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(54) Titre : TOXINES ET RADIONUCLEIDES COUPLES AUX LIGANDS DU RECEPTEUR IGF-1 POUR LE TRAITEMENT D'UN CANCER

(54) Title: TOXINS AND RADIONUCLIDES COUPLED TO IGF-1 RECEPTOR LIGANDS FOR TREATMENT OF CANCER

(57) Abrégé/Abstract:
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TOXINS AND RADIONUCLIDES COUPLED TO IGF-1 RECEPTOR LIGANDS FOR TREATMENT OF CANCER

Background

Currently 1.3 million people are diagnosed with cancer each year in the United States alone, and over 500,000 die. One method emerging as a new type of treatment for cancer is a type of radiation therapy using an antibody coupled to a therapeutic radionuclide, where the antibody recognizes a target that is specific for a particular type of cancer or found predominantly on cancerous cells. Two examples are currently approved for treatment in the United States: ibitrumomab tiuxetan (ZEVALIN®) and tositumomab (BEXAR®). Ibitrumomab tiuxetan recognizes the CD20 antigen, which is found on normal and malignant B cells. The antibody is coupled to a therapeutic Yttrium-90 radionuclide by a tiuxetan chelator moiety. Tositumomab also recognizes the CD20 antigen, but it is labelled with a I-131 radionuclide. Both are used to treat B-cell non-Hodgkin's lymphoma.

Anti-cancer agents involving toxins, such as diphtheria toxin or Pseudomonas exotoxin, coupled to antibodies that recognize targets found on cancerous cells have also been studied, although no agents of this type are currently approved in the United States.

New agents for treating cancer are needed. Preferably the agents would be targeted to cancer cells and largely spare healthy cells.

Summary

The invention provides new anti-cancer therapeutic agents involving ligands to the insulin-like growth factor-1 (IGF-1) receptor, such as IGF-1 itself, coupled to a therapeutic radionuclide or to a cellular toxin such as diphtheria toxin or Pseudomonas exotoxin.

In previous work, toxins and radionuclides have been coupled to antibodies to deliver them to antigens found specifically on cancer cells. One drawback to the use of antibodies is that antibodies are large molecules that often generate an immune response and hypersensitivity response in patients to whom they are administered. This can interfere with their use therapeutically. The size of antibodies also means they may not penetrate solid tumors efficiently. Antibodies also are not typically internalized by the

cells to which they bind. They simply sit on the cell surface. With radionuclide-labelled antibodies, it would be somewhat preferable if the radionuclide were internalized to the target cell and thus were closer to the nucleic acids of the cell, which are the therapeutic target of the radioactivity than it is on the surface of the cell. With toxin-conjugated antibodies, it is a larger problem if the antibody and toxin are not internalized into the target cancer cell, since toxins typically must be internalized by the cell in order to kill the cell.

The defining feature of cancer is that cancerous cells divide without appropriate control. Radiation is used in anti-cancer therapy because radiation is more toxic to actively dividing cells than to resting cells that are not dividing. But cancer cells are not always dividing. The effectiveness of radiation therapy could be increased if the cells could be induced to divide at or around the time they are exposed to radiation.

The IGF-1 receptor is significantly overexpressed in most tumors from almost all types of cancer. IGF-1 is a peptide of 70 amino acid residues having 40% identity with proinsulin. (Daughaday, W.H., et al., 1989, *Endocrine Revs*. 10:68.) IGF-1 is secreted by the liver into the circulatory system and stimulates growth of many cell types. IGF-1 is also produced by many cell types throughout the body, including many cancers, for autocrine and paracrine effects. IGF-1 production is stimulated by growth hormone. (Stewart, C.H., e t al., 1996, *Physiol. Revs*. 76:1005; Yakar, S., et al., 2002, *Endocrine* 19:239.) IGF-1 receptors were found to be 43 times more numerous on malignant breast cancer tissue than benign breast tissue (Jammes, H. et al. *Br. J. Cancer* 66:248-253).

IGF-1's biological role is to stimulate cell division. This is significant since radiation is more toxic to dividing cells than non-dividing cells. Thus, a radioactively labelled IGF-1 receptor agonist not only will be targeted with a high degree of specificity to cancer cells, but it may also cause the cells to divide as they are being irradiated, thus sensitizing them to the radiation.

