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Diagnose und Behandlung von Karzinomen und anderen Leiden

Diagnostic et traitement de cancers et d'autres maladies

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Description**TECHNICAL FIELD**

5 [0001] This specification describes diagnosis and treatment of diseases, including cancers. The types of diseases with which this specification is concerned include cancers derived from epithelial cells and malignant lymphoma. The specification describes other conditions, such as preneoplastic states, irritable bowel syndrome and viral and other infections. It is quite possible that the specification is also applicable to other diseases and conditions.

10 **BACKGROUND**

[0002] Adenosine triphosphate (ATP) can activate ligand-gated purinergic receptors known as P2X receptors. Receptor subtypes P2X₂ to P2X₇ have been identified. It is known that different P2X receptor subtypes are present in many cells, including epithelial cells and leukocytes, including lymphocytes, thymocytes, macrophages and dendritic cells.

15 [0003] P2X receptors are permeable to calcium ions as well as some other cations, such as potassium and sodium. An influx of calcium ions into a cell via a P2X receptor can be associated with cell death.

20 [0004] It is believed that the P2X₇ subtype is involved in apoptosis, or programmed cell death, in many cell types. In the presence of ATP, the P2X₇ receptor expressed on the surface of a cell is capable, within a second, of opening-calcium channels through the cell membrane. Continued exposure to ATP can lead to the formation of large pores, within a few seconds to tens of seconds, that enable the cell to be flooded with excess calcium, inducing apoptosis.

25 [0005] The amino acid sequences of the human and rat P2X₇ receptors are known, for example, from US patent No. 6,133,434 (Buell et al). Refer also to Figure 1 herein.

[0006] Exposure to ATP does not generally result in apoptosis in the case of epithelial cancer cells, for example. It has been found that such cells express P2X₇ receptors that are unable to form pores. These are regarded as non-functional receptors.

30 [0007] In human cancer cell lines, such as prostate PC3 and breast MCF7, as well as in animal cell lines including rodent hybridomas, the P2X₇ receptor is found on the cell surface in a non-functional conformation.

35 [0008] The B-cells of patients with malignant lymphoma express non-functional P2X₇ receptors. Lymphoma develops from malignant clones that escape cytolytic destruction. This process leads to the progressive accumulation of malignant B-lymphocytes and thus lymphadenopathy and/or splenomegaly.

40 [0009] The specification describes a probe for detection of a disease or condition, the probe being adapted to distinguish between functional P2X₇ receptors and non-functional P2X₇ receptors. Preferably, the probe distinguishes between functional and non-functional P2X₇ receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors or by detecting change in binding of one or more proteins necessary for pore formation in P2X₇ receptors.

45 [0010] In an alternate embodiment, the probe detects one or more parts of the P2X₇ receptor exposed in the absence of bound ATP. Such receptor part may include a P2X₇ monomer.

50 [0011] The specification describes a method for detecting a disease or condition, the method including the steps of using the probe of the invention to distinguish between functional P2X₇ receptors and non-functional P2X₇ receptors, providing a receptor expression profile, and comparing the receptor expression profile with that of a normal profile. The change may be detected, for example, as indicated above in connection with the probe itself.

55 [0012] The probe may be natural or artificial. Preferably, the probe is an antibody, which may be polyclonal, monoclonal, recombinant, a humanised antibody or an appropriate fragment thereof. The antibody is preferably directed against an epitope located in an extracellular domain adjacent to a site for binding ATP. In the case of human P2X₇ receptors, the probe is preferably adapted to distinguish between functional receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 210 is in the cis conformation.

[0013] The probe may distinguish between functional and non-functional receptors through detection of other conformational changes occurring at a site for binding ATP. For example, the change detected may be in an amino acid other than the proline referred to above. An example of such an amino acid is Pro199. As another example, the change detected may be in some other respect.

[0014] The probe may also be adapted to detect other regions of the P2X₇ receptor unchanged by functional state. The conformation of the monomeric subunits lacking bound ATP may be detectable using the probe, as the epitope chosen may specifically detect the shape of a region of the surface of the receptor accessible only when ATP is not bound. The probe may detect change in binding of one or more proteins, such as accessory or other proteins, necessary for pore formation. Non-limiting examples of such proteins are laminin, integrin, beta-actin, alpha-actinin and supervillin.

[0015] In the present invention, a P2X₇ subtype-specific antibody can be used to specifically detect or bind to non-functional P2X₇ receptors expressed in or on cells forming part of preneoplastic tissue, very early neoplastic tissue,

advanced neoplastic tissue and on any neoplastic cell expressing non-functional P2X₇ receptors. Thus, the P2X₇ receptor is detected or bound only when in the close-gated or non-functional conformation, even though it may be normally expressed in the cell membranes and may otherwise be partially able to function as a channel.

[0016] Further, the conformation of the monomeric subunits lacking bound ATP is also detectable with the antibody, because the epitope chosen specifically detects the shape of a region of the surface accessible only when ATP is not bound.

[0017] The non-functional P2X₇ receptors can be detected or bound by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors, so that the cause of the conformational change in the receptors relates to the interaction of the receptors with ATP. As set out above, the ATP molecules act as receptor agonists, so that when ATP is bound to the receptors, they are able to open a channel through the cell membrane for the inflow of calcium ions. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors, for reasons that may include a deficit in the local availability of ATP through production deficit or increase in the rate of degradation. If ATP binding to the receptors is disrupted, the receptor conformation is altered. This can be detected by using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP.

[0018] In the case of human P2X₇ receptors, the specific sequence involved in the conformational change may include Pro210, which undergoes a change in conformation from the trans form to the cis form in the absence of bound ATP. Thus, in the case of human receptors, an appropriate epitope sequence against which an antibody must be raised may include Pro210, and may extend either side of this residue, to an appropriate extent necessary to induce an antibody response. By way of non-limiting example, this may include a segment extending from Gly200 to Thr215. Further, a homologous segment from other mammals, such as rat, may be used where this cross-reacts with human tissue. As an example, the same segment Gly200 to Thr215 in rat may be used, although there are two amino acid substitutions in the rat sequence compared with the human sequence (refer US patent No. 6,133,434, for example).

[0019] In the case of non-human receptors, the specific sequence may be ascertained by suitable experiment.

[0020] The detection of non-functional P2X₇ receptors may show a distribution pattern in which functional receptors (and hence normal cells) may remain essentially unlabelled. However, non-functional conformations of P2X₇ receptors may be detected, initially in the nuclei and cytoplasm of cells, at a very early stage in preneoplasia. For example, in the case of epithelial cell cancer, using the method of the invention it may be possible to detect preneoplasia several years prior to the normal pathological appearance of cancer as detected by haematoxylin and eosin ("H & E") stained slides of biopsied tissues. Thus, cancers such as prostate, skin and breast may be detected far earlier than is currently the case, with the advantages of introduction of early therapy.

[0021] The full scope of the diseases and conditions which may be detected by the probe and method described herein has not yet been ascertained. However, it is believed that these include epithelial cell cancers, such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, as well as blood cancers including malignant lymphoma, irritable bowel syndrome and infection by viruses such as HIV or other pathological organisms, such as *Mycobacterium tuberculosis*. Infection may cause non-functional receptors to be expressed either directly through inhibition of co-factors required for functionality, or through the up-regulation of co-factors acting to inhibit P2X₇ function on epithelial or other cells, so rendering the infected cell less amenable to destruction by apoptosis.

[0022] Unless otherwise indicated, the term "disease or condition" as used herein is intended to include all those specific diseases and conditions set out in the preceding paragraph.

