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(54) CYTOTOXIC ANTIBODY AGAINST A C-TERMINAL PEPTIDE OF GPC3

ZYTOTOXISCHER ANTIKÖRPER GEGEN EIN C-TERMINALE PEPTID VON GPC3

ANTICORPS CYTOTOXIQUE DIRIGES CONTRE UN PEPTIDE DE GPC3 C-TERMINAL

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(73) Proprietor: **CHUGAI SEIYAKU KABUSHIKI KAISHA**
Tokyo, 115-8543 (JP)

(72) Inventors:
- **ABURATANI, H.**
  Res. Center for Adv. Scien. and Tech
  Meguro-ku,
  Tokyo 153-8 (JP)
- **MIDORIKAWA, Y.**
  Res. Center for Adv. Scie. and Tech
  Meguro-ku,
  Tokyo 153-8 (JP)
- **NAKANO, Kiyotaka,**
  Chugai Seiyaku Kabushiki Kaisha
  Gotenba-shi,
  Shizuoka 412-8513 (JP)
- **OHIZUMI, Iwao,**
  Chugai Seiyaku Kabushiki Kaisha
  Gotenba-shi,
  Shizuoka 412-8513 (JP)
- **ITO, Yukio,**
  Perseus Proteomics Inc.
  Tokyo 153-0041 (JP)
- **TOKITA, Susumu,**
  Perseus Proteomics Inc.
  Tokyo 153-0041 (JP)

(74) Representative: **Woods, Geoffrey Corlett**
J.A. Kemp & Co.
14 South Square
Gray's Inn
London
WC1R 5JJ (GB)

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Description

Technical Field

[0001] The present invention relates to an antibody against a C-terminal peptide of GPC3. The invention relates to an antibody against a GPC3 C-terminal peptide of about 30 kDa as found in the soluble form of the GPC3 core protein.

Background Art

[0002] The presence of the glypican family is reported as a new family of heparan sulfate proteoglycan existing on cell surface. Up to now, it is reported that five types of glypican (glypican 1, glypican 2, glypican 3, glypican 4 and glypican 5) exist. The members of the family have a core protein of a uniform size (about 60 kDa) and have unique cysteine residues well conserved in common, and are bound to cell membrane via glycosyphosphatidylinositol (GPI) anchor.

[0003] Glypican 3 (GPC3) is known to be deeply involved in cell division during development and the control of the pattern thereof. Additionally, it is known that the GPC3 gene is highly expressed in hepatoma cell and that the GPC3 gene is possibly used as a marker of hepatocellular carcinoma.

[0004] The present inventors previously found that an anti-GPC3 antibody had an ADCC activity and a CDC activity and was useful as the therapeutic treatment of hepatoma and filed a patent application (Japanese Patent Application 2001-189443).

[0005] However, GPC3 is a membrane-bound protein and it has not been reported that a GPC3 protein of secreted form existed. Thus, no examination has been made about the use of the GPC3 protein itself as a tumor marker in blood.

Disclosure of the Invention

[0006] The present inventors found a fact that glypican 3 (GPC3) is cleaved at an amino acid residue 358 thereof or at an amino acid residue 374 thereof or a region in the vicinity of the residues.

[0007] The inventors found that an antibody against the C terminus of GPC3 had a high cytotoxic activity and considered that the use of the anti-GPC3 antibody recognizing the C terminus would be preferable for disrupting cancer cell, i.e. for therapeutically treating cancer. Then, the inventors made an attempt of developing an antibody recognizing the C-terminal peptide of GPC3, and thus have achieved the invention.

[0008] The invention relates to an antibody against a peptide consisting of amino acid residues 375-580 of GPC3 wherein the antibody has cytotoxic activity

[0009] In one instance, the cytotoxic activity is a cytotoxic activity to HepG2 or HuH-7 cells.

[0010] Still further, the invention relates to the antibody, which is a monoclonal antibody.

[0011] Additionally, the invention relates to the antibody, which is a chimera antibody.

[0012] In addition, the invention relates to the antibody which is a humanised antibody.

[0013] Furthermore, the invention relates to the antibody which is a recombinant antibody

[0014] In addition, the invention relates to the antibody where the antibody has been produced in a mammalian cell. In one instance the mammalian cell may be one selected from a CHO, COS, myeloma, BHK, vero and Hela cell. The mammalian cell may be transformed with an expression vector comprising a gene encoding the antibody

[0015] In one instance, the invention relates to the antibody where the mammalian cell comprises:

(a) an expression vector comprising a gene encoding the antibody heavy (H) chain and a separate expression vector comprising a gene encoding the antibody light (L) chain; or
(b) a single expression vector encoding both the H and L chain.

[0016] In such circumstances, it may be that:

(a) the gene encoding the H chain of the antibody comprises the sequence of SEQ ID NO: 9; and/or
(b) the gene encoding the L chain of the antibody comprises the sequence of SEQ ID NO: 17.

[0017] It may also be that:

(a) the gene encoding the H chain of the antibody comprises the sequence of SEQ ID NO: 11; and/or
(b) the gene encoding the L chain of the antibody comprises the sequence of SEQ ID NO: 19.

[0018] Thus, the invention relates to the antibody for use in cell disruption, wherein the cells to be disrupted express GPC 3.

[0019] Additionally, the invention relates to the cell disrupting agent, where the cell is a cancer cell.

[0020] Further, the invention relates to an anti-cancer agent comprising the antibody. In particular, the antibody of the invention may be one for use in the treatment of cancer, where the cancer expresses GPC 3.

[0021] In one embodiment the cancer is hepatoma, pancreatic cancer, lung cancer, colon cancer, breast cancer, prostate cancer, leukemia or lymphoma.

[0022] The invention also relates to a pharmaceutical formulation comprising an antibody of the invention and a pharmaceutically acceptable carrier.

[0023] The invention is now described in detail hereinbelow.

[0024] Because the antibody against the C-terminal peptide of GPC3 in accordance with the invention has a high cytotoxic activity, the antibody can be used for disrupting cancer cells, i.e. for therapeutically treating cancer. Cancer possibly treated clinically using the antibody includes, but is not limited to, hepatoma, pancreatic cancer, lung cancer, colon cancer, breast cancer, prostate cancer, leukemia, and lymphoma. Preferably, the cancer is hepatoma.

1. Preparation of the anti-GPC3 antibody against the N-terminal peptide or the anti-GPC3 antibody against the C-terminal peptide

[0025] The amino acid sequence and nucleotide sequence of GPC3 are described in Lage, H. et al., Gene 188 (1997), 151-156 or GenBank: Z37987.

[0026] The anti-GPC3 antibody against the C-terminal peptide used in the invention should be capable of specifically binding to the C-terminal peptide of the GPC3 protein consisting of amino acid residues 375 to 580 of GPC3. The origin or type thereof (monoclonal, polyclonal) or the shape thereof is not specifically limited. Specifically, known antibodies such as mouse antibody, rat antibody, human antibody, chimera antibody and humanized antibody can be used.

[0027] When GPC3 is cleaved at a cleavage site, the GPC3 is cut into a peptide of about 40 kDa and a peptide of about 30 kDa, which are on the N-terminal side and the C-terminal side, respectively. The cleavage site of GPC3 is the amino acid reside 358, the amino acid residue 374 or a region in the vicinity thereof. The main cleavage site is believed to be the amino acid residue 358.

[0028] The C-terminal peptide of GPC3 is a C-terminal peptide of GPC3 and of about 30 kDa found in the soluble form of the GPC3 core protein. Based on the cleavage site mentioned above, the C-terminal peptide is a peptide of an amino acid sequence of from Val 375 to His 580. In accordance with the invention, fragments of such C-terminal peptide may be employed. In this specification, the C-terminal peptide is also referred to C-terminal fragment or C-terminal peptide fragment.

[0029] In other words, the antibody against the C-terminal peptide of GPC3 in accordance with the invention is an antibody recognizing an epitope existing on the C-terminal peptide of the GPC3 protein of amino acids 375 to 580, and the site of the epitope recognized is not limited.

[0030] The antibody may be a polyclonal antibodybut is preferably a monoclonal antibody.

[0031] The anti-GPC3 C-terminal peptide antibody for use in accordance with the invention can be obtained as a polyclonal antibody or a monoclonal antibody, using known techniques. The anti-GPC3 antibody for use in accordance with the invention is preferably a monoclonal antibody derived from mammals. The monoclonal antibody derived from mammals includes those produced by hybridoma, and those generated in hosts transformed with expression vectors carrying the antibody gene by genetic engineering technology.

[0032] Hybridoma producing a monoclonal antibody is prepared essentially using known techniques as follows. An animal is immunized by a conventional immunization method using GPC3 as a sensitizing antigen to obtain an immune cell, which is then fused to a known parent cell by a conventional cell fusion method. Fused cells are screened for monoclonal antibody-generating cells by a conventional screening method.

[0033] Specifically, a monoclonal antibody is prepared as follows.

[0034] First, GPC3 for use as a sensitizing antigen for obtaining antibody is prepared by expressing the GPC3 (MXR7) gene/amino acid sequence disclosed in Lage, H. et al., Gene 188 (1997), 151-156. Particularly, the gene sequence encoding GPC3 is inserted in a known expression vector to transform an appropriate host cell, then the intended human GPC3 protein is purified from the host cell or a culture supernatant thereof.

[0035] Additionally, naturally occurring GPC3 may also be purified and used.

[0036] Then, the purified GPC3 protein is used as a sensitizing antigen. The whole GPC3 protein may be used as a sensitizing antigen. Because an antibody against the N-terminal peptide of the GPC3 protein and an antibody against the C-terminal peptide thereof are also induced in this case, the antibody against the C-terminal peptide thereof may be separately selected. Alternatively, a partial C-terminal peptide thereof may also be used as a sensitizing antigen. In that case, such partial peptide may be obtained by chemical synthesis on the basis of the amino acid sequence of human GPC3 or by inserting a part of the GPC3 gene into an expression vector or by degrading naturally occurring GPC3 with proteases. A C-terminal peptide of GPC3 may be used as a partial peptide, and a smaller peptide fragment containing the epitope in the part may also be used.

[0037] Mammals for immunization with a sensitizing antigen are preferably selected, with taking account of the compatibility with parent cells for use in cell fusion. The mammals used for immunization preferably include, but are not limited to, rodents such as mouse, rat, hamster or rabbit or monkey.

[0038] For immunization of animals with a sensitizing antigen, known methods may be employed. Generally, for example, a sensitizing antigen is injected intraperitoneally or subcutaneously in mammals. Specifically, a sensitizing antigen is diluted with or suspended in PBS (phosphate-buffered saline) or physiological saline or the like, to an appropriate volume, and mixed with an appropriate volume of conventional adjuvants, such as Freund's complete adjuvant. After emulsification, the emulsified mixture is administered to mammals several times every 4 to 21 days. Additionally, an appropriate carrier may be used during the immunization with a sensitizing antigen. In case that a partial peptide of a very small molecular weight is to be used as a sensitizing antigen, the partial peptide may preferably be bound to carrier proteins, such as albumin and keyhole limpet hemocyanin upon immunization.

[0039] After mammals are immunized as above and the increase in the level of a desired antigen in serum is observed, immune cells are collected from the mammals, which are then subjected to cell fusion. Preferably, the immune cell is splenocyte.

[0040] As another parent cell to be fused to the immune cell, mammalian myeloma cell may be used. As the myeloma cell, known various cell lines are preferably used, including for example P3 (P3x63Ag8. 653) (J. Immunol. (1979) 123, 1548-1550), P3x63Ag8U. 1 (Current Topics in Microbiology and Immunology (1978) 81, 1-7), NS-1 (KohlerG. and Milstein, C. Eur. J. Immunol. (1976) 6, 511-519), MPC-11 (Margulies, D. H. et al., Cell (1976) 8, 405-415), SP2/0 (Shulman, M. et al., Nature (1978) 276, 269-270), F0 (de St. Groth, S. F. et al., J. Immunol. Methods (1980) 35, 1-21), S194 (Trowbridge, I. S. J. Exp. Med. (1978) 148, 313-323), andR210 (Galfre, G. et al., Nature (1979) 277, 131-133).

[0041] The cell fusion of the immune cell to the myeloma cell is essentially done by known methods, for example the method of Kohler & Milstein et al. (Kohler G. and Milstein C., Methods Enzymol. (1981) 73, 3-46).

[0042] More specifically, the cell fusion is carried out in conventional nutritious culture media in the presence of a cell fusion stimulator. Cell fusion stimulator includes, for example, polyethylene glycol (PEG) and Sendai virus (HVJ). If desired, auxiliary agents such as dimethylsulfoxide can be added and used so as to enhance the fusion efficiency.

[0043] The ratio of an immune cell and a myeloma cell to be used can appropriately be determined. For example, an immune cell at a ratio of 1- to 10-fold a myeloma cell is preferable. Culture medium for use in the cell fusion includes, for example, RPMI1640 and MEM, and other conventional culture media suitable for the growth of myeloma cell lines. Further, auxiliary serum agents such as fetal calf serum (FCS) may be used in combination.

[0044] The cell fusion can be done by thoroughly mixing predetermined amounts of immune cells and myeloma cells in the culture medium, adding the resulting mixture to a PEG solution (for example, mean molecular weight of about 1,000 to 6,000) preliminarily heated to about 37°C, generally to a concentration of 30 to 60 w/v %, and subsequently mixing the mixture to allow the intended fusion cell (hybridoma) to be formed.
Subsequently, a cell fusion agent and the like unpreferable for the growth of hybridoma are removed by adding appropriate culture medium sequentially and centrifuging the mixture to discard the supernatant, and repeating the procedures described above.