Furthermore, upon binding to its receptor, IGF-1 is internalized to the cell by receptor-mediated endocytosis. This brings a radionuclide attached to IGF-1 or to another IGF-1 receptor ligand into the cell and closer to the target nucleic acids. This factor is more important for toxin-IGF-1 receptor ligand conjugates, since it brings the toxin into the cell, and most toxins must penetrate the cell in order to exert their toxicity.

Accordingly, one embodiment of the invention provides an insulin-like growth

factor (IGF-1) receptor ligand carrying a therapeutic radionuclide.

Another embodiment of the invention provides an anti-cancer therapeutic agent comprising: an IGF-1 receptor ligand linked to a toxin.

Another embodiment of the invention provides a method of treating cancer in a mammal involving administering to the mammal a therapeutically effective amount of an IGF-1 receptor ligand carrying a therapeutic radionuclide.

Another embodiment of the invention provides a method of treating cancer in a mammal involving administering to the mammal a therapeutically effective amount of a therapeutic agent containing an IGF-1 receptor ligand linked to a toxin.

Another embodiment of the invention provides a method of inhibiting growth of cancer cells involving contacting the cancer cells with an IGF-1 receptor ligand carrying a therapeutic radionuclide.

Another embodiment of the invention provides a method of inhibiting growth of cancer cells involving contacting the cancer cells with a therapeutic agent containing an insulin-like growth factor-1 (IGF-1) receptor ligand linked to a toxin.

Another embodiment of the invention provides a method of screening a compound for anti-cancer activity involving contacting cancer cells with a compound comprising an IGF-1 receptor ligand carrying a therapeutic radionuclide.

Another embodiment of the invention provides a method of screening a compound for anti-cancer activity involving contacting cancer cells with a compound comprising an IGF-1 receptor ligand linked to a toxin.

Detailed Description

Definitions:

As used herein, the term "toxin" refers to a molecule or moiety that is generally lethal to all cells. This contrasts with traditional anti-cancer chemotherapy agents, which are selectively lethal to dividing cells and less lethal to non-dividing cells. Anti-cancer chemotherapy agents

that are selectively lethal to dividing cells and can be administered sytemically to treat cancer in mammals are excluded from the term "toxin" as used herein.

As used herein, the term "therapeutic radionuclide" refers to an atom that emits a form of radiation that is therapeutically useful to kill cancer cells.

As used herein, the term "containing" is open-ended, allowing the inclusion of other unnamed elements, and has the same meaning as "comprising."

Description:

One embodiment of the invention provides an IGF-1 receptor ligand carrying a therapeutic radionuclide. The radionuclide can be directly coupled to the ligand in some embodiments – for example by iodination of tyrosine or histidine residues on a protein ligand with I-131.

In other embodiments, the radionuclide is coupled to the ligand by a linker moiety. The linker moiety may include a chelator for holding the radionuclide. An example of a chelator is tiuxetan [N-[2-bis(carboxymethyl)amino]-(p-isothiocyanateophenyl)-propyl]-[N-[2-bis(carboxymethyl)amino]-2-(methyl)-ethyl]glycine]. Another example of a ligand for coupling is diethelyenetriaminepentaacetic acid (DTPA). Methods of radiolabelling IGF-1 ligands directly or with chelator linkers are described in greater detail in Example 1.

Examples of suitable radionuclides are Iodine-131, Yttrium-90, Indium-111, Rhenium-186, and Lutetium-177. These are all beta or gamma emitters with short half lives. With IGF-1 receptor ligands that are internalized into the cell, alpha emitters may also be therapeutically effective radionuclides. Alpha particles are absorbed in such short distances that they are ordinarily not therapeutically useful when emitted from the surface of a cancer cell or the outer surface of a tumor. But many ligands to IGF-1 receptor will be internalized by receptor-mediated endocytosis. This will bring the radionuclide into the nucleus of the cell, in direct contact with the DNA target. Thus, in that case an alpha emitting radioisotope may be useful.

The IGF-1 receptor ligand carrying a therapeutic radionuclide can be an IGF-1 receptor agonist. An agonist will stimulate cell division, and since radiation is more lethal for dividing cells than non-dividing cells, this will sensitize the cancerous cells to killing by the radiation. An example of a suitable IGF-1 receptor ligand that is an agonist is IGF-1 itself. The sequence of human IGF-1 is presented as SEQ ID NO:1.