[0023] In the specific case of irritable bowel syndromes ("IBS"), it has now been found that, in patients with this condition, the gut mucosa, that normally expresses P2X₇ receptors in the widely distributed lymphocytes present in the stroma beneath the epithelium, becomes up-regulated. In affected patients, this increased expression can be observed from duodenum to rectal mucosa. The increased expression may be found in isolated regions, or to be generally increased over the entire length of the intestinal tract in more extreme cases.

[0024] In the least affected cases, total P2X₇ receptors are up-regulated, but these are all functional and they do not penetrate into the epithelium. In more severe cases, total P2X₇ receptor expression is even higher, and the most affected areas of the gut exhibit receptors that are non-functional. These may be localised to caecal mucosa, for example, and may penetrate into the epithelium. The most severe cases are those in which total P2X₇ receptor expression is further increased and most of the receptors are non-functional with increased epithelial cell penetration.

[0025] As already discussed, non-functionality of P2X₇ receptors is caused by lack of appropriate binding of the ATP agonist to the receptors. The reasons for this may include a deficit in the local availability of ATP through production deficit or increase in rate of degradation through ecto-ATPase enzymatic degradation of ATP. If ATP binding to the receptors is disrupted, the receptor conformation is altered as already discussed, and this can be detected using the probe of the invention. However, the detection of total P2X₇ receptor distribution is best achieved using an epitope to other regions of the extracellular domain of the P2X₇ receptor that is not affected by ATP binding.

[0026] As described herein, one or two P2X₇ subtype-specific antibodies may be used to specifically distinguish

between total P2X₇ distribution and the proportion of receptors that are non-functional and expressed in gut mucosa. Thus the two antibodies used together can detect both total receptor count and those receptor channels present only in a close-gated or non-functional conformation. The first antibody is adapted to detect total P2X₇ receptor expression. The probe comprising or attached to the antibody of the invention can provide the second antibody for detection of IBS, not only distinguishing between functional and non-functional P2X₇ receptors, but also allowing for detection of other regions in which the receptor is unchanged by functional state. The antibodies may be used separately or together. Preferably, they are used in combination.

[0027] The detection of all P2X₇ receptors, separately from non-functional P2X₇ receptors, determines the severity of the condition. Expression of non-functional P2X₇ receptors in the gastrointestinal mucosa occurs in a pattern in which normal cells remain essentially unlabelled. Thereafter, the non-functional conformation of P2X₇ is first detected in the stroma underneath the epithelium ranging from isolated patches in mild cases of the syndrome to extensive expression throughout the length of the gastrointestinal tract with isolated patches of infiltration of non-functional receptors into the epithelium.

[0028] The specification describes a method of diagnosing irritable bowel syndrome, comprising detecting the P2X₇ expression profile of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells and/or tissue. Preferably, the detection of the P2X₇ expression profile includes use of one or more antibodies. Further, it is preferred that such antibody or antibodies are different from the probe of the invention in that they do not detect change in relation to binding of ATP to the P2X₇ receptors. The preparation of such antibodies will be readily apparent to one skilled in the art.

[0029] The specification describes use of one or more antibodies to diagnose irritable bowel syndrome.

[0030] The diagnostic can be used in standard microscopy employing standard immunohistochemical techniques.

[0031] Therapeutic treatment for this condition is discussed below, in connection with the third aspect of this invention.

[0032] Diagnosis using the probe and method described herein may be carried out using in situ imaging techniques to detect distribution in body tissues. In addition, standard microscopy, confocal microscopy and fluorescence activated cell sorting may be used. Normal immunohistochemical techniques for testing lymph, prostate, breast, skin, lung, uterus, bladder, cervix, stomach, oesophagus and similar biopsies, also fine needle aspirates of breast and other tissue and cell smears such as those taken for the detection of cervical cancer, may be used. Other techniques may be used with the probe and method described herein.

[0033] This specification describes an antibody for treating a disease or condition, the antibody being adapted to distinguish between functional P2X₇ receptors and non-functional P2X₇ receptors and being adapted to bind only to non-functional receptors. Preferably, the antibody distinguishes between the functional and non-functional receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors, or by detecting change in binding of one or more proteins necessary for pore formation in P2X₇ receptors and being adapted to bind only to non-functional receptors. In another embodiment, the antibody distinguishes between the functional and non-functional receptors by detecting parts of the receptor exposed in the absence of bound ATP.

[0034] The antibody for treating diseases and conditions may be the same as the antibody which may be used as the probe for diagnosing diseases and conditions. Such an antibody could be used to treat skin cancers topically, for example. For systemic treatment of cancer, the antibody or its active fragments should be humanised in order to minimise undesirable immune response side effects.

[0035] The antibody described herein may be used to treat diseases or conditions in mammals, including humans. Examples of the diseases or conditions have been set out above in connection with the probe described herein.

[0036] The specification describes an epitope capable of causing the generation of the antibody of the invention. The epitope preferably includes Pro210 and encompasses the segment Gly200 to Thr215 (in the P2X₇ sequence of the human receptor). The epitope should preferably have attached to the C-terminal end a Cys residue that is cross-linked to diphtheria toxin via the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS), so that the conformation adopted by the attached epitope peptide occupies a stable cis proline configuration.

[0037] This specific peptide conformation is intended to be presented to humans or animals with one or more diseases or conditions, especially epithelial cell cancers, such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, as well as malignant lymphoma, irritable bowel syndrome and infection by viruses such as HIV or other pathological organisms, such as *Mycobacterium tuberculosis*. The patient will preferably mount an immune response to the applied conjugated epitope and so generate antibodies recognising the non-functional P2X₇ receptors present on the surface of the affected cells, thus binding to them and alerting the appropriate immune cell to destroy the complexed cells. Other cells primed for cell death may also be affected.

[0038] It is to be understood that the sequence referred to above is not limiting on the scope of the invention, which includes alternate sequences and carriers and crosslinkers that similarly produce a specific immune response, preferably against only non-functional P2X₇ receptors, preferably ignoring all functional receptors expressed on cell surfaces, and so avoiding side effects.

[0039] The specification also describes the use of the antibody described herein as a therapeutic vehicle for treatment

of a disease or condition in a patient to regulate programmed cell death by targeting aberrant or non-functional P2X₇ receptors expressed on the surface of cells, while leaving all cells expressing normal (functional) receptors untouched. The specification describes the use of the epitope of the invention to cause the generation of the antibody, as above.

[0040] The specification describes a pharmaceutical composition for treatment or prevention of a disease or condition in a patient, the composition including a pharmaceutically effective amount of an antibody, or an epitope to cause the generation of such an amount, capable of regulating programmed cell death of cells having expressed on their surface aberrant or non-functional P2X₇ receptors.

[0041] The pharmaceutically effective amount of the antibody or epitope will vary according to the patient and the nature of the disease or condition. These variables can be ascertained by one skilled in the art.

[0042] The pharmaceutical composition may be administered in conjunction with a pharmaceutically acceptable carrier, which may be any of those known in the art or devised hereafter and suitable for the intended use. As well as carriers, the pharmaceutical compositions of the invention may include other ingredients, including dyes, preservatives, buffers and antioxidants, for example.

[0043] The pharmaceutical composition may take any desired form and may be administered, for example, in the form of an ointment, cream, solution, suspension, powder, tablet, capsule, suppository or pessary.

[0044] The pharmaceutical composition may be administered in any suitable way, which may include oral, parenteral, intravenous, intramuscular, subcutaneous or topical administration.

[0045] The specification describes treating or preventing a disease or condition in a patient, including administering to the patient a pharmaceutical composition as described herein.

