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(54) **ANTI-IGF-IR ANTIBODIES AND USES THEREOF**

ANTI-IGF-IR-ANTIKÖRPER UND IHRE VERWENDUNGEN

ANTICORPS ANTI-IGF-IR ET LEURS UTILISATIONS

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EP 2 507 263 B1

Description

CROSS-REFERENCE TO A RELATED APPLICATION

[0001] This application claims the benefit of U.S. provisional application Serial No. 61/266,681, filed December 4, 2009.

TECHNICAL FIELD

[0002] This disclosure pertains generally to potent neutralizing monoclonal antibodies against IGF-IR, and methods of using the same for cancer therapy.

BACKGROUND OF THE INVENTION

[0003] The insulin-like growth factors (IGFs) signaling system has been shown to play important roles in neoplasia. Insulin-receptors (IR) belong to the IGF signaling network. IGF receptor type 1 (IGF-IR) and its ligands (IGFI and II) are over-expressed in many types of solid and hematopoietic malignancies, including prostate cancer, breast cancer, liver cancer, and colon cancer, etc. There is substantial experimental and clinical evidence that targeting IGF-IR is a promising therapeutic strategy against cancer.

[0004] Strategies for down-regulating the IGF signaling system include reducing ligand bioavailability and inhibiting receptor function. Inhibition of IGF receptor function can be achieved using receptor-specific antibodies or small molecule tyrosine kinase inhibitors. A human-mouse chimeric antibody against the insulin-like growth factor-I receptor is described in Zhang et al. (mAbs, 1, 2009, 475-480). Desired IGF-IR-specific neutralizing antibodies can not only inhibit IGF-IR function, but also block the IGF-IR-mediated signaling pathway. There is a need to develop IGF-IR-specific antibodies that can inhibit cell-surface IGF-IR and block the binding of IGF-IR ligands to IGF-IR, thereby inhibiting ligand-induced receptor phosphorylation and the downstream signaling. IGF-IR-specific antibodies without cross-reactivity with IR are particularly desired.

BRIEF SUMMARY OF THE INVENTION

[0005] The subject invention provides antibodies and binding fragments thereof that specifically bind to human insulin-like growth factor 1 receptor (also known as human IGF receptor type 1). In one embodiment, the subject invention provides an antibody that binds to the extracellular domain of human IGF-IR, and thereby blocks the binding of both IGF-I and -II to IGF-IR and inhibits both IGF-I and IGF-II induced phosphorylation of IGF-IR and the downstream signaling, which lead to cancer cell proliferation and metastasis. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

[0006] In one embodiment, the subject invention provides an isolated antibody (H10), or a binding fragment thereof, comprising an amino acid sequence H10 V_H (SEQ ID NO: 1) and H10 V_L (SEQ ID NO: 2), wherein the antibody specifically binds to an epitope of IGF-IR.

[0007] The subject invention also includes pharmaceutical compositions comprising the antibody of the subject invention or a binding fragment thereof, and a medical use of the antibody in treating a tumor or cancer.

[0008] Additionally, the subject invention provides an isolated nucleic acid molecule encoding the antibody of the subject invention, or a binding fragment thereof, comprising a nucleic acid sequence selected from H10 V_H DNA (SEQ ID NO: 5), H10 V_L (SEQ ID NO: 6), H10 H3 (SEQ ID NO: 7), H10 L3 (SEQ ID NO: 8), or a combination thereof, wherein the nucleic acid molecule is optionally in a form of a vector, wherein the nucleic acid molecule or vector is optionally contained within a host cell, wherein the antibody or binding fragment thereof specifically binds to an epitope of IGF-IR. The disclosure also provides pharmaceutical compositions containing the nucleic acid molecules, and a medical use of the nucleic acid molecules in treating a tumor or cancer.

[0009] To the accomplishment of the foregoing and related ends, the invention comprises the features hereinafter fully described and particularly pointed out in the claims. The following description and the annexed drawings set forth in detail certain illustrative aspects and implementations of the invention. These are indicative of, however, a few of the various ways in which the principles of the invention may be employed. Other objects, advantages, and novel features of the invention will become apparent from the following detailed description of the invention when considered in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

Figure 1 illustrates amino acid sequences of various regions of the H10 antibody and nucleic acid sequences encoding these amino acid sequences. The amino acid sequences are presented from N-terminus to C-terminus (from upper left to lower right of the Figure) in accordance with convention. Sequences of HCDR1-3 and LCDR1-3 are underlined.

Figure 2 is a graph that illustrates an exemplified procedure of humanizing mouse monoclonal antibody Fab m590. H: human; m: mouse.

Figure 3 illustrates the binding of H10 to cell-associated IGF-IR on breast cancer MCF-7 cells in flow cytometry.

BRIEF DESCRIPTION OF THE SEQUENCES

[0011]

SEQ ID NO: 1 is an amino acid sequence of a V_H region of the H10 antibody of the subject invention.
SEQ ID NO: 2 is an amino acid sequence of a V_L region of the H10 antibody of the subject invention.
SEQ ID NO: 3 is an amino acid sequence of an H3 region (CDR3 of a V_H region) of the H10 antibody of the subject invention.
SEQ ID NO: 4 is an amino acid sequence of an L3 region (CDR3 of a V_L region) of the H10 antibody of the subject invention.
SEQ ID NO: 5 is a nucleic acid sequence of a V_H region of the H10 antibody of the subject invention.
SEQ ID NO: 6 is a nucleic acid sequence of a V_L region of the H10 antibody of the subject invention.
SEQ ID NO: 7 is a nucleic acid sequence of an H3 region (CDR3 of a V_H region) of the H10 antibody of the subject invention.
SEQ ID NO: 8 is a nucleic acid sequence of an L3 region (CDR3 of a V_L region) of the H10 antibody of the subject invention.
SEQ ID NO: 9 is an amino acid sequence of CDR1 of a V_H region of the H10 antibody of the subject invention.
SEQ ID NO: 10 is an amino acid sequence of CDR2 of a V_H region of the H10 antibody of the subject invention.
SEQ ID NO: 11 is an amino acid sequence of CDR1 of a V_L region of the H10 antibody of the subject invention.
SEQ ID NO: 12 is a nucleic acid sequence of CDR1 of a V_H region of the H10 antibody of the subject invention.
SEQ ID NO: 13 is a nucleic acid sequence of CDR2 of a V_H region of the H10 antibody of the subject invention.
SEQ ID NO: 14 is a nucleic acid sequence of CDR1 of a V_L region of the H10 antibody of the subject invention.
SEQ ID NO: 15 is an amino acid sequence of the human insulin-like growth factor 1 receptor (hIGF-IR) used according to the subject invention.

DETAILED DISCLOSURE OF THE INVENTION

[0012] The subject invention provides antibodies, or binding fragments thereof, that specifically bind to an epitope of the type 1 insulin-like growth factor receptor (IGF-IR). The disclosure more specifically provides antibodies, or binding fragments thereof, that bind to the extracellular domain of IGF-IR. Additionally, the disclosure provides epitopes that are recognized by the polypeptides (e.g., antibodies or binding fragments thereof) described herein, which epitopes can be used, in an embodiment in the development of cancer vaccine immunogens for the treatment of cancer.

[0013] The anti-IGF-IR antibodies can be used for cancer therapy, as well as to detect IGF-IR in an animal, including without limitation a human. The anti-IGF-IR antibodies of the subject invention can also be used to detect IGF-IR in a test sample. The test sample can be a tissue sample, a biopsy sample, and the like.

