Immunol., 147(1): 86-95). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos. 5,545, 807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., 1992, Bio/Technology, 10: 779-783; Lonberg et al., 1994, Nature, 368: 856-859; Morrison, 1994, Nature, 368: 812-13; Fishwild et al., 1996, Nature Biotechnology, 14: 845-51; Neuberger, 1996, Nature Biotechnology, 14: 826; Lonberg and Huszar, 1995, Intern. Rev. Immunol. 13 65-93.

[0100] Antibodies can be tested for anti-MFP activity using a variety of methods well-known in the art. Various techniques may be used for screening to identify antibodies having the desired specificity, including various immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), including direct and ligand-capture ELISAs, radioimmunoassays (RIAs), immunoblotting, and fluorescent activated cell sorting (FACS). Numerous protocols for competitive binding or immunoradiometric assays, using either polyclonal or monoclonal antibodies with established specificities, are well known in the art (see, e.g., Harlow and Lane, supra). Such immunoassays typically involve the measurement of complex formation between the target polypeptide and a specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the target polypeptide is preferred, but other assays, such as a competitive binding assay, may also be employed (see, e.g. Maddox et al., 1983, J Exp Med, 158: 1211).

[0101] Once made, the antibodies can be used to identify MFPs in a sample, e.g., from biopsied tissue, etc. The amount of MFPs or mRNAs encoding MRPs can be determined using methods well known in the art, including but not limited to, quantitative image analysis, and reverse transcriptase polymerase chain reaction (RT-PCR) using portions of the biopsied tissue. Quantitation of mRNA corresponding to MFPs, can be determined by a competition reaction using equal volumes of the patient sample run against a series of decreasing known concentrations, e.g., of a mimic or mutant cDNA fragment.

[0102] MFP antibodies as described herein, are capable of specifically binding to MFPs. By "specifically binding" herein is meant that the antibodies bind to the protein with a binding constant in the range of at least 10^{-4}-10^{-9} M^{-1}, preferably in the range of 10^{-4}-10^{-6} M^{-1}, with a preferred range being 10^{7}-10^{-9} M^{-1}.

[0103] In some embodiments, antibodies to MFPs are capable of reducing or eliminating the biological activity or function of the MFP(s). That is, the addition of anti-MFP antibodies (i.e., polyclonal or monoclonal) to SCC or neoplastic epithelial cells expressing a MFP reduces or eliminates the MFP activity. Generally, at least a 25% decrease in activity is preferred, with at least about 50% being particularly preferred and about a 95-100% decrease being especially preferred.

[0104] In some embodiments, antibodies to MFPs are conjugated to a therapeutic moiety. For example, the therapeutic moiety can be an agent that inhibits enzymatic activity such as protease or protein kinase activity associated with SCC. In other embodiments, the therapeutic moiety can be a cytotoxic agent. Cytotoxic agents are numerous and varied and include, but are not limited to, radiochemicals, cytotoxic drugs or toxins or active fragments of such toxins. Suitable toxins and their corresponding fragments include diphtheria A chain,

exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin and the like.

Squamous Cell Carcinomas

[0105] Squamous cells are flat cells which form the surface of an epithelium. They can be identified histologically by the fact that they look flattened and thin under a microscope. Epithelia lined by squamous cells can be classified as either simple squamous epithelium or stratified squamous epithelium.

[0106] Squamous cell carcinoma is a carcinoma that may occur in many different organs, including the skin, mouth, esophagus, lungs, and cervix. It is a malignant tumor of epithelium that shows squamous cell differentiation. Squamous cell carcinoma is usually developed in the epithelial layer of the skin and sometimes in various mucous membranes of the body. This type of cancer can be seen on the skin, lips, inside the mouth, throat or esophagus.

[0107] The most common noncutaneous tumor of the head and neck is squamous cell carcinoma of the larynx, followed by squamous cell carcinomas of the palatine tonsil, tongue, and floor of the mouth. Somewhat less common are tumors of the salivary gland, jaw, nose and paranasal sinuses, and ear. Tumors of the thyroid gland, eye, and skin are discussed elsewhere in the manual. Excluding the skin and thyroid gland, >90% of head and neck cancers are squamous cell (epidermoid) carcinomas, and 5% are melanomas, lymphomas, and sarcomas. The Epstein-Barr virus plays a role in the pathogenesis of nasopharyngeal cancer.

[0108] Oral squamous cell carcinoma affects about 30,000 Americans each year. Oral squamous cell carcinoma is the most common oral or pharyngeal cancer. The chief risk factors for oral squamous cell carcinoma are smoking and alcohol use. Squamous cell carcinoma of the tongue may also result from Plummer-Vinson syndrome, syphilis, or chronic trauma. About 40% of intraoral squamous cell carcinomas begin on the floor of the mouth or on the lateral and ventral surfaces of the tongue. About **38**% of all oral squamous cell carcinomas occur on the lower lip, and about 11% begin in the palate and tonsillar area.

[0109] About 90% of vulvar cancers are squamous cell carcinomas; about 5% are melanomas. Vulvar cancer most often occurs in elderly women. It usually. manifests as a palpable lesion. Diagnosis is by biopsy. Treatment includes excision and inguinal and femoral lymph node dissection. Vulvar cancer accounts for about 3 to 4% of gynecologic cancers in the US. Average age at diagnosis is about 70, and incidence increases with age. Risk factors include vulvar intraepithelial neoplasia (VIN), human papillomavirus infection, heavy cigarette smoking, lichen sclerosus, squamous hyperplasia, squamous carcinoma of vagina or cervix, and chronic granulomatous diseases. VIN is a precursor to vulvar cancer. VIN may be multifocal. Sometimes adenocarcinoma of the vulva, breast, or Bartholin's glands also develops.

[0110] Squamous cell carcinoma of the skin is a malignant tumor of epidermal keratinocytes that invades the dermis, usually occurring in sun-exposed areas. The incidence in the US is 80,000 to 100,000 cases annually, with 2000 deaths. Local destruction may be extensive, and metastases occur in advanced stages. Diagnosis is by biopsy. Treatment depends on the tumor's characteristics and may involve curettage and electrodesiccation, surgical excision, cryosurgery, or, occasionally, radiation therapy.

[0111] The clinical appearance is highly variable, but any nonhealing lesion on sun-exposed surfaces should be suspect. The tumor may begin as a red papule or plaque with a scaly or crusted surface and may become nodular, sometimes with a warty surface. In some, the bulk of the lesion may lie below the level of the surrounding skin. Eventually the tumor ulcerates and invades the underlying tissue. The percentage of squamous cell carcinomas on sun-exposed skin that metastasize is quite low. However, about ⅓ of lingual or mucosal cancers have metastasized before diagnosis.

[0112] About 80 to 85% of all cervical cancers are squamous cell carcinoma. Diagnosis is by screening cervical Papanicolaou (Pap) test and biopsy. Staging is clinical. Treatment usually includes surgical resection, radiation therapy, and, unless cancer is localized, chemotherapy; if cancer is widely metastasized, treatment is primarily chemotherapy. Cervical cancer results from cervical intraepithelial neoplasia (CIN), which appears to be caused by infection with human papillomavirus (HPV) type 16, 18, 31, 33, 35, or 39.

[0113] CIN is graded as 1 (mild cervical dysplasia), 2 (moderate dysplasia), or 3 (severe dysplasia and carcinoma in situ). CIN 3 is unlikely to regress spontaneously; if untreated, it may, over months or years, penetrate the basement membrane, becoming invasive carcinoma. Invasive cervical cancer usually spreads by direct extension into surrounding tissues or via the lymphatics to the pelvic and para-aortic lymph nodes. Hematogenous spread is possible.

[0114] In squamous cell carcinoma, distant metastases usually occur only when the cancer is advanced or recurrent. The 5-yr survival rates are 80 to 90% with stage I, 50 to 65% with stage II, 25 to 35% with stage III, and 0 to 15% with stage IV. Nearly 80% of recurrences manifest within 2 yr. Adverse prognostic factors include lymph node involvement, large tumor size and volume, deep cervical stromal invasion, parametrial invasion, vascular space invasion, and nonsquamous histology.

Diagnosis and Therapy

[0115] The MFSs and MFPs can be used in a variety of different ways. For example, the MFSs and MFPs can be used in diagnostic assays, screening assays, and in therapeutic application. In some embodiments, the MFPS and antibodies to MFPs are used as diagnostic markers for the detection of SCC. Detection of MFPs in putative SCC tissue or patients allows for a determination or diagnosis of SCC. To detect or diagnose SCC, baseline values for the expression or activity of MFPs are established in order to provide a basis for the diagnosis and/or prognosis of SCC in a patient. In some embodiments, this is accomplished by combining body fluids, tissue biopsies, or cell extracts taken from normal subjects with one or more antibody(ies) to a MFP under conditions suitable for complex formation. Such conditions are well known in the art. The amount of standard complex formation may be quantified by comparing levels of antibody-target complex in the normal sample with a dilution series of positive controls, in which a known amount of antibody is combined with known concentrations of purified MFP. Standard values obtained from normal samples may be compared with values obtained from samples from subjects suspected of having SCC. Deviation between standard and subject values establishes the presence of or predisposition to the disease state.

[0116] In other embodiments, the expression levels of genes are determined for different cellular states in the SCC

phenotype; that is, the expression levels of genes in normal tissue and in SCC tissue are evaluated to provide expression profiles. An expression profile of a particular cell state or point of development is essentially a "fingerprint" of the state; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is unique to the state of the cell. By comparing expression profiles of cells in different states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. Then, diagnosis may be done or confirmed: does tissue from a particular patient have the gene expression profile of normal or SCC tissue.

[0117] "Differential expression," or grammatical equivalents as used herein, refers to both qualitative as well as quantitative differences in the genes' temporal and/or cellular expression patterns within and among the cells. Thus, a differentially expressed gene can qualitatively have its expression altered, including an activation or inactivation, in, for example, normal versus lymphoma tissue. That is, genes may be turned on or turned off in a particular state, relative to another state. As is apparent to the skilled artisan, any comparison of two or more states can be made. Such a qualitatively regulated gene will exhibit an expression pattern within a state or cell type which is detectable by standard techniques in one such state or cell type, but is not detectable in both. Alternatively, the determination is quantitative in that expression is increased or decreased; that is, the expression of the gene is either upregulated, resulting in an increased amount of transcript, or downregulated, resulting in a decreased amount of transcript. The degree to which expression differs need only be large enough to quantify via standard characterization techniques as outlined below, such as by use of Affymetrix GeneChip™ expression arrays, Lockhart, Nature Biotechnology, 14:1675-1680 (1996), hereby expressly incorporated by reference. Other techniques include, but are not limited to, quantitative reverse transcriptase PCR, Northern analysis and RNase protection. As outlined above, preferably the change in expression (i.e. upregulation or downregulation) is at least about 50%, more preferably at least about 100%, more preferably at least about 150%, more preferably, at least about 200%, with from 300 to at least 1000% being especially preferred.

[0118] As will be appreciated by those in the art, this may be done by evaluation at either the gene transcript, or the protein level; that is, the amount of gene expression may be monitored using nucleic acid probes to the DNA or RNA equivalent of the gene transcript, and the quantification of gene expression levels, or, alternatively, the final gene product itself (protein) can be monitored, for example through the use of antibodies to the MF protein and standard immunoassays (ELISAs, etc.) or other techniques, including mass spectroscopy assays, 2D gel electrophoresis assays, etc. Thus, the proteins corresponding to MF genes, i.e. those identified as being important in a SCC phenotype, can be evaluated in a SCC diagnostic test.

[0119] Numerous methods known to those of ordinary skill in the art find use in diagnosing SCC; For example, in some embodiments, proteins can be obtained from a sample or a patient are separated by electrophoresis on a gel (typically a denaturing and reducing protein gel, but may be any other type of gel including isoelectric focusing gels and the like). Following separation of the proteins, MFPs can be detected by immunoblotting with antibodies raised against the MFPs. Methods of immunoblotting are well known to those of ordinary skill in the art.

[0120] In some embodiments, antibodies to the MFPs find use in in situ imaging techniques. In this method cells are contacted with from one to many antibodies to MFP(s). Following washing to remove non-specific antibody binding, the presence of the antibody or antibodies is detected. In one embodiment the antibody is detected by incubating with a secondary antibody that contains a detectable label. In another method the primary antibody to the MFP(s) contains a detectable label. In another preferred embodiment each one of multiple primary antibodies contains a distinct and detectable label. This method finds particular use in simultaneous screening for a plurality of MFPs. As will be appreciated by one of ordinary skill in the art, numerous other histological imaging techniques are useful in the invention.

[0121] In some embodiments the label is detected in a fluorometer which has the ability to detect and distinguish emissions of different wavelengths. In addition, a fluorescence activated cell sorter (FACS) can be used in the method.

[0122] In some embodiments, in situ hybridization of labeled MF nucleic acid probes to tissue arrays is done. For example, arrays of tissue samples, including SCC tissue and/or normal tissue, are made. In situ hybridization as is known in the art can then be done.

[0123] It is understood that when comparing the expression fingerprints between an individual and a standard, the skilled artisan can make a diagnosis as well as a prognosis. It is further understood that the genes which indicate the diagnosis may differ from those which indicate the prognosis.

[0124] In one embodiment, the MF proteins, antibodies, nucleic acids, and cells containing MF sequences are used in prognosis assays. In some embodiments, gene expression profiles can be generated that correlate to SCC severity, in terms of long term prognosis. Again, this may be done on either a protein or gene level, with the use of genes being preferred. In some embodiments, MF probes are attached to solid supports for the detection and quantification of MF sequences in a tissue or patient. The assays proceed as outlined for diagnosis.

[0125] The efficacy of therapeutic agents, such as antibodies and/or other candidate drugs also can be determined using the diagnostic assays described above. As will be appreciated by a person of skill in the art, assays to determine the efficacy of a therapeutic agent require the establishment of baseline values. In some embodiments, this is accomplished by combining body fluids, tissue biopsies, or cell extracts taken from a patient with SCC prior to treatment with the candidate drug with one or more antibody(ies) to a MFP under conditions suitable for complex formation. Such conditions are well known in the art. The amount of standard complex formation may be quantified by comparing levels of antibody-target complex in the normal sample with a dilution series of positive controls, in which a known amount of antibody is combined with known concentrations of purified MFP. Standard values obtained from a patient before treatment may be compared with values obtained from a patient after treatment. Deviation between standard and subject values establishes the efficacy of the drug.

Screening Assays

[0126] In some embodiments, the MF proteins, antibodies, nucleic acids, and cells containing the MF proteins are used in

screening assays. For example, screens for agents that modulate the SCC phenotype can be run. This can be done by screening for modulators of gene expression or for modulators of protein activity at the individual gene or protein level or by evaluating the effect of drug candidates on a "gene expression profile". In some embodiments, the expression profiles are used in conjunction with high throughput screening techniques to allow monitoring for expression profile genes after treatment with a candidate agent (see Zlokamik, et al., 1998, Science, 279: 84-8).

[0127] "Modulation" includes both an increase and a decrease in gene expression or activity. The preferred amount of modulation will depend on the original change of the gene expression in normal versus tumor tissue, with changes of at least 10%, preferably 50%, more preferably 100-300%, and in some embodiments 300-1000% or greater. If a gene exhibits a 4 fold increase in tumor compared to normal tissue, a decrease of about four fold is desired; a 10 fold decrease in tumor compared to normal tissue gives a 10 fold increase in expression for a candidate agent is desired, etc.

[0128] As will be appreciated by those in the art, this may be done by evaluation at either the gene or the protein level; that is, the amount of gene expression may be monitored using nucleic acid probes and the quantification of gene expression levels, or, alternatively, the level of the gene product itself can be monitored, for example through the use of antibodies to the MFPd and standard immunoassays. Alternatively, binding and bioactivity assays with the protein may be done as outlined below.

[0129] In some embodiments, gene expression monitoring is done and a number of genes, i.e. an expression profile, are monitored simultaneously. If desired, multiple protein expression monitoring can be done as well. In embodiments monitoring multiple genes or proteins, the corresponding MF probes are immobilized to solid supports. It is understood that immobilization can occur by any means, including for example; by covalent attachment, by electrostatic immobilization, by attachment through a ligand/ligand interaction, by contact or by depositing on the surface. "Solid support" or "solid substrate" refers to any solid phase material upon which a MF sequence, MFP, or antibody is synthesized, attached, ligated or otherwise immobilized. A solid support may be composed of organic polymers such as polystyrene, polyethylene, polypropylene, polyfluoroethylene, polyethyleneoxy, and polyacrylamide, as well as co-polymers and grafts thereof. A solid support may also be inorganic, such as glass, silica, controlled-pore-glass (CPG), or reverse-phase silica. The configuration of a solid support may be in the form of beads, spheres, particles, granules, a gel, or a surface. Surfaces may be planar, substantially planar, or non-planar. Solid supports may be porous or non-porous, and may have swelling or non-swelling characteristics. A solid support may be configured in the form of a well, depression or other container, vessel, feature or location. A plurality of solid supports may be configured in an array at various locations, addressable for robotic delivery of reagents, or by detection means including scanning by laser illumination and confocal or deflective light gathering.

[0130] Generally, a candidate bioactive agent is added prior to analysis. The term "candidate bioactive agent" or "drug candidate" or grammatical equivalents as used herein describes any molecule, e.g., protein, oligopeptide, small organic or inorganic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactive agents that are capable of directly or indirectly altering either the SCC phenotype, binding to and/or modulating the bioactivity of an MFP, or the expression of a MF sequence. In a particularly preferred embodiment, the candidate agent suppresses a SCC phenotype, for example to a normal tissue fingerprint. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

[0131] In one aspect, a candidate agent will neutralize the effect of an MFP. By "neutralize" is meant that activity of a protein is either inhibited or counter acted against so as to have substantially no effect on a cell.

[0132] Candidate agents encompass numerous chemical classes, though typically they are organic or inorganic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Preferred small molecules are less than 2000, or less than 1500 or less than 1000 or less than 500 D. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, proteins, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0133] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification to produce structural analogs.

[0134] In assays for altering the expression profile of one or more MF sequences, after the candidate agent has been added and the cells allowed to incubate for some period of time, the sample containing the MF sequences to be analyzed is added to a solid support. If required, the MF sequence is prepared using known techniques. For example, the sample may be treated to lyse the cells, using known lysis buffers, electroporation, etc., with purification and/or amplification such as PCR occurring as needed, as will be appreciated by those in the art.

[0135] Generally, one of the assay components is labeled to provide a means of detecting the binding complex of interest. By "labeled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the MF nucleic acids, proteins and

antibodies at any position. For example, the label should be capable of producing, either directly or indirectly, a detectable signal. The detectable moiety may be a radioisotope, such as .sup.3H, .sup.14C, .sup.32P, .sup.35S, or .sup.125I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the label may be employed, including those methods described by Hunter et al., 1962, Nature, 144: 945; David et al., 1974, Biochemistry, 13: 1014; Pain et al., 1981, J. Immunol. Meth., 40: 219; and Nygren, 1982, J. Histochem. and Cytochem., 30: 407. The label also can be an enzyme, such as, alkaline phosphatase or horseradish peroxidase, which when provided with an appropriate substrate produces a product that can be detected. Alternatively, the label can be a labeled compound or small molecule, such as an enzyme inhibitor, that binds but is not catalyzed or altered by the enzyme. The label also can be a moiety or compound, such as, an epitope tag or biotin which specifically binds to streptavidin. For the example of biotin, the streptavidin is labeled as described above, thereby, providing a detectable signal for the bound target sequence. As known in the art, unbound labeled streptavidin is removed prior to analysis.

[0136] As will be appreciated by those in the art, these assays can be direct hybridization assays or can comprise "sandwich assays", which include the use of multiple probes, as is generally outlined in U.S. Pat. Nos. 5,681,702, 5,597,909, 5,545,730, 5,594,117, 5,591,584, 5,571,670, 5,580,731, 5,571,670, 5,591,584, 5,624,802, 5,635,352, 5,594,118, 5,359,100, 5,124,246 and 5,681,697, all of which are hereby incorporated by reference.

[0137] A variety of hybridization conditions may be used in the present invention, including high, moderate and low stringency conditions as outlined above. The assays are generally run under stringency conditions which allows formation of the label probe hybridization complex only in the presence of target. Stringency can be controlled by altering a step parameter that is a thermodynamic variable, including, but not limited to, temperature, formamide concentration, salt concentration, chaotropic salt concentration pH, organic solvent concentration, etc.

[0138] These parameters may also be used to control non-specific binding, as is generally outlined in U.S. Pat. No. 5,681,697. Thus it may be desirable to perform certain steps at higher stringency conditions to reduce non-specific binding.

[0139] The reactions outlined herein may be accomplished in a variety of ways, as will be appreciated by those in the art. Components of the reaction may be added simultaneously, or sequentially, in any order, with preferred embodiments outlined below. In addition, the reaction may include a variety of other reagents may be included in the assays. These include reagents like salts, buffers, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal hybridization and detection, and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used, depending on the sample preparation methods and purity of the target. In addition, either solid phase or solution based (i.e., kinetic PCR) assays may be used.

[0140] Once the assay is run, the data is analyzed to determine the expression levels, and changes in expression levels as between states, of individual genes, or individual proteins, forming an expression profile.

[0141] In some embodiments, screening is done to alter the biological function of the expression product of an MF gene. Again, having identified the importance of a gene in a particular state, screening for agents that bind and/or modulate the biological activity of the gene product can be run as is more fully outlined below.

[0142] In some embodiments, screens are designed to first find candidate agents that can bind to MF proteins, and then these agents may be used in assays that evaluate the ability of the candidate agent to modulate the MFP activity and the SCC phenotype. As will be appreciated by those in the art, there are a number of different assays which may be run; binding assays and activity assays.