[0046] As defined in the claims, the invention also provides the use of the pharmaceutical composition of the invention, in the treatment or prevention of a disease or condition, in a patient.

[0047] It will be apparent to one skilled in the art that the pattern of use of the pharmaceutical composition of the invention may need to be altered for optimum effect. It may be necessary to take into account the nature of the disease or condition as well as its severity.

[0048] The specification describes the expression of ATPases (enzymes) that control the supply of ATP to P2X₇ receptors, for example in the B-cells of a patient having malignant lymphoma. Channel opening of P₂X₇ receptors on leukocytes is terminated through the rapid hydrolysis of ATP agonist by ecto-ATPases and ecto-ATPdiphosphohydrolases (ecto-ATPDases). These enzymes regulate numerous physiological processes that are dependent on ATP. Substrate specificity of ATPase and ATPDase activity on lymphocytes indicates the presence on the lymphocytes of more than one type on the cell surface, including CD39. Proliferation of one or more of these ATPases or ATPDases could limit the supply of ATP needed to control P2X₇ pore formation and the subsequent programmed cell death needed to regulate B-cell numbers.

[0049] Similarly, it is believed that, in the case of IBS, proliferation of ATPases may contribute to lack of appropriate binding of the agonist ATP to the P2X₇ receptors.

[0050] Accordingly, the specification describes a preparation for treatment or prevention of a disease or condition in a patient, the preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to P2X₇ receptors in the patient's cells or tissues. The specification describes treating or preventing a disease or condition in a patient, including the step of administering to the patient a preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to P2X₇ receptors in the cells or tissue of the patient.

[0051] Examples of such ATPases may be CD39 or CD73.

[0052] Such a substance may take the form of an ATP analogue, preferably non-hydrolysable, and specific for P2X₇, or another substance that inhibits the action of local ATPases depleting the availability of ATP for the P2X₇ binding site. The preparation may be in the form of a humanised antibody directed specifically against non-functional P2X₇ receptors.

[0053] The disease or condition is preferably malignant lymphoma or IBS but may also extend to other diseases or conditions, including other epithelial cell or blood cancers or viral and other pathological infections.

[0054] In the case of malignant lymphoma, the ATPases control the local supply of ATP to the P2X₇ receptors so as to reduce the concentration of ATP available for binding to the P2X₇ receptors and so deactivate them leading to a significant reduction in programmed B-cell death. These ATPases may be specifically expressed on the surface of the B-cells and appear to be up-regulated in malignant lymphoma. Preferably, application of a specific ATPase inhibitor may be used to regulate the availability of ATP on the P2X₇ receptors, so regulating programmed B-cell death.

[0055] For treatment of malignant lymphoma, the substance may include a synthetic agonist capable of blocking ATPases or ATPDases, of the form of non-hydrolysable P2X₇ agonist.

[0056] In relation to irritable bowel syndrome, administration of the preparation of the invention is intended to restore receptor function that may be depleted through overactivity of the muscle underlying the affected region of mucosa. The preparation described herein may act on the mucosa directly to remove these non-functional receptors and thereby restore local normal gastrointestinal secretory mechanisms. Therapeutic treatment is aimed at restoring the local supply of ATP to the non-functional receptors, so that normal receptor function is restored. The consequences of control of

receptor function include restoration of normal control of gastrointestinal secretions and peristalsis. This may be achieved by application of enteral or systemic supply of synthetic P2X₇-specific agonist, preferably non-hydrolysable by ATPases, by systemic application of an antibody directed against non-functional P2X₇ receptors, preferably a small humanised specific antibody to remove the non-functional receptors, leaving only functional receptors.

5 [0057] If abnormalities of peristalsis in the underlying smooth muscle are responsible for depleting the local availability of ATP for binding to the normal P2X₇ receptors, treatment may involve restoration of this natural supply of agonist by means of a limit on the uptake or use of ATP by the smooth muscle through application of a treatment to temporarily limit gut motility.

10 [0058] The specification describes a pharmaceutical composition for treatment of a disease or condition, the composition including a pharmaceutically effective amount of one or more substances adapted to regulate the expression of ATPases (enzymes) that control the supply of ATP to P2X₇ receptors.

15 [0059] The specification describes such similar applications that could be made in other medical conditions in which aberrant P2X₇ receptors are involved as a result of viral infection where the virus is protected in the infected cell by up-regulating non-functional P2X₇ receptor or where such receptors are up-regulated from the normal cell condition.

20 [0060] The specification describes treating irritable bowel syndrome, comprising administering to a patient a pharmaceutical composition as defined above.

[0061] The specification describes the use of such a pharmaceutical composition in the treatment of irritable bowel syndrome.

25 [0062] The pattern of use of one or more of the above pharmaceutically effective agents may need to be altered for optimum effect.

[0063] Expressed another way, the specification describes treating irritable bowel syndrome, including administering a composition adapted to restore P2X₇ receptor function. The receptor function may have been depleted through over-activity of the muscle underlying the affected region of mucosa. The composition may be the same as that set out above for the substance included in the preparation of the invention.

30 [0064] In a further aspect, the specification describes a method for distinguishing between different conformations of proteins by using an epitope capable of causing the generation of an antibody, or the antibody itself, to effect specific pharmaceutical outcomes (active as well as passive immunisation) from binding to all members of the proteins with a selected conformation. An example of this would be prion proteins in the conformation that leads to the condition vCJD. The abnormal form of the protein could be targeted by a Specific antibody or epitope causing the generation of the antibody, preferably humanised and reduced in size for optimum pharmacological effect.

BRIEF DESCRIPTION OF THE DRAWINGS

[0065]

35 Figure 1 shows the amino acid sequence of the human P2X₇ receptor (prior art). Sequences 65 to 81 and 200 to 216 are highlighted and are referred to below.

DETAILED DESCRIPTION OF THE INVENTION

40 [0066] To raise the antibody to non-functional P2X₇, the epitope used was the sequence 200 to 216 in Figure 1, containing a Cys at 216.

[0067] To raise the antibody to both functional and non-functional P2X₇, the epitope used was the sequence 65 to 81 in Figure 1, to which was added an N-terminal Cys.

45 [0068] The Cys residues on the epitopes were coupled via a maleimidocaproyl-N-hydroxysuccinimide (MCS) cross linker to diphtheria toxin (DT) carrier with ten peptide epitopes attached to each DT carrier, to maintain conformational stability and provide a larger antigenic structure. These conjugated epitopes were used as the antigens for injection into several animal species (sheep, rabbit and mouse) to raise antibodies specific to the epitopes, in the usual manner.

50 [0069] The procedure for raising antibodies is well documented in the prior art by use of antigen/adjuvant mixtures injected into animals at particular times. Specific examples for raising the antibodies are set out below:

Example 1

Sheep anti-P2X₇ antibodies

55 [0070] 500 µg of conjugate (approximately 100 µg of P2X₇ epitope) was diluted in phosphate-buffered saline (PBS) to 0.8 ml and was emulsified with 1.2 ml of Freund's Complete adjuvant. Sheep were injected at multiple sites both subcutaneously and intramuscularly with the antigen/adjuvant emulsion. Eight weeks later the sheep were again injected

with the same amount of conjugate emulsified with Freund's Incomplete adjuvant at multiple sites. This was repeated 4 weeks later and the animals were bled from the jugular vein. The serum collected was tested for antibody specificity. The sheep were then routinely injected and bled at eight week intervals to provide a pool of serum containing the specific antibodies.