[0045] The hybridoma thus obtained is selected by culturing in a conventional selective culture medium, such as HAT medium (containing hypoxanthine, aminopterin and thymidine). The culturing in the HAT medium is continued for a sufficient period of time (typically several days to several weeks) for killing cells (non-fused cells) other than the intended hybridoma cell. Then, a conventional limited dilution method is carried out for screening and single cloning of a hybridoma producing the intended antibody.

[0046] The screening and the single cloning of the hybridoma may be done by a screening method on the basis of known antigen-antibody reactions. The antigen is bound to carriers such as beads made of polystyrene and the like, or commercially available 96-well microtiter plates, and reacted with a culture supernatant of the hybridoma. After rinsing the carriers, an enzyme-labeled secondary antibody is added to the plate to determine whether an intended antibody reacting with the sensitizing antigen is contained in the culture supernatant. The hybridoma producing the intended antibody can be cloned by limited dilution method. The C-terminal peptide of GPC3 or a fragment thereof may be used as the antigen for screening.

[0047] In addition to obtaining hybridoma by immunizing an animal except humans with an antigen, a human antibody may be prepared by another method. Human lymphocyte is sensitized with GPC3 in vitro and is then fused to myeloma cell with a permanent division potency derived from humans, to obtain a desired human antibody with a binding activity to the C-terminal peptide of GPC3 (see JP-B-1-59878). Further, a human antibody against the C-terminal peptide of GPC3 may be obtained by administering GPC3 as an antigen to a transgenic animal bearing all the repertories of the genes of human antibodies to obtain a cell producing an anti-GPC3 antibody against the C-terminal peptide, and then immortalizing the cell (see International Publications WO 94/25585. WO 93/12227, WO 92/03918, and WO 94/02602).

[0048] The hybridoma producing the monoclonal antibody thus prepared can be subcultured in a conventional culture medium and can be stored in liquid nitrogen for a long period of time.

[0049] One method for obtaining the monoclonal antibody from the hybridoma involves culturing the hybridoma by a

conventional method and obtaining the monoclonal antibody from a culture supernatant thereof. Another method involves administering the hybridoma to an animal compatible to the hybridoma for proliferation and obtaining the monoclonal antibody in the form of ascites. The former method is suitable for obtaining the antibody at high purity, while the latter method is suitable for large-scale production of the antibody.

[0050] In accordance with the invention, a monoclonal antibody includes a recombinant antibody produced by gene recombinant technology. A recombinant antibody can be generated by cloning the gene of the antibody from the hybridoma, integrating the gene into an appropriate vector, introducing the gene into a host, and allowing the recombinant antibody to be produced by the host (see for example Vandamme, A. M. et al., Eur. J. Biochem. (1990) 192, 767-775, 1990). Specifically, mRNA encoding the variable (V) region of the anti-GPC3 C-terminal peptide is isolated from the hybridoma generating the hybridoma generating the anti-GPC3C-terminal peptide antibody, mRNA isolation can be done by known methods. For example, total RNA is prepared by guanidine ultra-centrifugation method (Chirgwin, J. M. et al., Biochemistry(1979)18, 5294-5299)or AGPC method(Chomczynski, P. et al., Anal. Biochem. (1987) 162, 156-159), from which the intended mRNA is prepared using the mRNA purification kit (manufactured by Pharmacia). Alternatively, mRNA can directly be prepared using QuickPrep mRNA purification kit (manufactured by Pharmacia).

[0051] cDNA of the V region of the antibody is synthesized from the resulting mRNA, using reverse transcriptase. cDNA can be synthesized, using AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (manufactured by Seikagaku Corporation). cDNA can also be synthesized and amplified using 5'-AmpliFinder Race Kit (manufactured by Clontech) and 5'-RACE method using PCR (Frohman, M.A. et al., Proc. Natl. Acad. Sci. USA (1988) 85, 8998-9002; Belyavsky, A. et al., Nucleic Acids Res. (1989) 17, 2919-2932).

[0052] The intended DNA fragment is purified from the resulting PCR product and linked to vector DNA. A recombinant vector is prepared from the vector DNA and introduced in Escherichia coli and the like to select a colony for preparation of a desired recombinant vector. Subsequently, the nucleotide sequence of the intended DNA can be confirmed by known methods, for example dideoxynucleotide chain termination method.

[0053] After DNA encoding the V region of the intended anti-GPC3 C-terminal peptide antibody is obtained, the DNA is inserted into an expression vector containing DNA encoding the desired constant region (C region) of the antibody.

[0054] So as to produce the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention, the gene of the antibody is introduced into an expression vector such that the gene is expressed under the control of an expression-regulating region, for example enhancer and promoter. Then, a host cell is transformed with the expression vector, to express the antibody.

[0055] The gene of the antibody may be expressed by separately inserting DNA encoding the heavy chain (H chain) of the antibody and DNA encoding the light chain (L chain) thereof in expression vectors to simultaneously transform a host cell, or by inserting DNAs encoding the H chain and the L chain in a single expression vector to transform a host cell (see WO 94/11523).

[0056] Additionally, not only such host cells but also transgenic animal can be used for generating a recombinant antibody. For example, the gene of the antibody is inserted intermediately into a gene encoding a protein (e.g., goat β casein) generated inherently in milk to prepare a fusion gene. The DNA fragment comprising the fusion gene with the gene of the antibody as inserted therein is injected in a goat embryo, which is introduced in a female goat. The desired antibody is obtained from the milk produced by a transgenic goat born from the goat having received the embryo or a progeny thereof. So as to increase the amount of milk containing the desired antibody as produced by the transgenic goat, hormone may appropriately be administered to the transgenic goat (Ebert, K. M. et al., Bio/Technology (1994) 12, 699-702)

[0057] In accordance with the invention, artificially modified recombinant antibodies, for example a chimera antibody (e.g., humanized antibody) may also be used. These modified antibodies can be produced, using existing methods. In case that the antibody of the invention is to be used as an antibody for therapeutic treatment, the genetic recombinant type antibody is preferably used.

[0058] Chimera antibody can be obtained by linking the DNA encoding the V region of the antibody as obtained in the manner described above to DNA encoding the C region of a human antibody, inserting the resulting DNA in an expression vector, and introducing the vector in a host for production of the antibody. Using this existing method, a chimera antibody useful in accordance with the invention can be obtained.

[0059] Humanized antibody is also referred to as reshaped human antibody and is prepared by transplanting the complementarity determining region (CDR) of an antibody of mammals except humans, for example mouse, into the complementarity determining region of a human antibody. General genetic recombination techniques thereof are also known in the art (see European Patent Application EP 125023; WO 96/02576).

[0060] Specifically, a DNA sequence designed such that the CDR of mouse antibody can be linked to the framework region (FR) of human antibody is synthetically prepared by PCR, using several oligonucleotides prepared in such a manner that the oligonucleotides might have parts overlapped with the terminal regions of both CDR and FR (see the method described in WO 98/13388).

[0061] The FR region of human antibody to be liked to CDR is selected such that the CDR can form a good antigen

binding site. If necessary, the amino acids in the FR in the V region of the antibody may be substituted, so that the CDR of the reshaped human antibody may form an appropriate antigen binding site (Sato, K. et al., Cancer Res. (1993) 53, 851-856).

[0062] As the C regions of chimera antibody and humanized antibody, those of human antibody are used; for example, Cγ1, Cγ2, Cγ3, and Cγ4 can be used for the H chain, while-Cκ and Cλ can be used for the L chain. So as to improve the stability of the antibody or the production thereof, the C region of human antibody may be modified.

[0063] Preferably, the chimera antibody contains a sequence of an antibody derived from mammals except humans in the V region, and contains a sequence derived from a human antibody in the C region.

[0064] Humanized antibody comprises the CDR of an antibody derived from mammals except humans, and the FR and C. regions derived from a human antibody. Because the antigenicity of chimera antibody such as humanized antibody is reduced in humans, chimera antibody is useful as an active component of a therapeutic agent of the invention.

[0065] The antibody for use in accordance with the invention is not only the whole antibody molecule but also a fragment of the ant ibody or a modif ied product thereof, including divalent antibody and monovalent antibody, as long as such fragment or such modified product can bind to the GPC3 C-terminal peptide. For example, the antibody fragment includes Fab, F(ab')2, Fv, Fab/C having one Fab and complete FC, or single chain Fv (scFv) where Fv of the H chain and the L chain are linked via an appropriate linker. Specifically, the antibody is treated with enzymes, for example papain and pepsin, to generate antibody fragments. Otherwise, genes encoding these antibody fragments are constructed, introduced in an expression vector and expressed in an appropriate host cell (see for example, Co, M. S. et al., J. Immunol. (1994) 152, 2968-2976; Better, M. & Horwitz, A. H. Methods in Enzymology (1989) 178, 476-496, Academic Press, Inc.; Plueckthun, A. & Skerra, A. Methods in Enzymology (1989) 178, 476-496, Academic Press. Inc. : Lamoyi, E., Methods in Enzymology (1989) 121, 652-663; Rousseaux, J. et al., Methods in Enzymology (1989) 121, 663-669; Bird, R. E. et al., TIBTECH (1991) 9, 132-137).

[0066] ScFv can be obtained by linking the V region of the H chain and the V region of the L chain of an antibody. In this scFv, the V region of the H chain and the V region of the L chain are linked together via a linker, preferably a peptide linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. U.S.A. (1988) 85, 5879-5883). The V region of the H chain and the V region of the L chain in scFv may be derived from any antibodies described herein. Any appropriate single-stranded peptide comprising 12 to 19 amino acid residues may be used as the peptide linker for linking the V regions.

[0067] DNA encoding scFv is obtained by first amplifying DNA encoding the H chain or the V region of the H chain and the DNA encoding the L chain or the V region of the L chain by using as a template a portion of DNA encoding all the sequences thereof or a desired amino acid sequence therein and a pair of primers defining both the ends, and then amplifying the DNA with DNA encoding the peptide linker and a pair of primers defined in such a manner that both the ends of the peptide linker may be linked respectively to the H chain and the L chain.

[0068] Once the DNA encoding scFv is prepared, an expression vector carrying the DNA and a host transformed with the expression vector can be obtained by conventional methods. scFv can be obtained using the host by conventional methods.

[0069] The antibody fragments can be generated by obtaining and expressing the gene in the same manner as described above and allowing a host to produce the fragments. The "antibody" in accordance with the invention includes such antibody fragments.

[0070] There may also be used a modified product of the antibody, for example, anti-glypican antibodies conjugated with various molecules such as labeling substances, toxin, and radioactive materials. The "antibody" in accordance with the invention includes these modified antibodies. Such modified antibodies can be obtained by chemical modification of an antibody.

[0071] Methods for modifying antibodies have already been established in the art.

[0072] Further, the antibody for use in accordance with the invention may be a bispecific antibody. The bispecific antibody may include those having antigen binding sites recognizing different epitopes on the C-terminal peptide of GPC3. Alternatively, one of the antigen binding sites recognizes the C-terminal peptide of GPC3, while the other antigen binding site may recognize a labeling substance and the like. Such bispecific antibody can be prepared or obtained by linking HL pairs of two types of antibodies or by fusing hybridomas generating different monoclonal antibodies together to prepare a fusion cell capable of producing a bispecific antibody. Further, such bispecific antibody can be prepared by genetic engineering technique.

[0073] In accordance with the invention, an antibody with a modified sugar chain may also be used for the purpose of enhancing cytotoxic activity. Modification technique of the sugar chain of antibody is known in the art(for example, WO 00/61739, WO 02/31140, etc.).

[0074] The antibody gene constructed in the manner described above can be expressed and obtained by known methods. In case of a mammalian cell, a conventional useful promoter, the antibody gene to be expressed and poly(A) signal downstream the 3' side thereof are functionally linked for the expression. For example, the promoter/enhancer includes human cytomegalovirus immediate early promoter/enhancer.

[0075] Additionally, the promoter/enhancer for use in the expression of the antibody for use in accordance with the

invention includes, for example, virus promoters including retrovirus, polyoma virus, adenovirus and simian virus 40 (SV40)/enhancer or promoters derived from mammalian cells such as human elongation factor Ia (HEFIa)/enhancer.

[0076] In case of using SV40 promoter/enhancer, gene expression can readily be done by the method of Mulligan et al. (Nature (1979) 277,108). In case of using the HEFI a promoter/enhancer, gene expression can readily be done by the method of Mizushima et al. (Nucleic Acids Res. (1990) 18, 5322).

[0077] In case of Escherichia coli, a useful conventional promoter, a signal sequence for antibody secretion and an antibody gene to be expressed are functionally linked for expressing the gene. The promoter includes for example lacz promoter and araB promoter. In case that lacz promoter is to be used, the gene can be expressed by the method of Ward et al. (Nature (1098), 341, 544-546; FASEBJ. (1992) 6, 2422-2427). In case that araB promoter is to be used, the gene can be expressed by the method of Better et al. (Science (1988) 240, 1041-1043).

[0078] As the signal sequence for antibody secretion, pe1B signal sequence (Lei, S. P. et al. J. Bacteriol. (1987) 169, 4379) may be used when the antibody is generated in the periplasm of Escherichia coli. After the antibody generated in the periplasm is separated, the structure of the antibody is appropriately refolded for use.

[0079] As the replication origin, those from SV40, polyoma virus, adenovirus and bovine papilloma virus (BPV) may be used. For amplification of the copy number of the gene in a host cell system, the expression vector may carry a selective marker, for example, aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, Escherichia coli xanthine guanine phosphoribosyl transferase (Ecogpt) gene and dehydrofolate reductase (dhfr) gene.