[0014] In one embodiment, the anti-IGF-IR antibody specifically binds to human insulin-like growth factor I receptor (also known as human insulin-like growth factor receptor type 1 (IGF-IR)). In a specific embodiment, the human IGF-IR has an amino acid sequence of SEQ ID NO: 15 (GenBank Accession No. P08069; GI: 124240). In an embodiment, the anti-IGF-IR antibody of the subject invention specifically binds to human IGF-IR expressed on cancer cells, such as for example, SKOV-3 and MCF-7 cells. In an embodiment, the anti-IGF-IR antibody of the subject invention specifically binds to human IGF-IR expressed on IGF-IR stably transfected Hela cells.

[0015] The term "binding specificity," "specificity," "specifically reacts," or "specifically interacts," as used herein, refers to the ability of an antibody or other agent to detectably bind an epitope presented on an antigen, such as an epitope of IGF-IR, while having relatively little detectable reactivity with other proteins or structures. Specificity can be relatively determined by binding or competitive assays, using e.g., Biacore instruments. Specificity can be exhibited by, e.g., an about 10:1, about 20:1, about 50:1, about 100:1, about 10,000:1 or greater ratio of affinity/avidity in binding to the specific antigen versus nonspecific binding to other irrelevant molecules.

[0016] In one embodiment, the disclosure provides an isolated polypeptide (e.g., antibody, or a binding fragment thereof), comprising the amino acid sequence selected from H10 V_H (SEQ ID NO: 1), H10 V_L (SEQ ID NO: 2), H10 H3 (CDR3 of a V_H region) (SEQ ID NO: 3), H10 L3 (CDR3 of a V_L region) (SEQ ID NO: 4), or a combination thereof, wherein

the polypeptide binds to an epitope of IGF-IR ectodomain. The polypeptide can comprise sequences selected from (a) the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2; (b) the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 4; (c) the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 4; or (d) the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 3.

[0017] In an embodiment, CDR1 of a V_H region of the anti-IGF-IR antibody comprises SEQ ID NO: 9. In an embodiment, CDR2 of a V_H region of the anti-IGF-IR antibody comprises SEQ ID NO: 10. In an embodiment, CDR3 of a V_H region of the anti-IGF-IR antibody comprises SEQ ID NO: 3. In a specific embodiment, a V_H region of the anti-IGF-IR antibody comprises SEQ ID NO: 1. In an embodiment, CDR1 of a V_L region of the anti-IGF-IR antibody comprises SEQ ID NO: 11. In an embodiment, CDR2 of a V_L region of the anti-IGF-IR antibody comprises GTS. In an embodiment, CDR3 of a V_L region of the anti-IGF-IR antibody comprises SEQ ID NO: 4. In a specific embodiment, a V_L region of the anti-IGF-IR antibody comprises SEQ ID NO: 2.

[0018] The disclosure also provides an isolated nucleic acid molecule encoding a polypeptide, or a binding fragment thereof, comprising H10 V_H DNA (SEQ ID NO: 5), H10 VL DNA (SEQ ID NO: 6), H10 H3 DNA (SEQ ID NO: 7), H10 L3 DNA (SEQ ID NO: 8), or a combination thereof, wherein the nucleic acid molecule is optionally in the form of a vector, wherein the nucleic acid molecule or vector is optionally contained within a host cell. In certain embodiments, the nucleic acid molecule can encode a polypeptide comprising (a) the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2; (b) the amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 4; (c) the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 4; or (d) the amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 3.

[0019] In an embodiment, CDR1 of a V_H region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 13. In an embodiment, CDR2 of a V_H region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 13. In an embodiment, CDR3 of a V_H region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 7. In a specific embodiment, a V_H region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 5. In an embodiment, CDR1 of a V_L region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 14. In an embodiment, CDR2 of a V_L region of the anti-IGF-IR antibody is encoded by a sequence comprising 5'ggcacgtcc 3'. In an embodiment, CDR3 of a V_L region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 8. In a specific embodiment, a V_L region of the anti-IGF-IR antibody is encoded by a sequence comprising SEQ ID NO: 6. The polypeptide can be any suitable polypeptide. For example, in one embodiment, the polypeptide is an antibody. Antibodies include both polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included are fragments or polymers of those immunoglobulin molecules, and human or humanized versions of immunoglobulin molecules or fragments thereof, as long as the molecules maintain the ability to bind with an epitope of the IGF-IR. The antibodies can be tested for their desired activity using the *in vitro* assays described herein, or by analogous methods, after which their *in vivo* therapeutic and/or diagnostic activities can be confirmed and quantified according to known clinical testing methods.

[0020] In another embodiment, the polypeptide is a monoclonal antibody or a binding fragment thereof. A monoclonal antibody refers to an antibody where individual antibodies within a population are identical.

[0021] The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric. The term "isolated polypeptide" is a polypeptide that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other proteins from the same species (3) is expressed by a cell of a species different from where the protein naturally originates, or (4) does not occur in nature. A polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates is also considered "isolated" from its naturally associated components. A polypeptide can also be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art.

[0022] The monoclonal antibodies can be made using any procedure known in the art. For example, monoclonal antibodies of the invention can be prepared using hybridoma methods, such as those described by Kohler et al., Nature, 256, 495-497 (1975).

[0023] The monoclonal antibodies also can be made by recombinant DNA methods, such as those described in U.S. Patent 4,816,567.

[0024] DNA encoding the disclosed monoclonal antibodies can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Libraries of antibodies or active antibody fragments can also be generated and screened using phage display techniques, e.g., as described in U.S. Patent 5,804,440 and U.S. Patent 6,096,441.

[0025] *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Examples of papain digestion are described in International Patent Application WO 94/29348 and U.S. Patent 4,342,566.

[0026] Papain digestion of antibodies typically produces two identical antigen-binding fragments - Fab fragments and a residual Fc fragment. Each Fab fragment has a single antigen binding site. Pepsin treatment yields a fragment that

has two antigen combining sites and is still capable of cross-linking antigen.

[0027] The disclosure encompasses chimeric antibodies and hybrid antibodies, with dual or multiple antigen or epitope specificities, single chain antibodies and fragments, such as, Fab', F(ab')2, Fab, scFv, and the like, including hybrid fragments. Such antibodies and fragments can be made by techniques known in the art and can be screened for specificity and activity according to general methods for producing antibodies and screening antibodies for specificity and activity (see, e.g., Harlow and Lane. Antibodies, A Laboratory Manual. Cold Spring Harbor Publications, New York, (1988).

[0028] The disclosure also encompasses human antibodies and/or humanized antibodies. Many non-human antibodies (e.g., those derived from mice, rats, or rabbits) are naturally antigenic in humans and, thus, can give rise to undesirable immune responses when administered to humans. Therefore, the use of human or humanized antibodies in the methods described herein serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

[0029] The human antibodies and humanized antibodies described herein can be prepared by any known technique. Examples of techniques for human monoclonal antibody production include those described by Boerner et al., J. Immunol., 147(1), 86-95 (1991).

[0030] Human antibodies described herein (and fragments thereof) can also be produced using phage display libraries (see, e.g., Marks et al., J. Mol. Biol., 222, 581-597 (1991)).

[0031] The human antibodies described herein can also be obtained from transgenic animals. For example, transgenic mutant mice that are capable of producing a full repertoire of human antibodies in response to immunization have been described (see, e.g., Jakobovits et al., PNAS, 90, 2551-255 (1993); and Jakobovits et al., Nature, 362, 255-258 (1993)).

[0032] Methods for humanizing non-human antibodies are known in the art. For example, humanized antibodies can be generated by substituting rodent complementarity-determining regions (CDRs) or CDR sequences for the corresponding sequences of a human antibody. Detailed procedures are disclosed in Jones et al., Nature, 321, 522-525 (1986); Riechmann et al., Nature, 332, 323-327 (1988); Verhoeyen et al., Science, 239, 1534-1536 (1988).