[0143] In some embodiments, binding assays are done. In general, purified or isolated MFPs are used. The methods comprise combining a MFP and a candidate bioactive agent, and determining the binding of the candidate agent to the MFP. Generally, the MFP or the candidate agent is non-diffusably bound to a solid support having isolated sample receiving areas (e.g. a microtiter plate, an array, etc.). Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the composition is not crucial so long as it is compatible with the reagents, maintains the activity of the composition and is nondiffusable. Preferred methods of binding include the use of antibodies (which do not sterically block either the ligand binding site or activation sequence when the protein is bound to the support), direct binding to "sticky" or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the protein or agent, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein or other moiety.

[0144] In some embodiments, the MFP is bound to the support, and a candidate bioactive agent is added to the assay. Alternatively, the candidate agent is bound to the support and the MFP is added. Novel binding agents include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, functional assays (phosphorylation assays, etc.) and the like.

[0145] The determination of the binding of the candidate bioactive agent to the MFP may be done in a number of ways. In a preferred embodiment, the candidate bioactive agent is labeled, and binding determined directly. For example, this may be done by attaching all or a portion of the MFP to a solid support, adding a labeled candidate agent (for example a fluorescent label), washing off excess reagent, and determining whether the label is present on the solid support. Various blocking and washing steps may be utilized as is known in the art.

[0146] In some embodiments, only one of the components is labeled. For example, the proteins (or proteinaceous candidate agents) may be labeled at tyrosine positions using .sup.125I, or with fluorophores. Alternatively, more than one

component may be labeled with different labels; using .sup. 125I for the proteins, for example, and a fluorophor for the candidate agents.

[0147] In some embodiments, the binding of the candidate bioactive agent is determined through the use of competitive binding assays. In this embodiment, the competitor is a binding moiety known to bind to the MFP, such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there may be competitive binding as between the bioactive agent and the binding moiety, with the binding moiety displacing the bioactive agent.

[0148] In some embodiments, the candidate bioactive agent is labeled. Either the candidate bioactive agent, or the competitor, or both, is added first to the protein for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40.degree. C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high through put screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

[0149] In some embodiments, the competitor is added first, followed by the candidate bioactive agent. Displacement of the competitor is an indication that the candidate bioactive agent is binding to the MFP and thus is capable of binding to, and potentially modulating, the activity of the MFP. In this embodiment, either component can be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the candidate bioactive agent is labeled, the presence of the label on the support indicates displacement.

[0150] In other embodiments, the candidate bioactive agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate that the bioactive agent is bound to the MFP with a higher affinity. Thus, if the candidate bioactive agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate that the candidate agent is capable of binding to the MFP.

[0151] In some embodiments, the methods comprise differential screening to identity bioactive agents that are capable of modulating the activity of the MFPs. In this embodiment, the methods comprise combining a MFP and a competitor in a first sample. A second sample comprises a candidate bioactive agent, a MFP and a competitor. The binding of the competitor is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of an agent capable of binding to the MFP and potentially modulating its activity. That is, if the binding of the competitor is different in the second sample relative to the first sample, the agent is capable of binding to the MFP.

[0152] In some embodiments, methods for screening for bioactive agents capable of modulating the activity of a MFP in a cell are provided. The methods comprise adding a candidate bioactive agent, as defined above, to a cell comprising MFPs. Typically, laminin-5 negative primary human keratinocytes are used. The cells can also contain recombinant nucleic acids that encode MF sequences, Ras and a stable NF-κB repressor mutant of IκBα (i.e. IKB) (see Dajee et al., 2003, Nature, 421: 630-643). Methods for culturing cells and for assaying cell scattering, adhesion and migration are described in Russell et al., 2003, J. Cell Sci., 116: 3543-3556, the entire contents of which are incorporated herein by reference.

[0153] In some embodiments, candidate agents can be introduced into immunodeficient mice that can subsequently be challenged with a MFPs and monitored for the development of tumors. For example, intraperitoneal injections of antibodies against one or more MFPS can be given to mice bearing human foreskin xenografts (see Examples; and Li et al., 2003, EMBO J., 22: 2400-2410). The mice can then be challenged with Ras/IKB transformed human keratinocytes and monitored for tumor growth and histology as described in Dajee et al., 2003, Nature, 421: 630-643.

[0154] Positive controls and negative controls may be used in the assays. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

[0155] A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding.

[0156] In one aspect, the assays are evaluated in the presence or absence or previous or subsequent exposure of physiological signals, for example hormones, antibodies, peptides, antigens, cytokines, growth factors, action potentials, pharmacological agents including chemotherapeutics, radiation, carcinogenics, or other cells (i.e. cell-cell contacts). In another example, the determinations are determined at different stages of the cell cycle process.

Pharmaceutical Compositions

[0157] Bioactive agents having pharmacological activity are identified as described above. By "pharmacological activity" herein is meant that the compounds are able to enhance or interfere with the activity of MFPs. The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a patient. A "patient" includes both humans and other animals, particularly mammals, and domestic animals. Thus, the methods are applicable to both human therapy and veterinary applications.

[0158] In some embodiments, bioactive agents are antibodies that recognize MFPs and that have been demonstrated to inhibit or modulate SCC as described herein. In other embodiments, bioactive agents include antisense compositions. These agents can be delivered directly or in pharmaceutical compositions along with suitable carriers or excipients, as well known in the art. Present methods of treatment include embodiments providing for administration of an effective amount of a compound or agent that inhibits the activity or expression of a MFP to a patient in need of treatment.

[0159] An effective amount of such agents can readily be determined by routine experimentation, as can the most effec-

tive and convenient route of administration and the most appropriate formulation. Various formulations and drug delivery systems are available in the art. (See, e.g., Remington's Pharmaceutical Sciences, supra.)

[0160] Suitable routes of administration may, for example, include oral, rectal, transmucosal, transdermal, nasal, or intestinal administration and parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. The agent or composition thereof may be administered in a local rather than a systemic manner. For example, a suitable agent can be delivered via injection or in a targeted drug delivery system, such as a depot or sustained release formulation.

[0161] The pharmaceutical compositions may be manufactured by any of the methods well-known in the art, such as by conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The compositions can include one or more physiologically acceptable carriers such as excipients and auxiliaries that facilitate processing of active molecules into preparations for pharmaceutical use. Proper formulation is dependent upon the route of administration chosen.

[0162] For example, for injection, the composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal or nasal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the agents can be formulated readily by combining the active agents with pharmaceutically acceptable carriers well known in the art. Such carriers enable the agents of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject. The agents may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0163] Pharmaceutical preparations for oral use can be obtained as solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0164] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active agent doses.

[0165] Pharmaceutical preparations for oral administration include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[0166] For administration by inhalation, the agents can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or any other suitable gas. In the case of a pressurized aerosol, the appropriate dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges for use in an inhaler or insufflator may be formulated. These typically contain a powder mix of the agent and a suitable powder base such as lactose or starch.

[0167] Compositions formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion can be presented in unit dosage form, e.g. in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Formulations for parenteral administration include aqueous solutions of the compound or agent to be administered, including in water-soluble form.

[0168] Suspensions of the active agents may also be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil and synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the agents to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0169] As mentioned above, the compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the present agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0170] Suitable carriers for the hydrophobic molecules of the invention are well-known in the art and include co-solvent systems comprising, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system is effective in dissolving hydrophobic agents and produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be

varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied. For example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

[0171] Alternatively, other delivery systems for hydrophobic molecules may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Liposomal delivery systems are discussed above in the context of gene-delivery systems. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the agents may be delivered using sustained-release systems, such as semi-permeable matrices of solid hydrophobic polymers containing the effective amount of the composition to be administered. Various sustained-release materials are established and available to those of skill in the art. Sustained-release capsules may, depending on their chemical nature, release the agents for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[0172] For any composition employed herein, a therapeutically effective dose can be estimated initially using a variety of techniques well-known in the art. For example, in a cell culture assay, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Where inhibition of MFP activity is desired, the concentration of the test agent that achieves a half-maximal inhibition of MFP activity can be determined. Dosage ranges appropriate for human subjects can be determined, using data obtained from cell culture assays and other animal studies.

[0173] A therapeutically effective dose of an agent refers to that amount of the agent that results in amelioration of symptoms or a prolongation of survival in a subject. Toxicity and therapeutic efficacy of such molecules can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the ratio LD_{50}/ED_{50}. Agents that exhibit high therapeutic indices are preferred.

[0174] Dosages preferably fall within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. Dosages may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration, and dosage should be chosen, according to methods known in the art, in view of the specifics of a subject's condition.

[0175] Dosage amount and interval may be adjusted individually to provide plasma levels or tissue levels of the active moiety which are sufficient to affect the expression or activity of MFPs, as desired, i.e. minimal effective concentration (MEC). The MEC will vary for each agent but can be estimated from, for example, in vitro data, such as the concentration necessary to achieve 50-90% inhibition of MFP activity using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Agents or compositions thereof should be administered using a regimen which maintains plasma levels above the MEC for about 10-90% of the duration of treatment, preferably about 30-90% of the duration of treatment, and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[0176] The amount of agent or composition administered will, of course, be dependent on a variety of factors, including the sex, age, and weight of the subject being treated, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

[0177] The present compositions may, if desired, be presented in a pack or dispenser device containing one or more unit dosage forms containing the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a agent of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of disorders or diseases, such as squamous cell carcinoma or other cancers and conditions associated with altered expression of MFPs.

EXAMPLES

Example 1

Requirement for G4 and/or G5 Domains in SCC Tumors

[0178] Laminin-5 undergoes processing of both its γ2 and α3 chains. As the α3 chain contains the primary integrin binding site(s), we performed further studies to examine the functional effects of α3 chain processing on SCC tumor development. We created truncations at the following sites: 1) amino acid residue 1337 (1337Tr), and 2) at amino acid residue 1450 (1450Tr). Keratinocytes from a junctional epidermolysis bullosa (JEB) patient with absent laminin □3 chain expression were transduced with LZRS retroviral vectors containing full length, 1450Tr or 1337Tr cDNA (Matsui et al., 1998, J. Exp. Med., 187: 1273-83). Each of the cDNAs restored trimeric laminin-5 expression in treated JEB keratinocytes, and each cDNA produced comparable levels of secreted laminin-5, as assessed by Western blot using laminin □3 specific antibody. While JEB keratinocytes with no laminin-5 expression (LacZ) were rounded, WT, 1337Tr and 1450Tr expressing keratinocytes showed flattening and spreading. While laminin-5 negative JEB keratinocytes (LacZ) were hypoproliferative, 1337Tr, and 1450Tr showed normal levels of proliferation, comparable to that of wild type (FIG. **5**).

[0179] Because laminin-5 processing is closely tied to migration, we studied the 1337Tr mutant in more detail, as truncation at this position simulated the effects of processing in vivo. We found that 1337Tr cells were capable of migration, in fact, 1337Tr cells migrated more efficiently in scratch assays compared to cells expressing wild type α3 chain (FIG. **6**).

[0180] We have previously described a model of human SCC which is obtained from SQ injection of Ras/IKB over expressing human keratinocytes in nude mice (Dajee et al., 2003, Nature, 421: 639-43). Tumors formed in these mice histologically and biochemically, were extremely similar to

human SCC tumors. We showed that while laminin-5 negative keratinocytes showed no tendency to form tumors after Ras/IKB transformation, retroviral transfer of laminin-5 cDNA restored both expression of laminin-5 and restored the capacity of these cells to form tumors. These results are significant in that they demonstrate that laminin-5 expression is absolutely required for SCC development.

[0181] As an extension of these tumor studies, we next studied the capacity of truncated laminin α3 chain to support SCC development. We Ras/IKB transformed JEB keratinocytes expressing α3 wild type (WT), 1337Tr or LacZ and injected SQ into nude mice. Through two sets of experiments with eight mice per condition, we found that the 1337Tr a3 chain expressing cells did not form any tumors and were most similar to LacZ negative controls (FIG. **7**). In addition, 1450Tr cells, though one set of experiments and four mice total per condition, fail to produce SCC tumors either. Despite a lack of tumor formation, 1337Tr and 1450Tr cells at injection sites clearly showed expression and extracellular deposition of mutant laminin-5 molecules. These results are significant in that they demonstrate that the G4-5 domain of laminin-5 is essential for SCC development.

Example 2

Cloning of G4 and/or G5 MF Sequences

[0182] The laminin α3 chain is processed at residues 1337-1338, according to N-terminal sequencing studies (Tsubota et al., 2000, Biochem. Biophys. Res. Commun., 278: 614-620). As shown in preliminary results, we have produced a human laminin a 3 cDNA (1337Tr) which codes for a protein truncated at amino acid 1337, simulating the cleavage product, and have also produced 1450Tr, an α3 cDNA truncated at amino acid 1450. We propose to produce another laminin α3 cDNA (1551Tr), truncated near the beginning of the G5 domain at amino acid 1551. PCR primers will be designed to produce a product that spans from nucleotide 2771 to nucleotide 4653 of the full length wild type laminin α3 cDNA. This will include a unique BstII site on the laminin α3 cDNA, which will be on the 5' end of the PCR product, and a NotI site will be engineered into the 3' side of the PCR product. The full length laminin α3 cDNA in pENTR1A.COPYRGT. (Invitrogen) Gateway plasmid will be cleaved with BstII and NotI enzymes and the PCR product described above will be ligated into the vector with the 3' end ligating with the BstII site in the laminin α3 cDNA, and the 3' end ligating with the pENTR1A multiple cloning site. This cloning product, which will be confirmed by'sequencing, will comprise cDNA coding for the laminin α3 chain amino acids 1-1551. The laminin α3 1551Tr cDNA will then be transferred from the pENTR1A plasmid to a Gateway adapted LSRZ retroviral vector through lambda phage recombination reactions.

[0183] Three cDNA constructs coding for laminin α3 G domain will be produced. One termed G4 will code for amino acids 1338 to 1560, one termed G5 will code for amino acids 1560 to 1713, and a third termed G4-5 will code for amino acids 1338 to 1713. We will produce each by PCR of the wild type laminin α3 cDNA. In one PCR experiment, we will engineer an EcoR1 tail at either end of each of the three PCR products for cloning into the bacterial expression vector pGEX (Amersham). These constructs will be confirmed by sequencing, and then utilized to produce purified G4, G5 and G4-5 domains in a bacterial expression system.

[0184] In another PCR experiment, an NheI tail is inserted on the 5' side and a Not I tail on the 3' side of each of the G4. G5 and G4/5 PCR products. These are cloned into the mammalian expression vector pCEP which contains a BM40 signal sequence to which cDNA can be cloned to via an NheI restriction site. We have previously used the BM40 signal sequence in this vector to successfully promote secretion of collagen XVII ectodomain (Areida et al., 2001, J. Biol. Chem., 276: 1594-601). The G4, G5 and G4-5 are cloned into pCEP vector, to pick up the BM40 signal sequence, then the BM40 signal sequence and laminin α3 G domain cDNA will be removed from the vector by KpnI and NotI restriction sites and ligated into the pENTR1A Gateway vector, and by lambda phage recombination, the laminin α3 G domain cDNAs with their BM40 signal sequences will be cloned into a Gateway adapted LZRS retroviral plasmid.

Example 3

Assays for Detecting Inhibition of SCC Tumorigenesis

[0185] It is tested whether G4-5 domain performs its function in SCC before or after it becomes processed and dissociated from laminin-5 by to restoring tumor generating capabilities in Ras/IKB transformed 1337Tr keratinocytes by adding exogenous G4-5 protein or G4-5 cDNA. If Ras/IKB transformed 1337Tr keratinocytes form tumors in nude mice after receiving G4-5 protein or cDNA, it is indicated that the G4-5 domain is active in SCC tumors in a soluble form.

[0186] Matrigel, which contains heparin sulfate proteoglycan as one of its primary constituents, is the material in which we suspend our Ras/IKB tumors cells in, during subcutaneous injection into nude mice. As the laminin α3 G4-5 domain has heparin binding properties (Amano et al., 2000, J. Biol. Chem., 275: 22728-35), we will suspend the G4-5 domain at 100 μg/250 μl into Matrigel and use it as a substrate for injection of Ras/IKB transformed 1337Tr keratinocytes. Laminin α3 G4-5 domain suspended in Matrigel is used as a control. We hypothesize that laminin G4-5 domain will remain localized to the Matrigel impregnated matrix surrounding tumor cells and will be slowly released as matrix is gradually remodeled by tumor cells. Four mice injected with Ras/IKB treated 1337 cells embedded in G4-5 domain containing Matrigel are tested, using four serial biopsies at 1 week intervals by IDIF using G4-5 domain specific antibodies to assess the persistence of G4-5 domain protein in injection/tumor sites.

[0187] A second set of experiments is performed injecting Ras/IKB transformed cells embedded in Matrigel containing either laminin α3 or laminin α3 G4-5 domain. These cells are injected into nude mice and assessed over the course of 4 weeks for tumor development. Wild type Ras/IKB transformed keratinocytes are used as a positive control and 6 mice per condition will be used.

[0188] Alternatively, the laminin G4 domain cDNA is delivered by gene therapy as described below. This technique should promote long term G4-5 domain expression in 1337 Tr cells over the course of the 4 week assay.

[0189] Laminin G4-5 domain cDNA is cloned into LZRS retroviral vector. LacZ or laminin G4-5 cDNA containing retrovirus are used to infect 1337Tr keratinocytes. Cells are selected with Blasticidin, transformed with Ras/IKB and then injected into nude mice. Six mice per condition are assessed over 4 weeks for tumor growth as previously described (Dajee

et al, 2003, Nature, 421: 639). Tumors are analyzed by IDIF using G4/5 or LacZ antibodies to verify secretion of retroviral cDNA products.

[0190] The effects of laminin α3 G4 and laminin α3 G5 antibodies on tumor development. Sufficient antibody is injected to maintain a circulating titer of 1:1000 as tested by dilution of mouse sera by Western blot analysis of G4-5 domain protein. Laminin α3 G4, G5 and G4-5 domains cloned into pGEX vector as outlined above is utilized to produce G4, G5 and G4-5 domain bacterial fusion proteins. Proteins are affinity purified on a GST column, and GST tags are subsequently removed by enterokinase (Invitrogen). Isolated G4 and G5 domain proteins are used to produce rabbit polyclonal antisera at Josman Labs, Napa, Calif., according to their recommended protocols.

[0191] Once high titer polyclonal antisera is obtained, additional G4, G5 and G4-5 protein are produced, affinity purified and coupled to a Sepharose CL-4B column at a concentration of 0.5 mg protein per ml of gel. G4 polyclonal antisera is affinity purified on a G4-sepharose column and G5 antisera is affinity purified on a G5 sepharose column. Affinity purified G4, G5 and G4-5 antibodies are dialyzed into PBS and filter sterilized. Initially, we the antibodies (G4, G5, G4-5) are tested by IP injection of immunodeficient mice bearing human foreskin xenografts by a technique utilized previously (Li et al., 2003, EMBO J., 22:2400-2410). Titers of circulating antibodies in treated mice are assessed at 3 day intervals using sera obtained from tail vein bleeds. The amount of antibody injected and the injection intervals is adjusted to maintain a titer sufficient to detect laminin G4-5 protein by Western blot at a 1:1000 serum dilution. We will clinically assess foreskin grafts and mouse skin over the course of three weeks of injections to determine whether epidermal separation is noted, and mice will be examined by autopsy to detect any epithelial sloughing of mucosa or internal organs.

[0192] Once the proper antibody dose and injection intervals are obtained, antibody inhibition of Ras/IKB wild type keratinocyte derived tumors is performed. In these studies, nude mice are administered periodic G4, G5 or G4-5 antibody injections to maintain a constant circulating antibody titer as described above. Once antibody titers are initiated, then mice receive SC injections of Ras/IKB transformed human keratinocytes. Three groups of 6 mice each are studied, using affinity purified laminin α3 G4 antibody, laminin α3 G5 antibody or mouse IgG. G domain antibody conditions are analyzed for tumor growth and tumor histology as previously described (Dajee et al., 2003; Nature, 421:639-43).

Example 4

Targeting a Tumor Specific Laminin Domain

[0193] Laminin-332 is critical for squamous cell carcinoma (SCC) tumorigenesis, but targeting it for cancer therapy was unachievable due to laminin-332's key role in promoting tissue integrity. Here, we show that a portion of laminin-332 termed G45, which is proteolytically removed and absent in normal tissues, is prominently expressed in most human SCC tumors and plays an important role in human SCC tumorigenesis. Primary human keratinocytes lacking G45 (ΔG45) showed alterations of basal receptor organization impaired matrix deposition, and increased migration. After SCC transformation, the absence of G45 domain in ΔG45 cells was associated with deficient ERK and phosphotidylinositol 3-kinase (PI3-K) pathway activation, impaired invasion, deficient metalloproteinase activity and absent tumorgenicity in vivo. Expression of G45 or activated PI3-K subunit in ΔG45 cells reversed these abnormalities. G45 antibody treatment induced SCC tumor apoptosis, decreased SCC tumor proliferation and markedly impaired human SCC tumorigenesis in vivo without affecting normal tissue adhesion. These results demonstrate a remarkable selectivity of expression and function for laminin-332 G45 in human SCC tumorigenesis and demonstrate its use as a specific target for anti-cancer therapy.

[0194] Squamous cell carcinoma (SCC) is a prevalent, invasive neoplasm arising in many tissues; causing significant morbidity and mortality. SCC is the most common cancer capable of metastasis and is second in frequency only to basal cell carcinoma. The incidence of SCC appears to be rising, and is more frequently affecting younger individuals. SCC tumors can show a high risk of recurrence, and those derived from mucosal sites often invade neighboring tissues and can also metastasize to the lymph nodes, lung and other distant sites. Chemotherapy for SCC has not been shown to significantly affect long term survival and most patients with advanced disease die despite currently available therapies. Results from the use of epidermal growth factor receptor inhibitory agents in clinical trials of advanced head and neck SCC in combination with conventional chemotherapy have been only modestly encouraging. All of these factors have led to the search for new and more specific agents in the treatment of SCC.