5 [0071] Other sheep were injected with the same dose of conjugated antigen similar to the schedule above but a different adjuvant was used. In these animals, 0.7 ml of the diluted antigen was mixed with 0.1 ml of a Quill A / DEAE Dextran solution (2.5 mg Quill A + 25 mg DE4E Dextran per ml of PBS) and 1.2 ml of ISA50 Montanide. The emulsion was injected at multiple sites both subcutaneously and intramuscularly. The antibodies produced using this adjuvant produced the same specificities as those produced using Freund's adjuvant.

10 Example 2

Rabbit anti-P2X₇ antibodies

15 [0072] Antibodies were raised in rabbits using the same two adjuvants as with the sheep and the same injection schedules, the only difference being that 300 µg amounts of the conjugate were used for the injection. The antibodies raised had the same specificities as those produced in the sheep and could readily discriminate between the epitopes against which they were raised.

20 Example 3

Mice anti-P2X₇ antibodies

25 [0073] Antibodies were raised in mice against the conjugated epitopes and also against the unconjugated epitope of the non-functional P2X₇ epitope (which is able to discriminate receptors that cannot form pores and thus fail to be apoptotic).

[0074] In these experiments, the adjuvant used was the QAI GEN Pty Ltd product, IMMUNEASY™ which contains the immuno-stimulatory product CpG DNA (trademark of Coley Pharmaceutical Group Inc.)

30 [0075] 5 µg of epitope or conjugated epitope was diluted in 70 µl of PBS and 30 µl of IMMUNEASY™ adjuvant. Mice were injected at multiple sites subcutaneously and intramuscularly. This regime was repeated two weeks later and again at a further two weeks. Mice were bled eight days after the third injection. Antibodies raised in mice by this method were again able to discriminate between the different P2X₇ epitopes and the antibodies against the P2X₇ non-functional epitope gave the same results as those raised in sheep and rabbits. In addition, antibodies raised against the unconjugated non-functional epitope were also able to recognise this epitope in tumour tissue.

35 [0076] As the above Examples illustrate, antibodies to various epitopes of the P2X₇ receptor in different species and using different adjuvants may be raised consistently. In particular, antibodies to an epitope of the P2X₇ receptor which identifies the receptor in the non-functional state, in which it cannot form a pore and carry out its apoptotic function under normal physiological conditions, may be raised routinely.

40 Example 4

[0077] The antibody detecting non-functional P2X₇ was tested by binding the antibody to cells expressing P2X₇ (human) with known function as revealed through the ability of the P2X₇ to take up ethidium or rubidium. These P2X₇ protein channels may have been mutated at base pair 1513, such that the channels would not form apoptotic pores. These and similar non-functional P2X₇ receptors expressed on malignant B lymphocytes also bound the antibody in flow cytometry and in standard immunohistochemistry while cells expressing normal functional P2X₇ (capable of taking up calcium, ethidium and rubidium with large fluxes) were unable to bind the antibody, because the epitope chosen to detect the non-functional receptors was unavailable in functional receptors. The Pro210 adopted a cis conformation in the non-functional receptors and it was specifically this conformation that was stabilised in the conjugated epitope used to raise the antibody. The Pro210 was in the trans conformation in the receptors that were shown to be functional. This was a result of the binding of ATP (adenosine triphosphate) to the P2X₇ receptor. When ATP was bound, the Pro210 on a segment immediately adjacent to the ATP binding site adopted a trans configuration.

[0078] This was verified using site directed mutagenesis to change the Pro210 to an Ala that was fixed in the trans configuration and this mutant protein was found to be fully functional and unable to bind the antibody raised to detect the non-functional receptor.

Example 5

[0079] Further verification of the specificity of the antibody to detect the non-functional receptor came in experiments that labelled macrophages expressing P2X₇. The macrophages bound antibody to the P2X₇ receptors using the P2X₇ universal antibody but did not bind the antibody to non-functional P2X₇ until they had been exposed to cancer cells such as mouse hybridoma cells. Contact between the macrophages and the hybridoma cells induced the expression on the macrophages of non-functional P2X₇ that was detected by the antibody to non-functional P2X₇ as well as the universal P2X₇ antibody.

[0080] The macrophages and B-cell lymphocytes extracted from patients with malignant lymphoma were tested and all these cells bound the antibody to universal P2X₇ as well as the antibody to the non-functional P2X₇ receptors, verifying that P2X₇ was non-functional in all the cancer cells detected, with the apoptotic pore formed by functional P2X₇ unable to form and thus induce apoptosis in cancer cells.

[0081] All such cancer cells from all epithelial cell cancers in humans such as prostate, breast, bowel, skin, stomach, cervix and others as well as malignant lymphoma, chronic lymphocytic leukaemia and brain tumours, as well as the same tumours in other mammals that were tested, including breast and prostate in dog and skin in cat as well as all mouse hybridoma cells, all express the same non-functional P2X₇. Sequence similarity between human, rat, cat, dog and mouse at the chosen epitopes is sufficient for positive identification to be made in all the above cases. This shows that the mechanism of cancer in these mammals is identical in that all cancer cells express non-functional P2X₇ receptors unable to form apoptotic pores that would normally kill the cell when activated. In this way the cancer cells become immortal, with apoptosis being switched off.

Example 6

[0082] As further verification that the cancer cells such as affected B-cell lymphocytes are unable to induce apoptosis through P2X₇ function, B cells from leukaemia patients containing non-functional P2X₇ receptors were incubated with 5 mM ATP for 2 hours in culture. The results were that all the non-functional receptors were forced by the excess ATP to open and induce apoptosis that killed the affected cells.

Example 7

[0083] As further verification that the antibody selectively binds cancer cells, skin from patients with basal cell carcinomas (BCC) were treated with the antibody to the non-functional P2X₇ receptors, suspended in an inert cream base and applied to the lesion and surrounding skin (refer Example 10, below). Within 1 week of daily application of the topical antibody, all trace of the BCCs had disappeared with no effect on surrounding skin since normal skin was devoid of the receptors.

DIAGNOSTIC APPLICATIONS

[0084] Descriptions are provided here by way of example, using the specific non-functional P2X₇ antibody in animals and demonstrating the universal application of the probe and method described herein to the diagnosis of most cancers in humans and other mammals.

[0085] In prostate tissue from humans and mammals, such as cats and dogs, when the antibody is used for diagnosis, no labelling is obtained in the absence of cancer or pre-cancerous lesions. However, the diagnostic method reveals first signs of neoplastic change while there is still no accompanying morphological changes detectable by H&E stain.

[0086] At this stage, it is necessary to stain for the receptor units first appearing in the nuclei of epithelial cells. These migrate to the cytoplasm in later stages of the disease, acting as a field effect throughout the prostate, so that less tissue need be biopsied to be certain of the existence of a tumour. In later stages of the disease, the staining becomes more confined to the apical epithelium.

[0087] Similarly, other epithelial cell cancers, like breast, lung, colon and skin in humans and in other mammals, such as cats and dogs, can be detected with margins as there is no longer a clear field effect in these other tissues.

[0088] The same stage development is seen in these other tissues, like breast and cervix, with nuclear stain preceding cytoplasmic stain, while normal tissue is unstained. Affected ducts and lobules in breast tissue are readily detected due to the local field effect within the individual affected duct system in the breast even where normal morphology suggests there is no cancer. Adjacent unaffected ducts appear unstained. Similarly, affected lymph nodes, directly draining tissue containing a tumour, show signs of the tumour through the field effect of affected lymphocytes. Thus, sentinel nodes can be detected without there being any metastatic cellular spread to the node.