[0033] Methods that can be used to produce humanized antibodies are also described in U.S. Patent 4,816,567; U.S. Patent 5,565,332; U.S. Patent 5,721,367; U.S. Patent 5,837,243; U.S. Patent 5, 939,598; U.S. Patent 6,130,364; and U.S. Patent 6,180,377.

[0034] The polypeptides, according to the subject invention also encompass bivalent antibodies, as well as fusion molecules and conjugates with other molecules that can enhance the inhibitory effect of the polypeptide. The generation of fusion molecules (e.g., proteins) and conjugates (e.g., through physical or chemical conjugation) is within the ordinary skill in the art and can involve the use of restriction enzyme or recombinant cloning techniques (see, e.g., U. S. Patent 5,31 4,995).

[0035] The fusion molecule (e.g., proteins and nucleic acid molecules) or conjugate can comprise one or more of SEQ ID NOs: 1-8 in combination with any suitable second molecule. For example, the fusion molecule or conjugate can comprise one or more of SEQ ID NOs: 1-8 in combination with a neutralizing scFv antibody fragment or a Fab fragment (e.g., that binds to an epitope of IGF-IR).

[0036] Toxins are poisonous substances that usually are produced by plants, animals, or microorganisms that, in sufficient doses, are lethal. Preferred toxin for use in the fusion molecules or conjugates described herein include Pseudomonas toxin, Diphtheria toxin tetanus toxoid, ricin, cholera toxin, Shiga-like toxin (SL T-I, SL T- II, SL T-10 IIV), L T toxin, C3 toxin, Shiga toxin, pertussis toxin, tetanus toxin, Pseudomonas exotoxin, alorin, saporin, modeccin, and gelanin. The polypeptide (e.g., antibody, or a binding fragment thereof), and the toxin can be linked in several ways. If the hybrid molecule is produced by expression of a fused gene, a peptide bond serves as the link between the toxin and the polypeptide.

[0037] Alternatively, the toxin and the polypeptide can be produced separately and later coupled (e.g., by means of a non-peptide covalent bond). For example, the covalent linkage may take the form of a disulfide bond. In this case, the nucleic acid molecule encoding the polypeptide can optionally contain an extra cysteine codon. The cysteine condon can be positioned so as to not interfere with the binding activity of the molecule. The toxin molecule can be derivatized with a sulfhydryl group reactive with the cysteine of the modified polypeptide. In the case of a peptide toxin, this optionally can be accomplished by inserting DNA encoding a cysteine codon into the nucleic acid molecule encoding the toxin. In another alternative, a sulfhydryl group, either by itself or as part of a cysteine residue, can be introduced into the polypeptide of the subject invention using solid phase polypeptide techniques.

[0038] Moreover, the polypeptides described herein can be combined with other well-known therapies already in use. The combination of the polypeptide described herein and one or more other therapeutic agents can provide a greater therapeutic effect than either agent alone, and preferably generate an additive or a synergistic effect with current treatments. For example, the polypeptide of the invention can be combined with other therapies targeting the IGF-IR, Her2 or other components in the IGF signaling network, including IGF-I, IGF-II, IGF binding proteins, such as anti-Her 2 monoclonal antibody herceptin, anti-IGF-IR monoclonal antibodies CP751,871 (Pfizer), MK-0646 (Pierre-Fabre/Merck), AmG479 (Amgen), IMC-A12 (ImClone), R1507 (Hoffmann LaRoche), robatumumab (Schering-Plough), and cytokine immune enhancement therapy (interleukin (IL)-2, IL-12, CD40L + IL-12, IL-7, and interferons (IFNs)). Such therapies

can be administered in the manner already in use for the known treatment providing a therapeutic effect. The polypeptide of the invention can be a neutralizing antibody against IGF-IR useful for cancer therapy. In an embodiment, the antibody (or binding fragment thereof) of the subject invention inhibits IGF-IR function and inhibits IGF-IR-mediated signaling. The antibody (or binding fragment thereof) of the subject invention has a high affinity for IGF-IR and is specific for IGF-IR.

[0039] In an embodiment, the antibody, or binding fragment thereof physically associates with other molecules (e.g., anti-IGF-IR antibodies, anti-Her2 antibodies) to inhibit IGF-IR- and Her2-mediated signaling. In other words, the polypeptide specifically binds, specifically reacts with, or specifically interacts with other target molecules (e.g. IGF-I, -II, IGF binding proteins). In an alternative embodiment, the polypeptide does not substantially physically associate with other molecules.

[0040] The epitopes recognized by the polypeptides described herein can be used as cancer vaccine immunogens, as active portions of cancer vaccine immunogens, and as targets for inhibitors of IGF signaling network. For example, the epitopes described herein (or polypeptides comprising the epitopes) can be used as targets to isolate antibodies that, other than those described herein, bind to the epitopes described herein. These antibodies can be used in the treatment and diagnosis of cancer.

[0041] While it is possible to administer (for example, as a vaccine) an epitope (or polypeptide comprising the epitope) that is recognized by the antibodies of the subject invention in a pure or substantially pure form, the epitope can be formulated into a pharmaceutical composition, formulation, or preparation. Accordingly, the disclosure encompasses a composition containing an epitope (or polypeptide comprising the epitope) recognized by the antibody described herein. The composition can further contain one or more pharmaceutically acceptable carriers (as described herein) and, optionally, other therapeutic ingredients. The composition comprising such epitope can be used therapeutically or to otherwise generate an immune response.

[0042] For example, a vaccine is provided to enhance the patient's own immune response to the antigens present due to tumorogenesis. Such vaccine, which acts as an immunogen, optionally can be a partially or substantially purified recombinant polypeptide containing the epitope or an analog thereof. The polypeptide comprising the epitope can be conjugated with one or more lipoproteins, administered in liposomal form, or with an adjuvant. Also encompassed by the disclosure are methods of developing vaccines or immunogenic compositions using the epitopes described herein.

[0043] The disclosure is also directed to methods of downregulating IGF-IR and inhibiting IGF-IR-mediated signaling in a mammal. The methods involve administering an effective amount of the polypeptide (e.g. the antibody or a binding fragment thereof that specifically binds to human IGF-IR), nucleic acid molecule that encodes the polypeptide, a vector comprising the nucleic molecule, a cell comprising the nucleic acid molecule and/or vector, or compositions comprising the foregoing, to the mammal, wherein cancer cell growth and cancer metastasis are reduced or inhibited. In one embodiment, the mammal is a human.

[0044] In one embodiment, the subject disclosure provides administering to a mammal a polypeptide (e.g. the antibody or a binding fragment thereof that specifically binds to human IGF-IR), a nucleic acid molecule, a vector containing the nucleic acid encoding the polypeptide, or a cell (e.g., a host cell) containing any of the above.

[0045] In one embodiment, the subject disclosure provides a method for treating tumor or cancer, comprising administering to a subject in need of such treatment an effective amount of an isolated antibody, or a binding fragment thereof, to a subject. In another embodiment, the subject invention embodies the administration of nucleic acid molecules, vectors, and host cells of the subject invention for tumor or cancer therapy. In a specific embodiment, the subject invention can be used to treat or ameliorate prostate cancer, breast cancer, liver cancer, and colon cancer.

[0046] The term "treatment" or any grammatical variation thereof (*e.g.,* treat, treating, and treatment *etc.*), as used herein, includes but is not limited to, ameliorating or alleviating a symptom of a disease or condition, reducing, suppressing, inhibiting, lessening, or affecting the progression, severity, and/or scope of a condition.