Other examples of IGF receptor agonists suitable for use in the invention include variants of IGF-1 that activate the receptor but have reduced affinity for the soluble IGF-1 binding proteins. Some examples are disclosed in U.S. Patent No. 4,876,242. IGF-1

binding proteins are natural serum proteins that bind to IGF-1, holding it in circulation and extending its biological half-life. It may be advantageous for radiolabelled and toxin-linked IGF-1 receptor ligands of this invention to have reduced binding to the IGF-1 binding proteins, because that reduced binding would accelerate the release of the agent to bind to the IGF-1 receptors. A particularly important variant of IGF-1 that binds to the IGF receptor but not to the soluble IGF binding proteins is the variant in which the first 16 residues of IGF-1 are replaced by the first 17 residues of the B-chain of insulin (U.S. Patent No. 4,876,242) (SEQ ID NO:2).

In another particular embodiment, the IGF-1 receptor agonist is IGF-2 (SEQ ID NO:8).

In particular embodiments of the invention, the toxin conjugates and radiolabelled agents include a variant IGF-1 protein that has reduced binding affinity for the soluble IGF-1 binding proteins. In particular embodiments, the variant has greater than 100-fold lower, more preferably greater than 1000-fold lower, binding affinity for the soluble IGF-1 binding proteins than does native IGF-1. Binding affinity for the soluble IGF-1 binding proteins can be assayed as described in Bayne, M.L. et al., 1988, *J. Biol. Chem.* 263:6233-6239; and Bayne, M.L. et al., 1987, *Proc. Natl. Acad. Sci. USA* 84:2638-2642. The technique involves measuring the variant IGF-1's inhibition constant for inhibiting ^{125}I-IGF-1 binding to the acid-stable protein fraction of human serum.

In particular embodiments of the invention, the IGF-1 receptor ligand that is radiolabelled or part of a toxin conjugate has a K_D for the IGF-1 receptor of less than less than 10 μM, less than 1 μM, less than 100 nM, less than 50 nM, less than 20 nM, less than 10 nM, less than 5 nM, less than 2 nM, or less than 1 nM. Preferably, the ligands have a K_D for the IGF-1 receptor of less than about 50 nM, more preferably less than about 20 nM. Binding affinity for the IGF-1 receptor can be determined as described, for instance, in Bayne, M.L. et al., 1988, *J. Biol. Chem.* 263:6233-6239; and Bayne, M.L. et al., 1987, *Proc. Natl. Acad. Sci. USA* 84:2638-2642, by measuring competition with radiolabelled IGF-1 for binding to placental membranes.

In some embodiments, the IGF-1 receptor ligand is an IGF-1 receptor antagonist. Some antagonist peptides are disclosed in U.S. published patent application No. 2004/0023887, including the peptide SFSCLESLVNGPAEKSRGQWDGCRKK (SEQ ID NO:3).

In particular embodiments, the IGF-1 receptor ligand has a greater affinity for the IGF-1 receptor than for the insulin receptor. In particular embodiments, the IGF-1 receptor ligand is not insulin. In particular embodiments, the IGF-1 receptor ligand has a higher affinity for the IGF-1 receptor than for the insulin receptor.

In particular embodiments, the IGF-1 receptor ligand is not an antibody or an antibody fragment. In other embodiments, the IGF-1 receptor ligand is an antibody or antibody fragment.

In particular embodiments, the IGF-1 receptor ligand is a polypeptide of fewer than 200 amino acid residues or fewer than 100 amino acid residues.

One embodiment of the invention is an anti-cancer therapeutic agent containing an IGF-1 receptor ligand linked to a toxin.

In particular embodiments of the toxin conjugates, the IGF-1 receptor ligand is an IGF-1 receptor agonist. For instance, it can be IGF-1 or one of the variant IGF-1s of U.S. Patent No. 4,876,242 that has reduced binding to the soluble IGF-1 binding proteins.

In some embodiments, the IGF-1 receptor ligand is an IGF-1 receptor antagonist. Some antagonist peptides are disclosed in U.S. published patent application No. 2004/0023887, including the peptide SFSCLESLVNGPAEKSRGQWDGCRKK (SEQ ID NO:3).