[0195] Laminins are a family of trimeric extracellular glycoproteins associated with the basement membrane zone (BMZ). Laminins interact with cell surface receptors and other BMZ components to provide cells with an interface to communicate with their surrounding extracellular environment. Laminin-332, a large molecule consisting of α3, β3, and γ2 chains, shows widespread expression in many epithelial tissues as well as in the tumor microenvironment of many carcinomas. Laminin-332 is required for tumorigenesis in a well characterized in vivo model of human SCC. In addition, laminin-332 expression in SCC tumors arising from a number of tissues correlates both with tumor invasiveness and patient prognosis. Soluble and insoluble laminin-332, furthermore, have been observed to induce the phosphoinositol-3-kinase (PI3-K) and mitogen-activated protein kinase (MAPK) pathways, which are known to mediate carcinogenesis.

[0196] Due to its critical role in SCC tumorigenesis, laminin-332 would represent an excellent candidate as an anti-tumor target, were it not for laminin-332's equally critical role in maintaining epithelial-mesenchymal cohesion across a broad range of normal tissues. For example, in the inherited disorder Herlitz's junctional epidermolysis bullosa (JEB), absence of laminin-332 expression due to laminin-332 gene mutations leads to widespread blistering and erosions and usually death during infancy. Therefore, any anti-cancer strategy targeting laminin-332 would need to address how to selectively disrupt laminin-332's pro-tumorigenic function without affecting its pro-cohesive function in normal tissues.

[0197] Towards this end, we focused on the α3 chain of laminin-332, which undergoes proteolytic cleavage shortly after secretion. This proteolytic event, which takes place in the large C-terminal globular (G) domain near the junction of the third and fourth EGF-like repeats termed G3 and G4 (FIG. **6***a*), converts the laminin α3 chain from a 200 kDa precursor to a 165 kDa processed product. The 37 kDa precursor region of the laminin α3 chain containing two EGF-like repeats G4 and G5 (G45), is removed during processing and as a result,

is absent in normal mature tissues. Any postnatal expression of unprocessed laminin α3 chain/G45 is only detectable transiently at healing wound edges.

[0198] There are many parallels between the process of wound healing, and tumorigenesis, including active synthesis of BMZ components, proliferation and cell migration. Because of these parallels, and because of the absence of unprocessed laminin α3 in normal mature tissues, we focused on the role of the laminin α3 G45 in human SCC. In this study, we demonstrate that while G45 is undetectable in normal mature tissues, it shows widespread expression in human SCC tumors, where it plays an important role in SCC tumorigenesis. Further we show that laminin α3 G45 can be selectively targeted in vivo by antibodies to inhibit human SCC tumorigenesis without disrupting normal tissues.

Materials And Methods

[0199] Cell lines. Primary human keratinocytes isolated from normal skin and a patient with junctional dystrophic epidermolysis bullosa lacking laminin-332 expression due to LAMA3 mutations were cultured in a 1:1 mix of defined keratinocyte serum free medium (SFM; Gibco, Carlsbad, Calif.) and Medium 154 (Cascade Biologics) at 37° C. in a humidified 5% CO_2 incubator. Modified human 293 PHOENIX cells (gift from Dr. G. Nolan, Stanford, Calif.) were cultured in DMEM supplemented with 10% fetal calf serum, 100 lU/ml penicillin and 100 μg/ml streptomycin at 37° C. in a humidified 10% CO_2 incubator.

[0200] Complementary DNA constructs. Human laminin α3 chain (GenBank NM227.2) is physiologically processed at residues 1337-1338, according to N-terminal sequencing studies. As there are no known mutations in laminin 332 alpha3 G45 domain in JEB patients, three cDNAs encoding HuLAMA3 were produced, one comprising the 200 kDa full-length α3 wild-type chain (WT), coding for residues 1-1713. The second comprised the 165 kDa α3 chain truncated at the physiologic processing site comprising residues 1-1337 (ΔG45), and the third comprised the 37 kDa cleaved G45 (G45), residues 1338-1713. These were generated by PCR, verified by direct sequence analysis and cloned into the retroviral vector backbone LZRS containing the encephalomyocarditis virus (EMCV)-IRES and blasticidin-resistance sequences and a GATEWAY® (Invitrogen, Carlsbad, Calif.) destination site, (pLZRS-GATEWAY). The BM40 signal sequence was incorporated upstream and in-frame of the mutant HuLAMA3 for directing expression. Retroviral expression vectors encoding either activated Ha-Ras, IκBα, or activated PI3-K p110-CAAX have been previously characterized. Amphotropic retroviral supernatant production and retroviral keratinocyte transduction were performed as described.

[0201] Statistical analysis. Student's t-test was employed to ascertain significant difference between data sets using Microsoft Excel. P-values are listed in figure legends.

[0202] WT and ΔG45 cDNA forward primer: 5'-AAAAAAGCTAGCATGGGTTGGCTTATA-3' α3WT cDNA reverse primer: 5'-CCCCCCGGGCCCGCGGC-CGCTTACAGGTCCTCCTC GCTMTCMlTTTTTGCTC-CTGGTCAGGACAACCATTCAGACTGAC-3' ΔG45 reverse primer 5'-MAACCAGGTTTAACMGACCAA-GACTTTTCGTATCAACCTGCTGTTGCTGGCCACA CCAGTGGCCTCCCCTAGGAGC-3' G45 forward primer: 5'-TTATGCTAGCGG ACACACCAGT-3' G45 reverse primer: 5'-TATTCTCGAGTTACTGGTCAGGAC-3'.

[0203] Antibodies. Anti-laminin α3 chain mouse mAb BM165 and G45 rabbit pAb, anti-laminin-332 rabbit pAb and laminin β3 chain mouse mAb K140 were previously characterized. We commercially obtained anti-p-ERK and p-Akt (Ser473) (Cell Signaling), anti α6 integrin mAb G0H3 (Chemicon), Ki67 mAb (LabVision) paxillin mAb (BD Biosciences Pharmingen) phalloidin pAb (Invitrogen) and β-actin mAb (Sigma).

[0204] Protein analysis. Laminin-332 deposition among WT, ΔG45, and ΔG45+G45 cells were studied over a 24 hr period. Proteins from whole cell lysates, conditioned media and matrix deposition were extracted and quantified by immunoblot as previously described. K140, an anti-laminin β3 mAb, was used for laminin deposition comparisons, which controlled for cell density by normalizing laminin β3 to actin bands. For measurement of AKT phosphorylation, near-confluent cells were extracted in RIPA buffer with protease and phosphatase inhibitors. Ratio of phospho-AKT to total AKT was calculated through densitometry. For ERK phosphorylation, Ras-IκBα (or Ras-IκBα+PI3-K) transformed cells were growth factor starved for 24 h then stimulated with 10 ng/ml EGF for 2 min before lysis in RIPA buffer for immunoblot analysis. Densitometric analysis was shown as phosphorylated with ERK1/2 normalized to untreated WT controls. NIH ImageJ software was used for densitometric analysis.

[0205] Immunofluorescence microscopy. Confocal analysis of cell adhesion proteins was performed exactly as previously described. Assaying ERK activation of keratinocytes after pulsing with 10 ng/mL EGF for two minutes has been described previously. Briefly, representative images from JEB^{null} keratinocytes expressing the indicated laminin α3 constructs were visualized by immunofluorescence microscopy using phospho-ERK antibody. Nuclear localization of phospho-ERK was quantified as % phospho-ERK staining nuclei over total nuclei. Imaging was carried out with a Zeiss LSM 510 confocal laser scanning microscope.

[0206] Immunohistochemistry. For immunoperoxidase staining, 5 μm paraffin sections of SCC tissue microarrays from skin (SK802) and multiple organs (BC00019) obtained from US Biomax Inc, Rockville, Md., were deparaffinized, rehydrated and antigen umnasked by boiling in 50 mM Tris-HCl, pH 9.5 for 15 min. Sections were then incubated with G45 rabbit pAb followed by biotin-conjugated secondary and DAB detection (LabVision). Tissues were counterstained with hematoxylin. G45 staining was graded by 2 independent blinded observers according to the percentage of number of tumor cells positive with staining; >75% (Strong), 25%-75% (Moderate) and <25% (Negative). For immunofluorescence, 5 μm cryosections were incubated with antibodies listed above and Hoescht-counterstained. Images were taken using a Zeiss Axiovert-100 microscope.

[0207] Cell adhesion, migration and invasion assays. G45 adhesion studies were carried out by coating purified LG4/5 fragment (10 μg/ml overnight at 4° C. Ras/IκBα transformed normal primary human keratinocytes were detached in PBS containing 10 mM EDTA and rinsed in serum-free medium. After washing with PBS and saturation of the wells with 1% BSA, the cell adhesion assays were performed in serum-free medium, as described in Shaw et al. (1997) Cell 91:949-60. Adhesion was determined after fixation with 1% glutaraldehyde in PBS and staining with 0.1% crystal violet, by absorbance at 570 nm using a MR5000 ELISA reader. A blank value corresponding to BSA-coated wells was subtracted.

Adhesion inhibition with G4/5 antibody (20 μg/ml) or heparin (10 μg/ml, Sigma), took place 60 minutes prior to as well as during the cell adhesion experiment. Cell detachment assays were carried out as described (Utani et al. (2003) J Biol Chem278:34483-90). Briefly, 2×10^4 cells/cm^2 were incubated for 24 h at 50% confluence. Detached cells were quantified at increasing time intervals after incubation in a 1:70 dilution of trypsin/EDTA in PBS (BioWhittaker). Each adhesion/detachment experiment was performed in triplicate.

[0208] Cell monolayer scratch assays=were performed by plating 10^6 cells into 60 mm tissue culture plates and incubating cells in SFM for 24 hr. Media was changed to SFM without additives for 16 hr. Fresh mitomycin-C (Sigma) was added at 10 μg/ml and cells incubated 3 hr. on ice. Cells were washed twice with SFMANA and scratched with a 1 mm cell scraper. Plates were washed three times with SFMINA and marked areas photographed using a Zeiss Axiovert 25 microscope (50× magnification). Migration was quantified by calculating percent change in the area between migrating cell sheets using NIH image software and >3 repeats per data point.

[0209] The in vitro invasion assays) were performed as previously described, briefly assays were performed in triplicate using chambers containing a polycarbonate membrane with eight micron pores, coated with Matrigel (Becton Dickinson). After 24 hours, invasive cells in the bottom chamber were lysed and quantified using CyQuant GR dye. Invasion index was quantified relative to percent invasion by JEB^{null} keratinocytes.

[0210] Zymography. 1 million keratinocytes were starved for 24 h and incubated in SFM media. Conditioned media were recovered and concentrated 80×. Samples were dissolved in nonreducing sample buffer (6% glycerol, 1% SDS, and 0.004% bromophenol blue), incubated in 37° C. water bath for 10 min, and loaded on a 10% gelatin gel for detection of MMP2 and MMP9 and 12% casein gel for detection of MMP1 (Invitrogen). The gel was run in Tris/glycine buffer for 2 h and then incubated in 2.5% Triton X-100 solution for 15 min twice to remove SDS. To detect MMP activity, the gel was incubated in reaction buffer containing 50 M Tris-HCl (pH 7.4), 0.2 M NaCl, 5 mM $CaCl_2$, and 1 μM $ZnCl_2$ overnight at 37° C. Protease activity was detected as translucent area in a Coomassie blue-stained gel. The scanned results of gels were calculated using NIH ImageJ software.

[0211] Tumorigenicity assay. Tumorgenicity assay was performed as previously described. Briefly, keratinocytes were incubated with LZRS-IRES-Blasticidin/H-Ras or LZRS-IRES-Blasticidin/IκBαM retroviral titer. Gene transfer was verified by immunoblotting of cell lysates.

[0212] One million Ras/IκBαM transformed cells suspended in 200 μl Matrigel (Beckton-Dickenson), were injected subcutaneously to the dorsal flank of 6 week nude mice, 5 mice were used per each condition. Tumors were measured on a weekly basis and analyzed at the end of four weeks. All animal studies were conducted in accordance with protocols approved by the Stanford Animal Use Committee. In some experiments, affinity purified G45 pAb, affinity purified mAb K140, or control rabbit IgG (Sigma) were injected intraperitoneally on a weekly basis, at a dose of 500 μg per mouse per week, which has been previously demonstrated to maintain high circulating antibody levels. Apoptotic tumor cells were detected using Roche's In Situ Cell Detection Kit. Proliferating SCC cells were detected with Ki67 and DAPI immunofluorescent staining. Proliferation and apoptosis were quantified as the ratio of staining of nuclear Ki67 and TUNEL, respectively, to total nuclear staining. NIH Image software was used to quantify the subset of apoptotic or proliferating tumor cells in five representative low-power fields on each tumor.

Results

[0213] Widespread accumulation of laminin-332 G45 in human SCC tumors. In an effort to determine whether unprocessed laminin α3 G45 domain accumulated in human SCC tumors, we examined frozen sections of four normal skin and four cutaneous SCC specimens obtained by Moh's surgery, using immunofluorescence microscopy. Using an antibody specific to the G45 domain and an antibody (BM165) which recognized the processed laminin-332 trimer (FIG. **6***a*), we found that G45, while consistently undetectable in normal skin, was abundantly present, and showed colocalization with mAb BM165 in human cutaneous SCC tumors from each of the four patient samples tested (FIG. **6***b*). In a more extensive survey of 75 cutaneous (FIG. **1***c*) and 56 extracutaneous (FIG. **6***d*) paraffin embedded SCC tumors, over 75% showed moderate to strong G45 expression. All G45 positive tumors also stained positively with total laminin-332 pAb. All but one of the G45 negative tumors were also negative for total laminin-332 expression. Thus G45 domain accumulation correlated well with total laminin-332 expression in SCC tumors.

[0214] Laminin-332 G45 domain facilitates the organization and function of matrix receptor complexes. To investigate laminin-332 G45 in SCC, we produced three laminin-332 α3 chain constructs (FIG. **7***a*), a full length wild type chain (WT), a mutant lacking G45 (ΔG45) and G45 (G45) itself. These constructs were stably expressed in laminin-332 null keratinocytes derived from a patient with junctional epidermolysis bullosa (JEB^{null}) with underlying LAMA3 gene mutations. As will be shown below, G45 separately expressed from the rest of the laminin332 molecule (ΔG45+G45) performed many of the same functions, albeit less efficiently (detailed below), as G45 synthesized as part of the laminin-332 molecule (WT). JEB^{null} keratinocytes expressing G45 without the ΔG45 construct, like untransduced JEB^{null} keratinocytes, did not adhere to culture surfaces and were not further analyzed further in vitro.

[0215] ΔG45 JEB^{null} cells synthesized, assembled and secreted trimeric laminin-332 as shown by non-reduced immunoblot analysis (FIG. **7***b*, left panel) and the ΔG45 chain was of equivalent apparent molecular weight compared to processed WT α3 chain (α3p) as shown by reduced immunoblot (FIG. **7***b*, center panel). The G45 construct was expressed in ΔG45 cells at levels similar to WT cells as clearly seen in conditioned cell medium (FIG. **7***b*, right panel), as well as cell lysate. We compared laminin-332 in these cells isolated from culture medium, or extracted from culture matrix as previously described, using actin from cell lysates as a control. Normally keratinocytes deposit more laminin-332 into their matrix than they secrete into their media, but ΔG45 cells secreted more laminin-332 into medium than matrix (FIG. **7***c*), consistent with previous observations. G45 synthesis in ΔG45 cells led to the majority of laminin-332 being deposited into matrix, indicating that G45 promoted laminin-332 deposition.

[0216] This was further examined by confocal microscopy (FIG. **8***a*). Analysis of laminin-332 antibody staining confirmed that the absence of G45 in ΔG45 cells correlated with a reduction in deposited laminin-332 which was again improved with the expression of G45 in ΔG45 cells (FIG. **8***a* top panel). Additional effects of G45 on basally located cell receptor complexes were also noted. WT cells showed characteristic peripheral focal adhesions (FA), containing paxillin

and central stable adhesions (SA) containing α6β4 integrin (FIG. **8***a* second panel). However SAs in ΔG45 cells were abnormally peripheral, adjacent to FAs (FIG. **8***a* third panel). G45 expression in ΔG45 cells restored some SAs to their normal central location. G45 co-localized with α6β4 integrin in SAs in WT and ΔG45 cells (FIG. **8***a,* bottom panel), suggesting interaction of G45 with SAs. In accordance with the analysis of SA formation, ΔG45 cells showed increased sensitivity to trypsin-induced detachment (FIG. **8***b*), which was corrected with G45 expression, suggesting that G45 induced laminin-332 deposition and SA formation led to increased stable cell adhesion. Previous studies have shown an inverse relationship between the rate of keratinocyte migration and laminin-332 deposition. In accordance with this, ΔG45 keratinocytes, with decreased laminin-332 deposition, migrated faster into scratches placed in confluent monolayers than WT cells with normal laminin-332 deposition (FIG. **8***c*). Expression of G45 construct in ΔG45 cells partially reversed these effects. We next determined whether these adhesion and migration abnormalities and these changes in extracellular matrix deposition and organization correlated with changes in tumorigenic potential.

[0217] Laminin-332 G45 promotes tumor invasion and metalloproteinase activity. SCC tumorigenesis was examined by retroviral transduction of primary human keratinocytes with oncogenic Ras and the NF-κB inhibitor IκBα, which produces transformed cells that generate human epidermal tumors indistinguishable from human SCC upon transfer to immunodeficient mice. Following Ras/IκBα transformation, ΔG45 cells showed impaired invasion into Matrigel, compared to WT cells which was partially corrected through G45 retroviral transduction (FIG. **9***a*). Carcinoma invasion has been linked to metalloproteinase activity and ΔG45 cells showed a striking deficiency of MMP-9 and MMP-1, which have been associated with SCC invasion (FIG. **9***b*). Metalloproteinase deficiencies in ΔG45 cells were reversed through expression of G45, although not to the levels of WT cells. This deficient MMP expression explains why, despite their increased migration in the untransformed state, transformed ΔG45 cells invaded Matrigel more poorly than transformed WT cells.

[0218] Activation of Phosphoinositol-3-kinase (PI3-K) and ERK pathways by laminin-332 G45. PI3-K pathway activation is critical for SCC invasion and ΔG45 cells showed decreased AKT phosphorylation suggesting that G45 was essential in promoting PI3-K pathway activation (FIG. **9***c*). G45 expressed as part of the laminin-332 molecule was more efficient at promoting AKT phosphorylation compared to G45 expressed separate from laminin-332 (≠G45+G45). We also found an impairment of ERK activation in transformed ΔG45 cells which was restored partially through overexpression of G45, but fully through overexpression of activated PI3-K p110 catalytic subunit (FIG. **9***c*). In addition, ΔG45 cells showed defective nuclear translocation of activated ERK, which was corrected partially through G45 expression but fully through activated p110 expression (FIG. **9***d*).

[0219] Laminin-332 G45 is required for in vivo Ras driven SCC tumorigenesis. We next examined G45 in human SCC tumorigenesis in vivo. After transfer to immunodeficient mice, transformed ΔG45 cells showed strikingly impaired tumorigenesis (FIG. **10***a,* top row). This was slightly improved when G45 was expressed in ΔG45 cells and tumor growth was more fully restored in ΔG45 cells through activated p110 expression. Transformed JEB^{null} cells overxpressing G45 alone produced no detectable tumors 4 weeks after injection, similar to what has been previously demonstrated for transformed JEB^{null} cells alone. While invasion into underlying muscle was consistently noted in tumors expressing wild type laminin α3 chain (WT), ΔG45 tumors showed a conspicuous invasive defect (FIG. **10***a,* second row). However, invasion into muscle was noted in ΔG45 cells tumors after expression of G45 or activated p110 subunit. Widespread apoptosis was present in ΔG45 tumors (FIG. **10***a,* third row, FIG. **10***b*). G45 expression diminished and p110 expression completely inhibited apoptosis in ΔG45 tumors. ΔG45 tumors showed deficient proliferation which was modestly increased with G45 domain expression, and fully restored to wild type levels with p110 expression (FIG. **10***a,* fourth row, FIG. **10***c*). Expression of G45 promoted laminin-332 deposition in the tumors, as did activated p110 subunit expression (FIG. **10***a,* fifth row), suggesting a possible link between G45 function, PI3-K pathway activation and laminin-332 deposition during SCC tumorigenesis.

[0220] Laminin-332 G45 antibody disrupted SCC tumorigenesis in vivo without affecting normal tissue integrity. Over the course of 4 weeks of treatment, G45 antibodies dramatically inhibited in vivo human SCC tumorigenesis (FIG. **11***a*). G45 antibody treated tumors produced both inhibition of proliferation and pronounced apoptosis compared to control antibody treated tumors (FIG. **11***b*) similar to the G45 genetic deletion experiments described above. Affinity purified G45 antibody was shown to specifically inhibit transformed keratinocyte adhesion to recombinant G45 protein (FIG. **11***c*). Interestingly heparin also disrupted this interaction suggesting that the heparan binding-properties of G45 were involved in its cellular interactions. Given its inhibitory effects on SCC tumor growth, we recognized the potential of the G45 antibody as an anti-cancer agent, and looked for possible toxic side effects of the antibodies in a survey of normal tissues. Interestingly, although the G45 polyclonal antibody specifically recognized native murine laminin-332, as shown by nonreduced immunoblot analysis of conditioned mouse keratinocyte culture medium (FIG. **11***c*), we found no blistering, epithelial-mesenchymal separation or other morphologic abnormalities observed in laminin-332 expressing tissues of mice treated with G45 antibodies, even after four weeks of antibody injections (FIG. **11***d*). Transmission electron microscopy of mouse skin after four weeks of antibody treatment showed no vesiculation or BMZ abnormalities (FIG. **11***d*).