[0089] Skin cancers, such as basal cell carcinoma and squamous cell carcinoma as well as malignant melanomas show positive staining for non-functional receptors and channel components (monomers) in keratinocyte and melanocyte

layers with clear margins beyond which normal skin is unlabelled on both epidermis and deep within the dermis.

[0090] All tested mammalian cancer cell lines such as human prostate (PC3) and breast (MCF7) and rodent hybridomas are positive for the non-functional receptors on the cell surface so that apoptosis is inhibited in these cancer cells. The general application of this diagnostic is seen by way of the same label on mouse hybridoma cells showing the ubiquitous nature of the receptor in other animal types besides human. Normal human B-cell lymphocytes show that functional P2X₇ receptors are expressed on the cell surface, so enabling apoptosis when necessary, while human B-cell lymphocytes from patients with malignant lymphoma show that non-functional P2X₇ receptors are expressed on the cell surface, so curtailing apoptosis.

10 THERAPEUTIC APPLICATIONS

[0091] Targeting this apparently ubiquitous P2X₇ non-functional conformer expressed on the cell surface of cancer cells attempting to undergo apoptosis may be used to treat most cancers in humans and other mammals. Examples are set out below:

15 Example 8

[0092] Mouse hybridoma cells were grown on a macrophage base both in the presence and absence of affinity purified antibody to non-functional P2X₇. Cell counts revealed that over 4 days while cells coincubated with purified normal IgG grew from 1×10^4 to 7×10^4 , coincubation with non-functional P2X₇ antibody kept the cell count to only 1.5×10^4 .

20 Example 9

[0093] This example shows that antibodies raised against the non-functional epitope of the P2X₇ receptor can inhibit tumour formation *in vivo*.

[0094] As shown above, antibodies raised in sheep against the non-functional P2X₇ epitope identified this non-functional P2X₇ apoptotic receptor on the surface of mouse hybridoma cells. Addition of this antibody to hybridoma cell cultures retarded the growth of the cells. Mouse hybridoma cells when injected into prepared inbreed mouse strains will cause tumour formation.

[0095] In this experiment, three groups of 10 Balb-c female mice each received the following treatments:

- Group 1: 10 mice each injected intraperitoneally (IP) with 1×10^6 hybridoma cells in 0.5 ml of cell culture medium on Day 1. On Days 2 and 3, they received an intraperitoneal injection of 0.5 ml of cell culture medium.
- Group 2: 10 mice each injected intraperitoneally (IP) with 1×10^6 hybridoma cells in 0.5 ml of cell culture medium containing 1 mg of purified sheep IgG on Day 1. On Days 2 and 3, they were injected with 0.5 ml of cell culture medium containing 1 mg of purified sheep IgG.
- Group 3: 10 mice each injected intraperitoneally (IP) with 1×10^6 hybridoma cells in 0.5 ml of cell culture medium containing 1 mg of purified sheep anti-P2X7 non-functional epitope IgG on Day 1. On Days 2 and 3, they received a further injection of 0.5 ml of cell culture medium containing 1 mg of purified sheep anti-P2X7 IgG.

[0096] Mice from all the groups were killed on Day 11 and examined for the presence of tumour. The tumours were excised and weighed.

[0097] The results were as follows:

Groups	Observations	Mean Tumour Weight per mice (\pm SD) (g)
1: Control 1	9 out of 10 mice had tumours.	3.98 ± 1.1
2: Control 2	10 out of 10 mice had tumours	2.93 ± 0.9
3: Experimental	9 out of 10 mice had tumours	1.13 ± 0.4

[0098] An analysis of variance showed a significant difference in tumour weight between the groups (probability < 0.01). The experimental group treated with the anti-P2X7 non-functional antibodies was significantly different ($p < 0.01$) from the two control groups. That is, treatment with antibodies against the P2X7 non-functional epitope significantly reduced the amount of tumour in the experimental animals.

Example 10

[0099] Specific affinity purified antibody (to greatly improve specificity) was applied to 3 human basal cell carcinomas ("BCC") either as a liquid held in place for 7 days or suspended in a dimethicone cream base. No trace of the BCC

5 lesions was detectable after treatment, while control skin was entirely unaffected due to the absence of the protein target.

[0100] It is believed that application to patients in general would involve production of a humanised monoclonal antibody (such as herceptin) so that internal cancers could be treated with the same efficacy as is revealed with topical application. All normal functional P2X₇ expressed on the cell surfaces of cells such as lymphocytes would need to remain unaffected by the presence of the antibody to avoid side effects. The antibody should therefore only bind to proteins expressed on 10 the cell surface of cells attempting to but unable to initiate apoptosis. Thus all cells targeted would be only those attempting to kill themselves through programmed cell death, including cancer cells. The P2X₇ receptors on these cells, particularly cancer cells, would be in a non-functional or ATP-depleted state.

ACTIVE IMMUNISATION

[0101] Active immunisation may also be used for therapeutic purposes. In this case the humans or other mammals need to be immunised against a specific epitope or epitopes that are in a conformation that mimics the conformation adopted only by the receptors in their non-functional (ATP-depleted) shape on the cell surface. Conformational flexibility that includes partial exposure of an epitope shape that is present in functional receptors should be avoided. The cis 15 configuration of the epitope Gly200-Thr215 as an example should be fixed before use by appropriate means. As added proof that this concept is sound is the observation that numerous animals including mice, rabbits and sheep used to raise the antibodies have not been inununo-compromised. None of these many animals have ever developed any tumours.

INDUSTRIAL APPLICABILITY

[0102] The invention in all its aspects has application to the fields of human and veterinary medicine and health, with the potential to enable early and accurate diagnosis of diseases and effective treatment, which in many cases is far less invasive or traumatic than those available in the prior art.

30 SEQUENCE LISTING

[0103]

35 <110> Intreat Pty Limited

<120> Antibodies to non-functional P2X7 receptor diagnosis and treatment of cancers and other conditions

40 <130> P31170EP-D1-PCT-MRM/PJG

<140> EP 02715313.9

<141> 2002-01-17

45 <150> PR2579 AU

<151> 2001-01-17

<150> PR5890 AU

<151> 2001-06-22

50 <150> PR5891 AU

<151> 2001-06-22

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<151> 2001-09-03

55 <150> PR7431 AU

<151> 2001-09-03

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20

Phe His Val Ile Ile Phe Ser Tyr Val Cys Phe Ala Leu Val Ser Asp
35 40 45

25

Lys Leu Tyr Gln Arg Lys Glu Pro Val Ile Ser Ser Val His Thr Lys
50 55 60

30

Val Lys Gly Ile Ala Glu Val Lys Glu Glu Ile Val Glu Asn Gly Val
65 70 75 80

35

Lys Lys Leu Val His Ser Val Phe Asp Thr Ala Asp Tyr Thr Phe Pro
85 90 95

40

45

50

55

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5 Gly Gln Glu Gln Arg Leu Cys Pro Glu Tyr Pro Thr Arg Arg Thr Leu
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10 Cys Ser Ser Asp Arg Gly Cys Lys Lys Gly Trp Met Asp Pro Gln Ser
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15 Lys Gly Ile Gln Thr Gly Arg Cys Val Val His Glu Gly Asn Gln Lys
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20 Pro Arg Pro Ala Leu Leu Asn Ser Ala Glu Asn Phe Thr Val Leu Ile
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25 Lys Asn Asn Ile Asp Phe Pro Gly His Asn Tyr Thr Thr Arg Asn Ile
 195 200 205

30 Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe His Lys Thr Gln Asn Pro
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Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile Phe Arg Glu Thr Gly Asp
 225 230 235 240