In particular embodiments of the toxin conjugates, the IGF-1 receptor ligand has a greater affinity for the IGF-1 receptor than for the insulin receptor. In particular embodiments, the IGF-1 receptor ligand is not insulin. In particular embodiments, the IGF-1 receptor ligand has a higher affinity for the IGF-1 receptor than for the insulin receptor.

In particular embodiments of the toxin conjugates, the IGF-1 receptor ligand is not an antibody or an antibody fragment. In other embodiments, the IGF-1 receptor ligand is an antibody or antibody fragment.

In particular embodiments of the toxin conjugates, the IGF-1 receptor ligand is a polypeptide of fewer than 200 amino acid residues or fewer than 100 amino acid residues.

In particular embodiments, the toxin is diphtheria toxin, Pseudomonas exotoxin, Clostridium perfringens enterotoxin, ricin, or a toxic fragment thereof.

In particular embodiments, the toxin portion of the IGF-1 receptor ligand-toxin

conjugate is a toxic fragment of a naturally occurring toxin. Most bacterial toxins, such as diphtheria toxin, Pseudomonas exotoxin, and Clostridium perfringens enterotoxin, include a receptor-binding moiety that targets the toxin to a particular cell-surface receptor, and a moiety that is responsible for the toxicity of the toxin protein. For instance, Clostridium perfringens enterotoxin binds to claudin-3 and claudin-4 on the cell surface. Clostridium perfringens enterotoxin (CPE) is a protein of 319 amino acid residues (SEQ ID NO:4). A peptide consisting of residues 290-319 of Clostridium perfringens enterotoxin binds to claudin-3 and claudin-4 but is not toxic (Hanna, P.C., et al., 1991, *J. Biol. Chem.* 266:11037-43). Approximately residues 45-116 of CPE are responsible for cytolysis of cells through forming large complexes in the cell membrane (Kokai-Kun, J.F. et al., 1996, *Infect. Immun.* 64:1020-25; Kokai-Kun, J.F. et al., 1997, *Clin. Infect. Dis.* 25 (Suppl.2):S165-S167; Kokai-Kun, J.F. et al., Infect. Immun. 65:1014-1022; Kokai-Kun, J.F. et al., 1999, *Infect. Immun.* 67:5634-5641; Hanna, P.C., et al., 1991, *J. Biol. Chem.* 266:11037-43). Deletion of just residues 315-319 is enough to abolish binding to the receptors (Kokai-Kun, J.F. et al., 1999, *Infect. Immun.* 67:5634-5641). Thus, in some embodiments of the present toxin conjugates, the toxin moiety of the conjugates is a fragment of CPE containing residues 45-116 of SEQ ID NO:4, but lacking residues 315-319 of SEQ ID NO:4. For instance, the toxin may be residues 1-314, 1-289, 1-116, 45-314, 45-289, or 45-116 of SEQ ID NO:4.

In other embodiments, the toxin is diphtheria toxin or a toxic fragment thereof. Diphtheria toxin is a protein of 535 amino acid residues (SEQ ID NO:5). It contains three domains. Residues 1-193 are the catalytic domain, having the ADP-ribosyl transferase activity that is responsible for inactivating elongation factor-2 in cells to kill them (Choe, S. et al., 1992, *Nature* 357:216-222). Approximately residues 203-378 are responsible for translocation of the toxin across the cell membrane (id). And approximately residues 386-535 are responsible for binding to the receptor (id). Fusions of interleukin-2 to residues 1-389 of diphtheria toxin have been found to be more cytotoxic against cells having interleukin-2 receptors than fusions to longer fragments of diphtheria toxin (Williams, D.P. et al., 1990, *J. Biol. Chem.* 265:11885-89); Kiyokawa, T. et al., 1991, *Protein Engineering* 4:463-468). Thus, in a particular embodiment, the toxin portion of the present conjugates is residues 1-389 of SEQ ID NO:5.

In one embodiment, the toxin-IGF-1 receptor ligand conjugate is or comprises

SEQ ID NO:6, which is residues 1-389 of diphtheria toxin coupled to the variant IGF-1 SEQ ID NO:2 that does not bind to the soluble IGF-binding proteins. The diphtheria toxin portion and IGF-1 portion of the conjugate in SEQ ID NO:6 are separated by a His-Ala linker.

In another embodiment, the toxin-IGF-1 receptor ligand conjugate is or comprises SEQ ID NO:7, which is residues 45-289 of Clostridium perfingens enterotoxin (CPE) coupled to SEQ ID NO:2. The CPE portion and IGF-1 portion of the conjugate in SEQ ID NO:7 are separated by a His-Ala linker.