[0080] So as to produce the antibody for use in accordance with the invention, an appropriate expression system, for example eukaryotic cell or prokaryotic cell system can be used. The eukaryotic cell includes for example established animal cell lines such as mammalian cell lines, insect cell lines, fungal cells and yeast cells. The prokaryotic cell includes for example bacterial cells such as Escherichia coli cell.

[0081] Preferably, the antibody for use in accordance with the invention is expressed in mammalian cells, for example CHO, COS, myeloma, BHK, Vero, and HeLa cell.

[0082] The transformed host cell is cultured in vitro or in vivo to produce the intended antibody. The host cell may be cultured by known methods. As the culture medium, for example, DMEM, MEM, RPMI 1640 and IMDM can be used. Auxiliary serum fluid such as fetal calf serum (FCS) may also be used in combination.

[0083] The antibody expressed and generated as described above can be separated from such cells or host animals and can then be purified to homogeneity. The antibody for use in accordance with the invention can be separated and purified using an affinity column. A protein A column includes, for example, Hyper D, POROS, Sepharose F. F. (manufactured by Pharmacia). Additionally, any separation and purification methods generally used for protein may be employed in the invention. For example, chromatography columns other than affinity column, filter, ultrafiltration, salting-out, and dialysis may be used in combination to separate and purify the antibody (Antibodies A Laboratory Manual, Ed. Harlow, David Lane, Cold Spring Harbor Laboratory, 1988).

2. Disruption of cancer cell using the anti-GPC3 C-terminal peptide antibody and cancer therapy using the same

(1) Determination of antibody activity

[0084] The antigen binding activity of the antibody for use in accordance with the invention may be assayed using known techniques (Antibodies A Laboratory Manual. Ed. Harlow, David Lane, Cold Spring Harbor Laboratory, 1988) and an activity of inhibiting the ligand-receptor binding thereof (Harada, A. et al., International Immunology (1993) 5, 681-690).

[0085] A method for assaying the antigen binding activity of the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention includs ELISA (enzyme-linked immunosorbent assay), EIA (enzyme immunoassay), RIA (radioimmunoassay) and fluorescent antibody method. In enzyme immunoassay, a sample containing the anti-GPC3 C-terminal peptide antibody, for example a culture supernatant of a cell producing the anti-GPC3 C-terminal peptide antibody or the purified antibody is added to a plate coated with the GPC3 C-terminal peptide consisting of amino acid residues 375-380 of GPC3. A secondary antibody labeled with an enzyme such as alkali phosphatase is added and the plate is incubated and rinsed, then an enzyme substrate such as p-nitrophenylphosphoric acid is added to measure the absorbance and assess the antigen binding activity.

[0086] So as to determine the activity of the antibody for use in accordance with the invention, the neutralization activity of the anti-GPC3 C-terminal peptide antibody is measured.

(2) Cytotoxicity

[0087] For therapeutic purpose, the antibody for use in accordance with the invention preferably has the ADCC activity or the CDC activity as cytotoxicity.

[0088] The ADCC activity can be assayed by mixing an effector cell, a target cell and the anti-GPC3 C-terminal peptide

antibody together and examining the ADCC level. As the effector cell, cell such as mouse splenocyte and mononuclear cell separated from human peripheral blood or bone marrow can be utilized. As the target cell, a human cell line such as human hepatoma line HuH-7 can be used. The target cells are preliminarily labeled with ^{51}Cr and incubated with the anti-GPC3 C-terminal peptide antibody, then effector cells at an appropriate ratio is added to the target cells and incubated. After incubation, the supernatant is collected to count the radioactivity in the supernatant, to assay the ADCC activity.

[0089] Further, the CDC activity can be assayed by mixing the labeled target cell described above with the anti-GPC3 C-terminal peptide antibody, subsequently adding complement, and counting the radioactivity in the supernatant after incubation.

[0090] The Fc moiety is needed for the antibody to exert the cytotoxicity. In case that the inhibitor of cell proliferation in accordance with the invention utilizes the cytotoxicity of the antibody, thus, the anti-GPC3 C-terminal peptide antibody for use in accordance with the invention preferably contains the Fc moiety.

(3) Cell disruption

[0091] The anti-GPC3 C-terminal peptide antibody of the invention may also be used for cell disruption, particularly the disruption of cancer cell. Further, the anti-GPC3 C-terminal peptide antibody of the invention can be used as an anticancer agent. Cancers to be therapeutically treated and prevented by the antibody of the invention include, but are not limited to, hepatoma, lung cancer, colon cancer, breast cancer, prostate cancer, pancreatic cancer and lymphoma, preferably Hepatoma.

(4) Administration method and pharmaceutical formulation

[0092] The cell disrupting agent or anticancer agent in accordance with the invention is used for the purpose of therapeutically treating or ameliorating diseases caused by abnormal cell growth, particularly cancer.

[0093] The effective dose is selected within a range of 0.001 mg to 1,000 mg per 1 kg body weight. Also the effective dose is selected within a range of 0.01 mg to 100,000 mg/body weight per patient. However, the dose of the therapeutic agents containing the anti-GPC3 C-terminal peptide antibody of the invention are not limited to the above doses.

[0094] The timing for administering the therapeutic agent of the invention is either before or after the onset of clinical symptoms of the diseases.

[0095] The therapeutic agent comprising the anti-GPC3 C-terminal-peptide antibody in accordance with the invention as an active component can be formulated by a conventional method (Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, USA), and may also contain pharmaceutically acceptable carriers and additives.

[0096] Examples of such carriers and pharmaceutical additives include water, pharmaceutically acceptable organic solvents, collagen, polyvinyl alcohol, polyvinyl pyrrolidone, carboxyvinyl polymer, carboxymethyl cellulose sodium, polyacrylate sodium, sodium alginate, water-soluble dextran, carboxymethyl starch sodium, pectin, methyl cellulose, ethyl cellulose, gum xanthan, gum arabic, casein, agar, polyethylene glycol, diglycerin, glycerin, propylene glycol, vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin (HSA), mannitol, sorbitol, lactose and surfactants acceptable as pharmaceutical additives.

[0097] In practice, an additive or a combination thereof is selected depending on the dosage form of the therapeutic agent of the invention. However, the additive is not limited to those described above. In case that the therapeutic agent is to be used in an injection formulation, the purified anti-GPC3 C-terminal peptide antibody of the invention is dissolved in a solvent, such as physiological saline, buffers, and glucose solution, and adsorption preventing agents such as Tween 80, Tween 20, gelatin and human serum albumin is added. Alternatively, the therapeutic agent is provided in a freeze-dried form as a dosage form to be dissolved and reconstituted prior to use. As excipients for freeze-drying, for example, sugar alcohols such as mannitol and glucose and sugars may be used.

Brief Description of the Drawings

[0098]

Fig. 1 shows bar graphs depicting the results of the analysis of GPC3 mRNA expression using Gene Chip, where Fig. 1A depicts GPC3 expression and Fig. 1B depicts the expression of alpha-fetoprotein (AFP). NL, CH, LC, WD, MD and PD on the holizontal axis represent normal liver, inflammatory lesion of hepatitis, lesion of liver cirrhosis, well-differentiated cancer, moderately differentiated cancer and poorly differentiated cancer, respectively.

Fig. 2 shows images of purified soluble GPC3 of heparan sulfate adduct type and the GPC3 core protein, as stained with CBB.

Fig. 3 shows bar graphs depicting the expression of the GPC3 gene in human hepatoma.

Fig. 4 shows the results of western blotting of the soluble form of the core protein using the anti-GPC3 antibody.
Fig. 5 shows the principle of sandwich ELISA using the anti-GPC3 antibody.
Fig. 6 is a graph of the standard curve for the GPC3 sandwich ELISA using M6B1 and M18D4.
Fig. 7 is a schematic view of the GPC3 structure.
Fig. 8 shows combinations of the anti-GPC3 antibodies employed in ELISA.
Fig. 9 is a graph of the standard curve for the GPC3 sandwich ELISA system using various combinations of the anti-GPC3 antibodies.
Fig. 10 shows the assay results of the ADCC activity of the anti-GPC3 antibody.
Fig. 11 shows the assay results of the CDC activity of the anti-GPC3 antibody.

Best Mode for Carrying out the Invention

[0099] The invention is now specifically described in the following Examples. However, the invention is not limited by the Examples.

[0100] In the Examples described in this specification, the following materials were used.

[0101] As expression vectors of the soluble form of GPC3 and the soluble form of the GPC3 core protein, pCXND2 and pCXND3 prepared by integrating the DHFR gene and the neomycin-resistant gene in pCAGGS were used.

[0102] DXB11 was purchased from ATCC. For culturing, 5 % FBS (GIBCO BRL CAT# 10099-141, Lot# A0275242/Minimum Essential Medium Alpha medium (αMEM (+)) (GIBCO BRL CAT# 12571-071)/1 % Penicillin-Streptomycin (GIBCO BRL CAT# 15140-122) was used. For selection of stable cell line of DXB11 expressing each protein, 500 μg/mL Geneticin (GIBCO BRL CAT# 10131-027)/5 % FBS/α MEM without ribonucleotides and deoxyribonucleotides (GIBCO BRL CAT# 12561-056)(αMEM(-))/PS was used alone or with supplemented with MTX to a final concentration of 25 nM.

[0103] HepG2 was purchased from ATCC and maintained in 10 % FBS/Dulbecco's modified Eagle medium (DMEM) (GIBCO BRL CAT# 11995-065)/PS.

[0104] The hybridoma was maintained in 10 % FBS/RPMI1640/1 × HAT media supplement (SIGMA CAT# H-0262) /0.5 × BM-Condimed H1 Hybridoma cloning supplement (Roche CAT# 1088947).

Example 1

Cloning and expression analysis of human GPC3 (GPC3) cDNA Cloning of full-length cDNA encoding human glypican 3 (GPC3 hereinafter)

[0105] The full-length cDNA encoding human GPC3 was amplified by PCR, using as a template a first strand cDNA prepared from a colon cancer cell line Caco2 by a general method and Advantage 2 kit (Clontech Cat. No. 8430-1). Specifically, 50 μl of a reaction solution containing Caco2-derived cDNA of 2 μl, 1 μl of a sense primer (SEQ ID NO: 1), 1 μl of an antisense primer (SEQ ID NO: 2), 5 μl of Advantage2 10 × PCR buffer, 8 μl of dNTP mix (1.25 mM) and 1.0 μl of Advantage polymerase Mix was subjected to 35 cycles of 94 °C for one minute, 63 °C for 30 seconds and 68 °C for 3 minutes. The amplified product from the PCR (inserted in TA vector pGEM-T easy using pGEM-T Easy Vector System I (Promega Cat No. A1360)) was sequenced using ABI3100 DNA sequencer to confirm that cDNA encoding the full-length human GPC3 was isolated. The sequence represented by SEQ ID NO: 3 indicates the nucleotide sequence of the human GPC3 gene, while the sequence represented by SEQ ID NO: 4 indicates the amino acid sequence of human GPC3 protein.

SEQ ID NO: 1: GATATC-ATGGCCGGGACCGTGCGCACCGCGT
SEQ ID NO: 2: GCTAGC-TCAGTGCACCAGGAAGAAGAAGCAC

Expression Analysis of human GPC3 mRNA using GeneChip

[0106] mRNA expression was analyzed in 24 cases with hepatoma lesions (well-differentiated cancer: WD; moderately differentiated cancer: MD; poorly differentiated cancer: PD), 16 hepatoma cases with non-cancer lesions (hepatitis lesion: CH, cirrhosis lesion : LC), 8 cases with normal liver: NL (informed consent acquired; available from Tokyo University, School of Medicine and Saitama Cancer Center), using GeneChip™ UG-95A Target (Affymetrix). Specifically, total RNA was prepared using ISOGEN (Nippon Gene) from the individual tissues, from which 15 μg each of total RNA was used for gene expression analysis according to the Expression Analysis Technical Manual (Affymetrix).

[0107] As shown in Fig.1, the mRNA expression level of human GPC3 gene (Probe Set ID: 39350_at) was apparently higher in many of the cases compared with the expression in normal liver tissue, despite the differentiation stages of hepatoma. Furthermore, comparison was made with the mRNA expression of alpha-fetoprotein (Probe Set ID: 40114_at) most commonly used as a diagnostic marker of hepatoma currently. It was shown that even in well-differentiated cancer showing almost no such mRNA expression of alpha-fetoprotein, sufficiently enhanced mRNA expression of GPC3 was

observed, and that the ratio of the activation of the mRNA expression of GPC3 was higher. Thus, it is considered that GPC3 detection is useful as a diagnostic method of hepatoma at an early stage.

Example 2

Preparation of anti-GPC3 antibody

Preparation of the soluble form of human GPC3

[0108] As a material for preparing anti-GPC3 antibody, the soluble form of the GPC3 protein lacking the hydrophobic region on the C-terminal side was prepared.

[0109] Using a plasmid DNA containing the complete full-length human GPC3 cDNA supplied from Tokyo University, Advanced Technology Institute, a plasmid DNA for expressing the soluble form of the GPC3 cDNA was constructed. PCR was conducted using a downstream primer (5'-ATA GAA TTC CAC CAT GGC CGG GAC CGT GCG C-3') (SEQ ID NO: 5) designed to remove the hydrophobic region on the C-terminal side (564-580 amino acid), and an upstream primer (5'-ATA GGA TCC CTT CAG CGG GGA ATG AAC GTT C-3') (SEQ ID NO.6) with the EcoRI recognition sequence and the Kozak's sequence having been added. The resulting PCR fragment (1711 bp) was cloned in pCXND2-Flag. The prepared expression plasmid DNA was introduced in a CHO cell line DXB11. Selection with 500 μg/mL Geneticin resulted in a CHO line highly expressing the soluble form of GPC3.