35 Asn Phe Ser Asp Val Ala Ile Gln Gly Gly Ile Met Gly Ile Glu Ile
 245 250 255

40 Tyr Trp Asp Cys Asn Leu Asp Arg Trp Phe His His Cys His Pro Lys
 260 265 270

Tyr Ser Phe Arg Arg Leu Asp Asp Lys Thr Thr Asn Val Ser Leu Tyr
 275 280 285

45 Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr Tyr Lys Glu Asn Asn Val
 290 295 300

50 Glu Lys Arg Thr Leu Ile Lys Val Phe Gly Ile Arg Phe Asp Ile Leu
 305 310 315 320

55 Val Phe Gly Thr Gly Gly Lys Phe Asp Ile Ile Gln Leu Val Val Tyr
 325 330 335

EP 1961 767 B1

Ile Gly Ser Thr Leu Ser Tyr Phe Gly Leu Ala Ala Val Phe Ile Asp
340 345 350

5 Phe Leu Ile Asp Thr Tyr Ser Ser Asn Cys Cys Arg His His Ile Tyr
355 360 365

10 Pro Trp Cys Lys Cys Cys Gln Pro Cys Val Val Asn Glu Tyr Tyr Tyr
370 375 380

Arg Lys Lys Cys Glu Ser Ile Val Glu Pro Lys Pro Thr Leu Lys Tyr
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15 Val Ser Phe Val Asp Glu Ser His Ile Arg Met Val Asn Gln Gln Leu
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20 Leu Gly Arg Ser Leu Gln Asp Val Lys Gly Gln Glu Val Pro Arg Pro
420 425 430

Ala Met Asp Phe Thr Asp Leu Ser Arg Leu Pro Leu Ala Leu His Asp
435 440 445

25 Thr Pro Pro Ile Pro Gly Gln Pro Glu Glu Ile Gln Leu Leu Arg Lys
450 455 460

30 Glu Ala Thr Pro Arg Ser Arg Asp Ser Pro Val Trp Cys Gln Cys Gly
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35 Ser Cys Leu Pro Ser Gln Leu Pro Glu Ser His Arg Cys Leu Glu Glu
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Leu Arg His Cys Ala Tyr Arg Cys Tyr Ala Thr Trp Arg Phe Gly Ser
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Cys

Claims

40 1. An isolated P2X7 receptor, comprising an amino acid sequence as shown in Figure 1, with the proviso that the
amino acid sequence of the isolated P2X7 receptor contains a proline corresponding to proline 210 shown in Figure
1, said proline being in a cis conformation.

45 2. An isolated P2X7 receptor according to Claim 1, having an amino acid sequence as shown in Figure 1, in which the
proline at position 210 is in a cis conformation.

50 3. A peptide having the amino acid sequence of a fragment of the receptor according to Claim 1, comprising residues
200(Gly) to 215(thr) shown in Figure 1, said sequence including proline at position 210 in a cis conformation, for
use in the prevention or treatment of a disease or condition.

55 4. A peptide for use according to Claim 3, further including an adjuvant.

5. A peptide for use according to Claim 3 or 4, wherein said disease is a cancer selected from the group consisting of
prostate, breast, skin, lung, cervical, uterus, stomach, oesophagus, bladder, colon, vaginal, and blood cancer, and
malignant lymphoma.

6. A sheep, rabbit or mouse antibody that binds to a peptide fragment of a P2X7 receptor having an amino acid
sequence as shown in residues 200-216 of Figure 1 in which the proline at position 210 is in a cis conformation,

wherein the Cys at position 216 of the peptide fragment is coupled to a diphtheria toxin via the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS);
 wherein the antibody does not bind to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 in which proline at position 210 is in a trans conformation.

5 7. A composition for use in the prevention or treatment of a disease or condition, wherein said composition is formulated for topical administration and comprises an antibody, wherein said antibody binds to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 in which proline at position 210 is in a cis conformation, and wherein the antibody does not bind to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 in which proline at position 210 is in a trans conformation.

10 8. A composition for use according to Claim 7, wherein the composition is formulated as a cream, solution, ointment, suspension, powder, tablet, capsule, suppository or pessary.

15 9. A composition for use according to Claim 7 or 8, wherein the disease or condition is skin cancer.

20 10. A composition for use according to any of Claims 7-9, wherein the antibody is affinity-purified, wherein the composition is formulated in a dimethicone cream base, and wherein the disease or condition is human basal cell carcinoma.

25 11. A reagent suitable for detecting a disease or condition by an *in vivo* imaging technique, said reagent comprising an antibody that binds to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 with the proviso that the proline at position 210 is in a cis conformation, wherein the antibody does not bind to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 in which proline at position 210 is in a trans conformation.

30 12. An *in vitro* method for determining whether an individual has a disease or condition, said method comprising:

 (i) contacting a cell or tissue sample from an individual with an antibody that binds to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 with the proviso that the proline at position 210 is in a cis conformation, wherein the antibody does not bind to an extracellular domain of a P2X7 receptor having an amino acid sequence as shown in Figure 1 in which proline at position 210 is in a trans conformation; and

 (ii) determining whether the cell or tissue is bound by the antibody;

35 wherein the cell or tissue sample is a lymph, prostate, breast, skin, lung, uterus, bladder, cervix, stomach or oesophagus sample.

40 13. An *in vitro* method according to Claim 12, wherein the disease or condition is a cancer selected from the group consisting of prostate, breast, skin, lung, cervical, colon, and blood cancer, and malignant lymphoma.

Patentansprüche

45 1. Isolierter P2X7 Rezeptor, umfassend eine Aminosäuresequenz wie in Figur 1 dargestellt, unter der Maßgabe, dass die Aminosäuresequenz des isolierten P2X7-Rezeptors ein Prolin enthält, welches dem Prolin 210, dargestellt in Figur 1, entspricht, wobei besagtes Prolin in einer cis-Konformation vorliegt.

50 2. Isolierter P2X7 Rezeptor gemäß Anspruch 1, welcher eine Aminosäuresequenz, wie in Figur 1, in der das Prolin an Position 210 in einer cis-Konformation vorliegt, dargestellt, hat.

55 3. Peptid, welches die Aminosäuresequenz eines Rezeptorfragments gemäß Anspruch 1, umfassend die Reste 200 (Gly) bis 215 (Thr) wie in Figur 1 dargestellt, wobei besagte Sequenz Prolin an der Position 210 in einer cis-Konformation beinhaltet, zur Verwendung bei der Prävention oder Behandlung einer Erkrankung oder eines Leidens, hat.

60 4. Peptid zur Verwendung gemäß Anspruch 3, weiterhin enthaltend ein Adjuvans.

65 5. Peptid zur Verwendung gemäß Anspruch 3 oder 4, wobei besagte Erkrankung eine Krebserkrankung ausgewählt

aus der Gruppe bestehend aus Prostata-, Brust-, Haut-, Lungen-, Gebärmutterhals-, Gebärmutter-, Magen-, Ösophagus-, Blasen-, Darm-, Vaginal-, und Blutkrebs, und malignem Lymphoma ist.

5 6. Schaf-, Kaninchen- oder Mausantikörper, welcher an ein Peptidfragment eines P2X7-Rezeptors, welcher eine Aminosäuresequenz wie in den Resten 200 bis 216 der Figur 1 dargestellt, in welcher das Prolin an Position 210 in einer cis-Konformation vorliegt, wobei das Cys an Position 216 des Peptidfragmentes durch den chemischen Vernetzer Maleimidocaproyl-N-hydroxysuccinimid (MCS) an ein Diphterietoxin gekoppelt ist, bindet; wobei der Antikörper nicht an eine extrazelluläre Domäne des P2X7-Rezeptors, welcher eine Aminosäuresequenz wie in Figur 1 dargestellt hat, in welcher Prolin an Position 210 in einer trans-Konformation vorliegt, bindet.