[0221] This study clearly indicates, through genetic and antibody mediated inhibition, a key role for laminin-332 G45 in SCC tumorigenesis. One of the striking aspects of this study was the stark contrast between undetectable G45 in normal mature tissues and prevalent accumulation of G45 in a wide array of squamous cell carcinomas. Over 75% of both cutaneous and non-cutaneous SCCs expressed laminin-332 and G45. Laminin-332 G45 was present in nearly 100% of human SCC tumors positive for total laminin-332 expression. Laminin-332 expression correlates with carcinoma invasiveness and a poor prognosis in SCC patients. As G45 persistence also correlates closely with laminin-332 expression in SCC tumors but is absent in normal tissues, G45, through additional clinical correlative studies, is an extremely useful marker to identify invasive SCC tumors.

[0222] Even though laminin-332 G45 is undetectable in mature tissues, it is transiently detectable at the leading edges of wounds. SCC invasion shares similarities with wound healing as both are processes of epithelial proliferation and extension which require the active synthesis and deposition of new BMZ components. These two processes differ in significant ways, however. In wounds, laminin-332 G45 becomes undetectable after closure, when synthesis of new BMZ components subsides and full processing and BMZ assembly is

completed. With SCC tumor invasion, the synthesis of BMZ components is not regulated by a closure event such as in wound healing, and thus the synthesis of BMZ components continues, without allowing for processing and maturation of the BMZ to occur to completion. This may account for the poor ultrastructural BMZ organization in invasive carcinomas, compared to normal tissues.

[0223] Why G45 accumulates at SCCs and at the leading edges of healing wounds may simply be a reflection of higher levels of total laminin-332 expression. The enzymes which process laminin α3 chain include plasmin and the C-proteinase family of enzymes, especially mammalian tolloid and bone morphogenic protein 1. There may be other mechanisms which control the rate of laminin α3 G45 processing, such as the tissue plasminogen proteolytic cascade. In addition, a group of enhancer proteins which modulate C-proteinase activity has also been described, and while one, termed PCPE-1 has not been shown to influence laminin-332 processing, other members of this family, including PCPE-2 remain to be evaluated. As the G45 appears to have potent pro-tumorigenic effects, factors which influence its proteolytic removal may have important bearing on SCC tumor progression.

[0224] Major pro-tumorigenic effects of G45 include its ability to enhance laminin-332 deposition in SCC tumors. Laminin332 G45 domain has heparin binding properties, and may have the ability to bind with extracellular heparan sulfate proteoglycan BMZ components such as perlecan or dystroglycan. However, even in the absence of G45, laminin-332 deposition was shown to be restored to near wild type levels in SCC tumors through activation of the PI3-K pathway. This suggests that rather than acting by anchoring, G45 likely promotes laminin-332 deposition by a signaling mechanism perhaps induced through interaction with another cell surface receptor. As we showed our tumor cell interactions with G45 could be blocked with heparin, a candidate which deserves further study is syndecan-1, a transmembrane heparan sulfate proteoglycan receptor which is expressed in epidermal cells and can directly bind the laminin α3 G45 domain. The G45 domain appears to provide unique pro-tumorigenic functions, including laminin deposition and proliferative stimulation which are not provided by the laminin P3 chain short arm.

[0225] While G45 is not known to directly bind α6β4 integrin, a number of observations suggest that G45 and α6β4 integrin functions in promoting tumorigenesis may be related. First we noted that G45 and α6β4 integrin localized together in basal keratinocyte receptor complexes termed SAs. While α6β4 integrin is well known to play a key role in the formation of these complexes, here we also noted a role for G45 in SA formation. Specifically the absence of G45 disrupted the localization of SA complexes, changing them from central to peripheral localization. In addition to co-localizing to and playing a role in the formation of SAs, α6β4 integrin and G45 also showed interesting parallels of relevance to SCC tumorigenesis. G45 was noted for its promotion of cellular invasion and its activation of PI3-K and ERK signaling pathways, leading to protection from apoptosis and increased proliferation. It is also well known that α6β4 integrin, like G45, promotes carcinoma invasion and PI3-K pathway activation and nuclear ERK translocation which leads to increased proliferation. Furthermore α6β4 integrin was shown to perform these functions in a laminin dependent manner though its substrate domain. Deletion of this substrate domain led to phosphorylated ERK which accumulated in the cytoplasm, similar to ΔG45 cells in our study. Thus it is likely that α6β4 integrin and signaling from its substrate domain is involved in the function of the laminin α3 G45 domain. It should also be noted that in our studies, G45 promoted pro-tumorigenic functions best when it was expressed as part of the laminin α3 chain, suggesting G45 may need to be associated with the rest of the laminin-332 trimer for optimal function. It is possible that the close proximity of the G45 domain to the α6β4 integrin (G1-3) binding site on the unprocessed laminin α3 chain (FIG. **6***a*) plays a role in this process.

[0226] G45 domain clearly modulates the expression of MMP-9 and MMP-1, two metalloproteinases known to play important roles in carcinoma invasion. Our observation extends earlier findings of the G45 domain's role in upregulating MMP-1) and MMP-9 in keratinocytes to squamous carcinoma cells. These results are consistent with other studies showing the role of extracellular matrix molecules changing MMP expression and activities. Overexpression of PI3-K has been previously shown to promote MMP-9 expression in carcinoma cells and G45's ability to promote activation of the PI3-K pathway may be related to its function in promoting MMP-9 activity in human SCC.

[0227] G45 is not required for maintenance of normal tissue cohesion, as no epithelial-mesenchymal disruption was seen in normal tissues repeatedly treated with G45 inhibitory antibodies. Overall, its striking pro-tumorigenic activity, its prevalent accumulation in human SCC tumors and its absence and lack of function in normal developed tissues collectively make G45 an attractive anti-cancer target.

[0228] While the foregoing has presented specific embodiments, it is to be understood that these embodiments have been presented by way of example only. It is expected that others will perceive and practice variations which, though differing from the foregoing, do not depart form the spirit and scope of the inventions as described and claimed herein. All patent applications, patents, and literature references cited in this specification are hereby incorporated by reference in their entirety. In case of conflict or inconsistency, the present description, including definitions, will control.

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    530                 535                 540
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Gln Met Pro Cys Ser Ser Val Thr Gly Gln Cys Glu Cys Arg Pro Gly
545                 550                 555                 560

Val Thr Gly Gln Arg Cys Asp Arg Cys Leu Ser Gly Ala Tyr Asp Phe
                565                 570                 575

Pro His Cys Gln Gly Ser Ser Ser Ala Cys Asp Pro Ala Gly Thr Ile
            580                 585                 590

Asn Ser Asn Leu Gly Tyr Cys Gln Cys Lys Leu His Val Glu Gly Pro
        595                 600                 605

Thr Cys Ser Arg Cys Lys Leu Leu Tyr Trp Asn Leu Asp Lys Glu Asn
    610                 615                 620

Pro Ser Gly Cys Ser Glu Cys Lys Cys His Lys Ala Gly Thr Val Ser
625                 630                 635                 640

Gly Thr Gly Glu Cys Arg Gln Gly Asp Gly Asp Cys His Cys Lys Ser
                645                 650                 655

His Val Gly Gly Asp Ser Cys Asp Thr Cys Glu Asp Gly Tyr Phe Ala
            660                 665                 670

Leu Glu Lys Ser Asn Tyr Phe Gly Cys Gln Gly Cys Gln Cys Asp Ile
        675                 680                 685

Gly Gly Ala Leu Ser Ser Met Cys Ser Gly Pro Ser Gly Val Cys Gln
    690                 695                 700

Cys Arg Glu His Val Val Gly Lys Val Cys Gln Arg Pro Glu Asn Asn
705                 710                 715                 720

Tyr Tyr Phe Pro Asp Leu His His Met Lys Tyr Glu Ile Glu Asp Gly
                725                 730                 735

Ser Thr Pro Asn Gly Arg Asp Leu Arg Phe Gly Phe Asp Pro Leu Ala
            740                 745                 750

Phe Pro Glu Phe Ser Trp Arg Gly Tyr Ala Gln Met Thr Ser Val Gln
        755                 760                 765

Asn Asp Val Arg Ile Thr Leu Asn Val Gly Lys Ser Ser Gly Ser Leu
    770                 775                 780

Phe Arg Val Ile Leu Arg Tyr Val Asn Pro Gly Thr Glu Ala Val Ser
785                 790                 795                 800

Gly His Ile Thr Ile Tyr Pro Ser Trp Gly Ala Ala Gln Ser Lys Glu
                805                 810                 815

Ile Ile Phe Leu Pro Ser Lys Glu Pro Ala Phe Val Thr Val Pro Gly
            820                 825                 830

Asn Gly Phe Ala Asp Pro Phe Ser Ile Thr Pro Gly Ile Trp Val Ala
        835                 840                 845

Cys Ile Lys Ala Glu Gly Val Leu Leu Asp Tyr Leu Val Leu Leu Pro
    850                 855                 860

Arg Asp Tyr Tyr Glu Ala Ser Val Leu Gln Leu Pro Val Thr Glu Pro
865                 870                 875                 880

Cys Ala Tyr Ala Gly Pro Pro Gln Glu Asn Cys Leu Leu Tyr Gln His
                885                 890                 895

Leu Pro Val Thr Arg Phe Pro Cys Thr Leu Ala Cys Glu Ala Arg His
            900                 905                 910

Phe Leu Leu Asp Gly Glu Pro Arg Pro Val Ala Val Arg Gln Pro Thr
        915                 920                 925

Pro Ala His Pro Val Met Val Asp Leu Ser Gly Arg Glu Val Glu Leu
    930                 935                 940
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His Leu Arg Leu Arg Ile Pro Gln Val Gly His Tyr Val Val Val Val
945                 950                 955                 960

Glu Tyr Ser Thr Glu Ala Ala Gln Leu Phe Val Val Asp Val Asn Val
                965                 970                 975

Lys Ser Ser Gly Ser Val Leu Ala Gly Gln Val Asn Ile Tyr Ser Cys
            980                 985                 990

Asn Tyr Ser Val Leu Cys Arg Ser  Ala Val Ile Asp His  Met Ser Arg
        995                 1000                1005

Ile Ala  Met Tyr Glu Leu Leu  Ala Asp Ala Asp Ile  Gln Leu Lys
    1010                1015                1020

Gly His  Met Ala Arg Phe Leu  Leu His Gln Val Cys  Ile Ile Pro
    1025                1030                1035

Ile Glu  Glu Phe Ser Ala Glu  Tyr Val Arg Pro Gln  Val His Cys
    1040                1045                1050

Ile Ala  Ser Tyr Gly Arg Phe  Val Asn Gln Ser Ala  Thr Cys Val
    1055                1060                1065

Ser Leu  Ala His Glu Thr Pro  Pro Thr Ala Leu Ile  Leu Asp Val
    1070                1075                1080

Leu Ser  Gly Arg Pro Phe Pro  His Leu Pro Gln Gln  Ser Ser Pro
    1085                1090                1095

Ser Val  Asp Val Leu Pro Gly  Val Thr Leu Lys Ala  Pro Gln Asn
    1100                1105                1110

Gln Val  Thr Leu Arg Gly Arg  Val Pro His Leu Gly  Arg Tyr Val
    1115                1120                1125

Phe Val  Ile His Phe Tyr Gln  Ala Ala His Pro Thr  Phe Pro Ala
    1130                1135                1140

Gln Val  Ser Val Asp Gly Gly  Trp Pro Arg Ala Gly  Ser Phe His
    1145                1150                1155

Ala Ser  Phe Cys Pro His Val  Leu Gly Cys Arg Asp  Gln Val Ile
    1160                1165                1170

Ala Glu  Gly Gln Ile Glu Phe  Asp Ile Ser Glu Pro  Glu Val Ala
    1175                1180                1185

Ala Thr  Val Lys Val Pro Glu  Gly Lys Ser Leu Val  Leu Val Arg
    1190                1195                1200

Val Leu  Val Val Pro Ala Glu  Asn Tyr Asp Tyr Gln  Ile Leu His
    1205                1210                1215

Lys Lys  Ser Met Asp Lys Ser  Leu Glu Phe Ile Thr  Asn Cys Gly
    1220                1225                1230

Lys Asn  Ser Phe Tyr Leu Asp  Pro Gln Thr Ala Ser  Arg Phe Cys
    1235                1240                1245

Lys Asn  Ser Ala Arg Ser Leu  Val Ala Phe Tyr His  Lys Gly Ala
    1250                1255                1260

Leu Pro  Cys Glu Cys His Pro  Thr Gly Ala Thr Gly  Pro His Cys
    1265                1270                1275

Ser Pro  Glu Gly Gly Gln Cys  Pro Cys Gln Pro Asn  Val Ile Gly
    1280                1285                1290

Arg Gln  Cys Thr Arg Cys Ala  Thr Gly His Tyr Gly  Phe Pro Arg
    1295                1300                1305

Cys Lys  Pro Cys Ser Cys Gly  Arg Arg Leu Cys Glu  Glu Met Thr
    1310                1315                1320

Gly Gln  Cys Arg Cys Pro Pro  Arg Thr Val Arg Pro  Gln Cys Glu
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    1325                1330                1335

Val Cys  Glu Thr His Ser Phe  Ser Phe His Pro Met  Ala Gly Cys
    1340                1345                1350

Glu Gly  Cys Asn Cys Ser Arg  Arg Gly Thr Ile Glu  Ala Ala Met
    1355                1360                1365

Pro Glu  Cys Asp Arg Asp Ser  Gly Gln Cys Arg Cys  Lys Pro Arg
    1370                1375                1380

Ile Thr  Gly Arg Gln Cys Asp  Arg Cys Ala Ser Gly  Phe Tyr Arg
    1385                1390                1395

Phe Pro  Glu Cys Val Pro Cys  Asn Cys Asn Arg Asp  Gly Thr Glu
    1400                1405                1410

Pro Gly  Val Cys Asp Pro Gly  Thr Gly Ala Cys Leu  Cys Lys Glu
    1415                1420                1425

Asn Val  Glu Gly Thr Glu Cys  Asn Val Cys Arg Glu  Gly Ser Phe
    1430                1435                1440

His Leu  Asp Pro Ala Asn Leu  Lys Gly Cys Thr Ser  Cys Phe Cys
    1445                1450                1455

Phe Gly  Val Asn Asn Gln Cys  His Ser Ser His Lys  Arg Arg Thr
    1460                1465                1470

Lys Phe  Val Asp Met Leu Gly  Trp His Leu Glu Thr  Ala Asp Arg
    1475                1480                1485

Val Asp  Ile Pro Val Ser Phe  Asn Pro Gly Ser Asn  Ser Met Val
    1490                1495                1500

Ala Asp  Leu Gln Glu Leu Pro  Ala Thr Ile His Ser  Ala Ser Trp
    1505                1510                1515

Val Ala  Pro Thr Ser Tyr Leu  Gly Asp Lys Val Ser  Ser Tyr Gly
    1520                1525                1530

Gly Tyr  Leu Thr Tyr Gln Ala  Lys Ser Phe Gly Leu  Pro Gly Asp
    1535                1540                1545

Met Val  Leu Leu Glu Lys Lys  Pro Asp Val Gln Leu  Thr Gly Gln
    1550                1555                1560

His Met  Ser Ile Ile Tyr Glu  Glu Thr Asn Thr Pro  Arg Pro Asp
    1565                1570                1575

Arg Leu  His His Gly Arg Val  His Val Val Glu Gly  Asn Phe Arg
    1580                1585                1590

His Ala  Ser Ser Arg Ala Pro  Val Ser Arg Glu Glu  Leu Met Thr
    1595                1600                1605

Val Leu  Ser Arg Leu Ala Asp  Val Arg Ile Gln Gly  Leu Tyr Phe
    1610                1615                1620

Thr Glu  Thr Gln Arg Leu Thr  Leu Ser Glu Val Gly  Leu Glu Glu
    1625                1630                1635

Ala Ser  Asp Thr Gly Ser Gly  Arg Ile Ala Leu Ala  Val Glu Ile
    1640                1645                1650

Cys Ala  Cys Pro Pro Ala Tyr  Ala Gly Asp Ser Cys  Gln Gly Cys
    1655                1660                1665

Ser Pro  Gly Tyr Tyr Arg Asp  His Lys Gly Leu Tyr  Thr Gly Arg
    1670                1675                1680

Cys Val  Pro Cys Asn Cys Asn  Gly His Ser Asn Gln  Cys Gln Asp
    1685                1690                1695

Gly Ser  Gly Ile Cys Val Asn  Cys Gln His Asn Thr  Ala Gly Glu
    1700                1705                1710
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His Cys  Glu Arg Cys Gln Glu  Gly Tyr Tyr Gly Asn  Ala Val His
    1715                1720                1725

Gly Ser  Cys Arg Ala Cys Pro  Cys Pro His Thr Asn  Ser Phe Ala
    1730                1735                1740

Thr Gly  Cys Val Val Asn Gly  Gly Asp Val Arg Cys  Ser Cys Lys
    1745                1750                1755

Ala Gly  Tyr Thr Gly Thr Gln  Cys Glu Arg Cys Ala  Pro Gly Tyr
    1760                1765                1770

Phe Gly  Asn Pro Gln Lys Phe  Gly Gly Ser Cys Gln  Pro Cys Ser
    1775                1780                1785

Cys Asn  Ser Asn Gly Gln Leu  Gly Ser Cys His Pro  Leu Thr Gly
    1790                1795                1800

Asp Cys  Ile Asn Gln Glu Pro  Lys Asp Ser Ser Pro  Ala Glu Glu
    1805                1810                1815

Cys Asp  Asp Cys Asp Ser Cys  Val Met Thr Leu Leu  Asn Asp Leu
    1820                1825                1830

Ala Thr  Met Gly Glu Gln Leu  Arg Leu Val Lys Ser  Gln Leu Gln
    1835                1840                1845

Gly Leu  Ser Ala Ser Ala Gly  Leu Leu Glu Gln Met  Arg His Met
    1850                1855                1860

Glu Thr  Gln Ala Lys Asp Leu  Arg Asn Gln Leu Leu  Asn Tyr Arg
    1865                1870                1875

Ser Ala  Ile Ser Asn His Gly  Ser Lys Ile Glu Gly  Leu Glu Arg
    1880                1885                1890

Glu Leu  Thr Asp Leu Asn Gln  Glu Phe Glu Thr Leu  Gln Glu Lys
    1895                1900                1905

Ala Gln  Val Asn Ser Arg Lys  Ala Gln Thr Leu Asn  Asn Asn Val
    1910                1915                1920

Asn Arg  Ala Thr Gln Ser Ala  Lys Glu Leu Asp Val  Lys Ile Lys
    1925                1930                1935

Asn Val  Ile Arg Asn Val His  Ile Leu Leu Lys Gln  Ile Ser Gly
    1940                1945                1950

Thr Asp  Gly Glu Gly Asn Asn  Val Pro Ser Gly Asp  Phe Ser Arg
    1955                1960                1965

Glu Trp  Ala Glu Ala Gln Arg  Met Met Arg Glu Leu  Arg Asn Arg
    1970                1975                1980

Asn Phe  Gly Lys His Leu Arg  Glu Ala Glu Ala Asp  Lys Arg Glu
    1985                1990                1995

Ser Gln  Leu Leu Leu Asn Arg  Ile Arg Thr Trp Gln  Lys Thr His
    2000                2005                2010

Gln Gly  Glu Asn Asn Gly Leu  Ala Asn Ser Ile Arg  Asp Ser Leu
    2015                2020                2025

Asn Glu  Tyr Glu Ala Lys Leu  Ser Asp Leu Arg Ala  Arg Leu Gln
    2030                2035                2040

Glu Ala  Ala Ala Gln Ala Lys  Gln Ala Asn Gly Leu  Asn Gln Glu
    2045                2050                2055

Asn Glu  Arg Ala Leu Gly Ala  Ile Gln Arg Gln Val  Lys Glu Ile
    2060                2065                2070

Asn Ser  Leu Gln Ser Asp Phe  Thr Lys Tyr Leu Thr  Thr Ala Asp
    2075                2080                2085
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Ser Ser  Leu Leu Gln Thr Asn  Ile Ala Leu Gln Leu  Met Glu Lys
    2090                 2095                 2100

Ser Gln  Lys Glu Tyr Glu Lys  Leu Ala Ala Ser Leu  Asn Glu Ala
    2105                 2110                 2115

Arg Gln  Glu Leu Ser Asp Lys  Val Arg Glu Leu Ser  Arg Ser Ala
    2120                 2125                 2130

Gly Lys  Thr Ser Leu Val Glu  Glu Ala Glu Lys His  Ala Arg Ser
    2135                 2140                 2145

Leu Gln  Glu Leu Ala Lys Gln  Leu Glu Glu Ile Lys  Arg Asn Ala
    2150                 2155                 2160

Ser Gly  Asp Glu Leu Val Arg  Cys Ala Val Asp Ala  Ala Thr Ala
    2165                 2170                 2175

Tyr Glu  Asn Ile Leu Asn Ala  Ile Lys Ala Ala Glu  Asp Ala Ala
    2180                 2185                 2190

Asn Arg  Ala Ala Ser Ala Ser  Glu Ser Ala Leu Gln  Thr Val Ile
    2195                 2200                 2205

Lys Glu  Asp Leu Pro Arg Lys  Ala Lys Thr Leu Ser  Ser Asn Ser
    2210                 2215                 2220

Asp Lys  Leu Leu Asn Glu Ala  Lys Met Thr Gln Lys  Lys Leu Lys
    2225                 2230                 2235