[0110] Using a 1700-cm^2 roller bottle, the CHO line highly expressing the soluble form of GPC3 was cultured at a large scale, and the culture supernatant was collected for purification. The culture supernatant was applied to DEAE Sepharose Fast Flow (Amersham CAT# 17-0709-01), washed, and eluted with a buffer containing 500 mM NaCl. Subsequently, the product was affinity purified using Anti-Flag M2 agarose affinity gel (SIGMA CAT# A-2220) and eluted with 200 μg/mL Flag peptide. After concentration with Centriprep-10 (Millipore Cat# 4304), the Flag peptide was removed by gel filtration with Superdex 200 HR 10/30 (Amersham CAT# 17-1088-01). Finally, the product was concentrated using DEAE Sepharose Fast Flow column, and eluted with PBS (containing 500 mM NaCl) containing no Tween 20 for replacement of the buffer.

Preparation of the soluble form of human GPC3 core protein

[0111] Using the wild type human GPC3 cDNA as template, cDNA was prepared by assembly PCR, where Ser 495 and Ser 509 were substituted with Ala. A primer was designed in such a fashion that His tag might be added to the C terminus. The resulting cDNA was cloned in pCXND3 vector. The prepared expression plasmidDNAwas introduced in a DXB11 line, followed by selection with 500 μg/mL Geneticin, to obtain the CHO line highly expressing the soluble form of the GPC3 core protein.

[0112] A large scale cultivation was done with a 1700-cm^2 roller bottle, and the culture supernatant was collected for purification. The supernatant was applied to Q sepharose Fast Flow (Amersham CAT# 17-0510-01), washed, and eluted with a phosphate buffer containing 500 mM NaCl. Subsequently, the product was affinity purified using Chelating Sepharose Fast Flow (Amersham CAT# 17-0575-01), and eluted with a gradient of 10-150 mM imidazole. Finally, the product was concentrated with Q sepharose Fast Flow and eluted with a phosphate buffer containing 500 mM NaCl.

[0113] SDS polyacrylamide gel electrophoresis showed a smear-like band of 50 to 300 kDa and a band of about 40 kDa. Fig.2 shows the results of the electrophoresis. GPC3 is a proteoglycan of 69 kDa and with a heparan sulfate-addition sequence at the C terminus. It was considered that the smear-like band corresponds to GPC3 modified with heparan sulfate. The results of amino acid sequencing indicated that the band of about 40 kDa had an origin in the N-terminal fragment. Thus, it was anticipated that GPC3 was more or less cleaved.

[0114] So as to remove antibodies against heparan sulfate in the following screening for hybridoma, the soluble form of the GPC3 core protein where a heparan sulfate-addition signal sequence Ser 495 and Ser 509 were substituted with Ala. CHO cell line highly expressing the protein was prepared as above, and the culture supernatant was affinity purified utilizing the His-tag. SDS polyacrylamide gel electrophoresis showed three bands of 70 kDa, 40 kDa and 30 kDa. Amino acid sequencing indicated that the band of 30 kDa was the C-terminal fragment of GPC3. The C-terminal fragment starts from serine 359 or from valine 375. Thus, it was anticipated that GPC3 received some enzymatic cleavage. The reason why the band of 30 kDa was not observed in the GPC3 of heparan sulfate-added type was that the fragment formed the smear-like band due to the addition of heparan sulfate. It is a novel finding that GPC3 receives enzymatic cleavage at a specific amino acid sequence, but the biological meaning thereof has not yet been elucidated.

[0115] The inventors made an assumption on the basis of the results that GPC3 on the membrane even in hepatoma patients would be cleaved and secreted as the soluble form in blood. Compared with AFP as a hepatoma marker, the expression of the gene of GPC3 was found higher in hepatoma patients at earlier stages (Fig. 1). So as to examine the possibility as a novel tumor marker with higher clinical utility than that of AFP, an anti-GPC3 antibody was prepared to

construct a sandwich ELISA system as described in Example 2 or below.

Preparation of anti-GPC3 antibody

[0116] Because the homology of human GPC3 with mouse GPC3 is as high as 94 % at the amino acid levels, it was considered that it might be difficult to obtain the anti-GPC3 antibody by the immunization of normal mouse with human GPC3. Thus, MRL/lpr mouse with autoimmune disease was used as an animal to be immunized. Five MRL/lpr mice (CRL) were immunized with the soluble form of GPC3. For the first immunization, the immunogen protein was adjusted to 100 μg/animal and was then emulsifiedusing FCA (Freund's complete adjuvant (H37 Ra), Difco (3113-60), Becton Dickinson (cat# 231131)), which was then subcutaneously administered to the mice. Two weeks later, the protein was adjusted to 50 μg/animal and emulsified with FIA (Freund's incomplete adjuvant, Difco (0639-60), Becton Dickinson (cat# 263910)) for subcutaneous administration to the mice. At one week interval since then, booster was carried out in total of 5 times. For final booster, the protein was diluted with PBS to 50 μg/animal, which was administered in the caudal vein. By ELISA using an immunoplate coated with the GPC3 core protein, it was confirmed that the serum antibody titer against GPC3 was saturated. A mouse myeloma cell P3U1 and mouse splenocyte were mixed together to allow for cell fusion in the presence of PEG1500 (Roche Diagnostics, cat# 783641). The resulting mixture was inoculated in a 96-well culture plate. From the next day, hybridoma was selected with the HAT medium, the culture supernatant was screened by ELISA. Positive clones were subjected to monocloning by limited dilution method. The resulted monoclone was cultured at an enlarged scale and the culture supernatant was collected. The screening by ELISA was done using the binding activity to the GPC3 core protein as a marker to obtain six clones of an anti-GPC3 antibody with a strong binding potency.

[0117] The antibody was purified using Hi Trap Protein G HP (Amersham CAT# 17-0404-01). The supernatant from the hybridoma culture was applied directly to a column, washed with a binding buffer (20 mM sodium phosphate, pH 7.0) and eluted with an elution buffer (0.1 M glycine-HCl, pH 2.7). The eluate was collected into a tube containing a neutralization buffer (1 M Tris-HCl. pH 9.0) for immediate neutralization. After antibody fractions were pooled, the resulting pool was dialyzed against 0.05 % Tween 20/PBS overnight and for a whole day for buffer replacement. NaN_3 was added to the purified antibody to 0.02 %. The antibody was stored at 4 °C.

Analysis of anti-GPC3 antibody

[0118] The antibody concentration was assayed by mouse IgG sandwich ELISA using goat anti-mouse IgG (gamma) (ZYMED CAT# 62-6600) and alkali phosphatase-goat anti-mouse IgG (gamma) (ZYMED CAT# 62-6622), along with a commercially available purified mouse IgG1 antibody (ZYMED CAT# 02-6100) as a standard.

[0119] The isotyping of the anti-GPC3 antibody was done with ImmunoPure Monoclonal Antibody Isotyping Kit II (PIERCE CAT# 37502) by the method according to the attached manual. The results of the isotyping indicated that all of the antibodies were of IgG1 type.

[0120] By western blotting using the GPC3 core protein, the epitopes of the anti-GPC3 antibody were classified. The soluble form of the GPC3 core protein was applied to 10 % SDS-PAGE mini (TEFCO CAT# 01-075) at 100 ng/lane for electrophoresis (60 V for 30 min; 120 V for 90 min), and subsequently transferred on Immobilon-P (Millipore CAT# IPVH R85 10) using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BIO-RAD) (15 V for 60 min). After the membrane was gently rinsed with TBS-T (0.05 % Tween 20, TBS), the membrane was shaken with 5 % skim milk-containing TBS-T for one hour (at ambient temperature) or overnight (at 4°C). After shaking with TBS-T for about 10 minutes, each anti-GPC3 antibody diluted with 1 % skim milk-containing TBS-T to 0.1 to 10 μg/ml was added for one-hour with shaking. The membrane was rinsed with TBS-T (10 minutes × three times) and shaken with HRP-anti-mouse IgG antibody (Amersham CAT# NA 931) diluted to 1.1000 with 1 % skim milk-containing TBS-T for one hour, and rinsed with TBS-T (10 minutes × three times). ECL-Plus (Amersham RPN 2132) was used for chromogenic reaction. Hyperfilm ECL (Amersham CAT# RPN 2103K) was used for detection. Fig.4 shows the results of the western blotting analysis. For the classification, it was determined that the antibody reacting with the band of 40 kDa has an epitope at the N terminus, while the antibody reacting with the band of 30 kDa has an epitope at the C terminus. As antibodies recognizing the N-terminal side, M6B1, M18D4, and M19B11 were obtained. As antibodies recognizing the C-terminal side, M3C11, M13B3, and M3B8 were obtained. The results of the analysis using BIACORE indicated that the KD values of the individual antibodies were in the range of from 0.2 to 17.6 nM.

Reference Example 1

Detection of the secreted form of GPC3

Mouse xenograft model

[0121] 3,000,000 human hepatoma HepG2 cells were transplanted under the abdominal skin in 6-weeks female SCID mice (Fox CHASE C. B-17/Icr-scidJcl, Japan Clair) andnudemice (BALB/cA Jcl-nu, Japan Clair). 53 days later when tumor was sufficiently formed, whole blood was drawn out from the posterior cava of HepG2-transplanted SCID mice #1, 3, and 4. Plasma was prepared in the presence of EDTA-2Na and aprotinin (Nipro Neotube vacuum blood tube, NIPRO, NT-EA0205) and stored at -20°C until assay date. In the case of the HepG2-transplanted SCID mouse #2, whole blood was taken 62 days after HepG2 transplantation. In the case of the HepG2-transplanted nude mice #1 and #2, whole blood was taken 66 days after HepG2 transplantation. As a control, plasma was prepared from normal SCID mouse of the same age by the same procedures.

Sandwich ELISA

[0122] So as to detect the secreted form of GPC3 in blood, a sandwich ELISA system of GPC3 was constructed. M6B1 was used as an antibody to be coated in a 96-well plate. M18D4 labeled with biotin was used as an antibody detecting GPC3 bound to M6B1. For chromogenic reaction, AMPAK of DAKO was used for achieving high detection sensitivity.

[0123] A 96-well immunoplate was coated with the anti-GPC3 antibody diluted with a coating buffer (0.1 M $NaHCO_3$, pH 9.6, 0.02 w/v % NaN_3) to obtain a concentration of 10 μg/mL, and incubated at 4 °C overnight. On the next day, the plate was rinsed three times with 300 μl/well of rinse buffer (0.05 v/v %, Tween 20, PBS) and 200 μl of dilution buffer (50 mM Tris-HCl, pH 8.1, 1 mM $MgCl_2$, 150 mM NaCl, 0.05 v/v % Tween 20, 0.02 w/v % NaN 3, 1 w/v % BSA) was added for blocking. After storage for several hours at ambient temperature or at 4 °C overnight, mouse plasma or the culture supernatant appropriately diluted with a dilution buffer was added and incubated at ambient temperature for one hour. After rinsing with RB at 300 μl/well three times, the biotin-labeled anti-GPC3 antibody diluted with a dilution buffer to 10 μg/mL was added, and incubated at ambient temperature for one hour. After rinsing with RB at 300 μl/well three times, AP-streptoavidin (ZYMED) diluted to 1/1000 with a dilution buffer was added, and incubated at ambient temperature for one hour. After rinsing with the rinse buffer at 300 μl/well five times, AMPAK (DAKO CAT# K6200) was added for chromogenic reaction according to the attached protocol, and the absorbance was measured with a microplate reader.

[0124] For biotinylation of the antibody, Biotin Labeling Kit (CAT# 1 418 165) of Roche was used. A spreadsheet software GlaphPad PRISM (GlaphPad software Inc. ver. 3.0) was used to calculate the concentration of the soluble form of GPC3 in a sample. Fig.5 shows the principle of the sandwich ELISA in this Example.

[0125] Using the purified soluble form of GPC3, a standard curve was prepared. Consequently, a system with a detection limit of several nanogams/mL could be constructed. Fig.6 shows a standard curve for the GPC3 sandwich ELISA using M6B1 and M18D4. Using the system, an attempt was made to detect the secreted form of GPC3 in the culture supernatant of HepG2 and the serum of a mouse transplanted with human hepatoma HepG2. The secreted form of GPC3 was detected in the culture supernatant of HepG2 and the serum of the mouse transplanted with human hepatoma HepG2, while the secreted form of GPC3 was below the detection limit in the control culture medium and the control mouse serum. On a concentration basis of the purified soluble form of GPC3, the soluble form of GPC3 was at 1.2 μg/mL in the culture supernatant of HepG2 and at 23 to 90 ng/mL in the serum of the mouse (Table 1).