[0047] The term "subject," as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the subject invention can be provided. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; and domesticated animals such as dogs, cats, horses, cattle, pigs, sheep, goats, chickens, mice, rats, guinea pigs, and hamsters.

[0048] Vectors include, for example, nucleic acid vectors, such as naked DNA and plasmids, and viral vectors, such as retroviral vectors, parvovirus-based vectors (e.g., adenoviral-based vectors and adeno-associated virus (AAV)-based vectors), lentiviral vectors (e.g., Herpes simplex (HSV)-based vectors), and hybrid or chimeric viral vectors, such as an adenoviral backbone with lentiviral components (see, e.g., Zheng et al., Nat. Biotech., 18(2), 176-80 (2000); International Patent Application WO 98/22143; International Patent Application WO 98/46778; and International Patent Application WO 00/17376) and an adenoviral backbone with AAV components (see, *e.g.,* Fisher et al., Hum. Gene Ther., 7, 2079-2087 (1996)).

[0049] Vectors and vector construction are known in the art (see, *e.g.,* Sam brook et al., Molecular Cloning: A Laboratory Manual, 2d edition, Cold Spring Harbor Laboratory, NY (1989); and Ausubel et al., Current Protocols in Molecular Biology, Green Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[0050] The vector can contain any suitable promoter and other regulatory sequences (e.g., transcription and translation initiation and termination codons, which are specific to the type of host) to control the expression of the nucleic acid sequence encoding the polypeptide. The promoter can be a native or nonnative promoter operably linked to the nucleic acid molecule described above. The selection of promoters, including various constitutive and regulatable promoters, is within the skill of an ordinary artisan.

[0051] Examples of regulatable promoters include inducible, repressible, and tissue-specific promoters. Specific examples include viral promoters, such as adenoviral promoters and AAV promoters. Additionally, combining the nucleic acid described above with a promoter is within the skill in the art.

[0052] Cells (e.g., isolated host cells) containing the above-described polypeptide or nucleic acid molecule encoding the polypeptide, optionally in the form of a vector, are also provided by the disclosure. Any suitable cell can be used. Examples include host cells, such as *E. coli* (e.g., *E. coli* Tb-1, TG-2, DH5a, XL-Blue MRF' (Stratagene), SA2821, and Y1090), *Bacillus subtilis, Salmonella typhimurium, Serratia marcescens,* Pseudomonas (e.g., *P. aerugenosa*), *N. grassa,* insect cells (e.g., Sf9, Ea4), yeast (*S. cerevisiae*) cells, and cells derived from a mammal, including human cell lines. Specific examples of suitable eukaryotic host cells include VERO, HeLa, 3T3, Chinese hamster ovary (CHO) cells, W138 BHK, COS-7, and MDCK cells. Alternatively, cells from a mammal, such as a human, to be treated in accordance with the methods described herein can be used as host cells.

[0053] Methods of introducing vectors into isolated host cells and the culture and selection of transformed host cells *in vitro* are known in the art and include the use of calcium chloride-mediated transformation, transduction, conjugation, triparental mating, DEAE, dextran-mediated transfection, infection, membrane fusion with liposomes, high velocity bombardment with DNA-coated microprojectiles, direct microinjection into single cells, and electroporation (see, e.g., Sambrook et al., Molecular Biology: A Laboratory Manual, Cold Spring Harbor Laboratory, NY (1989); Davis et al., Basic Methods in Molecular Biology (1986); and Neumann et al., EMBO J. 1, 841 (1982)

[0054] . In one embodiment, the cell containing the vector or nucleic acid molecule is transcribed and translated efficiently by the cell.

[0055] The nucleic acid molecules, vectors, cells, and polypeptides can be administered to a mammal alone, or in combination with a pharmaceutically acceptable carrier. By pharmaceutically acceptable is meant a material that is not biologically or otherwise undesirable (e.g., the material can be administered to a mammal, along with the nucleic acid, vector, cell, or polypeptide, without causing undesirable biological effects or interacting in a deleterious manner with other components of the pharmaceutical composition in which it is contained). The carrier is selected to minimize the degradation of the agent and to minimize adverse side effects in the mammal. The selection of carrier is well-known to one of ordinary skill in the art.

[0056] Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA (1995). Examples of pharmaceutical carriers include sterile water, saline, Ringer's solution, dextrose solution, and buffered solutions at physiological pH.

[0057] Typically, an appropriate amount of a pharmaceutically acceptable salt is used in the formulation to render the formulation isotonic. In one embodiment, the pH of the solution is from about 5 to about 8 (e.g., about 5.5, about 6, about 6.5, about 7, about 7.5, and ranges including any of these amounts therebetween), although pHs outside this range can be employed. In another embodiment, the pH is about 7 to about 7.5.

[0058] The pharmaceutical composition of the subject invention can also include sustained-release preparations, such as semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles (e.g., films, liposomes, or microparticles). It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and the concentration of composition being administered.

[0059] Examples of compositions (e.g., pharmaceutical compositions) containing the nucleic acid molecule, vector, cell, or polypeptide can include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like. The compositions can also include one or more active ingredients, such as anti-IGF-I, -II antibodies, chemotherapy drugs, and the like. The compositions described herein can be approved for use by the U.S. FDA or the equivalent in other countries. The composition (e.g., pharmaceutical composition) containing the nucleic acid molecule, vector, cell, or polypeptide can be administered in any suitable manner depending on whether local or systemic treatment is desired, and on the area to be treated.

[0060] If the composition is to be administered parenterally, the administration is generally by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for being prepared as a solution or suspension prior to injection, or as emulsions. Additionally, parental administration can involve the preparation of a slow-release or sustained-release system, such that a constant dosage is maintained (see, e.g., U.S. Patent 3,610,795).

[0061] Preparations for parenteral administration include sterile aqueous or nonaqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions,

emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives also can be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

[0062] The compositions can be in a form of acid- or base- addition salts, obtainable by reaction with inorganic acids, such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base, such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases, such as mono-, di-, trialkyl, and aryl amines and substituted ethanolamines.

[0063] The nucleic acid molecules, vectors, or polypeptides can be administered with a pharmaceutically acceptable carrier and can be delivered to the mammal's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well-known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis, and the like).

[0064] The exact amount of the compositions required to treat cancer may vary, depending on the species, age, gender, weight, and general conditions of the mammal, the particular polypeptide, nucleic acid, vector, or cell used, the route of administration, and whether other drugs are included in the regimen. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate, suitable amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect; however, the dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Dosage can vary, and can be administered in one or more (*e.g.,* two or more, three or more, four or more, or five or more) doses daily, for one or more days (or any suitable period of time to advance treatment). The composition can be administered immediately upon determination of cancer and continuously or intermittently administered.

[0065] The term "effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect. In an embodiment, an effective amount is an amount that is useful for treating or ameliorating tumor or cancer. In an embodiment, an effective amount enables an inhibition or reduction of cancer cell growth or metastasis in a subject. Effective dosages and schedules for administering the therapeutic agents and compositions described herein can be determined empirically, and making such determinations is routine to one of ordinary skill in the art.

[0066] The skilled artisan will understand that the dosage of the polypeptides varies, depending upon, for example, the route of administration, the particular polypeptide to be used, other drugs being administered, and the age, condition, gender and seriousness of the disease in the subject as described above. An effective dose of the polypeptide described herein generally ranges between about 1 μg/kg of body weight and 100 mg/kg of body weight. Examples of such dosage ranges are, e.g., about 1 μg - 100 μg/kg, about 100 μg - 1 mg/kg, about 1 mg/kg - 10 mg/kg, or about 10 mg - 100 mg/kg, once a month, a week, bi-weekly, daily, or two to four times daily.