Toxins can be chemically conjugated to proteinaceous IGF-1 receptor ligands by methods disclosed in U.S. provisional patent application serial no. 60/513,048 and international patent application WO2005/041865, which are incorporated by reference.

Where the toxins and IGF-1 receptor ligands are both proteins or peptides, the conjugates are preferably fusion proteins, expressed by recombinant DNA methods, of the toxins and the IGF-1 receptor ligands.

In particular embodiments of the toxin conjugates, the toxin is a calicheamicin or a derivative thereof (Merck Index 13th edition, #1722). Specifically it may be calicheamicin γ_1^I or a derivative thereof. Derivatization of the calicheamicins typically occurs on the trisulfide (Hamann, P.R. et al., 2005, Bioconjugate Chem. 16:346-353; Hinman, L.M. et al., pp. 87-106 in *Enediyne Antibiotics as Antitumor Agents*, D.B. Borders, T.W. Doyle, eds., Marcel Dekker Inc. New York, 1995). The calicheamicins are sometimes considered considered to be conventional anti-cancer chemotherapy agents in contradistinction to toxins. But they are treated as "toxins" herein because when administered systemically without conjugation to a targeting agent, at or below the doses that in the short term are most effective against tumors in an animal model, they exhibit almost 100% delayed lethality to the animals (Durr, F.E. et al. pp. 127-136 in *Enediyne Antibiotics as Antitumor Agents*, D.B. Borders, T.W. Doyle, eds., Marcel Dekker Inc. New York, 1995).

Preferably, an IGF-1 receptor ligand that is a protein (e.g., SEQ ID NO:1 or SEQ ID NO:2) is conjugated through one or more of its lysine residues to N-acetyl-calicheamicin γ_1^I. N-acetyl-calicheamicin γ_1^I can be made by acetylating calicheamicin γ_1^I in methanol in an excess of acetic anhydride. NAc-gamma calicheamicin dimethyl acid O-succinimidyl ester (compound 6 in Hamann, P.R. et al., 2005, *Bioconjugate*

Chem. 16:346-353) is prepared as described in Hamann, P.R. et al., 2002, *Bioconjugate Chem.* 13:40-46. This activated succinimidyl ester reacts with amine groups on proteins to form an amide bond conjugate between the IGF-1 receptor ligand and N-acetyl-calicheamicin γ_1^I.

One embodiment of the invention is a method of treating cancer in a mammal involving administering to the mammal a therapeutically effective amount of an IGF-1 receptor ligand carrying a therapeutic radionuclide. In particular embodiments, the cancer is non-small cell lung cancer, prostate cancer, colorectal cancer, breast cancer, pancreatic cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia, liver cancer, stomach cancer, ovarian cancer, uterine cancer, testicular cancer, brain cancer, or melanoma.

One embodiment of the invention is a method of treating cancer in a mammal involving administering to the mammal a therapeutically effective amount of a therapeutic agent containing an insulin-like growth factor-1 (IGF-1) receptor ligand linked to a toxin. In particular embodiments, the cancer is non-small cell lung cancer, prostate cancer, colorectal cancer, breast cancer, pancreatic cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia, liver cancer, stomach cancer, ovarian cancer, uterine cancer, testicular cancer, brain cancer, or melanoma.

One embodiment of the invention is a method of inhibiting growth of cancer cells involing contacting the cancer cells with an IGF-1 receptor ligand carrying a therapeutic radionuclide. The contacting can be in vivo or in vitro. In particular embodiments, the IGF-1 receptor ligand carrying the therapeutic radionuclide kills the cancer cells.

Another embodiment of the invention is a method of inhibiting growth of cancer cells involving contacting the cancer cells with a therapeutic agent containing an IGF-1 receptor ligand linked to a toxin. The contacting can be in vitro or in vivo. In particular embodiments, the cancer cells are killed.

Another embodiment of the invention is a method of screening a compound for anti-cancer activity involving contacting cancer cells with a compound comprising an IGF-1 receptor ligand carrying a therapeutic radionuclide. The method involves monitoring the growth or killing of the cancer cells. The contacting can be in vitro or in vivo.