Gln Glu  Val Ser Pro Ala Leu  Asn Asn Leu Gln Gln  Thr Leu Asn
    2240                 2245                 2250

Ile Val  Thr Val Gln Lys Glu  Val Ile Asp Thr Asn  Leu Thr Thr
    2255                 2260                 2265

Leu Arg  Asp Gly Leu His Gly  Ile Gln Arg Gly Asp  Ile Asp Ala
    2270                 2275                 2280

Met Ile  Ser Ser Ala Lys Ser  Met Val Arg Lys Ala  Asn Asp Ile
    2285                 2290                 2295

Thr Asp  Glu Val Leu Asp Gly  Leu Asn Pro Ile Gln  Thr Asp Val
    2300                 2305                 2310

Glu Arg  Ile Lys Asp Thr Tyr  Gly Arg Thr Gln Asn  Glu Asp Phe
    2315                 2320                 2325

Lys Lys  Ala Leu Thr Asp Ala  Asp Asn Ser Val Asn  Lys Leu Thr
    2330                 2335                 2340

Asn Lys  Leu Pro Asp Leu Trp  Arg Lys Ile Glu Ser  Ile Asn Gln
    2345                 2350                 2355

Gln Leu  Leu Pro Leu Gly Asn  Ile Ser Asp Asn Met  Asp Arg Ile
    2360                 2365                 2370

Arg Glu  Leu Ile Gln Gln Ala  Arg Asp Ala Ala Ser  Lys Val Ala
    2375                 2380                 2385

Val Pro  Met Arg Phe Asn Gly  Lys Ser Gly Val Glu  Val Arg Leu
    2390                 2395                 2400

Pro Asn  Asp Leu Glu Asp Leu  Lys Gly Tyr Thr Ser  Leu Ser Leu
    2405                 2410                 2415

Phe Leu  Gln Arg Pro Asn Ser  Arg Glu Asn Gly Gly  Thr Glu Asn
    2420                 2425                 2430

Met Phe  Val Met Tyr Leu Gly  Asn Lys Asp Ala Ser  Arg Asp Tyr
    2435                 2440                 2445

Ile Gly  Met Ala Val Val Asp  Gly Gln Leu Thr Cys  Val Tyr Asn
    2450                 2455                 2460

Leu Gly  Asp Arg Glu Ala Glu  Leu Gln Val Asp Gln  Ile Leu Thr
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    2465                2470                2475

Lys Ser  Glu Thr Lys Glu Ala  Val Met Asp Arg Val  Lys Phe Gln
    2480                2485                2490

Arg Ile  Tyr Gln Phe Ala Arg  Leu Asn Tyr Thr Lys  Gly Ala Thr
    2495                2500                2505

Ser Ser  Lys Pro Glu Thr Pro  Gly Val Tyr Asp Met  Asp Gly Arg
    2510                2515                2520

Asn Ser  Asn Thr Leu Leu Asn  Leu Asp Pro Glu Asn  Val Val Phe
    2525                2530                2535

Tyr Val  Gly Gly Tyr Pro Pro  Asp Phe Lys Leu Pro  Ser Arg Leu
    2540                2545                2550

Ser Phe  Pro Pro Tyr Lys Gly  Cys Ile Glu Leu Asp  Asp Leu Asn
    2555                2560                2565

Glu Asn  Val Leu Ser Leu Tyr  Asn Phe Lys Lys Thr  Phe Asn Leu
    2570                2575                2580

Asn Thr  Thr Glu Val Glu Pro  Cys Arg Arg Arg Lys  Glu Glu Ser
    2585                2590                2595

Asp Lys  Asn Tyr Phe Glu Gly  Thr Gly Tyr Ala Arg  Val Pro Thr
    2600                2605                2610

Gln Pro  His Ala Pro Ile Pro  Thr Phe Gly Gln Thr  Ile Gln Thr
    2615                2620                2625

Thr Val  Asp Arg Gly Leu Leu  Phe Phe Ala Glu Asn  Gly Asp Arg
    2630                2635                2640

Phe Ile  Ser Leu Asn Ile Glu  Asp Gly Lys Leu Met  Val Arg Tyr
    2645                2650                2655

Lys Leu  Asn Ser Glu Leu Pro  Lys Glu Arg Gly Val  Gly Asp Ala
    2660                2665                2670

Ile Asn  Asn Gly Arg Asp His  Ser Ile Gln Ile Lys  Ile Gly Lys
    2675                2680                2685

Leu Gln  Lys Arg Met Trp Ile  Asn Val Asp Val Gln  Asn Thr Ile
    2690                2695                2700

Ile Asp  Gly Glu Val Phe Asp  Phe Ser Thr Tyr Tyr  Leu Gly Gly
    2705                2710                2715

Ile Pro  Ile Ala Ile Arg Glu  Arg Phe Asn Ile Ser  Thr Pro Ala
    2720                2725                2730

Phe Arg  Gly Cys Met Lys Asn  Leu Lys Lys Thr Ser  Gly Val Val
    2735                2740                2745

Arg Leu  Asn Asp Thr Val Gly  Val Thr Lys Lys Cys  Ser Glu Asp
    2750                2755                2760

Trp Lys  Leu Val Arg Ser Ala  Ser Phe Ser Arg Gly  Gly Gln Leu
    2765                2770                2775

Ser Phe  Thr Asp Leu Gly Leu  Pro Pro Thr Asp His  Leu Gln Ala
    2780                2785                2790

Ser Phe  Gly Phe Gln Thr Phe  Gln Pro Ser Gly Ile  Leu Leu Asp
    2795                2800                2805

His Gln  Thr Trp Thr Arg Asn  Leu Gln Val Thr Leu  Glu Asp Gly
    2810                2815                2820

Tyr Ile  Glu Leu Ser Thr Ser  Asp Ser Gly Ser Pro  Ile Phe Lys
    2825                2830                2835

Ser Pro  Gln Thr Tyr Met Asp  Gly Leu Leu His Tyr  Val Ser Val
    2840                2845                2850
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Ile Ser  Asp Asn Ser Gly Leu  Arg Leu Leu Ile Asp  Asp Gln Leu
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Leu Arg  Asn Ser Lys Arg Leu  Lys His Ile Ser Ser  Ser Arg Gln
    2870                 2875                 2880

Ser Leu  Arg Leu Gly Gly Ser  Asn Phe Glu Gly Cys  Ile Ser Asn
    2885                 2890                 2895

Val Phe  Val Gln Arg Leu Ser  Leu Ser Pro Glu Val  Leu Asp Leu
    2900                 2905                 2910

Thr Ser  Asn Ser Leu Lys Arg  Asp Val Ser Leu Gly  Gly Cys Ser
    2915                 2920                 2925

Leu Asn  Lys Pro Pro Phe Leu  Met Leu Leu Lys Gly  Ser Thr Arg
    2930                 2935                 2940

Phe Asn  Lys Thr Lys Thr Phe  Arg Ile Asn Gln Leu  Leu Gln Asp
    2945                 2950                 2955

Thr Pro  Val Ala Ser Pro Arg  Ser Val Lys Val Trp  Gln Asp Ala
    2960                 2965                 2970

Cys Ser  Pro Leu Pro Lys Thr  Gln Ala Asn His Gly  Ala Leu Gln
    2975                 2980                 2985

Phe Gly  Asp Ile Pro Thr Ser  His Leu Leu Phe Lys  Leu Pro Gln
    2990                 2995                 3000

Glu Leu  Leu Lys Pro Arg Ser  Gln Phe Ala Val Asp  Met Gln Thr
    3005                 3010                 3015

Thr Ser  Ser Arg Gly Leu Val  Phe His Thr Gly Thr  Lys Asn Ser
    3020                 3025                 3030

Phe Met  Ala Leu Tyr Leu Ser  Lys Gly Arg Leu Val  Phe Ala Leu
    3035                 3040                 3045

Gly Thr  Asp Gly Lys Lys Leu  Arg Ile Lys Ser Lys  Glu Lys Cys
    3050                 3055                 3060

Asn Asp  Gly Lys Trp His Thr  Val Val Phe Gly His  Asp Gly Glu
    3065                 3070                 3075

Lys Gly  Arg Leu Val Val Asp  Gly Leu Arg Ala Arg  Glu Gly Ser
    3080                 3085                 3090

Leu Pro  Gly Asn Ser Thr Ile  Ser Ile Arg Ala Pro  Val Tyr Leu
    3095                 3100                 3105

Gly Ser  Pro Pro Ser Gly Lys  Pro Lys Ser Leu Pro  Thr Asn Ser
    3110                 3115                 3120

Phe Val  Gly Cys Leu Lys Asn  Phe Gln Leu Asp Ser  Lys Pro Leu
    3125                 3130                 3135

Tyr Thr  Pro Ser Ser Ser Phe  Gly Val Ser Ser Cys  Leu Gly Gly
    3140                 3145                 3150

Pro Leu  Glu Lys Gly Ile Tyr  Phe Ser Glu Glu Gly  Gly His Val
    3155                 3160                 3165

Val Leu  Ala His Ser Val Leu  Leu Gly Pro Glu Phe  Lys Leu Val
    3170                 3175                 3180

Phe Ser  Ile Arg Pro Arg Ser  Leu Thr Gly Ile Leu  Ile His Ile
    3185                 3190                 3195

Gly Ser  Gln Pro Gly Lys His  Leu Cys Val Tyr Leu  Glu Ala Gly
    3200                 3205                 3210

Lys Val  Thr Ala Ser Met Asp  Ser Gly Ala Gly Gly  Thr Ser Thr
    3215                 3220                 3225
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```
Ser Val  Thr Pro Lys Gln Ser  Leu Cys Asp Gly Gln  Trp His Ser
    3230                3235                3240

Val Ala  Val Thr Ile Lys Gln  His Ile Leu His Leu  Glu Leu Asp
    3245                3250                3255

Thr Asp  Ser Ser Tyr Thr Ala  Gly Gln Ile Pro Phe  Pro Pro Ala
    3260                3265                3270

Ser Thr  Gln Glu Pro Leu His  Leu Gly Gly Ala Pro  Ala Asn Leu
    3275                3280                3285

Thr Thr  Leu Arg Ile Pro Val  Trp Lys Ser Phe Phe  Gly Cys Leu
    3290                3295                3300

Arg Asn  Ile His Val Asn His  Ile Pro Val Pro Val  Thr Glu Ala
    3305                3310                3315

Leu Glu  Val Gln Gly Pro Val  Ser Leu Asn Gly Cys  Pro Asp Gln
    3320                3325                3330
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<210> SEQ ID NO 5
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

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<211> LENGTH: 1724
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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Gln Arg Val Pro Phe Leu Gln Pro Pro Gly Gln Ser Gln Leu Gln Ala
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Ser Tyr Val Glu Phe Arg Pro Ser Gln Gly Cys Ser Pro Gly Tyr Tyr
    50                  55                  60

Arg Asp His Lys Gly Leu Tyr Thr Gly Arg Cys Val Pro Cys Asn Cys
65                  70                  75                  80

Asn Gly His Ser Asn Gln Cys Gln Asp Gly Ser Gly Ile Cys Val Asn
                85                  90                  95

Cys Gln His Asn Thr Ala Gly Glu His Cys Glu Arg Cys Gln Glu Gly
            100                 105                 110

Tyr Tyr Gly Asn Ala Val His Gly Ser Cys Arg Ala Cys Pro Cys Pro
        115                 120                 125

His Thr Asn Ser Phe Ala Thr Gly Cys Val Val Asn Gly Gly Asp Val
    130                 135                 140

Arg Cys Ser Cys Lys Ala Gly Tyr Thr Gly Thr Gln Cys Glu Arg Cys
145                 150                 155                 160

Ala Pro Gly Tyr Phe Gly Asn Pro Gln Lys Phe Gly Gly Ser Cys Gln
                165                 170                 175

Pro Cys Ser Cys Asn Ser Asn Gly Gln Leu Gly Ser Cys His Pro Leu
            180                 185                 190

Thr Gly Asp Cys Ile Asn Gln Glu Pro Lys Asp Ser Ser Pro Ala Glu
        195                 200                 205

Glu Cys Asp Asp Cys Asp Ser Cys Val Met Thr Leu Leu Asn Asp Leu
    210                 215                 220

Ala Thr Met Gly Glu Gln Leu Arg Leu Val Lys Ser Gln Leu Gln Gly
225                 230                 235                 240

Leu Ser Ala Ser Ala Gly Leu Leu Glu Gln Met Arg His Met Glu Thr
                245                 250                 255

Gln Ala Lys Asp Leu Arg Asn Gln Leu Leu Asn Tyr Arg Ser Ala Ile
            260                 265                 270

Ser Asn His Gly Ser Lys Ile Glu Gly Leu Glu Arg Glu Leu Thr Asp
        275                 280                 285

Leu Asn Gln Glu Phe Glu Thr Leu Gln Glu Lys Ala Gln Val Asn Ser
    290                 295                 300

Arg Lys Ala Gln Thr Leu Asn Asn Asn Val Asn Arg Ala Thr Gln Ser
305                 310                 315                 320

Ala Lys Glu Leu Asp Val Lys Ile Lys Asn Val Ile Arg Asn Val His
                325                 330                 335

Ile Leu Leu Lys Gln Ile Ser Gly Thr Asp Gly Glu Gly Asn Asn Val
            340                 345                 350

Pro Ser Gly Asp Phe Ser Arg Glu Trp Ala Glu Ala Gln Arg Met Met
        355                 360                 365

Arg Glu Leu Arg Asn Arg Asn Phe Gly Lys His Leu Arg Glu Ala Glu
    370                 375                 380

Ala Asp Lys Arg Glu Ser Gln Leu Leu Leu Asn Arg Ile Arg Thr Trp
385                 390                 395                 400

Gln Lys Thr His Gln Gly Glu Asn Asn Gly Leu Ala Asn Ser Ile Arg
                405                 410                 415

Asp Ser Leu Asn Glu Tyr Glu Ala Lys Leu Ser Asp Leu Arg Ala Arg
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Leu Gln Glu Ala Ala Ala Gln Ala Lys Gln Ala Asn Gly Leu Asn Gln
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Glu Asn Glu Arg Ala Leu Gly Ala Ile Gln Arg Gln Val Lys Glu Ile
    450                 455                 460

Asn Ser Leu Gln Ser Asp Phe Thr Lys Tyr Leu Thr Thr Ala Asp Ser
465                 470                 475                 480

Ser Leu Leu Gln Thr Asn Ile Ala Leu Gln Leu Met Glu Lys Ser Gln
                485                 490                 495

Lys Glu Tyr Glu Lys Leu Ala Ala Ser Leu Asn Glu Ala Arg Gln Glu
            500                 505                 510

Leu Ser Asp Lys Val Arg Glu Leu Ser Arg Ser Ala Gly Lys Thr Ser
        515                 520                 525

Leu Val Glu Glu Ala Glu Lys His Ala Arg Ser Leu Gln Glu Leu Ala
    530                 535                 540

Lys Gln Leu Glu Glu Ile Lys Arg Asn Ala Ser Gly Asp Glu Leu Val
545                 550                 555                 560

Arg Cys Ala Val Asp Ala Ala Thr Ala Tyr Glu Asn Ile Leu Asn Ala
                565                 570                 575

Ile Lys Ala Ala Glu Asp Ala Ala Asn Arg Ala Ala Ser Ala Ser Glu
            580                 585                 590

Ser Ala Leu Gln Thr Val Ile Lys Glu Asp Leu Pro Arg Lys Ala Lys
        595                 600                 605

Thr Leu Ser Ser Asn Ser Asp Lys Leu Leu Asn Glu Ala Lys Met Thr
    610                 615                 620

Gln Lys Lys Leu Lys Gln Glu Val Ser Pro Ala Leu Asn Asn Leu Gln
625                 630                 635                 640

Gln Thr Leu Asn Ile Val Thr Val Gln Lys Glu Val Ile Asp Thr Asn
                645                 650                 655

Leu Thr Thr Leu Arg Asp Gly Leu His Gly Ile Gln Arg Gly Asp Ile
            660                 665                 670

Asp Ala Met Ile Ser Ser Ala Lys Ser Met Val Arg Lys Ala Asn Asp
        675                 680                 685

Ile Thr Asp Glu Val Leu Asp Gly Leu Asn Pro Ile Gln Thr Asp Val
    690                 695                 700

Glu Arg Ile Lys Asp Thr Tyr Gly Arg Thr Gln Asn Glu Asp Phe Lys
705                 710                 715                 720

Lys Ala Leu Thr Asp Ala Asp Asn Ser Val Asn Lys Leu Thr Asn Lys
                725                 730                 735

Leu Pro Asp Leu Trp Arg Lys Ile Glu Ser Ile Asn Gln Gln Leu Leu
            740                 745                 750

Pro Leu Gly Asn Ile Ser Asp Asn Met Asp Arg Ile Arg Glu Leu Ile
        755                 760                 765

Gln Gln Ala Arg Asp Ala Ala Ser Lys Val Ala Val Pro Met Arg Phe
    770                 775                 780

Asn Gly Lys Ser Gly Val Glu Val Arg Leu Pro Asn Asp Leu Glu Asp
785                 790                 795                 800

Leu Lys Gly Tyr Thr Ser Leu Ser Leu Phe Leu Gln Arg Pro Asn Ser
                805                 810                 815

Arg Glu Asn Gly Gly Thr Glu Asn Met Phe Val Met Tyr Leu Gly Asn
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Lys Asp Ala Ser Arg Asp Tyr Ile Gly Met Ala Val Val Asp Gly Gln
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Leu Thr Cys Val Tyr Asn Leu Gly Asp Arg Glu Ala Glu Leu Gln Val
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Asp Gln Ile Leu Thr Lys Ser Glu Thr Lys Glu Ala Val Met Asp Arg
865                 870                 875                 880

Val Lys Phe Gln Arg Ile Tyr Gln Phe Ala Arg Leu Asn Tyr Thr Lys
                885                 890                 895

Gly Ala Thr Ser Ser Lys Pro Glu Thr Pro Gly Val Tyr Asp Met Asp
            900                 905                 910

Gly Arg Asn Ser Asn Thr Leu Leu Asn Leu Asp Pro Glu Asn Val Val
        915                 920                 925

Phe Tyr Val Gly Gly Tyr Pro Pro Asp Phe Lys Leu Pro Ser Arg Leu
    930                 935                 940

Ser Phe Pro Pro Tyr Lys Gly Cys Ile Glu Leu Asp Asp Leu Asn Glu
945                 950                 955                 960

Asn Val Leu Ser Leu Tyr Asn Phe Lys Lys Thr Phe Asn Leu Asn Thr
                965                 970                 975

Thr Glu Val Glu Pro Cys Arg Arg Arg Lys Glu Glu Ser Asp Lys Asn
            980                 985                 990

Tyr Phe Glu Gly Thr Gly Tyr Ala  Arg Val Pro Thr Gln  Pro His Ala
        995                 1000                1005

Pro Ile  Pro Thr Phe Gly Gln  Thr Ile Gln Thr Thr  Val Asp Arg
    1010                1015                1020

Gly Leu  Leu Phe Phe Ala Glu  Asn Gly Asp Arg Phe  Ile Ser Leu
    1025                1030                1035

Asn Ile  Glu Asp Gly Lys Leu  Met Val Arg Tyr Lys  Leu Asn Ser
    1040                1045                1050

Glu Leu  Pro Lys Glu Arg Gly  Val Gly Asp Ala Ile  Asn Asn Gly
    1055                1060                1065

Arg Asp  His Ser Ile Gln Ile  Lys Ile Gly Lys Leu  Gln Lys Arg
    1070                1075                1080

Met Trp  Ile Asn Val Asp Val  Gln Asn Thr Ile Ile  Asp Gly Glu
    1085                1090                1095

Val Phe  Asp Phe Ser Thr Tyr  Tyr Leu Gly Gly Ile  Pro Ile Ala
    1100                1105                1110

Ile Arg  Glu Arg Phe Asn Ile  Ser Thr Pro Ala Phe  Arg Gly Cys
    1115                1120                1125

Met Lys  Asn Leu Lys Lys Thr  Ser Gly Val Val Arg  Leu Asn Asp
    1130                1135                1140

Thr Val  Gly Val Thr Lys Lys  Cys Ser Glu Asp Trp  Lys Leu Val
    1145                1150                1155

Arg Ser  Ala Ser Phe Ser Arg  Gly Gly Gln Leu Ser  Phe Thr Asp
    1160                1165                1170

Leu Gly  Leu Pro Pro Thr Asp  His Leu Gln Ala Ser  Phe Gly Phe
    1175                1180                1185

Gln Thr  Phe Gln Pro Ser Gly  Ile Leu Leu Asp His  Gln Thr Trp
    1190                1195                1200

Thr Arg  Asn Leu Gln Val Thr  Leu Glu Asp Gly Tyr  Ile Glu Leu
    1205                1210                1215
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Ser Thr  Ser Asp Ser Gly Ser  Pro Ile Phe Lys Ser  Pro Gln Thr
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Tyr Met  Asp Gly Leu Leu His  Tyr Val Ser Val Ile  Ser Asp Asn
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Ser Gly  Leu Arg Leu Leu Ile  Asp Asp Gln Leu Leu  Arg Asn Ser
    1250                 1255                 1260

Lys Arg  Leu Lys His Ile Ser  Ser Ser Arg Gln Ser  Leu Arg Leu
    1265                 1270                 1275

Gly Gly  Ser Asn Phe Glu Gly  Cys Ile Ser Asn Val  Phe Val Gln
    1280                 1285                 1290

Arg Leu  Ser Leu Ser Pro Glu  Val Leu Asp Leu Thr  Ser Asn Ser
    1295                 1300                 1305

Leu Lys  Arg Asp Val Ser Leu  Gly Gly Cys Ser Leu  Asn Lys Pro
    1310                 1315                 1320