10 7. Zusammensetzung zur Verwendung bei der Prävention oder Behandlung einer Erkrankung oder eines Leidens, wobei besagte Zusammensetzung für die topische Verabreichung formuliert ist und einen Antikörper, wobei besagter Antikörper an eine extrazelluläre Domäne eines P2X7-Rezeptors, welcher eine Aminosäuresequenz wie in Figur 1 dargestellt, in welcher Prolin an Position 210 in cis-Konformation vorliegt, bindet, und wobei der Antikörper nicht an eine extrazelluläre Domäne des P2X7-Rezeptors, welcher eine Aminosäuresequenz, wie in Figur 1 dargestellt in welcher Prolin an Position 210 in trans-Konformation vorliegt, hat, bindet, umfasst.

15 8. Zusammensetzung zur Verwendung gemäß Anspruch 7, wobei die Zusammensetzung als eine Creme, Lösung, Salbe, Suspension, Puder, Tablette, Kapsel, Zäpfchen oder Pessar formuliert ist.

20 9. Zusammensetzung zur Verwendung gemäß Anspruch 7 oder 8, wobei die Erkrankung oder das Leiden Hautkrebs ist.

25 10. Zusammensetzung zur Verwendung gemäß einem der Ansprüche 7 bis 9, wobei der Antikörper affinitätschromatographisch gereinigt ist, wobei die Zusammensetzung als eine Dimetikoncremebasis formuliert ist, und worin die Erkrankung oder das Leiden menschliches Basalzellkarzinom ist.

30 11. Reagens geeignet zum Nachweis einer Erkrankung oder eines Leidens durch ein *in vivo* bildgebendes Verfahren, wobei besagtes Reagens einen Antikörper umfasst, der an eine extrazelluläre Domäne eines P2X7-Rezeptors, welcher eine Aminosäuresequenz wie in Figur 1 dargestellt, unter der Voraussetzung, dass das Prolin an Position 210 in einer cis-Konformation vorliegt, hat, wobei der Antikörper nicht an eine extrazelluläre Domäne eines P2X7-Rezeptors, welcher eine Aminosäuresequenz, wie in Figur 1 dargestellt, in der Prolin an Position 210 in einer trans-Konformation vorliegt, bindet, hat.

35 12. *In vitro* Methode zum Nachweis ob ein Individuum eine Erkrankung oder ein Leiden aufweist, wobei die Methode umfasst:

40 (i) in Verbindung bringen einer Zelle oder eines Gewebes eines Individuums mit einem Antikörper, der an eine extrazelluläre Domäne eines P2X7-Rezeptors, welcher eine Aminosäuresequenz wie in Figur 1 dargestellt, unter der Maßgabe, dass das Prolin an Position 210 in einer cis-Konformation vorliegt, hat, wobei der Antikörper nicht an eine extrazelluläre Domäne des P2X7-Rezeptors, welcher eine Aminosäuresequenz wie in Zeichnung 1 dargestellt, in der Prolin an Position 210 in einer trans-Konformation vorliegt, hat, bindet, bindet; und

45 (ii) Nachweis, ob die Zelle oder das Gewebe durch den Antikörper gebunden wird; wobei die Zell- oder Gewebeprobe eine Lymph-, Prostata- Brust-, Haut-, Lungen-, Gebärmutter-, Blasen-, Gebärmutterhals-, Magen- oder Ösophagusprobe ist.

50 13. *In vitro* Methode gemäß Anspruch 12, wobei die Erkrankung oder das Leiden eine Krebserkrankung ausgewählt aus der Gruppe bestehend aus Prostata-, Brust-, Haut-, Lungen-, Gebärmutterhals-, Darm-, und Blutkrebs, und malignem Lymphom ist.

Revendications

55 1. Récepteur P2X7 isolé, comprenant une séquence d'aminoacides telle que présentée sur la figure 1, à condition que la séquence d'aminoacides du récepteur P2X7 isolé contienne une proline correspondant à la proline 210 présentée sur la figure 1, ladite proline étant dans une conformation cis.

2. Récepteur P2X7 isolé selon la revendication 1, ayant une séquence d'aminoacides telle que présentée sur la figure 1, dans laquelle la proline en position 210 est dans une conformation cis.

3. Peptide ayant la séquence d'aminoacides d'un fragment du récepteur selon la revendication 1, comprenant les résidus 200 (Gly) à 215 (Thr) présentés sur la figure 1, ladite séquence comprenant la proline en position 210 dans une conformation cis, à utiliser dans la prévention ou le traitement d'une maladie ou d'une pathologie.

5 4. Peptide à utiliser selon la revendication 3, comprenant en outre un adjuvant.

5 5. Peptide à utiliser selon la revendication 3 ou 4, ladite maladie étant un cancer choisi dans le groupe constitué par le cancer de la prostate, du sein, de la peau, du poumon, du col de l'utérus, de l'utérus, de l'estomac, de l'oesophage, de la vessie, du côlon, du vagin et du sang, et le lymphome malin.

10 6. Anticorps de mouton, de lapin ou de souris, qui se lie à un fragment peptidique d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée dans les résidus 200-216 de la figure 1, dans laquelle la proline en position 210 est dans une conformation cis, le résidu Cys en position 216 du fragment peptidique étant couplé à une toxine diptérique par l'intermédiaire du réticulant chimique maléimidocaproyl-N-hydroxysuccinimide (MCS); l'anticorps ne se liant pas au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1 dans laquelle la proline en position 210 est dans une conformation trans.

15 7. Composition à utiliser dans la prévention ou le traitement d'une maladie ou d'une pathologie, ladite composition étant formulée en vue de l'administration topique et comprenant un anticorps, où ledit anticorps se lie au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1 dans laquelle la proline en position 210 est dans une conformation cis, et ledit anticorps ne se lie pas au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1 dans laquelle la proline en position 210 est dans une conformation trans.

20 8. Composition à utiliser selon la revendication 7, la composition étant formulée sous forme d'une crème, d'une solution, d'une pommade, d'une suspension, d'une poudre, d'un comprimé, d'une capsule, d'un suppositoire ou d'un ovule.

9. Composition à utiliser selon la revendication 7 ou 8, où la maladie ou la pathologie est un cancer de la peau.

30 10. Composition à utiliser selon l'une quelconque des revendications 7-9, où l'anticorps est purifié par affinité, la composition est formulée dans une base de crème diméthicone, et la maladie ou la pathologie est le carcinome basocellulaire humain.

35 11. Réactif convenant pour la détection d'une maladie ou d'une pathologie par une technique d'imagerie *in vivo*, ledit réactif comprenant un anticorps qui se lie au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1, à condition que la proline en position 210 soit dans une conformation cis, l'anticorps ne se liant pas au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1 dans laquelle la proline en position 210 est dans une conformation trans.

40 12. Procédé *in vitro* pour déterminer si un individu a une maladie ou une pathologie, ledit procédé comprenant:

45 (i) la mise en contact d'un échantillon de cellules ou de tissu d'un individu avec un anticorps qui se lie au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1, à condition que la proline en position 210 soit dans une conformation cis, l'anticorps ne se liant pas au domaine extracellulaire d'un récepteur P2X7 ayant une séquence d'aminoacides telle que présentée sur la figure 1 dans laquelle la proline en position 210 est dans une conformation trans; et

50 (ii) la détermination de la liaison éventuelle de la cellule ou du tissu à l'anticorps; l'échantillon de cellules ou de tissu étant un échantillon de lymphé, de prostate, de sein, de peau, de poumon, d'utérus, de vessie, de col de l'utérus, d'estomac ou d'oesophage.