Another embodiment of the invention involves screening a compound for anti-

cancer activity involving contacting cancer cells with a compound comprising an IGF-1 receptor ligand linked to a toxin. The method involves monitoring the growth or killing of the cancer cells. The contacting can be in vitro or in vivo.

The invention will now be illustrated by the following non-limiting examples.

Example 1

Treatment of a Mouse Breast Cancer Model with ^{131}I-IGF-1 and ^{90}Y-IGF-1

Materials and Methods

IGF is radioiodinated with I-131 (Amersham Biosciences) by use of the IODO-GEN method (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril; Pierce Biotechnology, Inc). Briefly, IGF-1 is incubated at room temperature in 85 µl PBS (0.1 M, pH 7.4) in a glass vial coated with 50-100 µg of IODO-GEN. After 10 min, the reaciton is stopped by the additon of 100 µl of a saturated tyrosine solution. The reaction mixture then is separated on a PD--10 column (Amersham Biosciences), eluting with PBS and 0.5% bovine serum albumin. The specific activity is approximately 80 kBq/µg (Koppe, M.J. et al. 2004. *J. Nucl. Med.* 45:1224-1232).

To label with Y-90, IGF-1 is conjugated with isothiocyanato-benzyl-diethylenetriaminepentaacetic acid (ITC-DTPA, Macrocyclics, Inc.). ITC-DTPA is conjugated to IGF-1 in $NaHCO_3$ buffer (0.1 M, pH 8.2) by use of a 100-fold molar excess of ITC-DTPA as described by Ruegg et al. (Ruegg, C.L. et al., 1990, *Cancer Res.* 50:4221-26) for 1 hour at room temperature. The DTPA-IGF-1 conjugate is purified by dialysis against ammoinum acetate buffer (0.1M, pH 5.0). The number of DTPA ligands per IGF-1 molecule can be determined by the method of Hnatowich, D.J. et al. 1983, *J. Immunol. Methods* 65:147-157. Up to three ligands may be conjugated per IGF-1. The purified DTPA-IGF-1 conjugate (0.8 mg/ml) is inclubated with Y-90 (Perkin-Elmer Corp.) in ammonium acetate buffer (0.1 M, pH 5.4) at room temperature for 20 minutes. The specific activity is approximately 370 kBq/µg (Koppe, M.J. et al. 2004. *J. Nucl. Med.* 45:1224-1232).

The radiolabelled preprations are purified by gel filtartion on a PD-10 column (Amersham), eluting with PBS with 0.5% bovine serum albumin. The amount of free radioisotope is determined by thin-layer chromatography with silica gel strips and citrate

buffer (0.1 M, pH 6.0) as the mobile phase. Less than 5% of the label should be unconjugated to IGF-1.

The maximal tolerated dose of unlabelled IGF-1, ^{131}I-IGF-1, and ^{90}Y-IGF-1 is determined in six-week old female nude mice (nu/nu, Sprague Dawley, Madison, Wisconsin).

MCF-7 is a human breast cancer cell line that is responsive to IGF-1 (Dupont, J., et al., 2003, *J. Biol. Chem.* 278:37256). MCF-7 cells are cultured in F12/DME medium supplemented with 5% fetal calf serum (FCS) and 10 μg/ml insulin in 95% air, 5% CO_2 at 37°C (Karey, K.P. et al., 1988, *Cancer Res.* 48:4083-4092.) Cells are transferred every 4-6 days and seeded at 1.75 x 10^6 cells/plate in 20 ml medium in a 10 cm dish.

MCF-7 cells are cultured as described above. Six-week-old female nude mice (nu/nu, Sprague Dawley, Madison, Wisconsin) are injected subcutaneously in the back with 5 x 10^6 MCF-7 cells in 0.05 ml serum-free medium. Estrogen production in the mice is inadequate to support growth of MCF-7, so the mice are given injections of beta-estradiol dissolved in sesame oil (0.1 mg/0.05 ml oil s.c.) beginning one day before injection of the cancer cells and weekly thereafter. Tumors are allowed to grow until a diameter of 5 mm. (Hardman, W.E., et al., 1999, *Anticancer Res.* 19:2269.)

When the tumors reach 5 mm, IGF-1, ^{131}I-IGF-1, or ^{90}Y-IGF-1 is injected at half its maximal tolerated dose in 5 mice each. Five control mice receive no agent.

Tumor size is monitored every 3 days.