Pro Phe  Leu Met Leu Leu Lys  Gly Ser Thr Arg Phe  Asn Lys Thr
    1325                 1330                 1335

Lys Thr  Phe Arg Ile Asn Gln  Leu Leu Gln Asp Thr  Pro Val Ala
    1340                 1345                 1350

Ser Pro  Arg Ser Val Lys Val  Trp Gln Asp Ala Cys  Ser Pro Leu
    1355                 1360                 1365

Pro Lys  Thr Gln Ala Asn His  Gly Ala Leu Gln Phe  Gly Asp Ile
    1370                 1375                 1380

Pro Thr  Ser His Leu Leu Phe  Lys Leu Pro Gln Glu  Leu Leu Lys
    1385                 1390                 1395

Pro Arg  Ser Gln Phe Ala Val  Asp Met Gln Thr Thr  Ser Ser Arg
    1400                 1405                 1410

Gly Leu  Val Phe His Thr Gly  Thr Lys Asn Ser Phe  Met Ala Leu
    1415                 1420                 1425

Tyr Leu  Ser Lys Gly Arg Leu  Val Phe Ala Leu Gly  Thr Asp Gly
    1430                 1435                 1440

Lys Lys  Leu Arg Ile Lys Ser  Lys Glu Lys Cys Asn  Asp Gly Lys
    1445                 1450                 1455

Trp His  Thr Val Val Phe Gly  His Asp Gly Glu Lys  Gly Arg Leu
    1460                 1465                 1470

Val Val  Asp Gly Leu Arg Ala  Arg Glu Gly Ser Leu  Pro Gly Asn
    1475                 1480                 1485

Ser Thr  Ile Ser Ile Arg Ala  Pro Val Tyr Leu Gly  Ser Pro Pro
    1490                 1495                 1500

Ser Gly  Lys Pro Lys Ser Leu  Pro Thr Asn Ser Phe  Val Gly Cys
    1505                 1510                 1515

Leu Lys  Asn Phe Gln Leu Asp  Ser Lys Pro Leu Tyr  Thr Pro Ser
    1520                 1525                 1530

Ser Ser  Phe Gly Val Ser Ser  Cys Leu Gly Gly Pro  Leu Glu Lys
    1535                 1540                 1545

Gly Ile  Tyr Phe Ser Glu Glu  Gly Gly His Val Val  Leu Ala His
    1550                 1555                 1560

Ser Val  Leu Leu Gly Pro Glu  Phe Lys Leu Val Phe  Ser Ile Arg
    1565                 1570                 1575

Pro Arg  Ser Leu Thr Gly Ile  Leu Ile His Ile Gly  Ser Gln Pro
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Gly Lys  His Leu Cys Val Tyr  Leu Glu Ala Gly Lys  Val Thr Ala
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Ser Met  Asp Ser Gly Ala Gly  Gly Thr Ser Thr Ser  Val Thr Pro
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Lys Gln  Ser Leu Cys Asp Gly  Gln Trp His Ser Val  Ala Val Thr
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Ile Lys  Gln His Ile Leu His  Leu Glu Leu Asp Thr  Asp Ser Ser
    1640                1645                1650

Tyr Thr  Ala Gly Gln Ile Pro  Phe Pro Pro Ala Ser  Thr Gln Glu
    1655                1660                1665

Pro Leu  His Leu Gly Gly Ala  Pro Ala Asn Leu Thr  Thr Leu Arg
    1670                1675                1680

Ile Pro  Val Trp Lys Ser Phe  Phe Gly Cys Leu Arg  Asn Ile His
    1685                1690                1695

Val Asn  His Ile Pro Val Pro  Val Thr Glu Ala Leu  Glu Val Gln
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Gly Pro  Val Ser Leu Asn Gly  Cys Pro Asp Gln
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<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

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<210> SEQ ID NO 8
<211> LENGTH: 1725
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 8

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Ile Phe Gly Ala Ala Leu Gly Gln Cys Leu Gly Tyr Gly Ser Glu Gln
            20                  25                  30

Gln Arg Val Ala Phe Leu Gln His Pro Gly Gln Asn His Leu Gln Ala
        35                  40                  45

Ser Tyr Met Glu Leu Arg Pro Ser Gln Gly Cys Arg Pro Gly Tyr Tyr
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    50                  55                  60

Arg Asp Ile Lys Ser Phe Pro Ala Gly Arg Ser Val Pro Cys Asn Cys
65                  70                  75                  80

Asn Gly His Ser Asn Arg Cys Gln Asp Gly Ser Gly Val Cys Ile Asn
                85                  90                  95

Cys Gln His Asn Thr Ala Gly Glu His Cys Glu Arg Cys Lys Arg Gly
            100                 105                 110

Tyr Tyr Gly Ser Ala Ile His Gly Ser Cys Arg Val Cys Pro Cys Pro
        115                 120                 125

His Thr Asn Ser Phe Ala Thr Gly Cys Ala Val Asp Gly Gly Ala Val
    130                 135                 140

Arg Cys Ala Cys Lys Pro Gly Tyr Thr Gly Ala Gln Cys Glu Arg Cys
145                 150                 155                 160

Ala Pro Gly Tyr Phe Gly Asn Pro Gln Lys Phe Gly Gly Ser Cys Gln
                165                 170                 175

Pro Cys Asn Cys Asn Ser Asn Gly Gln Phe Gly Thr Cys Asp Pro Leu
            180                 185                 190

Thr Gly Asp Cys Val Ser Gln Glu Pro Lys Asp Gly Ser Pro Ala Glu
        195                 200                 205

Glu Cys Asp Asp Cys Asp Ser Cys Val Met Thr Leu Leu Asn Asp Leu
    210                 215                 220

Val Pro Met Gly Glu Glu Leu Ala Leu Val Lys Ser Lys Leu Gln Gly
225                 230                 235                 240

Leu Ser Val Asn Thr Gly Ser Leu Glu Gln Ile Arg His Val Glu Met
                245                 250                 255

Gln Ala Lys Asp Leu Arg Asn Gln Leu Leu Gly Phe Arg Ser Ala Ile
            260                 265                 270

Ser Ser His Gly Ser Gln Met Asp Gly Leu Glu Lys Glu Leu Ser His
        275                 280                 285

Leu Tyr Gln Glu Phe Glu Thr Leu Gln Glu Lys Ala Gln Val Asn Ser
    290                 295                 300

Arg Lys Ala Gln Thr Leu Tyr Asn Asn Ile Asp Thr Thr Ile Gln Asn
305                 310                 315                 320

Ala Lys Glu Leu Asp Met Lys Ile Lys Asn Ile Leu Thr Asn Val His
                325                 330                 335

Ile Leu Leu Lys Gln Ile Ala Arg Pro Gly Gly Glu Gly Met Asp Leu
            340                 345                 350

Pro Val Gly Asp Trp Ser Arg Glu Ser Ala Glu Ala Gln Arg Met Leu
        355                 360                 365

Arg Glu Leu Arg Gly Arg Asp Phe Lys Lys His Leu Gln Glu Ala Glu
    370                 375                 380

Ala Gln Lys Met Glu Ala Gln Leu Leu Leu Asn Arg Ile Arg Thr Trp
385                 390                 395                 400

Leu Glu Ser His Gln Val Glu Asn Asn Gly Leu Leu Lys Asn Ile Arg
                405                 410                 415

Asp Ser Leu Asn Asp Tyr Glu Ala Lys Leu Gln Asp Leu Arg Ser Val
            420                 425                 430

Leu Gln Glu Ala Ala Ala Gln Gly Lys Gln Ala Thr Gly Leu Asn His
        435                 440                 445

Glu Asn Glu Gly Val Leu Gly Ala Ile Gln Arg Gln Met Lys Glu Met
    450                 455                 460
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Asp Ser Leu Lys Lys Tyr Leu Thr Glu His Leu Ala Thr Ala Asp Ala
465                 470                 475                 480

Ser Leu Leu Gln Thr Asn Ser Leu Leu Gln Arg Met Asp Thr Ser Gln
                485                 490                 495

Lys Glu Tyr Glu Ser Leu Ala Ala Ala Leu Asn Gly Ala Arg Gln Glu
            500                 505                 510

Leu Asn Asp Gln Val Arg Glu Leu Ser Arg Ser Gly Gly Lys Ala Pro
        515                 520                 525

Leu Val Ala Glu Ala Glu Lys His Ala Gln Ser Leu Gln Glu Leu Ala
    530                 535                 540

Lys Gln Leu Glu Glu Ile Lys Arg Asn Thr Ser Gly Asp Glu Ser Val
545                 550                 555                 560

Arg Cys Val Val Asp Ala Ala Thr Ala Tyr Glu Ser Ile Leu Asn Ala
                565                 570                 575

Ile Arg Ala Ala Glu Asp Ala Ala Gly Lys Ala Asp Ser Ala Ser Glu
            580                 585                 590

Ser Ala Phe Gln Thr Val Ile Lys Glu Asp Leu Pro Arg Arg Ala Lys
        595                 600                 605

Thr Leu Ser Ser Asp Ser Glu Glu Leu Leu Asn Glu Ala Lys Met Thr
    610                 615                 620

Arg Lys Arg Leu Gln Gln Glu Ile Asn Pro Ala Leu Asn Ser Leu Gln
625                 630                 635                 640

Gln Thr Leu Lys Thr Val Ser Val Gln Lys Asp Leu Leu Asp Ala Asn
                645                 650                 655

Val Thr Ala Val Arg Asn Asp Leu Arg Gly Ile Gln Arg Gly Asp Ile
            660                 665                 670

Asp Ser Val Val Ser Gly Ala Lys Ser Met Val Arg Lys Ala Asn Gly
        675                 680                 685

Ile Thr Ser Glu Val Leu Asp Gly Leu Ser Pro Ile Gln Thr Asp Leu
    690                 695                 700

Gly Arg Ile Lys Asp Ser Tyr Gly Ser Thr Arg His Glu Asp Phe Asn
705                 710                 715                 720

Lys Ala Leu Ile Asp Ala Asn Asn Ser Val Lys Lys Leu Thr Lys Lys
                725                 730                 735

Leu Pro Asp Leu Phe Val Lys Ile Glu Ser Ile Asn Gln Gln Leu Leu
            740                 745                 750

Pro Leu Gly Asn Ile Ser Asp Asn Val Asp Arg Ile Arg Glu Leu Ile
        755                 760                 765

Thr Gln Ala Arg Asp Ala Ala Asn Lys Val Ala Ile Pro Met Arg Phe
    770                 775                 780

Asn Gly Lys Ser Gly Val Glu Val Arg Leu Pro Asn Asp Leu Glu Asp
785                 790                 795                 800

Leu Lys Gly Tyr Thr Ser Leu Ser Leu Phe Leu Gln Arg Pro Asp Leu
                805                 810                 815

Arg Glu Asn Gly Gly Thr Glu Asp Met Phe Val Met Tyr Leu Gly Asn
            820                 825                 830

Lys Asp Ala Ser Lys Asp Tyr Ile Gly Met Ala Val Val Asp Gly Gln
        835                 840                 845

Leu Thr Cys Val Tyr Asn Leu Gly Asp Arg Glu Ala Glu Val Gln Ile
    850                 855                 860
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Asp Gln Val Leu Thr Glu Ser Glu Ser Gln Glu Ala Val Met Asp Arg
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Val Lys Phe Gln Arg Ile Tyr Gln Phe Ala Lys Leu Asn Tyr Thr Lys
                885                 890                 895

Glu Ala Thr Ser Asn Lys Pro Lys Ala Pro Ala Val Tyr Asp Leu Glu
            900                 905                 910

Gly Gly Ser Ser Asn Thr Leu Leu Asn Leu Asp Pro Glu Asp Ala Val
        915                 920                 925

Phe Tyr Val Gly Gly Tyr Pro Pro Asp Phe Glu Leu Pro Ser Arg Leu
    930                 935                 940

Arg Phe Pro Pro Tyr Lys Gly Cys Ile Glu Leu Asp Asp Leu Asn Glu
945                 950                 955                 960

Asn Val Leu Ser Leu Tyr Asn Phe Lys Thr Thr Phe Asn Leu Asn Thr
                965                 970                 975

Thr Glu Val Glu Pro Cys Arg Arg Arg Lys Glu Glu Ser Asp Lys Asn
            980                 985                 990

Tyr Phe Glu Gly Thr Gly Tyr Ala  Arg Ile Pro Thr Gln  Pro Asn Ala
        995                 1000                1005

Pro Phe  Pro Asn Phe Ile Gln  Thr Ile Gln Thr Thr  Val Asp Arg
    1010                1015                1020

Gly Leu  Leu Phe Phe Ala Glu  Asn Gln Asp Asn Phe  Ile Ser Leu
    1025                1030                1035

Asn Ile  Glu Asp Gly Asn Leu  Met Val Arg Tyr Lys  Leu Asn Ser
    1040                1045                1050

Glu Pro  Pro Lys Glu Lys Gly  Ile Arg Asp Thr Ile  Asn Asp Gly
    1055                1060                1065

Lys Asp  His Ser Ile Leu Ile  Thr Ile Gly Lys Leu  Gln Lys Arg
    1070                1075                1080

Met Trp  Ile Asn Val Asn Glu  Arg Ser Val Arg Ile  Glu Gly Glu
    1085                1090                1095

Ile Phe  Asp Phe Ser Thr Tyr  Tyr Leu Gly Gly Ile  Pro Ile Ala
    1100                1105                1110

Ile Arg  Glu Arg Phe Asn Ile  Ser Thr Pro Ala Phe  Gln Gly Cys
    1115                1120                1125

Met Lys  Asn Leu Lys Lys Thr  Ser Gly Val Val Arg  Leu Asn Asp
    1130                1135                1140

Thr Val  Gly Val Thr Lys Lys  Cys Ser Glu Asp Trp  Lys Leu Val
    1145                1150                1155

Arg Thr  Ala Ser Phe Ser Arg  Gly Gly Gln Met Ser  Phe Thr Asn
    1160                1165                1170

Leu Asp  Val Pro Ser Thr Asp  Arg Phe Gln Leu Ser  Phe Gly Phe
    1175                1180                1185

Gln Thr  Phe Gln Pro Ser Gly  Thr Leu Leu Asn His  Gln Thr Arg
    1190                1195                1200

Thr Ser  Ser Leu Leu Val Thr  Leu Glu Asp Gly His  Ile Glu Leu
    1205                1210                1215

Ser Thr  Arg Asp Ser Asn Ile  Pro Ile Phe Lys Ser  Pro Gly Thr
    1220                1225                1230

Tyr Met  Asp Gly Leu Leu His  His Val Ser Val Ile  Ser Asp Thr
    1235                1240                1245

Ser Gly  Leu Arg Leu Leu Ile  Asp Asp Gln Val Leu  Arg Arg Asn
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Gln Arg  Leu Pro Ser Phe Ser  Asn Ala Gln Gln Ser  Leu Arg Leu
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Gly Gly  Gly His Phe Glu Gly  Cys Ile Ser Asn Val  Leu Val Gln
    1280                1285                1290

Arg Phe  Ser Gln Ser Pro Glu  Val Leu Asp Leu Ala  Ser Lys Ser
    1295                1300                1305

Thr Lys  Lys Asp Ala Ser Leu  Gly Gly Cys Ser Leu  Asn Lys Pro
    1310                1315                1320

Pro Phe  Leu Met Leu Phe Lys  Ser Pro Lys Arg Phe  Asn Lys Gly
    1325                1330                1335

Arg Ile  Phe Asn Val Asn Gln  Leu Met Gln Asp Ala  Pro Gln Ala
    1340                1345                1350

Thr Arg  Ser Thr Glu Ala Trp  Gln Asp Gly Arg Ser  Cys Leu Pro
    1355                1360                1365

Pro Leu  Asn Thr Lys Ala Ser  His Arg Ala Leu Gln  Phe Gly Asp
    1370                1375                1380

Ser Pro  Thr Ser His Leu Leu  Leu Lys Leu Pro Gln  Glu Leu Leu
    1385                1390                1395

Lys Pro  Arg Ser Gln Phe Ser  Leu Asp Ile Gln Thr  Thr Ser Pro
    1400                1405                1410

Lys Gly  Leu Val Phe Tyr Ala  Gly Thr Lys Asp Ser  Phe Leu Ala
    1415                1420                1425

Leu Tyr  Val Ala Asp Gly Arg  Val Val Phe Ala Leu  Gly Ala Gly
    1430                1435                1440

Gly Lys  Lys Leu Arg Leu Arg  Ser Lys Glu Arg Tyr  His Asp Gly
    1445                1450                1455

Lys Trp  His Thr Val Val Phe  Gly Leu Asn Gly Gly  Lys Ala Arg
    1460                1465                1470

Leu Val  Val Asp Gly Leu Arg  Ala Gln Glu Gly Ser  Leu Pro Gly
    1475                1480                1485

Asn Ser  Thr Ile Ser Pro Arg  Glu Gln Val Tyr Leu  Gly Leu Pro
    1490                1495                1500

Leu Ser  Arg Lys Pro Lys Ser  Leu Pro Gln His Ser  Phe Val Gly
    1505                1510                1515

Cys Leu  Arg Asp Phe Gln Leu  Asn Ser Lys Pro Leu  Asp Ser Pro
    1520                1525                1530

Ser Ala  Arg Phe Gly Val Ser  Pro Cys Leu Gly Gly  Ser Leu Glu
    1535                1540                1545

Lys Gly  Ile Tyr Phe Ser Gln  Gly Gly Gly His Val  Ile Leu Ala
    1550                1555                1560

Asn Ser  Val Ser Leu Gly Pro  Glu Leu Lys Leu Thr  Phe Ser Ile
    1565                1570                1575

Arg Pro  Arg Ser Leu Thr Gly  Val Leu Ile His Val  Gly Ser Gln
    1580                1585                1590

Ser Gly  Gln Arg Leu Ser Val  Tyr Met Glu Ala Gly  Lys Val Thr
    1595                1600                1605

Thr Ser  Val Ser Ser Asp Ala  Gly Gly Ser Val Thr  Ser Ile Thr
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Pro Lys  Gln Ser Leu Cys Asp  Gly Gln Trp His Ser  Val Ala Val
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Ser Ile  Lys Gln Arg Ile Leu  His Leu Glu Leu Asp  Thr Asp Ser
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Ser Tyr  Thr Val Ala Pro Leu  Ser Phe Ser Pro Asn  Ser Thr Arg
    1655                 1660                 1665

Gly Ser  Leu His Val Gly Gly  Val Pro Asp Lys Leu  Lys Met Leu
    1670                 1675                 1680

Thr Leu  Pro Val Trp Asn Ser  Phe Phe Gly Cys Leu  Lys Asn Ile
    1685                 1690                 1695

Gln Val  Asn His Val Pro Val  Pro Ile Thr Glu Ala  Thr Glu Val
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Gln Gly  Ser Val Ser Leu Asn  Gly Cys Pro Asp His
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<211> LENGTH: 7323
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

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tccagaaaag cacaaacatt atataacaac attgatcaga caatccaaag tgccaaagaa 5940

ctggacatga agattaaaaa catcgttcag aatgtgcaca ttctcctgaa gcagatggcg 6000

aggccaggtg gagaaggcac ggacttgcca gtgggtgact ggtccaggga gctggccgaa 6060

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gctcaacgca tgatgcgaga cctgcgaagc cgagacttta aaaagcacct ccaagaagca 6120

gaggccgaga aaatggaagc ccagctctta ctgcaccgga tcaggacctg gctggaatcc 6180

caccaggtgg agaacaacgg actgctaaag aatattcggg actccttaaa tgattatgaa 6240

gacaaacttc aggacctacg ttccatcctc caggaggcag ctgcccaggc aaagcaggcc 6300

actggcatca accatgaaaa tgaggggttt ctcggagcca tccagagaca aatgaaagaa 6360

atggattccc tgaagaatga cttcaccaag tacctggcca cagccgactc ttccctgctg 6420

cagaccaaca atctactgca gcagatggac aaaagccaga aggaatatga aagcttagct 6480

gctgctttaa atggagcaag acaggaactg agtgacagag tgcgagaact gtccagatcg 6540

ggtggcaaag caccgctggt ggtggaggca gagaagcatg cacagtcttt acaggagctg 6600

gcaaagcagc tggaagagat aaagagaaac accagcgggg atgagctggt gcgttgtgct 6660

gtggatgctg ccacggccta tgagaacatc ctcaatgcca tcagagcagc agaggatgca 6720

gccagcaagg ccaccagtgc ctccaagtct gccttccaaa cagtgataaa ggaagacctt 6780

ccaaaaagag ctaagaccct gagttctgac agcgaggaac tgttaaatga agccaagatg 6840

acacagaaaa ggctacagca agtcagtcca gctctcaaca gcctacaaca aaccctgaag 6900

actgtatcag ttcagaagga cctgctagat gccaacctca ctgttgcccg tgatgatctt 6960

catgggatac agagaggtga tatcgacagt gtggtgatcg gtgcaaagag catggtcagg 7020

gaagccaacg gaataacaag cgaggtcctg gacgggctca accccatcca gacagatttg 7080

ggaaggatta aggacagcta tgagagcgca cggcgtgaag acttcagcaa ggctctggtc 7140

gatgccaata actcagtaaa gaaattaacc aggaagttgc ctgatctttt tatcaagatt 7200

gaaagtatca accaacagtt gctgcccctg gggaacatct ctgacaatgt ggaccgaatc 7260

cgagaactca ttcagcaggc cagagatgct gcaaacaagg tgggtattcc catttggctc 7320

tag 7323

<210> SEQ ID NO 10
<211> LENGTH: 2440
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Met Ser Glu Gly Ile Cys Cys Arg Ala Gly Ala Leu Cys Lys Ser Gly
1 5 10 15