Table 1

Assay of the secreted form of GPC3 in the plasma of a mouse transplanted with HepG2 (ng/mL)						
	Tumor volume (mm^3)	M6B01 (N)-M 1 BD4(N)	M19B11 (N)-M18D4(N)	M6B1 (N)-BioM3C1 1(C)	M13B3(C)-Bi oM18D4(N)	M13B3(C)-Bi oM3B8(C)
Culture supernatan t of HepG2		1190	1736	224	234	<1

(continued)

	Tumor volume (mm^3)	M6B01 (N)-M1 BD4(N)	M19B11 (N)-M18D4(N)	M6B1 (N)-BioM3C11(C)	M13B3(C)-BioM18D4(N)	M13B3(C)-BioM3B8(C)
HepG2-transplanted SCID mouse #1	2022	65.4	76.9	<10	<10	<10
HepG2-transplanted SCID mouse #2	1706	71.7	94.8	<10	<10	<10
HepG2-transplanted SCID mouse #3	2257	90.3	113.9	<10	<10	<10
HepG2-transplanted SCID mouse #4	2081	87.3	107.3	<10	15.0	<10
HepG2-transplanted nude mouse #1	1994	58.7	53.6	19.7	35.5	102.2
HepG2-transplanted nude mouse #2	190 & 549	22.9	33.6	<10	11.5	40.6
Normal SCID mouse #1	0	<10	<10	<10	<10	<10
Normal SCID mouse #2	0	<10	<10	<10	<10	<10
Normal SCID mouse #3	0	<10	<10	<10	<10	<10

Structure of secreted form of GPC3

[0126] It was examined whether or not the blood-secreted GPC3 has the structure of the N-terminal fragment as preliminarily assumed. In case that the secreted form of GPC3 was the N-terminal fragment, it is considered that the secreted form of GPC3 will not be detected by sandwich ELISA with a combination of an antibody recognizing the N terminus and an antibody recognizing the C terminus. Using three types of each antibody recognizing the N-terminal fragment and each antibody recognizing the C-terminal fragment, sandwich ELISA systems with various combinations were constructed. Fig.7 shows the structure of the secreted form of GPC3 and Fig.8 shows combinations of the antibodies. Fig. 9 shows a standard curve of the sandwich ELISA. Table 1 shows the assay results. As shown in Table 1, the secreted form of GPC3 was detected at higher values in the culture supernatant of HepG2 and the serum of a mouse transplanted with human hepatoma HepG2 with combinations of antibodies recognizing the N-terminal fragment, while it was detected below the detection limit in many samples from the mice with the systems containing antibodies recognizing the C-terminal fragment. Thus, it was anticipated that the secreted form of GPC3 dominantly comprises the N-terminal fragment. Accordingly, it was suggested that the blood-secreted GPC3 was possibly detected at a high sensitivity by using an

antibody against the amino acid sequence comprising the amino acid residue 1 to the amino acid residue 374 of GPC3.

Example 3

Preparation of anti-GPC3 mouse-human chimera antibody

[0127] Using total RNA extracted from a hybridoma producing an antibody capable of binding to human GPC3 (human GPC3-antibody recognizing C-terminus: M3C11, M1E07; human GPC3-antibody recognizing N terminus: M19B11, M18D04, M5B09, M10D02), the cDNA of variable region of the antibody was amplified by RT-PCR. The total RNA was extracted from the hybridoma of 1×10^7 cells, using RNeasy Plant Mini Kits (manufactured by QIAGEN). Using 1 μg of the total RNA and also using SMART RACE cDNA Amplification Kit (manufactured by CLONTECH), a synthetic oligonucleotide MHC-IgG1 (SEQ ID NO:7) complementary to the mouse IgG1 constant region sequence or a synthetic oligonucleotide kappa (SEQ ID NO:8) complementary to the nucleotide sequence of the mouse κ chain constant region, a 5'-terminal fragment of the gene was amplified. The reverse-transcription was done at 42 °C for one hour and 30 minutes. 50 μl of the PCR solution contained 5 μl of 10 × Advantage 2 PCR Buffer, 5 μl of 10 × Universal Primer A Mix, 0.2 mM dNTPs (dATP, dGTP, dCTP, dTTP), 1 μl of Advantage 2 Polymerase Mix (all manufactured by CLONTECH), 2.5 μl of the reverse-transcription product, and 10 pmole of the synthetic oligonucleotide MHC-IgG1 or kappa. After the initial temperature at 94 °C for 30 seconds, a cycle of 94 °C for 5 seconds and 72 °C for 3 minutes was repeated five times; a cycle of 94 °C for 5 seconds, 70 °C for 10 seconds and 72 °C for 3 minutes was repeated five times; and a cycle of 94 °C for 5 seconds, 68 °C for 10 seconds and 72 °C for 3 minutes was repeated 25 times. Finally, the reaction product was heated at 72 °C for 7 minutes. After the individual PCR products were purified from agarose gel using QIAquick Gel Extraction Kit (manufactured by QIAGEN), the products were cloned in pGEM-T Easy vector (manufactured by Promega), and the nucleotide sequence was determined.

[0128] Then, the sequences of the variable regions of the H chain and L chain were linked to the constant regions of the human H chain and L chain. PCR was done using a synthetic oligonucleotide complementary to the 5'-terminal nucleotide sequence of the H chain variable region of each antibody and having the Kozak's sequence and a synthetic oligonucleotide complementary to the 3'-terminal nucleotide sequence and having an NheI site. The resulting PCR products were cloned in a pB-CH vector with the human IgG1 constant region inserted in pBluescript KS+ vector (manufactured by TOYOBO). The mouse H chain variable region and the human H chain (yl chain) constant region are liked together via the NheI site. The prepared H chain gene fragment was cloned in an expression vector pCXND3. The scheme of the construction of the vector pCXND3 is described below. So as to divide the gene encoding the antibody H chain and the vector sequence from DHFR-DE-rvH-PM1-f (see WO 92/19759), the vector was digested at the restriction enzyme EcoRI/SmaI sites to recover only the vector sequence. Subsequently, the vector sequence was cloned in EcoRI-NotI-BamHI adaptor (manufactured by Takara Shuzo Co., Ltd.). This vector was designated as pCHO1. A region from pCHO1 expressing the DHFR gene was cloned in pCXN at the restriction enzyme HindIII site (Niwa et al., Gene 1991: 108: 193-200). The resulting vector was designated as pCXND3. The nucleotide sequences of the H chains of the anti-GPC3 mouse-human chimera antibodies (M3C11, M1E07, M19B11, M18D04) contained in each plasmid are shown as SEQ ID NOS: 9, 11, 13 and 15, respectively. The amino acid sequences thereof are shown as SEQ ID NOS: 10, 12, 14, and 16, respectively. Additionally, PCR was done using a synthetic oligonucleotide complementary to the 5'-terminal nucleotide sequence of the L chain variable region of each antibody and having the Kozak's sequence and a synthetic oligonucleotide complementary to the 3'-terminal nucleotide sequence and having a BsiWI site. The resulting PCR products were cloned in a pB-CL vector, where the human kappa chain constant region was preliminarily inserted in pBluescript KS+vector(manufactured by TOYOBO). The human L chain variable region and the constant region were linked together via the BsiWI site. The prepared L chain gene fragment was cloned in an expression vector pUCAG. The vector pUCAG is a vector prepared by digesting pCXN (Niwa et al., Gene 1991: 108: 193-200) with restriction enzyme BamHI to obtain a 2.6-kbp fragment, which is then cloned into the restriction enzyme BamHI site of pUC19 vector (manufactured by TOYOBO). The nucleotide sequences of the L chains of the anti-GPC3 mouse-human chimera antibodies (M3C11, M1E07. M19B11, M18D04) contained in each plasmid are shown as SEQ ID NOS: 17, 19, 21 and 23, respectively. The amino acid sequences thereof are shown as SEQ ID NOS: 18, 20, 22 and 24, respectively.

[0129] So as to prepare an expression vector of the anti-GPC3 mouse-human chimera antibody, a gene fragment obtained by digesting the pUCAG vector having the L chain gene fragment inserted therein with restriction enzyme HindIII (manufactured by Takara Shuzo Co., Ltd.) was cloned into the restriction enzyme HindIII cleavage site of pCXND3 having the H chain gene inserted therein. The plasmid will express the neomycin-resistant gene, the DHFR gene and the anti-GPC3 mouse-human chimera antibody gene in animal cells.

[0130] A CHO-based cell line for stable expression (DG44 line) was prepared as follows. The gene was introduced by electroporation method using Gene PulserII (manufactured by Bio Rad). 25 μg of each expression vector of the anti-GPC3 mouse-human chimera antibody and 0.75 ml of CHO cells (1 x 10^7 cells/ml) suspended in PBS were mixed together, and cooled on ice for 10 minutes, which was then transferred into a cuvette and received a pulse at 1.5 kV

and 25 μFD. After a recovery time at ambient temperature for 10 minutes, the cells treated by the electroporation were suspended in 40 mL of a CHO-S-SFMII culture medium (manufactured by Invitrogen) containing 1 × HT supplement (manufactured by Invitrogen). A 50-fold dilution was prepared using the same culture medium, and added at 100 μl/well in a 96-well culture plate. After culturing in a CO_2 incubator (5 % CO_2) for 24 hours, Geneticin (manufactured by Invitrogen) was added to 0.5 mg/mL, and continued cultivation for 2 weeks. The IgG in the culture supernatant from the wells of colonies of a Geneticin resistance transformant cell was assayed by the following concentration assay method. A cell line with high productivity was expanded at an enlarged scale. The cell line stably expressing the anti-GPC3 mouse-human chimera antibody was cultured in a large-scale culturing and the culture supernatant was collected.

[0131] The IgG concentration in the culture supernatant was assayed by human IgG sandwich ELISA using Goat Anti-human IgG (manufactured by BIOSORCE) and Goat Anti-human IgG alkaline phosphatase conjugated(manufactured by BIOSORCE) and compared with the commercially available purified human IgG (manufactured by Cappel).

[0132] Each anti-GPC3 mouse-human chimera antibody was purified using Hi Trap Protein G HP (manufactured by Amersham). A culture supernatant of a CHO cell line producing the anti-GPC3 mouse-human chimera antibody was directly applied to a column and eluted with elution buffer (0.1 M glycine-HC1, pH 2.7). Eluate was collected into a tube containing a neutralization buffer (1 M Tris-HC1, pH 9.0) for immediate neutralization. Antibody fractions were pooled and dialyzed against 0.05% Tween 20/PBS overnight and for a whole day to replace the buffer. NaN_3 was added to the purified antibody to 0.02 % and stored at 4 °C.

Example 4

Preparation of a CHO cell line stably expressing the full length GPC3

[0133] Human GPC3 cDNA was obtained by digesting pGEM-T Easy vector with the full-length human GPC3 cDNA cloned therein with restriction enzyme EcoRI (manufactured by Takara Shuzo Co., Ltd.) and cloned in an expression vector pCOS2. The scheme of the construction of the vector pCOS2 is described below. So as to divide the gene of the antibody H chain of DHFR-ΔE-rvH-PM1-f (see WO 92/19759) from the vector, the vector was digested at the restriction enzyme EcoRI/SmaI sites, to recover only the vector sequence. Subsequently, the vector sequence was cloned in EcoRI-NotI-BamHI adaptor (manufactured by Takara Shuzo Co., Ltd.). This vector was designated as pCHO1. A region from pCH01 expressing the DHFR gene was removed, into which the sequence of the neomycin resistant gene in HEF-VH-gγ1 (Sato et al., Mol. Immunol. 1994: 31: 371-381) was inserted. The vector was designated as pCOS2.

[0134] A cell line stably expressing the full-length human GPC3 was prepared as follows. 10 μl of the full-length human GPC3 gene-expressing vector and 60 μl of SuperFect (manufactured by QIAGEN) were mixed together, to form a complex, which was then added to a CHO cell line DXB11 to introduce the gene. After culturing in a CO_2 incubator (5 % CO_2) for 24 hours, αMEM (manufactured by GIBCO BRL) containing Geneticin (manufactured by Invitrogen) to a final concentration of 0.5 mg/mL and 10 % FBS (manufactured by GIBCO BRL) was used to start selection. The resulting Geneticin-resistant colonies were collected and cell cloning was done by limited dilution method. Individual cell clones were solubilized to confirm the expression of the full-length human GPC3 by western blotting using the anti-GPC3 antibody. A cell strain stably expressing human GPC3 was obtained.

Example 5

ADCC assay using PBMC derived from human peripheral blood (1) Preparation of human PBMC

[0135] Peripheral blood was collected from normal subjects with heparinized syringes, and diluted to 2 fold with PBS (-), and overlaid on Ficoll-Paque™PLUS (Amersham Pharmacia Biotech AB). This was centrifuged (500 × g, 30 minutes, 20°C), and collected the intermediate layer as a mononuclear cell fraction. After rinsing three times, the resulting fraction was suspended in 10% FBS/RPMI to prepare a human PBMC solution.

(2) Preparation of target cell

[0136] HepG2 cell cultured in 10 % FBS/RPMI 1640 culture medium was detached from the dish using trypsin-EDTA (Invitrogen Corp), divided in each well at 1×10^4 cells/well in a U-bottom 96-well plate (Falcon), and cultured for 2 days. After culturing, 5.55 MBq of chromium-51 was added and the cells were incubated in a 5 % CO_2 gas incubator at 37 °C for one hour. The resulting cells were rinsed once with the culture medium, to which 50 μl of 10% FBS/RPMI 1640 culture medium was added to prepare a target cell.

(3) Chromium release test (ADCC activity)

[0137] 50 μl of an antibody solution prepared to each concentration was added to the target cell on ice for 15 minutes. Subsequently, 100 μl of a human PBMC solution was added (5×10^5 cells/well), and incubated in a 5% CO_2 gas incubator at 37 °C for 4 hours. After incubation, the plate was centrifuged and the radioactivity in 100 μl of the culture supernatant was counted with a gamma counter. The specific chromium release ratio was determined by the following formula:

$$\text{Specific chromium release ratio (\%)} = (A-C) \times 100/(B-C)$$

"A" represents the mean radioactivity value (cpm) in each well; "B" represents the mean radioactivity value (cpm) in a well where 100 μl of aqueous 2 % NP-40 solution (Nonidet P-40, Code No. 252-23, Nakarai Tesque) and 50 μl of 10 % FBS/RPMI culture medium were added to the target cell; and "C" represents the mean radioactivity value (cpm) in a well where 150 μl of 10 % FBS/RPMI culture medium was added to the target cell. The test was done in triplicate to calculate the mean of the ADCC activity (%) and the standard error.