[0067] Guidance in selecting appropriate doses for anti-IGF-IR antibodies, such as the polypeptides described herein, is found in the literature on therapeutic uses of antibodies (see, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985); and Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977).

[0068] A typical daily dosage of the polypeptide used might range from about 1 μg/kg to up to about 100 mg/kg of body weight or more per day, depending on the factors mentioned above. For example, the range can be from about 100 mg to about 1 g per dose. Nucleic acids, vectors, and host cells should be administered so as to result in comparable levels of production of polypeptides.

[0069] The disclosure also includes kits comprising the polypeptides, nucleic acid molecules, vectors, cells, epitopes, or compositions of the foregoing. The kits can include a separate container containing a suitable carrier, diluent, or excipient. The kits also can include an adjuvant, cytokine, antiviral agent, immunoassay reagents, PCR reagents, radiolabels, and the like. Additionally, the kits can include instructions for mixing or combining ingredients and/or administration.

[0070] The disclosure also provides a method of detecting IGF-IR in a mammal comprising contacting a sample obtained from the mammal with the polypeptide described herein. If an antigen is present in the mammal, to which the polypeptide can bind, a complex forms between the polypeptide and the antigen. Detection of the complex indicates the presence of elevated IGF-IR in the mammal.

[0071] The sample from the mammal can be of any suitable sample to detect the presence of IGF-IR. The complex can be detected by any suitable manner. The polypeptides described herein are utilizable as labeled molecules employed in radioimmunoassay (RIA) or enzyme immunoassay (EIA), particularly enzyme linked immunosorbent assay (ELISA), by introducing thereto radioactive substances such as 1125, 1131, H3 (tritium), C14, and the like; various enzyme reagents such as peroxidase (POX), chymotripsinogen, procarboxypeptidase, glyceraldehyde-3-phosphate dehydroge-

nase, amylase, phosphorylase, D-Nase, P-Nase, i3-galactosidase, glucose-6-phosphate dehydrogenase, ornithine decarboxylase, and the like. The radioactive substance can be introduced in a conventional manner. For example, the introduction of radioactive iodine, 1125, can be carried out by the oxidative ionization method using chloramine T (see, e.g., Hunter et al., Nature, 194, 495-496 (1962)) or by using the Bolten-Hunter reagent (1125-iodinated p-hydroxyphenyl propionic acid N-hydroxy-succinimide ester).

[0072] The label for use in the method can be any suitable label known in the art, such as biotinylated proteins or peptides.

EXAMPLE

Binding of H10 to cell-associated IGF-IR on breast cancer MCF-7 cells in flow cytometry

[0073] MCF-7 cells expressing IGF-IR were incubated with PBS buffer containing 1% FBS (filled curve) or with 10 μg/ml of recombinant H10 (empty curve) at 4°C for lh. The bound antibodies were detected using phycoerythrin conjugated to goat anti-human IgG, F(ab')2 (1:500) (ImmunoJackson). A FACSArray plate reader (BD biosciences) was used for fluorescence-activated cell sorting (FACS) and FlowJo software for data interpretation. The result is shown in figure 3.

SEQUENCE LISTING

[0074]

<110> University of Hong Kong Zhang, Mei-Yun

<120> ANTI-IGF-IR ANTIBODIES AND USES THEREOF

<130> UHK.153X

<150> 61/266,681
<151> 2009-12-14

<160> 15

<170> PatentIn version 3.5

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Ser Val Arg Val Ser Cys Gln Val Ser Gly Tyr Ser Phe Thr Ala Tyr
            20                  25                  30

Tyr Val Ser Trp Val Arg Gln Thr Pro Gly His Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Asn Pro Asp Asn Gly Gly Asn Asn Tyr Ala Gln Lys Phe
    50                  55                  60

His Gly Arg Val Thr Phe Ile Ala Asp Glu Ser Thr Arg Thr Val His
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Lys Ser Thr Ser Tyr Asp Tyr Asp Gly Tyr Trp Tyr Phe Asp Val
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Leu
            20                  25                  30

Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Asn
        35                  40                  45

Gly Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr
                85                  90                  95

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cctggacacg ggcttgagtg gatgggaggg attaatcctg acaatggtgg taacaactac 180

gcacagaagt ttcacggccg agtgacattt atcgccgacg agtccacgag gacagtccac 240

atggaactgc gcagcctgag atctgaggac acggccgtct atttttgtgc aaagtcaacc 300

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aaagccccta agctcctgat caatggcacg tccagtttgc aaagtggggt cccatcaagg 180

ttcagcggca gtggatctgg gacagatttc actctcacca tcagcagcct gcagcctgaa 240

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            20                  25                  30

Cys Gly Pro Gly Ile Asp Ile Arg Asn Asp Tyr Gln Gln Leu Lys Arg
        35                  40                  45

Leu Glu Asn Cys Thr Val Ile Glu Gly Tyr Leu His Ile Leu Leu Ile
    50                  55                  60

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

Ile Thr Glu Tyr Leu Leu Leu Phe Arg Val Ala Gly Leu Glu Ser Leu
                85                  90                  95

Gly Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Trp Lys Leu Phe
            100                 105                 110

Tyr Asn Tyr Ala Leu Val Ile Phe Glu Met Thr Asn Leu Lys Asp Ile
        115                 120                 125

Gly Leu Tyr Asn Leu Arg Asn Ile Thr Arg Gly Ala Ile Arg Ile Glu
    130                 135                 140
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Lys Asn Ala Asp Leu Cys Tyr Leu Ser Thr Val Asp Trp Ser Leu Ile
145                 150                 155                 160

Leu Asp Ala Val Ser Asn Asn Tyr Ile Val Gly Asn Lys Pro Pro Lys
                165                 170                 175

Glu Cys Gly Asp Leu Cys Pro Gly Thr Met Glu Glu Lys Pro Met Cys
            180                 185                 190

Glu Lys Thr Thr Ile Asn Asn Glu Tyr Asn Tyr Arg Cys Trp Thr Thr
        195                 200                 205

Asn Arg Cys Gln Lys Met Cys Pro Ser Thr Cys Gly Lys Arg Ala Cys
    210                 215                 220

Thr Glu Asn Asn Glu Cys Cys His Pro Glu Cys Leu Gly Ser Cys Ser
225                 230                 235                 240

Ala Pro Asp Asn Asp Thr Ala Cys Val Ala Cys Arg His Tyr Tyr Tyr
                245                 250                 255

Ala Gly Val Cys Val Pro Ala Cys Pro Pro Asn Thr Tyr Arg Phe Glu
            260                 265                 270

Gly Trp Arg Cys Val Asp Arg Asp Phe Cys Ala Asn Ile Leu Ser Ala
        275                 280                 285

Glu Ser Ser Asp Ser Glu Gly Phe Val Ile His Asp Gly Glu Cys Met
    290                 295                 300

Gln Glu Cys Pro Ser Gly Phe Ile Arg Asn Gly Ser Gln Ser Met Tyr
305                 310                 315                 320

Cys Ile Pro Cys Glu Gly Pro Cys Pro Lys Val Cys Glu Glu Glu Lys
                325                 330                 335

Lys Thr Lys Thr Ile Asp Ser Val Thr Ser Ala Gln Met Leu Gln Gly
            340                 345                 350

Cys Thr Ile Phe Lys Gly Asn Leu Leu Ile Asn Ile Arg Arg Gly Asn
        355                 360                 365