Gln Gln Val Ser Thr Val Val Val Val Asp Pro Pro Asn His Ala Ser
20 25 30

Gly Met Arg Thr Glu Cys Ser Pro Pro Glu His Val His Thr Cys Ile
35 40 45

Lys Glu Pro Gln Asn Gln Leu Phe His Val Ala Tyr Ile Leu Ile Lys
50 55 60

Phe Ala Asn Ser Pro Arg Pro Asp Leu Trp Ile Leu Glu Arg Ser Val
65 70 75 80

Asp Phe Gly Ser Thr Tyr Ser Pro Trp Gln Tyr Phe Ala His Ser Arg
85 90 95

Arg Asp Cys Val Glu Gln Phe Gly Gln Glu Ala Asn Met Ala Ile Thr
100 105 110

Gln Asp Asp Gln Met Leu Cys Val Thr Glu Tyr Ser Arg Ile Val Pro
115 120 125

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Leu Glu Asn Gly Glu Ile Val Val Ser Leu Ile Asn Gly Arg Pro Gly
    130                 135                 140

Ala Lys Lys Phe Ala Phe Ser Asp Thr Leu Arg Glu Phe Thr Lys Ala
145                 150                 155                 160

Thr Asn Ile Arg Leu Arg Phe Leu Arg Thr Asn Thr Leu Leu Gly His
                165                 170                 175

Leu Ile Ser Lys Ala Glu Arg Asp Pro Thr Val Thr Arg Arg Tyr Tyr
            180                 185                 190

Cys Met Glu Ala Asp Asp Ala Leu Phe Ser Val Leu Gln Tyr Tyr Tyr
        195                 200                 205

Ser Ile Lys Asp Ile Ser Val Gly Gly Arg Cys Val Cys Asn Gly His
    210                 215                 220

Ala Glu Ala Cys Ser Ala Asp Asn Pro Glu Lys Gln Phe Arg Cys Glu
225                 230                 235                 240

Cys Gln His His Thr Cys Gly Asp Thr Cys Asn Arg Cys Cys Ala Gly
                245                 250                 255

Tyr Asn Gln Arg Arg Trp Gln Pro Ala Gly Gln Glu Gln His Asn Glu
            260                 265                 270

Cys Glu Ala Cys Asn Cys His Gly His Ala Val Asp Cys Tyr Tyr Asp
        275                 280                 285

Pro Asp Val Glu His Gln Gln Ala Ser Leu Asn Ser Lys Gly Val Tyr
    290                 295                 300

Ala Gly Gly Gly Val Cys Ile Asn Cys Gln His Asn Thr Ala Gly Val
305                 310                 315                 320

Asn Cys Glu Lys Cys Ala Lys Gly Tyr Phe Arg Pro His Gly Val Pro
                325                 330                 335

Val Asp Ala Leu His Gly Cys Ile Pro Cys Ser Cys Asp Pro Glu Arg
            340                 345                 350

Ala Asp Asp Cys Asp Gln Gly Ser Gly His Cys His Cys Lys Pro Asn
        355                 360                 365

Phe Ser Gly Asp Tyr Cys Glu Thr Cys Ala Asp Gly Tyr Tyr Asn Phe
    370                 375                 380

Pro Phe Cys Leu Arg Ile Pro Val Phe Pro Asn Tyr Thr Pro Ser Pro
385                 390                 395                 400

Glu Asp Pro Val Ala Gly Asn Ile Lys Gly Lys Asp Pro Gly Thr Leu
                405                 410                 415

Asp Pro Pro Val Ile Ala Asn Gly Ala Tyr Leu Gly Ala Ser Arg Leu
            420                 425                 430

Glu Gln Gly Ala Thr Gly Gln Gly Ser Pro Ala Glu Arg Val Thr His
        435                 440                 445

Thr Asn Ser Trp Leu Ser Ser Ser Met Pro Met Leu Gln Val Arg Ala
    450                 455                 460

Ala Ile His Glu Ala Lys Cys Tyr Ser Leu Cys Phe Cys Met Tyr Val
465                 470                 475                 480

Glu His Ser Gly Thr Val Pro Pro Ala Leu Gly Ser Gly Tyr Thr Gly
                485                 490                 495

Asp Ser Glu Pro Lys Thr Gly Thr Gln Ala Lys Arg Gly Cys Asp Cys
            500                 505                 510

Asn Leu Glu Gly Val Leu Pro Glu Ile Cys Asp Asp Arg Gly Arg Cys
        515                 520                 525
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Leu Cys Arg Pro Gly Val Glu Gly Pro Gln Cys Asp Ser Cys Arg Ser
    530                 535                 540

Gly Ser Tyr Ser Phe Pro Ile Cys Gln Ala Cys Gln Cys Ser Thr Ile
545                 550                 555                 560

Gly Ser Tyr Pro Val Pro Cys Asp Pro Gly Asn Gly Gln Cys Asp Cys
                565                 570                 575

Leu Pro Gly Ile Thr Gly Arg Gln Cys Asp Arg Cys Leu Ser Gly Ala
            580                 585                 590

Tyr Asp Phe Pro Tyr Cys Gln Gly Lys Glu Ala Gly Ser Met Leu Glu
        595                 600                 605

Ala Arg Ser Ser Ser Glu Trp Val Gln Leu Thr Ser Trp Arg Ser Leu
    610                 615                 620

Gly Tyr Cys Gln Cys Lys Gln His Val Ala Ser Pro Thr Cys Ser Val
625                 630                 635                 640

Cys Lys Pro Leu Tyr Trp Asn Leu Ala Lys Glu Asn Pro Arg Gly Cys
                645                 650                 655

Ser Glu Cys Gln Cys His Glu Ala Gly Thr Leu Ser Gly Ile Gly Glu
            660                 665                 670

Cys Gly Gln Glu Asp Gly Asp Cys Ser Cys Lys Ala His Val Thr Gly
        675                 680                 685

Asp Ala Cys Asp Thr Cys Glu Asp Gly Phe Phe Ser Leu Glu Lys Ser
    690                 695                 700

Asn Tyr Phe Gly Cys Gln Gly Cys Gln Cys Asp Ile Gly Gly Ala Leu
705                 710                 715                 720

Thr Thr Met Cys Ser Gly Pro Ser Gly Val Cys Gln Cys Arg Glu His
                725                 730                 735

Val Glu Gly Lys Gln Cys Gln Arg Pro Glu Asn Asn Tyr Tyr Phe Pro
            740                 745                 750

Asp Leu His His Met Lys Tyr Glu Val Glu Asp Gly Thr Gly Pro Asn
        755                 760                 765

Gly Arg Asn Leu Arg Phe Gly Phe Asp Pro Leu Val Phe Pro Glu Phe
    770                 775                 780

Ser Trp Arg Gly Tyr Ala Pro Met Thr Ser Val Gln Val Tyr Met Ser
785                 790                 795                 800

Glu Cys Val Cys Pro Leu His Cys Met Leu Phe Trp Gly Thr Phe Gln
                805                 810                 815

Asn Glu Val Arg Val Arg Leu Ser Val Arg Gln Ser Ser Leu Ser Leu
            820                 825                 830

Phe Arg Ile Val Leu Arg Tyr Ile Ser Pro Gly Thr Glu Ala Ile Ser
        835                 840                 845

Gly Arg Ile Thr Leu Tyr Ser Ser Gln Gly Asp Ser Asp Ala Leu Gln
    850                 855                 860

Ser Arg Lys Ile Thr Phe Pro Pro Ser Lys Glu Pro Ala Phe Val Thr
865                 870                 875                 880

Val Pro Gly Asn Gly Phe Ala Gly Pro Phe Ser Ile Thr Pro Gly Thr
                885                 890                 895

Trp Ile Ala Cys Ile Gln Val Glu Gly Val Leu Leu Asp Tyr Leu Val
            900                 905                 910

Leu Leu Pro Arg Asp Tyr Tyr Glu Ala Phe Thr Leu Gln Val Pro Val
        915                 920                 925

Thr Glu Pro Cys Ala His Thr Gly Ser Pro Gln Asp Asn Cys Leu Leu
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    930                 935                 940

Tyr Gln His Leu Pro Leu Thr Ala Phe Ser Cys Thr Leu Ala Cys Glu
945                 950                 955                 960

Ala Arg His Phe Leu Leu Asp Gly Glu Leu Arg Pro Leu Ala Met Arg
                965                 970                 975

Gln Pro Thr Pro Thr His Pro Ala Met Val Asp Leu Ser Gly Arg Glu
            980                 985                 990

Val Glu Leu Gln Leu Arg Leu Arg  Val Pro Gln Val Gly  His Tyr Val
        995                 1000                1005

Val Leu  Leu Glu Tyr Ala Thr  Glu Val Glu Gln Leu  Phe Val Val
    1010                1015                1020

Asp Val  Asn Leu Lys Ser Ser  Gly Ser Ala Leu Ala  Gly Gln Val
    1025                1030                1035

Asn Ile  Tyr Ser Cys Lys Tyr  Ser Ile Pro Cys Arg  Ser Val Val
    1040                1045                1050

Ile Asp  Ser Leu Ser Arg Thr  Ala Val His Glu Leu  Leu Ala Asp
    1055                1060                1065

Ala Asp  Ile Gln Leu Lys Ala  His Met Ala His Phe  Leu Leu Tyr
    1070                1075                1080

His Ile  Cys Ile Ile Pro Ala  Glu Glu Phe Ser Thr  Glu Tyr Leu
    1085                1090                1095

Arg Pro  Gln Val His Cys Ile  Ala Ser Tyr Arg Gln  His Ala Asn
    1100                1105                1110

Pro Ser  Ala Ser Cys Val Ser  Leu Ala His Glu Thr  Pro Pro Thr
    1115                1120                1125

Ala Ser  Ile Leu Asp Ala Thr  Ser Arg Gly Leu Phe  Ser Ala Leu
    1130                1135                1140

Pro His  Glu Pro Ser Ser Pro  Ala Asp Gly Val Thr  Leu Lys Ala
    1145                1150                1155

Pro Gln  Ser Gln Val Thr Leu  Lys Gly Leu Ile Pro  His Leu Gly
    1160                1165                1170

Arg His  Val Phe Val Ile His  Phe Tyr Gln Ala Glu  His Pro Gly
    1175                1180                1185

Phe Pro  Thr Glu Val Ile Val  Asn Gly Gly Arg Gln  Trp Ser Gly
    1190                1195                1200

Ser Phe  Leu Ala Ser Phe Cys  Pro His Leu Leu Gly  Cys Arg Asp
    1205                1210                1215

Gln Val  Ile Ser Asp Gly Gln  Val Glu Phe Asp Ile  Ser Glu Ala
    1220                1225                1230

Glu Val  Ala Val Thr Val Lys  Ile Pro Asp Gly Lys  Ser Leu Thr
    1235                1240                1245

Leu Val  Arg Val Leu Val Val  Pro Ala Glu Asn Tyr  Asp Tyr Gln
    1250                1255                1260

Ile Leu  His Lys Thr Thr Val  Asp Lys Ser Ser Glu  Phe Ile Ser
    1265                1270                1275

Ser Cys  Gly Gly Asp Ser Phe  Tyr Ile Asp Pro Gln  Ala Ala Ser
    1280                1285                1290

Gly Phe  Cys Lys Asn Ser Ala  Arg Ser Leu Val Ala  Phe Tyr His
    1295                1300                1305

Asn Gly  Ala Ile Pro Cys Glu  Cys Asp Pro Ala Gly  Thr Ala Gly
    1310                1315                1320
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His His  Cys Ser Pro Glu Gly  Gly Gln Cys Pro Cys  Arg Pro Asn
    1325                1330                1335

Val Ile  Gly Arg Gln Cys Ser  Arg Cys Ala Thr Gly  Tyr Tyr Gly
    1340                1345                1350

Phe Pro  Tyr Cys Lys Pro Cys  Asn Cys Gly Arg Arg  Leu Cys Glu
    1355                1360                1365

Glu Val  Thr Gly Lys Cys Leu  Cys Pro Pro His Thr  Val Arg Pro
    1370                1375                1380

Gln Cys  Glu Val Cys Glu Met  Asn Ser Phe Asn Phe  His Pro Val
    1385                1390                1395

Ala Gly  Cys Asp Val Cys Asn  Cys Ser Arg Lys Gly  Thr Ile Glu
    1400                1405                1410

Ala Ala  Val Ser Glu Cys Asp  Arg Asp Ser Gly Gln  Cys Arg Cys
    1415                1420                1425

Lys Pro  Arg Val Thr Gly Gln  Gln Cys Asp Lys Cys  Ala Pro Gly
    1430                1435                1440

Phe Tyr  Gln Phe Pro Glu Cys  Val Pro Cys Ser Cys  Asn Arg Asp
    1445                1450                1455

Gly Thr  Glu Pro Ser Val Cys  Asp Pro Glu Thr Gly  Ala Cys Met
    1460                1465                1470

Cys Lys  Glu Asn Val Glu Gly  Pro Gln Cys Gln Leu  Cys Arg Glu
    1475                1480                1485

Gly Ser  Phe Tyr Leu Asp Pro  Thr Asn Pro Lys Gly  Cys Thr Lys
    1490                1495                1500

Cys Phe  Cys Phe Gly Val Asn  Thr Asp Cys Gln Ser  Ser His Lys
    1505                1510                1515

Gln Arg  Ala Lys Phe Val Asp  Met Met Gly Trp Arg  Leu Glu Thr
    1520                1525                1530

Ala Asp  Gly Val Asp Val Pro  Val Ser Phe Asn Pro  Gly Ser Asn
    1535                1540                1545

Ser Met  Val Ala Asp Leu Gln  Glu Leu Pro Pro Ser  Val His Ser
    1550                1555                1560

Ala Ser  Trp Val Ala Pro Pro  Ser Tyr Leu Gly Asp  Lys Val Ser
    1565                1570                1575

Ser Tyr  Gly Gly Tyr Leu Thr  Tyr His Ala Lys Ser  Phe Gly Leu
    1580                1585                1590

Pro Gly  Asp Met Val Leu Leu  Gly Lys Gln Pro Asp  Val Gln Leu
    1595                1600                1605

Thr Gly  Gln His Met Ser Leu  Ile His Lys Glu Pro  Ser Asp Pro
    1610                1615                1620

Arg Pro  Asp Arg Leu His His  Gly Arg Val Gln Val  Ile Glu Gly
    1625                1630                1635

Asn Phe  Arg His Glu Gly Ser  Ser Ala Pro Val Ser  Arg Glu Glu
    1640                1645                1650

Leu Met  Thr Val Leu Ser Arg  Leu Glu Arg Leu His  Ile Arg Gly
    1655                1660                1665

Leu His  Phe Thr Glu Thr Gln  Arg Leu Thr Leu Gly  Glu Val Gly
    1670                1675                1680

Leu Glu  Glu Ala Ser Asp Thr  Gly Ser Gly Pro Arg  Ala His Leu
    1685                1690                1695
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Val Glu  Met Cys Ala Cys Pro  Pro Asp Tyr Thr Gly  Asp Ser Cys
    1700                 1705                 1710

Gln Gly  Cys Arg Pro Gly Tyr  Tyr Trp Asp Asn Lys  Ser Leu Pro
    1715                 1720                 1725

Val Gly  Arg Cys Val Pro Cys  Asn Cys Asn Gly His  Ser Asn Arg
    1730                 1735                 1740

Cys Gln  Asp Gly Ser Gly Ile  Cys Ile Asn Cys Gln  His Asn Thr
    1745                 1750                 1755

Ala Gly  Glu His Cys Glu Arg  Cys Gln Ala Gly His  Tyr Gly Asn
    1760                 1765                 1770

Ala Ile  His Gly Ser Cys Arg  Val Cys Pro Cys Pro  His Thr Asn
    1775                 1780                 1785

Ser Phe  Ala Thr Gly Cys Ala  Val Asp Gly Gly Ala  Val Arg Cys
    1790                 1795                 1800

Ala Cys  Lys Pro Gly Tyr Thr  Gly Thr Gln Cys Glu  Arg Cys Ala
    1805                 1810                 1815

Pro Gly  Tyr Phe Gly Asn Pro  Gln Lys Phe Gly Gly  Ser Cys Gln
    1820                 1825                 1830

Pro Cys  Asn Cys Asn Ser Asn  Gly Gln Leu Gly Pro  Cys Asp Pro
    1835                 1840                 1845

Leu Thr  Gly Asp Cys Val Asn  Gln Glu Pro Lys Asp  Gly Ser Pro
    1850                 1855                 1860

Ala Glu  Glu Cys Asp Asp Cys  Asp Ser Cys Val Met  Thr Leu Leu
    1865                 1870                 1875

Asn Asp  Leu Ala Ser Met Gly  Glu Glu Leu Arg Leu  Val Lys Ser
    1880                 1885                 1890

Lys Leu  Gln Gly Leu Ser Val  Ser Thr Gly Ala Leu  Glu Gln Ile
    1895                 1900                 1905

Arg His  Met Glu Thr Gln Ala  Lys Asp Leu Arg Asn  Gln Leu Leu
    1910                 1915                 1920

Gly Phe  Arg Ser Ala Thr Ser  Ser His Gly Ser Lys  Met Asp Asp
    1925                 1930                 1935

Leu Glu  Lys Glu Leu Ser His  Leu Asn Arg Glu Phe  Glu Thr Leu
    1940                 1945                 1950

Gln Glu  Lys Ala Gln Val Asn  Ser Arg Lys Ala Gln  Thr Leu Tyr
    1955                 1960                 1965

Asn Asn  Ile Asp Gln Thr Ile  Gln Ser Ala Lys Glu  Leu Asp Met
    1970                 1975                 1980

Lys Ile  Lys Asn Ile Val Gln  Asn Val His Ile Leu  Leu Lys Gln
    1985                 1990                 1995

Met Ala  Arg Pro Gly Gly Glu  Gly Thr Asp Leu Pro  Val Gly Asp
    2000                 2005                 2010

Trp Ser  Arg Glu Leu Ala Glu  Ala Gln Arg Met Met  Arg Asp Leu
    2015                 2020                 2025

Arg Ser  Arg Asp Phe Lys Lys  His Leu Gln Glu Ala  Glu Ala Glu
    2030                 2035                 2040

Lys Met  Glu Ala Gln Leu Leu  Leu His Arg Ile Arg  Thr Trp Leu
    2045                 2050                 2055

Glu Ser  His Gln Val Glu Asn  Asn Gly Leu Leu Lys  Asn Ile Arg
    2060                 2065                 2070

Asp Ser  Leu Asn Asp Tyr Glu  Asp Lys Leu Gln Asp  Leu Arg Ser
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    2075                2080                2085

Ile Leu  Gln Glu Ala Ala Ala  Gln Ala Lys Gln Ala  Thr Gly Ile
    2090                2095                2100

Asn His  Glu Asn Glu Gly Val  Leu Gly Ala Ile Gln  Arg Gln Met
    2105                2110                2115

Lys Glu  Met Asp Ser Leu Lys  Asn Asp Phe Thr Lys  Tyr Leu Ala
    2120                2125                2130

Thr Ala  Asp Ser Ser Leu Leu  Gln Thr Asn Asn Leu  Leu Gln Gln
    2135                2140                2145

Met Asp  Lys Ser Gln Lys Glu  Tyr Glu Ser Leu Ala  Ala Ala Leu
    2150                2155                2160

Asn Gly  Ala Arg Gln Glu Leu  Ser Asp Arg Val Arg  Glu Leu Ser
    2165                2170                2175

Arg Ser  Gly Gly Lys Ala Pro  Leu Val Val Glu Ala  Glu Lys His
    2180                2185                2190

Ala Gln  Ser Leu Gln Glu Leu  Ala Lys Gln Leu Glu  Glu Ile Lys
    2195                2200                2205

Arg Asn  Thr Ser Gly Asp Glu  Leu Val Arg Cys Ala  Val Asp Ala
    2210                2215                2220

Ala Thr  Ala Tyr Glu Asn Ile  Leu Asn Ala Ile Arg  Ala Ala Glu
    2225                2230                2235

Asp Ala  Ala Ser Lys Ala Thr  Ser Ala Ser Lys Ser  Ala Phe Gln
    2240                2245                2250

Thr Val  Ile Lys Glu Asp Leu  Pro Lys Arg Ala Lys  Thr Leu Ser
    2255                2260                2265

Ser Asp  Ser Glu Glu Leu Leu  Asn Glu Ala Lys Met  Thr Gln Lys
    2270                2275                2280

Arg Leu  Gln Gln Val Ser Pro  Ala Leu Asn Ser Leu  Gln Gln Thr
    2285                2290                2295

Leu Lys  Thr Val Ser Val Gln  Lys Asp Leu Leu Asp  Ala Asn Leu
    2300                2305                2310

Thr Val  Ala Arg Asp Asp Leu  His Gly Ile Gln Arg  Gly Asp Ile
    2315                2320                2325

Asp Ser  Val Val Ile Gly Ala  Lys Ser Met Val Arg  Glu Ala Asn
    2330                2335                2340

Gly Ile  Thr Ser Glu Val Leu  Asp Gly Leu Asn Pro  Ile Gln Thr
    2345                2350                2355

Asp Leu  Gly Arg Ile Lys Asp  Ser Tyr Glu Ser Ala  Arg Arg Glu
    2360                2365                2370

Asp Phe  Ser Lys Ala Leu Val  Asp Ala Asn Asn Ser  Val Lys Lys
    2375                2380                2385

Leu Thr  Arg Lys Leu Pro Asp  Leu Phe Ile Lys Ile  Glu Ser Ile
    2390                2395                2400

Asn Gln  Gln Leu Leu Pro Leu  Gly Asn Ile Ser Asp  Asn Val Asp
    2405                2410                2415