[0138] The results are shown in Fig.10. Among the six types of anti-GPC3 chimera antibodies, the antibodies ch.M3C11 and ch.M1E07 recognizing the C terminus exerted the ADCC activity, while the antibodies ch. M19B11, ch. M18D04, ch. M5E09 and ch. M10D02 recognizing the N terminus hardly exerted the ADCC activity. The above results indicate that the ADCC activities of the chimera antibodies depend on the recognition sites of the antibodies. Further, it was expected that the antibodies recognizing the C terminus of GPC3 were possibly useful in clinical applications since the antibodies recognizing the C terminal sides from the cleavage sites exerted the ADCC activity.

Example 6

Assay of complement-dependent cytotoxic activity (CDC activity)

(1) Preparation of human albumin veronal buffer (HAVB)

[0139] 12.75 g of NaCl (superior grade; Wako Pure Chemical Industries, Ltd.), 0.5625 g of Na-barbital (superior grade; Wako Pure Chemical Industries, Ltd.), and 0.8625 g of barbital (superior grade; Wako Pure Chemical Industries, Ltd.) were dissolved in Milli Q water to 200 mL, and autoclaved (121 °C, 20 minutes). 100 mL of autoclaved warm Milli Q water was added. Then, it was confirmed that the resulting mixture was at pH 7.43 (pH 7.5 recommended). This was defined as 5 × Veronal Buffer. 0.2205 g of $CaCl_2$-$2H_2O$ (superior grade; Wako Pure Chemical Industries, Ltd.) was dissolved in 50 mL of Milli Q water to 0.03 mol/L. The resulting solution was defined as $CaCl_2$ solution. 1.0165 g of $MgCl_2$-6 H_2O (superior grade; Wako Pure Chemical Industries, Ltd.) was dissolved in 50 mL of Milli Q water to 0.1 mol/L. The resulting solution was defined as $MgCl_2$ solution. 100 mL of 5 × Veronal Buffer, 4 mL of human serum albumin (Buminate^R 25 %, 250 mg/mL of human serum albumin concentration, Baxter), 2.5 mL of the $CaCl_2$ solution, 2.5 mL of the $MgCl_2$ solution, 0.1 g of KC1 (superior grade; Wako Pure Chemical Industries , Ltd..), and 0.5 g of glucose (D (+)-glucose, anhydrous glucose, superior grade; Wako Pure Chemical Industries, Ltd.) were dissolved in Milli Q water to 500 mL. This was defined as HAVB. After filtration and sterilization, the resulting solution was stored at a set temperature of 5 °C.

(2) Preparation of target cell

[0140] CHO cell expressing GPC3 on the cell membrane as prepared in Example 4 was cultured in alpha-MEM nucleic acid (+) culture medium (GIBCO) supplemented with 10 % FBS and 0.5 mg/mL Geneticin (GIBCO) , detached from the dish using a cell dissociation buffer (Invitrogen Corp), and divided at 1×10^4 cells/well in each well of a 96-well flat bottom plate (Falcon), for culturing for 3 days. After culturing, 5.55 MBq of chromium-51 was added, and incubated in a 5 % CO_2 gas incubator at 37°C for one hour. The resulting cell was rinsed twice with HAVB, to which 50 μl of HAVB was added to prepare a target cell.

(3) Chromium release test (CDC activity)

[0141] Each chimera antibody was diluted with HAVB to prepare an antibody solution of 40 μg/mL. The antibody solution was added in a 50 μl-portion to the target cell, which was then left on ice for 15 minutes. Subsequently, baby rabbit compliment (Cedarlane) diluted with HAVB was added in 100 μl portions to each well to a final concentration of

30 % (final antibody concentration of 10 μg/mL), and incubated in a 5 % CO_2 gas incubator at 37°C for 90 minutes. After centrifugation of the plate, a 100-μl portion of the supernatant was recovered from each well, and the radioactivity was measured with a gamma counter. The specific chromium release ratio was determined by the following formula:

$$\text{Specific chromium release ratio (\%)} = (A-C) \times 100/(B-C)$$

"A" represents the mean radioactivity value (cpm) in each well; "B" represents the mean radioactivity value (cpm) in a well where 100 μl of aqueous 2% NP-40 solution (Nonidet P-40, Code No. 252-23, Nakarai Tesque) and 50 μl of HAVB were added to the target cell; and "C" represents the mean radioactivity value (cpm) in a well where 150 μl of HAVB was added to the target cell. The test was done in triplicate to calculate the mean of the CDC activity (%) and the standard error.

[0142] The results are shown in Fig.11. Among the six types of the anti-GPC3 chimera antibodies, the antibodies ch.M3C11 and M1E07 recognizing the C terminus exerted the CDC activity, while the antibodies ch. M19B11, ch. M18D04. ch. M5E09 and ch. M10D02 recognizing the N terminus exerted low CDC activities. The above results indicate that the CDC activities of the chimera antibodies depend on the recognition sites of the antibodies. Further, it was expected that the antibodies recognizing the C terminus of GPC3 were possibly useful in clinical applications since the antibodies recognizing the C terminal sides from the cleavage sites exerted the CDC activity.

Industrial Applicability

[0143] As shown in the Examples, it was suggested such that a portion of GPC3 highly expressed in hepatoma cells may exist as a secreted form in blood. It is observed that GPC3 is expressed in cancer cell lines other than hepatoma cell lines, such as lung cancer, colon cancer, breast cancer, prostate cancer, pancreatic cancer and lymphoma. If antibodies recognizing the C-terminal fragment with the ADCC activity and/or the CDC activity are used for treating hepatoma, the antibodies can efficiently reach hepatoma cell without being trapped by the secreted form of GPC3 present in blood. Thus, such antibodies are useful as agents for disrupting cancer cells and as anti-cancer agents.

[0144] The contents of all the publications listed in this specification are entirely included in the specification. Additionally, a person skilled in the art will readily understand that various modifications and variations of the invention are possible without departure from the technical scope and inventive range described in the attached claims. It is intended that the invention also encompasses such modifications and variations.

SEQUENCE LISTING

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Val Val Glu Ile Asp Lys Tyr Trp Arg Glu Tyr Ile Leu Ser Leu Glu
    290                 295                 300
Glu Leu Val Asn Gly Met Tyr Arg Ile Tyr Asp Met Glu Asn Val Leu
305                 310                 315                 320
Leu Gly Leu Phe Ser Thr Ile His Asp Ser Ile Gln Tyr Val Gln Lys
                325                 330                 335
Asn Ala Gly Lys Leu Thr Thr Thr Ile Gly Lys Leu Cys Ala His Ser
            340                 345                 350
Gln Gln Arg Gln Tyr Arg Ser Ala Tyr Tyr Pro Glu Asp Leu Phe Ile
        355                 360                 365
Asp Lys Lys Val Leu Lys Val Ala His Val Glu His Glu Glu Thr Leu
    370                 375                 380
Ser Ser Arg Arg Arg Glu Leu Ile Gln Lys Leu Lys Ser Phe Ile Ser
385                 390                 395                 400
Phe Tyr Ser Ala Leu Pro Gly Tyr Ile Cys Ser His Ser Pro Val Ala
                405                 410                 415
Glu Asn Asp Thr Leu Cys Trp Asn Gly Gln Glu Leu Val Glu Arg Tyr
            420                 425                 430
Ser Gln Lys Ala Ala Arg Asn Gly Met Lys Asn Gln Phe Asn Leu His
        435                 440                 445
Glu Leu Lys Met Lys Gly Pro Glu Pro Val Val Ser Gln Ile Ile Asp
    450                 455                 460
Lys Leu Lys His Ile Asn Gln Leu Leu Arg Thr Met Ser Met Pro Lys
465                 470                 475                 480
Gly Arg Val Leu Asp Lys Asn Leu Asp Glu Glu Gly Phe Glu Ser Gly
                485                 490                 495
```

```
Asp Cys Gly Asp Asp Glu Asp Glu Cys Ile Gly Gly Ser Gly Asp Gly
                500                 505                 510
Met Ile Lys Val Lys Asn Gln Leu Arg Phe Leu Ala Glu Leu Ala Tyr
            515                 520                 525
Asp Leu Asp Val Asp Asp Ala Pro Gly Asn Ser Gln Gln Ala Thr Pro
        530                 535                 540
Lys Asp Asn Glu Ile Ser Thr Phe His Asn Leu Gly Asn Val His Ser
545                 550                 555                 560
Pro Leu Lys Leu Leu Thr Ser Met Ala Ile Ser Val Val Cys Phe Phe
                565                 570                 575
Phe Leu Val His
            580
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<210> 5
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 5
atagaattcc accatggccg ggaccgtgcg c 31

<210> 6
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 6
ataggatccc ttcagcgggg aatgaacgtt c 31

<210> 7
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 7
gggccagtgg atagacagat g 21

<210> 8
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic DNA

<400> 8
gctcactgga tggtgggaag atg 23

<210> 9
<211> 1392
<212> DNA
<213> Artificial Sequence <220>
<221> CDS
<222> (1)..(1389)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M3C11 H chain)

<400> 9

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atg aac ttc ggg ctc acc ttg att ttc ctt gtc ctt act tta aaa ggt    48
Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
  1               5                  10                  15
gtc cag tgt gag gtg caa ctg gtg gag tct ggg gga ggc tta gtg aag    96
Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
             20                  25                  30
cct gga gga tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc act ttc   144
Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
         35                  40                  45
agt cgc tat gcc atg tct tgg gtt cgc cag att cca gag aag ata ctg   192
Ser Arg Tyr Ala Met Ser Trp Val Arg Gln Ile Pro Glu Lys Ile Leu
     50                  55                  60
gag tgg gtc gca gcc att gat agt agt ggt ggt gac acc tac tat tta   240
Glu Trp Val Ala Ala Ile Asp Ser Ser Gly Gly Asp Thr Tyr Tyr Leu
 65                  70                  75                  80
gac act gtg aag gac cga ttc acc atc tcc aga gac aat gcc aat aat   288
Asp Thr Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn
                 85                  90                  95
acc ctg cac ctg caa atg cgc agt ctg agg tct gag gac aca gcc ttg   336
Thr Leu His Leu Gln Met Arg Ser Leu Arg Ser Glu Asp Thr Ala Leu
            100                 105                 110
tat tac tgt gta aga cag ggg ggg gct tac tgg ggc caa ggg act ctg   384
Tyr Tyr Cys Val Arg Gln Gly Gly Ala Tyr Trp Gly Gln Gly Thr Leu
        115                 120                 125
gtc act gtc tct gca gct agc acc aag ggc cca tcg gtc ttc ccc ctg   432
Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
    130                 135                 140
gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc   480
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
145                 150                 155                 160
ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca   528
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
                165                 170                 175
ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc   576
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
            180                 185                 190
tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc   624
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
        195                 200                 205
ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac   672
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
    210                 215                 220
acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gac aaa act cac   720
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
225                 230                 235                 240
aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc   768
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
                245                 250                 255
ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc   816
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270
```

```
cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag    864
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285
gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag    912
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300
aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc    960
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305                 310                 315                 320
gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag   1008
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335
tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc   1056
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350
tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc   1104
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365
cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg   1152
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380
gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat   1200
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400
ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc   1248
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                405                 410                 415
gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg   1296
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430
tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg   1344
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445
cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga   1392
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
```

<210> 10
<211> 463
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M3C11 H chain)

<400> 10

```
Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
  1               5                  10                  15
Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
             20                  25                  30
Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
         35                  40                  45
Ser Arg Tyr Ala Met Ser Trp Val Arg Gln Ile Pro Glu Lys Ile Leu
     50                  55                  60
Glu Trp Val Ala Ala Ile Asp Ser Ser Gly Gly Asp Thr Tyr Tyr Leu
 65                  70                  75                  80
Asp Thr Val Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn
                 85                  90                  95
Thr Leu His Leu Gln Met Arg Ser Leu Arg Ser Glu Asp Thr Ala Leu
            100                 105                 110
```

```
Tyr Tyr Cys Val Arg Gln Gly Gly Ala Tyr Trp Gly Gln Gly Thr Leu
        115                 120                 125
Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
    130                 135                 140
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
145                 150                 155                 160
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
                165                 170                 175
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
            180                 185                 190
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
        195                 200                 205
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
    210                 215                 220
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
225                 230                 235                 240
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
                245                 250                 255
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            260                 265                 270
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        275                 280                 285
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    290                 295                 300
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
305                 310                 315                 320
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                325                 330                 335
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
            340                 345                 350
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        355                 360                 365
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
    370                 375                 380
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
385                 390                 395                 400
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
                405                 410                 415
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
            420                 425                 430
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
        435                 440                 445
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
```

<210> 11
<211> 1413
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(1410)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M1E07 H chain)

<400> 11

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atg gga tgg aac tgg atc ttt att tta atc ctg tca gta act aca ggt   48
Met Gly Trp Asn Trp Ile Phe Ile Leu Ile Leu Ser Val Thr Thr Gly
```

```
  1               5                  10                  15
gtc cac tct gag gtc cag ctg cag cag tct gga cct gag ctg gtg aag    96
Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
            20                  25                  30
cct ggg gct tca gtg aag ata tcc tgc aag gct tct ggt tac tca ttc   144
Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe
        35                  40                  45
act ggc tac tac atg cac tgg gtg aag caa agt cct gaa aag agc ctt   192
Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser Pro Glu Lys Ser Leu
    50                  55                  60
gag tgg att gga gag att aat cct agc act ggt ggt act acc tac aac   240
Glu Trp Ile Gly Glu Ile Asn Pro Ser Thr Gly Gly Thr Thr Tyr Asn
65                  70                  75                  80
cag aag ttc aag gcc aag gcc aca ttg act gta gac aaa tcc tcc agc   288
Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
                85                  90                  95
aca gcc tac atg cag ctc aag agc ctg aca tct gag gac tct gca gtc   336
Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110
tat tac tgt gca agg agg ggc gga tta act ggg acg agc ttc ttt gct   384
Tyr Tyr Cys Ala Arg Arg Gly Gly Leu Thr Gly Thr Ser Phe Phe Ala
        115                 120                 125
tac tgg ggc caa ggg act ctg gtc act gtc tct gca gct agc acc aag   432
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys
    130                 135                 140
ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg   480
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145                 150                 155                 160
ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg   528
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                 170                 175
gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc   576
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
            180                 185                 190
ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtg   624
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                 200                 205
gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac   672
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210                 215                 220
gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc   720
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225                 230                 235                 240
aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa   768
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                245                 250                 255
ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac   816
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
            260                 265                 270
acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac   864
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                 280                 285
gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc   912
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                 295                 300
gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac   960
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                 310                 315                 320
agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg  1008
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                 330                 335
```

```
ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca   1056
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                 345                 350
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa   1104
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365
cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac   1152
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
    370                 375                 380
cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc   1200
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400
gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc   1248
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
                405                 410                 415
acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag   1296
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
            420                 425                 430
ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc   1344
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
        435                 440                 445
tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc   1392
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    450                 455                 460
tcc ctg tct ccg ggt aaa tga                                      1413
Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 12
<211> 470
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (MIE07 H chain)

<400> 12