Results:

It is determined that mice harboring MCF-7 tumors and receiving ^{131}I-IGF-1 or ^{90}Y-IGF-1 survive longer and have slower tumor growth than control mice receiving no treatment or receiving unlabelled IGF-1.

Example 2

Treatment of a Mouse Breast Cancer Model with IGF-1-dgRicin A conjugate

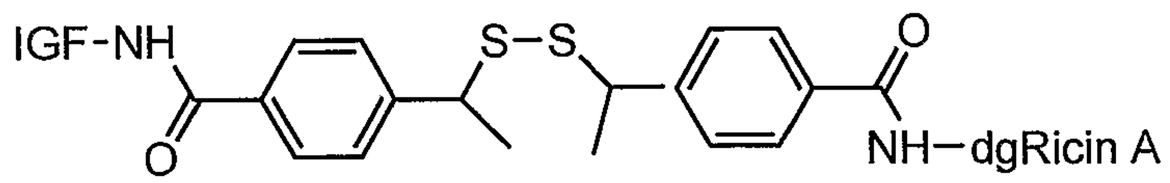
Materials and Methods:

Ricin A chain is prepared as described in Gregg, E.O. et al. (1987, *J. Immunol.* 138:4502-08). Ricin A chain is deglycosylated as described in Blakey, D.C. et al. (1987, *Cancer Res.* 47:947-952).

The conjugation procedure is generally as described in Thorpe, P.E. et al. (1982, *Immunol. Rev.* 62:119-158) and Huang, X. et al. (2004, *Prostate* 61:1-11). To a solution of IGF-1 (1 ml, 10 mg/ml) in 50 mM borate buffer (pH 9.0) was added 1.2 mg 4-succinimidyloxycarbonyl-methyl-α-[2-pyridyldithio]toluene (SMPT, Pierce Biotechnology) dissolved in 10 µl dimethylformamide. That is approximately a 3:1 molar ratio of SMPT to IGF-1. After 30 minutes stirring at room temperature, the reaction mixture is passed through a Sephadex G-25 column equilibrated with 0.1 M sodium acetate, 0.1 M NaCl, pH 4.5. The derivatized protein is eluted with the same buffer. Deglycosylated ricin A chain is derivatized with SMPT by the same procedure at a ratio of 2.6 SMPT per ricin A chain molecule. The derivatized deglycosylated ricin A is separated from reagents by passing the solution through a SEPHADEX G-25 column equilibrated with 0.1 M sodium acetate, 0.1 M NaCl, pH 4.5.

The derivatized IGF-1 is concentrated to 2.5 ml, and 10 mg of dithiothreitol is added. After stirring for 30 minutes at room temperature, the solution is passed through a SEPHADEX G-25 column equilibrated with nitrogen-flushed PBS. The eluted IGF-1 is run directly into a solution of the derivatized deglycosylated ricin A to react in a mixture with a 1:1.5 ratio of IGF-1 to deglycosylated ricin A (dgA). The reaction forms an IGF-1-dgA conjugate with 1 dgA per IGF-1.

Conjugates with 1 dgA per IGF-1 are purified by size-exclusion chromatography on SUPERDEX 200. The conjugate is characterized by SDS-PAGE to verify that the material consists of purified conjugate with 1 IGF-1 linked to 1 deglycosylated ricin A chain. The structure of the conjugate is shown below, where the linker is attached to lysine side chains or the N-terminal alpha-amino groups of the proteins.



Mouse treatment: MCF-7 cells are cultured as described above. Six-week-old female nude mice (nu/nu, Sprague Dawley, Madison, Wisconsin) are injected subcutaneously in the back with 5 x 10^6 MCF-7 cells in 0.05 ml serum-free medium. Estrogen production in the mice is inadequate to support growth of MCF-7, so the mice are given injections of beta-estradiol dissolved in sesame oil (0.1 mg/0.05 ml oil s.c.)

beginning one day before injection of the cancer cells and weekly thereafter. Tumors are allowed to grow until a diameter of 5 mm. (Hardman, W.E., et al., 1999, *Anticancer Res.* 19:2269.)

The maximal tolerated dose of the conjugate is determined by i.p. injection of a range of doses of the conjugate into mice. Then half the maximal tolerated dose of the conjugate is injected i.p. into 5 mice with 5 mm tumors. Five control mice with 5 mm tumors are untreated. Tumor size is monitored every 3 days thereafter.