Arg Ile  Arg Glu Leu Ile Gln  Gln Ala Arg Asp Ala  Ala Asn Lys
    2420                2425                2430

Val Gly  Ile Pro Ile Trp Leu
    2435                2440
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<210> SEQ ID NO 11

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<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Cys Ser Pro Leu Pro Lys Thr Gln Ala Asn His Gly Ala Leu Gln Phe
1 5 10 15

Gly Asp Ile Pro Thr Ser His Leu Leu Phe Lys Leu Pro Gln Glu Leu
20 25 30

Leu Lys Pro Arg Ser Gln Phe Ala Val Asp Met Gln Thr Thr Ser Ser
35 40 45

Arg Gly Leu Val Phe His Thr Gly Thr Lys Asn Ser Phe Met Ala Leu
50 55 60

Tyr Leu Ser Lys Gly Arg Leu Val Phe Ala Leu Gly Thr Asp Gly Lys
65 70 75 80

Lys Leu Arg Ile Lys Ser Lys Glu Lys Cys Asn Asp Gly Lys Trp His
85 90 95

Thr Val Val Phe Gly His Asp Gly Glu Lys Gly Arg Leu Val Val Asp
100 105 110

Gly Leu Arg Ala Arg Glu Gly Ser Leu Pro Gly Asn Ser Thr Ile Ser
115 120 125

Ile Arg Ala Pro Val Tyr Leu Gly Ser Pro Pro Ser Gly Lys Pro Lys
130 135 140

Ser Leu Pro Thr Asn Ser Phe Val Gly Cys Leu Lys Asn Phe Gln Leu
145 150 155 160

Asp Ser Lys Pro Leu Tyr Thr Pro Ser Ser Ser Phe Gly Val Ser Ser
165 170 175

Cys Leu Gly Gly Pro Leu Glu Lys Gly Ile Tyr Phe Ser Glu Glu Gly
180 185 190

Gly His Val Val Leu Ala His Ser Val Leu Leu Gly Pro Glu Phe Lys
195 200 205

Leu Val Phe Ser Ile Arg Pro Arg Ser Leu Thr Gly Ile Leu Ile His
210 215 220

Ile Gly Ser Gln Pro Gly Lys His Leu Cys Val Tyr Leu Glu Ala Gly
225 230 235 240

Lys Val Thr Ala Ser Met Asp Ser Gly Ala Gly Gly Thr Ser Thr Ser
245 250 255

Val Thr Pro Lys Gln Ser Leu Cys Asp Gly Gln Trp His Ser Val Ala
260 265 270

Val Thr Ile Lys Gln His Ile Leu His Leu Glu Leu Asp Thr Asp Ser
275 280 285

Ser Tyr Thr Ala Gly Gln Ile Pro Phe Pro Pro Ala Ser Thr Gln Glu
290 295 300

Pro Leu His Leu Gly Gly Ala Pro Ala Asn Leu Thr Thr Leu Arg Ile
305 310 315 320

Pro Val Trp Lys Ser Phe Phe Gly Cys Leu Arg Asn Ile His Val Asn
325 330 335

His Ile Pro Val Pro Val Thr Glu Ala Leu Glu Val Gln Gly Pro Val
340 345 350

Ser Leu Asn Gly Cys Pro Asp Gln
355 360

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<210> SEQ ID NO 12
<211> LENGTH: 1371
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

tgctcaccac ttcccaagac ccaggccaat catggagccc tccagtttgg ggacattccc 60

accagccact tgctattcaa gcttcctcag gagctgctga aacccaggtc acagtttgct 120

gtggacatgc agacaacatc ctccagagga ctggtgtttc acacgggcac taagaactcc 180

tttatggctc tttatctttc aaaaggacgt ctggtctttg cactggggac agatgggaaa 240

aaattgagga tcaaaagcaa ggagaaatgc aatgatggga aatggcacac ggtggtgttt 300

ggccatgatg gggaaaaggg gcgcttggtt gtggatggac tgagggcccg ggagggaagt 360

ttgcctggaa actccaccat cagcatcaga gcgccagttt acctgggatc acctccatca 420

gggaaaccaa agagcctccc cacaaacagc tttgtgggat gcctgaagaa ctttcagctg 480

gattcaaaac ccttgtatac cccttcttca agcttcgggg tgtcttcctg cttgggtggt 540

cctttggaga aaggcattta tttctctgaa gaaggaggtc atgtcgtctt ggctcactct 600

gtattgttgg ggccagaatt taagcttgtt ttcagcatcc gcccaagaag tctcactggg 660

atcctaatac acatcggaag tcagcccggg aagcacttat gtgtttacct ggaggcagga 720

aaggtcacgg cctctatgga cagtggggca ggtgggacct caacgtcggt cacaccaaag 780

cagtctctgt gtgatggaca gtggcactcg gtggcagtca ccataaaaca acacatcctg 840

cacctggaac tggacacaga cagtagctac acagctggac agatcccctt cccacctgcc 900

agcactcaag agccactaca ccttggaggt gctccagcca atttgacgac actgaggatc 960

cctgtgtgga aatcattctt tggctgtctg aggaatattc atgtcaatca catccctgtc 1020

cctgtcactg aagccttgga agtccagggg cctgtcagtc tgaatggttg tcctgaccag 1080

taacccaagc ctatttcaca gcaaggaaat tcaccttcaa aagcactgat tacccaatgc 1140

acctccctcc ccagctcgag atcattcttc aattaggaca caaaccagac aggtttaata 1200

gcgaatctaa ttttgaattc tgaccatgga tacccatcac tttggcattc agtgctacat 1260

gtgtatttta tataaaaatc ccatttcttg aagataaaaa aattgttatt caaattgtta 1320

tgcacagaat gtttttggta atattaattt ccactaaaaa attaaatgtc t 1371

<210> SEQ ID NO 13
<211> LENGTH: 184
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Cys Leu Gly Gly Pro Leu Glu Lys Gly Ile Tyr Phe Ser Glu Glu Gly
1 5 10 15

Gly His Val Val Leu Ala His Ser Val Leu Leu Gly Pro Glu Phe Lys
20 25 30

Leu Val Phe Ser Ile Arg Pro Arg Ser Leu Thr Gly Ile Leu Ile His
35 40 45

Ile Gly Ser Gln Pro Gly Lys His Leu Cys Val Tyr Leu Glu Ala Gly
50 55 60

Lys Val Thr Ala Ser Met Asp Ser Gly Ala Gly Gly Thr Ser Thr Ser
65 70 75 80

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Val Thr Pro Lys Gln Ser Leu Cys Asp Gly Gln Trp His Ser Val Ala
                85                  90                  95

Val Thr Ile Lys Gln His Ile Leu His Leu Glu Leu Asp Thr Asp Ser
            100                 105                 110

Ser Tyr Thr Ala Gly Gln Ile Pro Phe Pro Pro Ala Ser Thr Gln Glu
        115                 120                 125

Pro Leu His Leu Gly Gly Ala Pro Ala Asn Leu Thr Thr Leu Arg Ile
    130                 135                 140

Pro Val Trp Lys Ser Phe Phe Gly Cys Leu Arg Asn Ile His Val Asn
145                 150                 155                 160

His Ile Pro Val Pro Val Thr Glu Ala Leu Glu Val Gln Gly Pro Val
                165                 170                 175

Ser Leu Asn Gly Cys Pro Asp Gln
            180
```

<210> SEQ ID NO 14
<211> LENGTH: 843
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

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tgcttgggtg gtcctttgga gaaaggcatt tatttctctg aagaaggagg tcatgtcgtc     60

ttggctcact ctgtattgtt ggggccagaa tttaagcttg ttttcagcat ccgcccaaga    120

agtctcactg ggatcctaat acacatcgga agtcagcccg ggaagcactt atgtgtttac    180

ctggaggcag gaaaggtcac ggcctctatg gacagtgggg caggtgggac ctcaacgtcg    240

gtcacaccaa agcagtctct gtgtgatgga cagtggcact cggtggcagt caccataaaa    300

caacacatcc tgcacctgga actggacaca gacagtagct acacagctgg acagatcccc    360

ttcccacctg ccagcactca agagccacta caccttggag gtgctccagc caatttgacg    420

acactgagga tccctgtgtg gaaatcattc tttggctgtc tgaggaatat tcatgtcaat    480

cacatccctg tccctgtcac tgaagccttg gaagtccagg ggcctgtcag tctgaatggt    540

tgtcctgacc agtaacccaa gcctatttca cagcaaggaa attcaccttc aaaagcactg    600

attacccaat gcacctccct ccccagctcg agatcattct tcaattagga cacaaaccag    660

acaggtttaa tagcgaatct aattttgaat tctgaccatg gatacccatc actttggcat    720

tcagtgctac atgtgtattt tatataaaaa tcccatttct tgaagataaa aaaattgtta    780

ttcaaattgt tatgcacaga atgtttttgg taatattaat ttccactaaa aaattaaatg    840

tct                                                                  843
```

<210> SEQ ID NO 15
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

```
Pro Leu Pro Lys Thr Gln Ala Asn His Gly Ala
1               5                   10
```

<210> SEQ ID NO 16
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 16

cacttcccaa gacccaggcc aatcatggag c 31

<210> SEQ ID NO 17
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Ser His Leu Leu Phe Lys Leu Pro Gln Glu Leu Leu Lys Pro Arg Ser
1 5 10 15

<210> SEQ ID NO 18
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

gccacttgct attcaagctt cctcaggagc tgctgaaacc caggtca 47

<210> SEQ ID NO 19
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Thr Ser Ser Arg Gly Leu Val Phe His Thr Gly Thr Lys Asn Ser Phe
1 5 10 15

Met Ala Leu Tyr Leu Ser Lys Gly Arg Leu Val Phe Ala Leu Gly Thr
20 25 30

Asp Gly Lys Lys Leu Arg Ile Lys Ser Lys Glu Lys Cys Asn Asp Gly
35 40 45

Lys Trp His Thr Val Val Phe Gly His Asp Gly Glu Lys Gly Arg Leu
50 55 60

Val Val Asp Gly Leu Arg Ala Arg Glu Gly Ser Leu Pro Gly Asn Ser
65 70 75 80

Thr Ile Ser Ile Arg Ala Pro Val Tyr Leu Gly Ser Pro Pro Ser Gly
85 90 95

Lys Pro Lys Ser Leu Pro Thr Asn Ser Phe Val Gly Cys Leu Lys Asn
100 105 110

Phe Gln Leu Asp Ser Lys Pro Leu Tyr Thr Pro Ser Ser Ser Phe Gly
115 120 125

Val Ser Ser
130

<210> SEQ ID NO 20
<211> LENGTH: 398
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

gacaacatcc tccagaggac tggtgtttca cacgggcact aagaactcct ttatggctct 60

ttatctttca aaaggacgtc tggtctttgc actggggaca gatgggaaaa aattgaggat 120

caaaagcaag gagaaatgca atgatgggaa atggcacacg gtggtgtttg gccatgatgg 180

ggaaaagggg cgcttggttg tggatggact gagggcccgg gagggaagtt tgcctggaaa 240

-continued

ctccaccatc agcatcagag cgccagttta cctgggatca cctccatcag ggaaaccaaa 300

gagcctcccc acaaacagct ttgtgggatg cctgaagaac tttcagctgg attcaaaacc 360

cttgtatacc ccttcttcaa gcttcggggt gtcttcct 398

<210> SEQ ID NO 21
<211> LENGTH: 315
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Thr Ser Ser Arg Gly Leu Val Phe His Thr Gly Thr Lys Asn Ser Phe
1 5 10 15

Met Ala Leu Tyr Leu Ser Lys Gly Arg Leu Val Phe Ala Leu Gly Thr
20 25 30

Asp Gly Lys Lys Leu Arg Ile Lys Ser Lys Glu Lys Cys Asn Asp Gly
35 40 45

Lys Trp His Thr Val Val Phe Gly His Asp Gly Glu Lys Gly Arg Leu
50 55 60

Val Val Asp Gly Leu Arg Ala Arg Glu Gly Ser Leu Pro Gly Asn Ser
65 70 75 80

Thr Ile Ser Ile Arg Ala Pro Val Tyr Leu Gly Ser Pro Pro Ser Gly
85 90 95

Lys Pro Lys Ser Leu Pro Thr Asn Ser Phe Val Gly Cys Leu Lys Asn
100 105 110

Phe Gln Leu Asp Ser Lys Pro Leu Tyr Thr Pro Ser Ser Ser Phe Gly
115 120 125

Val Ser Ser Cys Leu Gly Gly Pro Leu Glu Lys Gly Ile Tyr Phe Ser
130 135 140

Glu Glu Gly Gly His Val Val Leu Ala His Ser Val Leu Leu Gly Pro
145 150 155 160

Glu Phe Lys Leu Val Phe Ser Ile Arg Pro Arg Ser Leu Thr Gly Ile
165 170 175

Leu Ile His Ile Gly Ser Gln Pro Gly Lys His Leu Cys Val Tyr Leu
180 185 190

Glu Ala Gly Lys Val Thr Ala Ser Met Asp Ser Gly Ala Gly Gly Thr
195 200 205

Ser Thr Ser Val Thr Pro Lys Gln Ser Leu Cys Asp Gly Gln Trp His
210 215 220

Ser Val Ala Val Thr Ile Lys Gln His Ile Leu His Leu Glu Leu Asp
225 230 235 240

Thr Asp Ser Ser Tyr Thr Ala Gly Gln Ile Pro Phe Pro Pro Ala Ser
245 250 255

Thr Gln Glu Pro Leu His Leu Gly Gly Ala Pro Ala Asn Leu Thr Thr
260 265 270

Leu Arg Ile Pro Val Trp Lys Ser Phe Phe Gly Cys Leu Arg Asn Ile
275 280 285

His Val Asn His Ile Pro Val Pro Val Thr Glu Ala Leu Glu Val Gln
290 295 300

Gly Pro Val Ser Leu Asn Gly Cys Pro Asp Gln
305 310 315

<210> SEQ ID NO 22

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<211> LENGTH: 935
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

catcctccag aggactggtg tttcacacgg gcactaagaa ctcctttatg gctctttatc 60

tttcaaaagg acgtctggtc tttgcactgg ggacagatgg gaaaaaattg aggatcaaaa 120

gcaaggagaa atgcaatgat gggaaatggc acacggtggt gtttggccat gatggggaaa 180

aggggcgctt ggttgtggat ggactgaggg cccgggaggg aagtttgcct ggaaactcca 240

ccatcagcat cagagcgcca gtttacctgg gatcacctcc atcagggaaa ccaaagagcc 300

tccccacaaa cagctttgtg ggatgcctga agaactttca gctggattca aaacccttgt 360

ataccccttc ttcaagcttc ggggtgtctt cctgcttggg tggtcctttg gagaaaggca 420

tttatttctc tgaagaagga ggtcatgtcg tcttggctca ctctgtattg ttggggccag 480

aatttaagct tgttttcagc atccgcccaa gaagtctcac tgggatccta atacacatcg 540

gaagtcagcc cgggaagcac ttatgtgttt acctggaggc aggaaaggtc acggcctcta 600

tggacagtgg ggcaggtggg acctcaacgt cggtcacacc aaagcagtct ctgtgtgatg 660

gacagtggca ctcggtggca gtcaccataa aacaacacat cctgcacctg gaactggaca 720

cagacagtag ctacacagct ggacagatcc ccttcccacc tgccagcact caagagccac 780

tacaccttgg aggtgctcca gccaatttga cgacactgag gatccctgtg tggaaatcat 840

tctttggctg tctgaggaat attcatgtca atcacatccc tgtccctgtc actgaagcct 900

tggaagtcca ggggcctgtc agtctgaatg gttgt 935

<210> SEQ ID NO 23
<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Phe Lys Leu Val Phe Ser Ile Arg Pro Arg Ser Leu Thr Gly Ile Leu
1 5 10 15

Ile His Ile Gly Ser Gln Pro Gly Lys His Leu Cys Val Tyr Leu Glu
20 25 30

Ala Gly Lys Val Thr Ala Ser Met Asp Ser Gly Ala Gly Gly Thr Ser
35 40 45

Thr Ser Val Thr Pro Lys Gln Ser Leu Cys Asp Gly Gln Trp His Ser
50 55 60

Val Ala Val Thr Ile Lys Gln His Ile Leu His Leu Glu Leu Asp Thr
65 70 75 80

Asp Ser Ser Tyr Thr Ala Gly Gln Ile Pro Phe Pro Pro Ala Ser Thr
85 90 95

Gln Glu Pro Leu His Leu Gly Gly Ala Pro Ala Asn Leu Thr Thr Leu
100 105 110

Arg Ile Pro Val Trp Lys Ser Phe Phe Gly Cys Leu Arg Asn Ile His
115 120 125

Val Asn His Ile Pro Val Pro Val Thr Glu Ala Leu Glu Val Gln Gly
130 135 140

-continued

```
Pro Val Ser Leu Asn Gly Cys Pro Asp Gln
145                 150


<210> SEQ ID NO 24
<211> LENGTH: 462
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

ttaagcttgt tttcagcatc cgcccaagaa gtctcactgg gatcctaata cacatcggaa     60

gtcagcccgg gaagcactta tgtgtttacc tggaggcagg aaaggtcacg gcctctatgg    120

acagtggggc aggtgggacc tcaacgtcgg tcacaccaaa gcagtctctg tgtgatggac    180

agtggcactc ggtggcagtc accataaaac aacacatcct gcacctggaa ctggacacag    240

acagtagcta cacagctgga cagatcccct tcccacctgc cagcactcaa gagccactac    300

accttggagg tgctccagcc aatttgacga cactgaggat ccctgtgtgg aaatcattct    360

ttggctgtct gaggaatatt catgtcaatc acatccctgt ccctgtcact gaagccttgg    420

aagtccaggg gcctgtcagt ctgaatggtt gtcctgacca gt                       462
```

What is claimed is:

1. A method of treating squamous cell carcinoma (SCC) in a patient comprising administering a therapeutically effective amount of one or more antibodies in a pharmaceutically acceptable carrier, wherein one or more of said antibodies is capable of specifically binding laminin G4 and/or G5 domain or subdomain, and inhibiting SCC tumorigenesis.

2. A method according to claim **1**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:13.

3. A method according to claim **1**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:15.

4. A method according to claim **1**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:17.

5. A method according to claim **1**, wherein said antibody binds to a binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:19.

6. A method according to claim **1**, wherein said antibody binds to a MFP comprising the amino acid sequence of SEQ ID NO:21.

7. A method according to claim **1**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:23.

8. A method according to claim **1**, wherein said antibody is a polyclonal antibody.

9. A method according to claim **1**, wherein said antibody is a monoclonal antibody.

10. A method according to claim **1**, wherein said SCC is selected from the group consisting of skin cancer, lung cancer, head cancer, gastric cancer, colorectal, throat cancer, cancer of the urinary tract, cancer of the reproductive tract, esophageal cancer, and bronchiogenic carcinoma.

11. A method for diagnosing the presence of SCC comprising the steps of: a) contacting a sample suspected of comprising neoplastic epithelial cells with an antibody capable of specifically binding laminin 5 G4 and/or G5 domain or subdomain, b) detecting the binding of said antibody; and, c) diagnosing therefrom the presence or absence of SCC in said sample.

12. A method according to claim **11**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:13.

13. A method according to claim **1 1**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:15.

14. A method according to claim **11**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:17.

15. A method according to claim **11**, wherein said antibody binds to a binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:19.

16. A method according to claim **11**, wherein said antibody binds to a MFP comprising the amino acid sequence of SEQ ID NO:21.

17. A method according to claim **11**, wherein said antibody binds to a polypeptide comprising the amino acid sequence of SEQ ID NO:23.

18. A method according to claim **11**, wherein said antibody further comprises a detectable label.

19. The method according to claim **11**, wherein said epithelial cells are selected from the group consisting of squamous cells, keratinocytes, mucosal epithelial cells, gastrointestinal epithelial cells, corneal epithelia of the eye, and epithelial cells of the urinary and reproductive tract.

20. The method according to claim **11**, wherein said sample is a tissue sample.

21. The method according to claim **11**, wherein said sample is a urine sample.

22. The method according to claim **11**, wherein said sample is a blood sample.

23. A method of evaluating the effect of a candidate SCC drug in a patient comprising the steps of: a) detecting the presence of laminin 5 G4 and/or G5 domain or subdomain in a tissue sample from a patient diagnosed with a SCC tumor prior to treatment with a candidate drug; and b) detecting the

presence of a said laminin 5 G4 and/or G5 domain or subdomain in a tissue sample from said patient following treatment with said candidate drug; wherein a decrease in said laminin 5 G4 and/or G5 domain or subdomain following treatment with said candidate drug indicates that said candidate drug is effective in treating said SCC in said patient.

24. A method according to claim **23**, wherein said candidate agent is selected from the group consisting of antibodies and fragments thereof, small molecules, polypeptides, and aptamers.

* * * * *

专利名称(译)	用于抑制鳞状细胞癌的组合物和方法		
公开(公告)号	US20080233125A1	公开(公告)日	2008-09-25
申请号	US12/082906	申请日	2008-04-14
[标]申请(专利权)人(译)	PETER MARINKOVICH中号		
申请(专利权)人(译)	PETER MARINKOVICH中号		
当前申请(专利权)人(译)	PETER MARINKOVICH中号		
[标]发明人	PETER MARINKOVICH M		
发明人	PETER, MARINKOVICH M.		
IPC分类号	A61K39/395 G01N33/53 A61P35/00		
CPC分类号	A61K9/0019 A61K2039/505 G01N2333/78 C07K2317/73 G01N33/57484 C07K16/30 A61P35/00		
其他公开文献	US7875277		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及使用靶向层粘连蛋白5α3G4-G5结构域的试剂检测和抑制鳞状细胞癌的组合物和方法。