Asn Ile Ala Ser Glu Leu Glu Asn Phe Met Gly Leu Ile Glu Val Val
    370                 375                 380

Thr Gly Tyr Val Lys Ile Arg His Ser His Ala Leu Val Ser Leu Ser
385                 390                 395                 400
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Phe Leu Lys Asn Leu Arg Leu Ile Leu Gly Glu Glu Gln Leu Glu Gly
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Asn Tyr Ser Phe Tyr Val Leu Asp Asn Gln Asn Leu Gln Gln Leu Trp
            420                 425                 430

Asp Trp Asp His Arg Asn Leu Thr Ile Lys Ala Gly Lys Met Tyr Phe
        435                 440                 445

Ala Phe Asn Pro Lys Leu Cys Val Ser Glu Ile Tyr Arg Met Glu Glu
    450                 455                 460

Val Thr Gly Thr Lys Gly Arg Gln Ser Lys Gly Asp Ile Asn Thr Arg
465                 470                 475                 480

Asn Asn Gly Glu Arg Ala Ser Cys Glu Ser Asp Val Leu His Phe Thr
                485                 490                 495

Ser Thr Thr Thr Ser Lys Asn Arg Ile Ile Ile Thr Trp His Arg Tyr
            500                 505                 510

Arg Pro Pro Asp Tyr Arg Asp Leu Ile Ser Phe Thr Val Tyr Tyr Lys
        515                 520                 525

Glu Ala Pro Phe Lys Asn Val Thr Glu Tyr Asp Gly Gln Asp Ala Cys
    530                 535                 540

Gly Ser Asn Ser Trp Asn Met Val Asp Val Asp Leu Pro Pro Asn Lys
545                 550                 555                 560

Asp Val Glu Pro Gly Ile Leu Leu His Gly Leu Lys Pro Trp Thr Gln
                565                 570                 575

Tyr Ala Val Tyr Val Lys Ala Val Thr Leu Thr Met Val Glu Asn Asp
            580                 585                 590

His Ile Arg Gly Ala Lys Ser Glu Ile Leu Tyr Ile Arg Thr Asn Ala
        595                 600                 605

Ser Val Pro Ser Ile Pro Leu Asp Val Leu Ser Ala Ser Asn Ser Ser
    610                 615                 620

Ser Gln Leu Ile Val Lys Trp Asn Pro Pro Ser Leu Pro Asn Gly Asn
625                 630                 635                 640

Leu Ser Tyr Tyr Ile Val Arg Trp Gln Arg Gln Pro Gln Asp Gly Tyr
                645                 650                 655
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Leu Tyr Arg His Asn Tyr Cys Ser Lys Asp Lys Ile Pro Ile Arg Lys
            660                 665                 670

Tyr Ala Asp Gly Thr Ile Asp Ile Glu Glu Val Thr Glu Asn Pro Lys
        675                 680                 685

Thr Glu Val Cys Gly Gly Glu Lys Gly Pro Cys Cys Ala Cys Pro Lys
    690                 695                 700

Thr Glu Ala Glu Lys Gln Ala Glu Lys Glu Glu Ala Glu Tyr Arg Lys
705                 710                 715                 720

Val Phe Glu Asn Phe Leu His Asn Ser Ile Phe Val Pro Arg Pro Glu
                725                 730                 735

Arg Lys Arg Arg Asp Val Met Gln Val Ala Asn Thr Thr Met Ser Ser
            740                 745                 750

Arg Ser Arg Asn Thr Thr Ala Ala Asp Thr Tyr Asn Ile Thr Asp Pro
        755                 760                 765

Glu Glu Leu Glu Thr Glu Tyr Pro Phe Phe Glu Ser Arg Val Asp Asn
    770                 775                 780

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

Ile Asp Ile His Ser Cys Asn His Glu Ala Glu Lys Leu Gly Cys Ser
                805                 810                 815

Ala Ser Asn Phe Val Phe Ala Arg Thr Met Pro Ala Glu Gly Ala Asp
            820                 825                 830

Asp Ile Pro Gly Pro Val Thr Trp Glu Pro Arg Pro Glu Asn Ser Ile
        835                 840                 845

Phe Leu Lys Trp Pro Glu Pro Glu Asn Pro Asn Gly Leu Ile Leu Met
    850                 855                 860

Tyr Glu Ile Lys Tyr Gly Ser Gln Val Glu Asp Gln Arg Glu Cys Val
865                 870                 875                 880

Ser Arg Gln Glu Tyr Arg Lys Tyr Gly Gly Ala Lys Leu Asn Arg Leu
                885                 890                 895

Asn Pro Gly Asn Tyr Thr Ala Arg Ile Gln Ala Thr Ser Leu Ser Gly
```

900 905 910

Asn Gly Ser Trp Thr Asp Pro Val Phe Phe Tyr Val Gln Ala Lys Thr
915 920 925

Gly Tyr Glu Asn Phe Ile His Leu Ile Ile Ala Leu Pro Val Ala Val
930 935 940

Leu Leu Ile Val Gly Gly Leu Val Ile Met Leu Tyr Val Phe His Arg
945 950 955 960

Lys Arg Asn Asn Ser Arg Leu Gly Asn Gly Val Leu Tyr Ala Ser Val
965 970 975

Asn Pro Glu Tyr Phe Ser Ala Ala Asp Val Tyr Val Pro Asp Glu Trp
980 985 990

Glu Val Ala Arg Glu Lys Ile Thr Met Ser Arg Glu Leu Gly Gln Gly
995 1000 1005

Ser Phe Gly Met Val Tyr Glu Gly Val Ala Lys Gly Val Val Lys
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Asp Glu Pro Glu Thr Arg Val Ala Ile Lys Thr Val Asn Glu Ala
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Ala Ser Met Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val
1040 1045 1050

Met Lys Glu Phe Asn Cys His His Val Val Arg Leu Leu Gly Val
1055 1060 1065

Val Ser Gln Gly Gln Pro Thr Leu Val Ile Met Glu Leu Met Thr
1070 1075 1080

Arg Gly Asp Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Met
1085 1090 1095

Glu Asn Asn Pro Val Leu Ala Pro Pro Ser Leu Ser Lys Met Ile
1100 1105 1110

Gln Met Ala Gly Glu Ile Ala Asp Gly Met Ala Tyr Leu Asn Ala
1115 1120 1125

Asn Lys Phe Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val
1130 1135 1140

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Ala Glu  Asp Phe Thr Val Lys  Ile Gly Asp Phe Gly  Met Thr Arg
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Asp Ile  Tyr Glu Thr Asp Tyr  Tyr Arg Lys Gly Gly  Lys Gly Leu
    1160                1165                1170

Leu Pro  Val Arg Trp Met Ser  Pro Glu Ser Leu Lys  Asp Gly Val
    1175                1180                1185

Phe Thr  Thr Tyr Ser Asp Val  Trp Ser Phe Gly Val  Val Leu Trp
    1190                1195                1200

Glu Ile  Ala Thr Leu Ala Glu  Gln Pro Tyr Gln Gly  Leu Ser Asn
    1205                1210                1215

Glu Gln  Val Leu Arg Phe Val  Met Glu Gly Gly Leu  Leu Asp Lys
    1220                1225                1230

Pro Asp  Asn Cys Pro Asp Met  Leu Phe Glu Leu Met  Arg Met Cys
    1235                1240                1245

Trp Gln  Tyr Asn Pro Lys Met  Arg Pro Ser Phe Leu  Glu Ile Ile
    1250                1255                1260

Ser Ser  Ile Lys Glu Glu Met  Glu Pro Gly Phe Arg  Glu Val Ser
    1265                1270                1275

Phe Tyr  Tyr Ser Glu Glu Asn  Lys Leu Pro Glu Pro  Glu Glu Leu
    1280                1285                1290

Asp Leu  Glu Pro Glu Asn Met  Glu Ser Val Pro Leu  Asp Pro Ser
    1295                1300                1305

Ala Ser  Ser Ser Ser Leu Pro  Leu Pro Asp Arg His  Ser Gly His
    1310                1315                1320

Lys Ala  Glu Asn Gly Pro Gly  Pro Gly Val Leu Val  Leu Arg Ala
    1325                1330                1335

Ser Phe  Asp Glu Arg Gln Pro  Tyr Ala His Met Asn  Gly Gly Arg
    1340                1345                1350

Lys Asn  Glu Arg Ala Leu Pro  Leu Pro Gln Ser Ser  Thr Cys
    1355                1360                1365
```

Claims

1. An isolated humanized antibody or a binding fragment thereof that specifically binds to human insulin-like growth factor I receptor (IGF-IR), wherein a V_H region which comprises SEQ ID NO: 1 and wherein a V_L region which comprises SEQ ID NO: 2.