55 13. Procédé *in vitro* selon la revendication 12, où la maladie ou la pathologie est un cancer choisi dans le groupe constitué par le cancer de la prostate, du sein, de la peau, du poumon, du col de l'utérus, du côlon et du sang, et le lymphome malin.

1 MET-PRO-ALA-CYS-CYS-SER-CYS-SER-ASP-VAL-PHE-GLN-TYR-GLE-THR-ASN-LYS-VAL-THR-ARG
 2 ILE-GLN-SER-MET-ASN-TYR-GLY-THR-ILE-LYS-TRP-PHE-PHE-HIS-VAL-ILE-ILE-PHE-SER-TYR
 41 VAL-CYS-PHE-ALA-LEU-VAL-SER-ASP-LYS-LEU-TYR-GLN-ARG-LYS-GLU-PRO-VAL-ILE-SER-SER
 61 VAL-HIS-THR-LYS-VAL-LYS-GLY-ILE-ALA-GLU-VAL-LYS-GLU-GLU-ILE-VAL-GLU-ASN-GLY-VAL
 81 LYS-LYS-LEU-VAL-HIS-SER-VAL-PHE-ASP-THR-ALA-ASP-TYR-THR-PHE-PRO-LEU-GLN-GLY-ASN
 101 SER-PHE-PHE-VAL-MET-THR-ASN-PHE-LEU-LYS-THR-GLU-GLY-GLN-GLU-GLN-ARG-LEU-CYS-PRO
 121 GLU-TYR-PRO-THR-ARG-ARG-THR-LEU-CYS-SER-SER-ASP-ARG-GLY-CYS-LYS-LYS-GLY-TRP-MET
 141 ASP-PRO-GLN-SER-LYS-GLY-ILE-GLN-THR-GLY-ARG-CYS-VAL-VAL-HIS-GLU-GLY-ASN-GLN-LYS
 161 THR-CYS-GLU-VAL-SER-ALA-TRP-CYS-PRO-ILE-GLU-ALA-VAL-GLU-GLU-ALA-PRO-ARG-PRO-ALA
 181 LEU-LEU-ASN-SER-ALA-GLU-ASN-PHE-THR-VAL-LEU-ILE-LYS-ASN-ASN-ILE-ASP-PHE-PRO-GLY
 201 HIS-ASN-TYR-THR-THR-ARG-ASN-ILE-LEU-PRO-GLY-LEU-ASN-ILE-THR-CYS-THR-PHE-HIS-LYS
 221 THR-GLN-ASN-PRO-GLN-CYS-PRO-ILE-PHE-ARG-LEU-GLY-ASP-ILE-PHE-ARG-GLU-THR-GLY-ASP
 241 ASN-PHE-SER-ASP-VAL-ALA-ILE-GLN-GLY-GLY-ILE-MET-GLY-ILE-GLU-ILE-TYR-TRP-ASP-CYS
 261 ASN-LEU-ASP-ARG-TRP-PHE-HIS-HIS-CYS-HIS-PRO-LYS-TYR-SER-PHE-ARG-ARG-LEU-ASP-ASP
 281 LYS-THR-THR-ASN-VAL-SER-LEU-TYR-PRO-GLY-TYR-ASN-PHE-ARG-TYR-ALA-LYS-TYR-TYR-LYS
 301 GLU-ASN-ASN-VAL-GLU-LYS-ARG-THR-LEU-ILE-LYS-VAL-PHE-GLY-ILE-ARG-PHE-ASP-ILE-LEU
 321 VAL-PHE-GLY-THR-GLY-GLY-LYS-PHE-ASP-ILE-ILE-GLN-LEU-VAL-VAL-TYR-ILE-GLY-SER-THR
 341 LEU-SER-TYR-PHE-GLY-LEU-ALA-ALA-VAL-PHE-JLE-ASP-PHE-LEU-ILE-ASP-THR-TYR-SER-SER
 361 ASN-CYS-CYS-ARG-HIS-HIS-ILE-TYR-PRO-TRP-CYS-LYS-CYS-CYS-GLN-PRO-CYS-VAL-VAL-ASN
 381 GLU-TYR-TYR-TYR-ARG-LYS-LYS-CYS-GLU-SER-ILE-VAL-GLU-PRO-LYS-PRO-THR-LEU-LYS-TYR
 401 VAL-SER-PHE-VAL-ASP-GLU-SER-HIS-ILE-ARG-MET-VAL-ASN-GLN-GLN-LEU-LEU-GLY-ARG-SER
 421 LEU-GLN-ASP-VAL-LYS-GLY-GLN-GLU-VAL-PRO-ARG-PRO-ALA-MET-ASP-PHE-THR-ASP-LEU-SER
 441 ARG-LEU-PRO-LEU-ALA-LEU-HIS-ASP-THR-PRO-PRO-ILE-PRO-GLY-GLN-PRO-GLU-GLU-ILE-GLN
 461 LEU-LEU-ARG-LYS-GLU-ALA-THR-PRO-ARG-SER-ARG-ASP-SER-PRO-VAL-TRP-CYS-GLN-CYS-GLY
 481 SER-CYS-LEU-PRO-SER-GLN-LEU-PRO-GLU-SER-HIS-ARG-CYS-LEU-GLU-GLU-LEU-CYS-CYS-ARG
 501 LYS-LYS-PRO-GLY-ALA-CYS-ILE-THR-THR-SER-GLU-LEU-PHE-ARG-LYS-LEU-VAL-LEU-SER-ARG
 521 HIS-VAL-LEU-GLN-PHE-LEU-LEU-TYR-GLN-GLU-PRO-LEU-LEU-ALA-LEU-ASP-VAL-ASP-SER
 541 THR-ASN-SER-ARG-LEU-ARG-HIS-CYS-ALA-TYR-ARG-CYS-TYR-ALA-THR-TRP-ARG-PHE-GLY-SER
 561 GLN-ASP-MET-ALA-ASP-PHE-ALA-ILE-LEU-PRO-SER-CYS-CYS-ARG-TRP-ARG-ILE-ARG-LYS-GLU
 581 PHE-PRO-LYS-SER-GLU-GLY-GLN-TYR-SER-GLY-PHE-LYS-SER-PRO-TYR

FIGURE 1

Sequence of human P2X₇ receptor.

REFERENCES CITED IN THE DESCRIPTION

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摘要(译)

提供了分离的P2X7受体，其包含与图1所示序列具有同源性的氨基酸序列，其中分离的P2X7受体的氨基酸序列含有对应于图1中所示的脯氨酸210的脯氨酸，所述脯氨酸位于顺式构象。

Met Pro Ala Cys Cys Ser Cys Ser Asp Val Phe Gln Tyr Glu Thr Asn
1 5 10 15

Lys Val Thr Arg Ile Gln Ser Met Asn Tyr Gly Thr Ile Lys Trp Phe
20 25 30

Phe His Val Ile Ile Phe Ser Tyr Val Cys Phe Ala Leu Val Ser Asp
35 40 45

Lys Leu Tyr Gln Arg Lys Glu Pro Val Ile Ser Ser Val His Thr Lys
50 55 60

Val Lys Gly Ile Ala Glu Val Lys Glu Glu Ile Val Glu Asn Gly Val
65 70 75 80

Lys Lys Leu Val His Ser Val Phe Asp Thr Ala Asp Tyr Thr Phe Pro
85 90 95