```
Met Gly Trp Asn Trp Ile Phe Ile Leu Ile Leu Ser Val Thr Thr Gly
  1               5                  10                  15
Val His Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys
             20                  25                  30
Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe
         35                  40                  45
Thr Gly Tyr Tyr Met His Trp Val Lys Gln Ser Pro Glu Lys Ser Leu
     50                  55                  60
Glu Trp Ile Gly Glu Ile Asn Pro Ser Thr Gly Gly Thr Thr Tyr Asn
 65                  70                  75                  80
Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
                 85                  90                  95
Thr Ala Tyr Met Gln Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110
Tyr Tyr Cys Ala Arg Arg Gly Gly Leu Thr Gly Thr Ser Phe Phe Ala
        115                 120                 125
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys
    130                 135                 140
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145                 150                 155                 160
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                 170                 175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
```

```
                180                 185                 190
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                 200                 205
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210                 215                 220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225                 230                 235                 240
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                245                 250                 255
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
            260                 265                 270
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                 280                 285
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                 295                 300
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                 310                 315                 320
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                 330                 335
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                 345                 350
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
    370                 375                 380
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
                405                 410                 415
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
            420                 425                 430
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
        435                 440                 445
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    450                 455                 460
Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 13
<211> 1416
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(1413)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M19B11 H chain)

<400> 13

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atg aac ttc ggg ctc acc ttg att ttc ctc gtc ctt act tta aaa ggt   48
Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
  1               5                  10                  15
gtc cag tgt gag gtg cag ctg gtg gag tct ggg gga gac tta gtg aag   96
Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys
            20                  25                  30
cct gga ggg acc ctg aaa ctc tcc tgt gca gcc tct gga tcc act ttc   144
Pro Gly Gly Thr Leu Lys Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe
        35                  40                  45
```

```
agt aac tat gcc atg tct tgg gtt cgc cag act cca gag aag agg ctg   192
Ser Asn Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
     50                  55                  60
gag tgg gtc gca gcc att gat agt aat gga ggt acc acc tac tat cca   240
Glu Trp Val Ala Ala Ile Asp Ser Asn Gly Gly Thr Thr Tyr Tyr Pro
 65                  70                  75                  80
gac act atg aag gac cga ttc acc att tcc aga gac aat gcc aag aac   288
Asp Thr Met Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
                 85                  90                  95
acc ctg tac ctg caa atg aac agt ctg agg tct gaa gac aca gcc ttt   336
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Phe
            100                 105                 110
tat cac tgt aca aga cat aat gga ggg tat gaa aac tac ggc tgg ttt   384
Tyr His Cys Thr Arg His Asn Gly Gly Tyr Glu Asn Tyr Gly Trp Phe
        115                 120                 125
gct tac tgg ggc caa ggg act ctg gtc act gtc tct gca gct agc acc   432
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr
    130                 135                 140
aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct   480
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160
ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa   528
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175
ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac   576
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190
acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc   624
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205
gtg gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc   672
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220
aac gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag   720
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
225                 230                 235                 240
ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct   768
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255
gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag   816
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270
gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg   864
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285
gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac   912
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300
ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac   960
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320
aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac   1008
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335
tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc   1056
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350
cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga   1104
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365
gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag   1152
```

```
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
    370                 375                 380
aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac   1200
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385                 390                 395                 400
atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag   1248
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
                405                 410                 415
acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc   1296
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
            420                 425                 430
aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca   1344
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
        435                 440                 445
tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc   1392
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
    450                 455                 460
ctc tcc ctg tct ccg ggt aaa tga                                  1416
Leu Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 14
<211> 471
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M19B11 H chain)

<400> 14

```
Met Asn Phe Gly Leu Thr Leu Ile Phe Leu Val Leu Thr Leu Lys Gly
  1               5                  10                  15
Val Gln Cys Glu Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys
            20                  25                  30
Pro Gly Gly Thr Leu Lys Leu Ser Cys Ala Ala Ser Gly Ser Thr Phe
        35                  40                  45
Ser Asn Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu
    50                  55                  60
Glu Trp Val Ala Ala Ile Asp Ser Asn Gly Gly Thr Thr Tyr Tyr Pro
65                  70                  75                  80
Asp Thr Met Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
                85                  90                  95
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ser Glu Asp Thr Ala Phe
            100                 105                 110
Tyr His Cys Thr Arg His Asn Gly Gly Tyr Glu Asn Tyr Gly Trp Phe
        115                 120                 125
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr
    130                 135                 140
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
145                 150                 155                 160
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
                165                 170                 175
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
            180                 185                 190
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
        195                 200                 205
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
    210                 215                 220
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
225                 230                 235                 240
```

```
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
                245                 250                 255
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
            260                 265                 270
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
        275                 280                 285
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
    290                 295                 300
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
305                 310                 315                 320
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
                325                 330                 335
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
            340                 345                 350
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
        355                 360                 365
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
    370                 375                 380
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
385                 390                 395                 400
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
                405                 410                 415
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
            420                 425                 430
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
        435                 440                 445
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
    450                 455                 460
Leu Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 15
<211> 1413
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(1410)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M18D04 H chain)

<400> 15

```
atg gaa tct aac tgg ata ctt cct ttt att ctg tcg gta gct tca ggg   48
Met Glu Ser Asn Trp Ile Leu Pro Phe Ile Leu Ser Val Ala Ser Gly
  1               5                  10                  15
gtc tac tca gag gtt cag ctc cag cag tct ggg act gtg ctg gca agg   96
Val Tyr Ser Glu Val Gln Leu Gln Gln Ser Gly Thr Val Leu Ala Arg
             20                  25                  30
cct ggg gct tca gtg aag atg tcc tgc aag gct tct ggc tac acc ttt   144
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
         35                  40                  45
act ggc tac tgg atg cgc tgg gta aaa cag agg cct gga cag ggt ctg   192
Thr Gly Tyr Trp Met Arg Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
     50                  55                  60
gaa tgg att ggc gct att tat cct gga aat agt gat aca aca tac aac   240
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Thr Tyr Asn
 65                  70                  75                  80
cag aag ttc aag ggc aag gcc aaa ctg act gca gtc aca tct gtc agc   288
```

```
Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Val Ser
                85                  90                  95
act gcc tac atg gaa ctc agc agc ctg aca aat gag gac tct gcg gtc   336
Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val
            100                 105                 110
tat tac tgt tca aga tcg ggg gac cta act ggg ggg ttt gct tac tgg   384
Tyr Tyr Cys Ser Arg Ser Gly Asp Leu Thr Gly Gly Phe Ala Tyr Trp
        115                 120                 125
ggc caa ggg act ctg gtc act gtc tct aca gcc aaa gct agc acc aag   432
Gly Gln Gly Thr Leu Val Thr Val Ser Thr Ala Lys Ala Ser Thr Lys
    130                 135                 140
ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct ggg   480
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145                 150                 155                 160
ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg   528
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                 170                 175
gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac acc   576
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
            180                 185                 190
ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc gtg   624
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                 200                 205
gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc aac   672
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210                 215                 220
gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc   720
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225                 230                 235                 240
aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa   768
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                245                 250                 255
ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac   816
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
            260                 265                 270
acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac   864
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                 280                 285
gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc   912
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                 295                 300
gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac   960
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                 310                 315                 320
agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg  1008
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                 330                 335
ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca  1056
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                 345                 350
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa  1104
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365
cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac  1152
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
    370                 375                 380
cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc  1200
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400
gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc  1248
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
```

```
                    405                 410                 415
acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag   1296
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
                420                 425                 430
ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc   1344
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
            435                 440                 445
tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc   1392
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
        450                 455                 460
tcc ctg tct ccg ggt aaa tga                                      1413
Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 16
<211> 470
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M18D04 H chain)

<400> 16

```
Met Glu Ser Asn Trp Ile Leu Pro Phe Ile Leu Ser Val Ala Ser Gly
 1               5                  10                  15
Val Tyr Ser Glu Val Gln Leu Gln Gln Ser Gly Thr Val Leu Ala Arg
            20                  25                  30
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45
Thr Gly Tyr Trp Met Arg Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    50                  55                  60
Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asn Ser Asp Thr Thr Tyr Asn
65                  70                  75                  80
Gln Lys Phe Lys Gly Lys Ala Lys Leu Thr Ala Val Thr Ser Val Ser
                85                  90                  95
Thr Ala Tyr Met Glu Leu Ser Ser Leu Thr Asn Glu Asp Ser Ala Val
            100                 105                 110
Tyr Tyr Cys Ser Arg Ser Gly Asp Leu Thr Gly Gly Phe Ala Tyr Trp
        115                 120                 125
Gly Gln Gly Thr Leu Val Thr Val Ser Thr Ala Lys Ala Ser Thr Lys
    130                 135                 140
Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145                 150                 155                 160
Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
                165                 170                 175
Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
            180                 185                 190
Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
        195                 200                 205
Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
    210                 215                 220
Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
225                 230                 235                 240
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
                245                 250                 255
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
            260                 265                 270
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
        275                 280                 285
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
    290                 295                 300
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305                 310                 315                 320
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
                325                 330                 335
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
            340                 345                 350
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
        355                 360                 365
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
    370                 375                 380
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385                 390                 395                 400
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
                405                 410                 415
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
            420                 425                 430
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
        435                 440                 445
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
    450                 455                 460
Ser Leu Ser Pro Gly Lys
465                 470
```

<210> 17
<211> 717
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(714)

<220> <223> Description of Artificial Sequence: Mouse-human chimeric antibody (M3C11 L chain)

<400> 17

```
atg agt cct gcc cag ttc ctg ttt ctg tta gtg ctc tgg att cgg gaa    48
Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg Glu
  1               5                  10                  15
acc aac ggt gat gtt gtg atg acc cag act cca ctc act ttg tcg gtt    96
Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val
             20                  25                  30
acc att gga caa cca gcc tcc atc tct tgc aag tca agt cag agc ctc   144
Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu
         35                  40                  45
tta gat agt gat gga aag aca tat ttg aat tgg ttg tta cag agg cca   192
Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro
     50                  55                  60
ggc cag tct cca aag cgc cta atc tat ctg gtg tct aaa ttg gac tct   240
Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser
 65                  70                  75                  80
gga gcc cct gac agg ttc act ggc agt gga tca ggg aca gat ttc aca   288
Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
                 85                  90                  95
ctg aaa atc agt aga gtg gag gct gag gat ttg gga att tat tat tgc   336
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys
            100                 105                 110
tgg caa ggt aca cat ttt ccg ctc acg ttc ggt gct ggg acc aag ctg   384
Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
        115                 120                 125
gag ctg aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg cca   432
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140
tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg   480
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145                 150                 155                 160
aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat aac   528
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
                165                 170                 175
gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac agc   576
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
            180                 185                 190
aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa gca   624
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195                 200                 205
gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc   672
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210                 215                 220
ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tga       717
Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 18
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M3C11 L chain)

<400> 18

```
Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg Glu
 1               5                  10                  15
Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val
            20                  25                  30
Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu
        35                  40                  45
Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro
    50                  55                  60
Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser
65                  70                  75                  80
Gly Ala Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr
                85                  90                  95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys
            100                 105                 110
Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
        115                 120                 125
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145                 150                 155                 160
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
                165                 170                 175
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
            180                 185                 190
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195                 200                 205
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210                 215                 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 19
<211> 717
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(714)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M1E07 L chain)

<400> 19

```
atg agt cct gtc cag ttc ctg ttt ctg tta atg ctc tgg att cag gaa    48
Met Ser Pro Val Gln Phe Leu Phe Leu Leu Met Leu Trp Ile Gln Glu
  1               5                  10                  15
acc aac ggt gat gtt gtg atg acc cag act cca ctg tct ttg tcg gtt    96
Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val
             20                  25                  30
acc att gga caa cca gcc tct atc tct tgc aag tca agt cag agc ctc   144
Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu
         35                  40                  45
tta tat agt aat gga aag aca tat ttg aat tgg tta caa cag agg cct   192
Leu Tyr Ser Asn Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro
     50                  55                  60
ggc cag gct cca aag cac cta atg tat cag gtg tcc aaa ctg gac cct   240
Gly Gln Ala Pro Lys His Leu Met Tyr Gln Val Ser Lys Leu Asp Pro
 65                  70                  75                  80
ggc atc cct gac agg ttc agt ggc agt gga tca gaa aca gat ttt aca   288
Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Glu Thr Asp Phe Thr
                 85                  90                  95
ctt aaa atc agc aga gtg gag gct gaa gat ttg gga gtt tat tac tgc   336
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys
            100                 105                 110
ttg caa agt aca tat tat ccg ctc acg ttc ggt gct ggg acc aag ctg   384
Leu Gln Ser Thr Tyr Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
        115                 120                 125
gag ctg aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg cca   432
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140
tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg   480
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145                 150                 155                 160
aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat aac   528
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
                165                 170                 175
gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac agc   576
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
            180                 185                 190
aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa gca   624
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195                 200                 205
gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc   672
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210                 215                 220
ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tga       717
Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 20
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M1E07 L chain)

<400> 20