2. The isolated humanized antibody or a binding fragment thereof according to claim 1, which specifically binds to human IGF-IR expressed on cancer cells.

3. The isolated humanized antibody or a binding fragment thereof according to one of the claims 1-2, which specifically binds to the extracellular domain of human IGF-IR.

4. The isolated humanized antibody or a binding fragment thereof according to one of the claims 1-3, which is a monoclonal antibody.

5. The isolated humanized antibody or a binding fragment thereof according to one of the claims 1-4, which comprises an Fab, Fab', $F(ab')_2$, or scFv.

6. A pharmaceutical composition comprising an isolated humanized antibody or a binding fragment thereof according to any of the preceding claims and a pharmaceutically acceptable carrier.

7. A nucleic acid molecule encoding an isolated humanized antibody or a binding fragment thereof according to any one of the claims 1-5.

8. The nucleic acid molecule according to claim 7, which comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and 5'ggcacgtcc 3'.

9. A pharmaceutical composition comprising a nucleic acid molecule according to one of the claims 7 or 8 and a pharmaceutically acceptable carrier.

10. An isolated humanized antibody or a binding fragment thereof as defined by any one of the claims 1-5 for use in treating a tumor or cancer, comprising administering to a subject in need of such treatment an effective amount of said isolated antibody or binding fragment thereof.

11. The isolated humanized antibody or a binding fragment thereof for use of claim 10, wherein the subject is a human.

12. The isolated humanized antibody or a binding fragment thereof for use of claim 10 or 11, wherein the tumor or cancer is prostate cancer, breast cancer, liver cancer, or colon cancer.

13. An isolated nucleic acid molecule as defined by claim 7 or 8 for use in treating a tumor or cancer, comprising administering to a subject in need of such treatment an effective amount of said isolated nucleic acid molecule.

14. The isolated nucleic acid molecule for use of claim 13, wherein the tumor or cancer is prostate cancer, breast cancer, liver cancer, or colon cancer.

Patentansprüche

1. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente, das insbesondere an einen humanen insulinartigen Wachstumsfaktor 1-Rezeptor (IGF-IR) bindet, wobei eine V_H-Region SEQ ID NO:1 und wobei eine V_L-Region SEQ ID NO:2 aufweist.

2. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente nach Anspruch 1, das insbesondere an humanen, auf Krebszellen expressierten IGF-IR bindet.

3. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente nach einem der Ansprüche 1-2, das insbesondere an die extrazelluläre Domäne von humanem IGF-IR bindet.

4. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente nach einem der Ansprüche 1-3, welcher ein monoklonaler Antikörper ist.

5. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente nach einem der Ansprüche 1-4, welcher ein Fab, Fab', F(ab')2 oder scFv aufweist.

6. Pharmazeutische Zusammensetzung aufweisend einen isolierten humanisierten Antikörper oder eines seiner Bindungsfragmente nach einem der vorstehenden Ansprüche und einen pharmazeutisch akzeptablen Träger.

7. Nukleinsäurenmolekül, das einen isolierten humanisierten Antikörper oder eines seiner Bindungsfragmente nach einem der Ansprüche 1-5 kodiert.

8. Nukleinsäurenmolekül nach Anspruch 7, das eine Nukleinsäuresequenz aufweist, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14 und 5' ggcacgtcc 3'.

9. Pharmazeutische Zusammensetzung, die ein Nukleinsäurenmolekül gemäß einem der Ansprüche 7 oder 8 und einen pharmazeutisch akzeptablen Träger aufweist.

10. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente wie in einem der Ansprüche 1-5 definiert zur Verwendung bei der Behandlung von Krebs oder eines Tumors, aufweisend Verabreichen einer wirksamen Menge des besagten isolierten Antikörpers oder eines seiner Bindungsfragmente an ein Subjekt, das eine solche Behandlung benötigt.

11. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente zur Verwendung nach Anspruch 10, wobei das Subjekt ein Mensch ist.

12. Isolierter humanisierter Antikörper oder eines seiner Bindungsfragmente zur Verwendung nach Anspruch 10 oder 11, wobei der Tumor oder Krebs Prostatakrebs, Brustkrebs, Leberkrebs oder Darmkrebs ist.

13. Isoliertes Nukleinsäurenmolekül nach Anspruch 7 oder 8 zur Verwendung bei der Behandlung von Krebs oder eines Tumors, aufweisend das Verabreichen einer wirksamen Menge des besagten isolierten Nukleinsärenmoleküls an ein Subjekt, das eine solche Behandlung benötigt.

14. Isoliertes Nukleinsäuremolekül zur Verwendung nach Anspruch 13, wobei der Tumor oder Krebs Prostatakrebs, Brustkrebs, Leberkrebs oder Darmkrebs ist.

Revendications

1. Anticorps humanisé isolé ou fragment de liaison de celui-ci qui se lie spécifiquement au récepteur de facteur de croissance insulinomimétique I humain (IGF-IR), dans lequel une région V_H comprend la SEQ ID NO : 1, et dans lequel une région V_L comprend la SEQ ID NO : 2.

2. Anticorps humanisé isolé ou fragment de liaison de celui-ci selon la revendication 1, qui se lie spécifiquement à l'IGF-IR humain exprimé sur des cellules cancéreuses.

3. Anticorps humanisé isolé ou fragment de liaison de celui-ci selon la revendication 1 ou 2, qui se lie spécifiquement au domaine extracellulaire de l'IGF-IR humain.

4. Anticorps humanisé isolé ou fragment de liaison de celui-ci selon l'une quelconque des revendications 1 à 3, qui est un anticorps monoclonal.

5. Anticorps humanisé isolé ou fragment de liaison de celui-ci selon l'une quelconque des revendications 1 à 4, lequel comprend Fab, Fab', $F(ab')_2$ ou scFv.

6. Composition pharmaceutique comprenant un anticorps humanisé isolé ou fragment de liaison de celui-ci selon l'une quelconque des revendications précédentes et un porteur acceptable sur le plan pharmaceutique.

7. Molécule d'acide nucléique codant un anticorps humanisé isolé ou fragment de liaison de celui-ci selon l'une quelconque des revendications 1 à 5.

8. Molécule d'acide nucléique selon la revendication 7, laquelle comprend une séquence d'acide nucléique sélectionnée parmi le groupe consistant en SEQ ID NO : 5, SEQ ID NO : 6, SEQ ID NO : 7, SEQ ID NO : 8, SEQ ID NO : 12, SEQ ID NO : 13, SEQ ID NO : 14 et 5'ggcacgtcc3'.