Results:

It is determined that mice receiving the IGF-1-dgA conjugate survive longer and have slower tumor growth than control untreated mice.

All cited patents, patent documents, and other references are incorporated by reference.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 13

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JUMBO APPLICATIONS/PATENTS

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NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

What is claimed is:

1. An insulin-like growth factor-1 (IGF-1) receptor ligand carrying a therapeutic radionuclide.

2. The IGF-1 receptor ligand of claim 1 wherein the ligand is coupled to the radionuclide by a linker moiety.

3. The IGF-1 receptor ligand of claim 1 wherein the linker moiety comprises a chelator.

4. The IGF-1 receptor ligand of claim 1 wherein the therapeutic radionuclide is Iodine-131, Yttrium-90, Indium-111, Rhenium-186, or Lutetium-177.

5. The IGF-1 receptor ligand of claim 1 wherein the ligand is an IGF-1 receptor agonist.

6. The IGF-1 receptor ligand of claim 5 wherein the ligand is IGF-1.

7. The IGF-1 receptor ligand of claim 5 wherein the ligand is an IGF-1 receptor antagonist.

8. The IGF-1 receptor ligand of claim 1 wherein the ligand has a greater affinity for the IGF-1 receptor than for the insulin receptor.

9. An anti-cancer therapeutic agent comprising:
an insulin-like growth factor-1 (IGF-1) receptor ligand linked to
a toxin.

10. The anti-cancer therapeutic agent of claim 9 wherein the IGF-1 receptor ligand is an IGF-1 receptor agonist.

11. The anti-cancer therapeutic agent of claim 10 wherein the IGF-1 receptor ligand is IGF-1.

12. The anti-cancer therapeutic agent of claim 9 wherein the IGF-1 receptor ligand is an IGF-1 receptor antagonist.

13. The anti-cancer therapeutic agent of claim 9 wherein the IGF-1 receptor ligand has a greater affinity for the IGF-1 receptor than for the insulin receptor.

14. The anti-cancer therapeutic agent of claim 9 wherein the toxin is diphtheria toxin, Pseudomonas exotoxin, Clostridium perfringens enterotoxin, ricin, or a toxic fragment thereof.

15. A method of treating cancer in a mammal comprising:

administering to the mammal a therapeutically effective amount of an IGF-1 receptor ligand carrying a therapeutic radionuclide.

16. The method of claim 15 wherein the cancer is non-small cell lung cancer, prostate cancer, colorectal cancer, breast cancer, pancreatic cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia, liver cancer, stomach cancer, ovarian cancer, uterine cancer, testicular cancer, brain cancer, or melanoma.

17. A method of treating cancer in a mammal comprising:

administering to the mammal a therapeutically effective amount of a therapeutic agent containing an insulin-like growth factor-1 (IGF-1) receptor ligand linked to a toxin.

18. The method of claim 17 wherein the cancer is non-small cell lung cancer, prostate cancer, colorectal cancer, breast cancer, pancreatic cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia, liver cancer, stomach cancer, ovarian cancer, uterine cancer, testicular cancer, brain cancer, or melanoma.

19. A method of inhibiting growth of cancer cells comprising:

contacting the cancer cells with an IGF-1 receptor ligand carrying a therapeutic radionuclide.

20. The method of claim 19 wherein the contacting is in vitro.

21. The method of claim 19 wherein the contacting is in vivo.

22. A method of inhibiting growth of cancer cells comprising:

contacting the cancer cells with a therapeutic agent containing an insulin-like growth factor-1 (IGF-1) receptor ligand linked to a toxin.

23. The method of claim 22 wherein the contacting is in vitro.

24. The method of claim 22 wherein the contacting is in vivo.

25. A method of screening a compound for anti-cancer activity comprising:

contacting cancer cells with a compound comprising an IGF-1 receptor ligand carrying a therapeutic radionuclide.

26. The method of claim 25 wherein the contacting is in vitro.

27. The method of claim 25 wherein the contacting is in vivo.

28. A method of screening a compound for anti-cancer activity comprising:

contacting cancer cells with a compound comprising an IGF-1 receptor ligand linked to a toxin.

29. The method of claim 28 wherein the contacting is in vitro.

30. The method of claim 28 wherein the contacting is in vivo.