9. Composition pharmaceutique, comprenant une molécule d'acide nucléique selon la revendication 7 ou 8 et un porteur acceptable sur le plan pharmaceutique.

10. Anticorps humanisé isolé ou fragment de liaison de celui-ci selon l'une quelconque des revendications 1 à 5, destiné à être utilisé dans le traitement d'une tumeur ou d'un cancer, comprenant l'administration à un sujet nécessitant un traitement de ce type d'une quantité efficace dudit anticorps humanisé isolé ou fragment de liaison de celui-ci.

11. Anticorps humanisé isolé ou fragment de liaison de celui-ci pour l'utilisation selon la revendication 10, dans lequel le sujet est humain.

12. Anticorps humanisé isolé ou fragment de liaison de celui-ci destiné à une utilisation selon la revendication 10 ou 11, dans lequel la tumeur ou le cancer est un cancer de la prostate, un cancer du sein, un cancer du foie ou un cancer du côlon.

13. Molécule d'acide nucléique isolée selon la revendication 7 ou 8, destinée à être utilisée dans le traitement d'une tumeur ou d'un cancer, comprenant l'administration à un sujet nécessitant un traitement de ce type d'une quantité efficace de ladite molécule d'acide nucléique isolée.

14. Molécule d'acide nucléique isolée destiné à une utilisation selon la revendication 13, dans laquelle la tumeur ou le cancer est un cancer de la prostate, un cancer du sein, un cancer du foie ou un cancer du côlon.

Figure 1

H10 VH (SEQ ID No: 1):
EVQLLESGAEVKRPGSSVRVSCQVSGYSFTAYYVSWVRQTPGHGLEWMGGINPDNG
GNNYAQKFHGRVTFIADESTRTVHMELRSLRSEDTAVYFCAKSTSYDYDGYWYFDVW
GQGTAVTVFSS

H10 VL (SEQ ID NO: 2):
ELQMTQSPSSVSASVGDRVTITCRASSSVSYLAWYQQKPGKAPKLLINGTSSLQSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQRSSYPFTFGGGTKVEIKR

H10 H3 (SEQ ID No: 3): AKSTSYDYDGYWYFDV

H10 L3 (SEQ ID NO: 4): QQRSSYPFT

H10 VH DNA (SEQ ID NO: 5):
gaggtgcagctgctcgagtctggggctgaggtgaagaggcctgggtcctcggtgagagtctcctgccaagtttctggttact
cattcactgcctactacgtcagttgggtgcgacagacccctggacacgggcttgagtggatgggagggattaatcctgaca
atggtggtaacaactacgcacagaagtttcacggccgagtgacatttatcgccgacgagtccacgaggacagtccacat
ggaactgcgcagcctgagatctgaggacacggccgtctatttttgtgcaaagtcaacctcctatgattacgacggttactggt
acttcgatgtctggggccaagggaccgcggtcaccgtcttctcctca

H10 VL DNA (SEQ ID NO: 6):
gagctccagatgacccagtctccatcttccgtgtctgcatctgtcggagacagagtcaccatcacttgtcgggcgagttcaa
gtgtaagttacttagcctggtatcagcagaaaccagggaaagcccctaagctcctgatcaatggcacgtccagtttgcaaa
gtggggtcccatcaaggttcagcggcagtggatctgggacagatttcactctcaccatcagcagcctgcagcctgaagatt
ttgcgacttactattgtcagcaaaggagtagttacccattcacgttcggcggagggaccaaggtggagatcaaacga

H10 H3 DNA (SEQ ID NO: 7): gcaaagtcaacctcctatgattacgacggttactggtacttcgatgtc

H10 L3 DNA (SEQ ID NO: 8): cagcaaaggagtagttacccattcacg

Figure 2

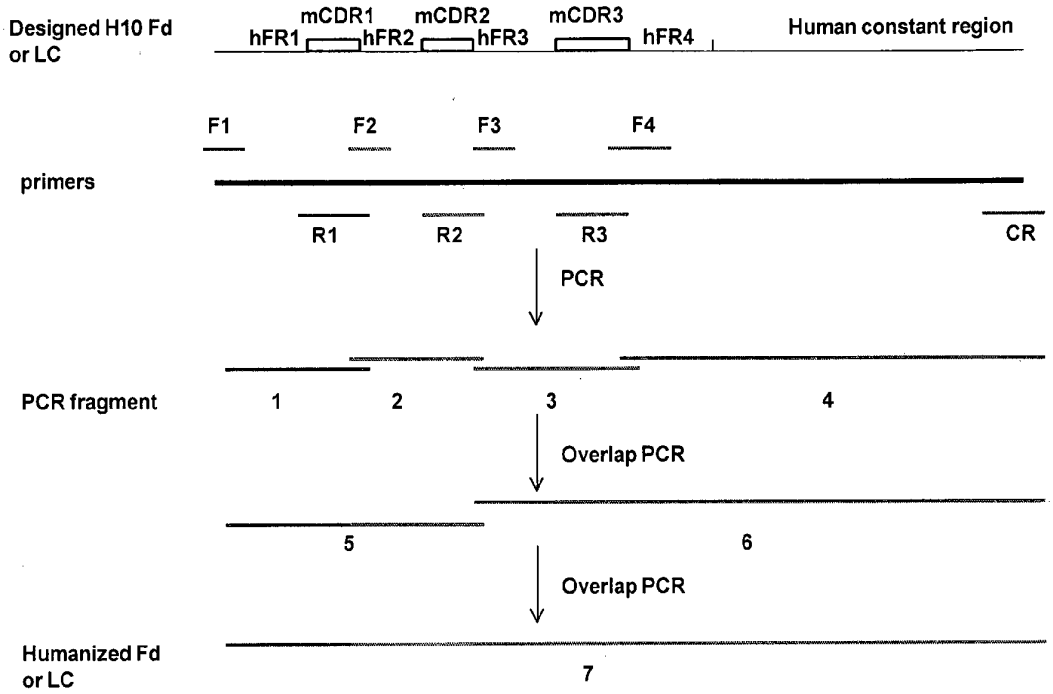
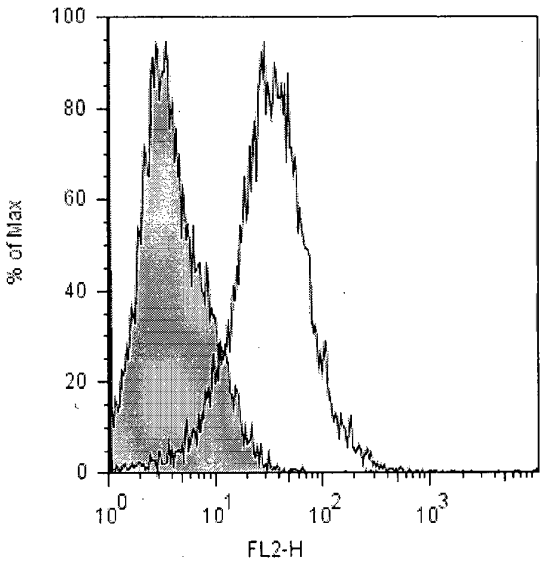

Designed H10 Fd or LC
mCDR1
mCDR2
mCDR3
hFR1
hFR2
hFR3
hFR4
Human constant region
F1
F2
F3
F4
primers
R1
R2
R3
CR
PCR
PCR fragment
1
2
3
4
Overlap PCR
5
6
Overlap PCR
Humanized Fd or LC
7

Figure 3



Filled curve: 2nd antibody control
Empty curve: 10 µg/ml of recombinant H10

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