```
Met Ser Pro Val Gln Phe Leu Phe Leu Leu Met Leu Trp Ile Gln Glu
 1               5                  10                  15
Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val
            20                  25                  30
Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu
        35                  40                  45
Leu Tyr Ser Asn Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro
    50                  55                  60
Gly Gln Ala Pro Lys His Leu Met Tyr Gln Val Ser Lys Leu Asp Pro
65                  70                  75                  80
Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Glu Thr Asp Phe Thr
                85                  90                  95
Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys
            100                 105                 110
Leu Gln Ser Thr Tyr Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
        115                 120                 125
Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140
Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145                 150                 155                 160
Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
                165                 170                 175
Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
            180                 185                 190
Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195                 200                 205
Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210                 215                 220
Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 21
<211> 705
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(702)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M19B11 L chain)

<400> 21

```
atg aga ccc tcc att cag ttc ctg ggg ctc ttg ttg ttc tgg ctt cat   48
Met Arg Pro Ser Ile Gln Phe Leu Gly Leu Leu Leu Phe Trp Leu His
 1               5                  10                  15
ggt gtt cag tgt gac atc cag atg aca cag tct cca tcc tca ctg tct   96
```

```
Gly Val Gln Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
            20                  25                  30
gca tct ctg gga ggc aaa gtc acc atc act tgc aag gca agt cag gac   144
Ala Ser Leu Gly Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
         35                  40                  45
att aac aag aat ata gtt tgg tac caa cac aag cct gga aaa ggt cct   192
Ile Asn Lys Asn Ile Val Trp Tyr Gln His Lys Pro Gly Lys Gly Pro
     50                  55                  60
agg ctg ctc ata tgg tac aca tct aca tta cag cca ggc atc cca tca   240
Arg Leu Leu Ile Trp Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser
 65                  70                  75                  80
agg ttc agt gga agt ggg tct ggg aga gat tat tcc ttc agc atc agc   288
Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser
                85                  90                  95
aac ctg gag cct gaa gat att gca act tat tac tgt cta cag tat gat   336
Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp
            100                 105                 110
aat ctt cca cgg acg ttc ggt gga ggc acc aaa ctg gaa atc aaa cgt   384
Asn Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
        115                 120                 125
acg gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag   432
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
    130                 135                 140
ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat   480
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145                 150                 155                 160
ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg   528
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
                165                 170                 175
ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc   576
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
            180                 185                 190
tac agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa   624
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
        195                 200                 205
cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc   672
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
    210                 215                 220
gtc aca aag agc ttc aac agg gga gag tgt tga                       705
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230
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<210> 22
<211> 234
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M19B11 L chain)

<400> 22

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 1               5                  10                  15
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            20                  25                  30
Ala Ser Leu Gly Gly Lys Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
         35                  40                  45
Ile Asn Lys Asn Ile Val Trp Tyr Gln His Lys Pro Gly Lys Gly Pro
     50                  55                  60
Arg Leu Leu Ile Trp Tyr Thr Ser Thr Leu Gln Pro Gly Ile Pro Ser
```

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 65                  70                  75                  80
Arg Phe Ser Gly Ser Gly Ser Gly Arg Asp Tyr Ser Phe Ser Ile Ser
                85                  90                  95
Asn Leu Glu Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln Tyr Asp
            100                 105                 110
Asn Leu Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
        115                 120                 125
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
    130                 135                 140
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145                 150                 155                 160
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
                165                 170                 175
Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
            180                 185                 190
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
        195                 200                 205
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
    210                 215                 220
Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230
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<210> 23
<211> 720
<212> DNA
<213> Artificial Sequence

<220>
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<222> (1)..(717)

<220>
<223> Description of Artificial Sequence: Mouse-human chimeric antibody (M18D04 L chain)

<400> 23

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  1               5                  10                  15
gga tcc act gca gat att gtg atg acg cag gct gca ttc tcc aat cca   96
Gly Ser Thr Ala Asp Ile Val Met Thr Gln Ala Ala Phe Ser Asn Pro
            20                  25                  30
gtc act ctt gga aca tca act tcc atc tcc tgc agg tct agt aag agt   144
Val Thr Leu Gly Thr Ser Thr Ser Ile Ser Cys Arg Ser Ser Lys Ser
        35                  40                  45
ctc cta cat agt aat ggc atc act tat ttg tat tgg tat ctg cag aag   192
Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
    50                  55                  60
cca ggc cag tct cct cag ctc ctg att tat cag atg tcc aac ctt gcc   240
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala
65                  70                  75                  80
tca gga gtc cca gac agg ttc agt agc agt ggg tca gga act gat ttc   288
Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
                85                  90                  95
aca ctg aga atc agc aga gtg gag gct gag gat gtg ggt gtt tat tac   336
Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
            100                 105                 110
tgt gct caa aat cta gaa ctt ccg tat acg ttc gga tcg ggg acc aag   384
Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Ser Gly Thr Lys
        115                 120                 125
ctg gaa ata aaa cgt acg gtg gct gca cca tct gtc ttc atc ttc ccg   432

Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
    130                 135                 140
cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg   480
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160
ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat   528
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                165                 170                 175
aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag gac   576
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190
agc aag gac agc acc tac agc ctc agc agc acc ctg acg ctg agc aaa   624
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
        195                 200                 205
gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc acc cat cag   672
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
    210                 215                 220
ggc ctg agc tcg ccc gtc aca aag agc ttc aac agg gga gag tgt tga   720
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
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<210> 24
<211> 239
<212> PRT
<213> Artificial Sequence

<220> <223> Description of Artificial Sequence: Mouse-human chimeric antibody (M18D04 L chain)

<400> 24

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            20                  25                  30
Val Thr Leu Gly Thr Ser Thr Ser Ile Ser Cys Arg Ser Ser Lys Ser
        35                  40                  45
Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys
    50                  55                  60
Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala
65                  70                  75                  80
Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe
                85                  90                  95
Thr Leu Arg Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
            100                 105                 110
Cys Ala Gln Asn Leu Glu Leu Pro Tyr Thr Phe Gly Ser Gly Thr Lys
        115                 120                 125
Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
    130                 135                 140
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160
Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                165                 170                 175
Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190
Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
        195                 200                 205
Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
    210                 215                 220
Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

Claims

1. An antibody against a peptide consisting of amino acid residues 375-580 of GPC 3, wherein the antibody has a cytotoxic activity.

2. The antibody of claim 1, wherein the cytotoxic activity is a cytotoxic activity to HepG2 or HuH-7 cells.

3. The antibody of claims 1 or 2, wherein the antibody is:

   a monoclonal antibody; and/or
   is a chimera antibody; and/or
   is a humanised antibody.

4. The antibody of any one of the preceding claims, wherein the cytotoxic activity is ADCC activity.

5. The antibody of any one of the preceding claims, wherein the cytotoxic activity is CDC activity.

6. The antibody of any one of the preceding claims, where the antibody is a recombinant antibody.

7. The antibody of claim 6, wherein the antibody has been produced in a mammalian cell.

8. The antibody of claim 7, wherein the mammalian cell is selected from a CHO, COS, myeloma, BHK, vero and Hela cell.

9. The antibody of claim 7 or 8, wherein the mammalian cell is transformed with an expression vector comprising a gene encoding the antibody.

10. The antibody of claim 9, wherein the mammalian cell comprises:

(a) an expression vector comprising a gene encoding the antibody heavy (H) chain and a separate expression vector comprising a gene encoding the antibody light (L) chain; or
(b) a single expression vector encoding both the H and L chain.

11. The antibody of claim 9 or 10, wherein:

(a) the gene encoding the H chain of the antibody comprises the sequence of SEQ ID NO: 9; and/or
(b) the gene encoding the L chain of the antibody comprises the sequence of SEQ ID NO: 17.

12. The antibody of claim 9 or 10, wherein:

(a) the gene encoding the H chain of the antibody comprises the sequence of SEQ ID NO: 11; and/or
(b) the gene encoding the L chain of the antibody comprises the sequence of SEQ ID NO: 19.

13. The antibody of any one of the preceding claims which is a humanized antibody.

14. An antibody as defined in any one of the preceding claims for use in the treatment of cancer, where the cancer expresses GPC 3.

15. An antibody as defined in any one of claims 1 to 13 for use in cell disruption, wherein the cells to be disrupted express GPC 3.

16. An antibody according to claim 15, wherein the cell is a cancer cell.

17. An antibody according to claim 14 or 16, wherein the cancer is hepatoma, pancreatic cancer, lung cancer, colon cancer, breast cancer, prostate cancer, leukemia or lymphoma.

18. A pharmaceutical formulation comprising the antibody as defined in any one of claims 1 to 13 and a pharmaceutically acceptable carrier and/or additive.

Patentansprüche

1. Antikörper gegen ein Peptid bestehend aus den Aminosäureresten 375-580 von GPC 3, wobei der Antikörper eine zytotoxische Aktivität aufweist.

2. Der Antikörper nach Anspruch 1, wobei die zytotoxische Aktivität eine zytotoxische Aktivität gegen HepG2 oder HuH-7 Zellen ist.

3. Der Antikörper nach Anspruch 1 oder 2, wobei der Antikörper:

ein monoklonaler Antikörper; und/oder
ein chimärer Antikörper; und/oder
ein humanisierter Antikörper ist.

4. Der Antikörper nach einem der vorangegangenen Ansprüche, wobei die zytotoxische Aktivität eine ADCC Aktivität ist.

5. Der Antikörper nach einem der vorangegangenen Ansprüche, wobei die zytotoxische Aktivität eine CDC Aktivität ist.

6. Der Antikörper nach einem der vorangegangenen Ansprüche, wobei der Antikörper ein rekombinanter Antikörper ist.

7. Der Antikörper nach Anspruch 6, wobei der Antikörper in einer Säugetierzelle produziert wurde.

8. Der Antikörper nach Anspruch 7, wobei die Säugetierzelle ausgewählt ist aus CHO, COS, Myelom, BHK, Vero und Hela-Zellen.

9. Der Antikörper nach Anspruch 7 oder 8, wobei die Säugetierzelle mit einem Expressionsvektor, umfassend ein Gen, das den Antikörper kodiert, transformiert ist.

10. Der Antikörper nach Anspruch 9, wobei die Säugetierzelle umfasst:

(a) einen Expressionsvektor, umfassend ein Gen, das die schwere Kette (H) des Antikörpers kodiert und einen separaten Expressionsvektor, umfassend ein Gen, das die leichte Kette (L) des Antikörpers kodiert; oder
(b) einen einzelnen Expressionsvektor, der sowohl die H- als auch die L-Kette kodiert.

11. Der Antikörper nach Anspruch 9 oder 10, wobei:

(a) das Gen, das die H-Kette des Antikörpers kodiert, die Sequenz mit der SEQ ID Nr: 9 umfasst; und/oder
(b) das Gen, das die L-Kette des Antikörpers kodiert, die Sequenz mit der SEQ ID Nr: 17 umfasst.

12. Der Antikörper nach Anspruch 9 oder 10, wobei:

(a) das Gen, das die H-Kette des Antikörpers kodiert, die Sequenz mit der SEQ ID Nr: 11 umfasst; und/oder
(b) das Gen, das die L-Kette des Antikörpers kodiert, die Sequenz mit der SEQ ID Nr: 19 umfasst.

13. Der Antikörper nach einem der vorangegangenen Ansprüche, der ein humanisierter Antikörper ist.

14. Antikörper wie in einem der vorangegangenen Ansprüche definiert, zur Verwendung in der Behandlung von Krebs, wobei der Krebs GPC 3 exprimiert.

15. Antikörper wie in einem der Ansprüche 1 bis 13 definiert, zur Verwendung bei Zellaufschlüssen, wobei die aufzuschließende Zelle GPC 3 exprimiert.

16. Ein Antikörper nach Anspruch 15, wobei die Zelle eine Krebszelle ist.

17. Ein Antikörper nach Anspruch 14 oder 16, wobei der Krebs ein Hepatom, Bauchspeicheldrüsenkrebs, Lungenkrebs, Dickdarmkrebs, Brustkrebs, Prostatakrebs, Leukämie oder ein Lymphom ist.

18. Pharmazeutische Formulierung umfassend den Antikörper wie in einem der Ansprüche 1 bis 13 definiert und einen pharmazeutisch verträglichen Träger und/oder Additiv.

Revendications

1. Anticorps dirigé contre un peptide consistant en les résidus d'acides aminés 375-580 de GPC 3, où l'anticorps possède une activité cytotoxique.

2. Anticorps selon la revendication 1, où l'activité cytotoxique est une activité cytotoxique contre des cellules HepG2 ou HuH-7.

3. Anticorps selon les revendications 1 ou 2, où l'anticorps est :

un anticorps monoclonal ; et/ou
est un anticorps chimérique ; et/ou
est un anticorps humanisé.

4. Anticorps selon l'une quelconque des revendications précédentes, où l'activité cytotoxique est une activité ADCC.

5. Anticorps selon l'une quelconque des revendications précédentes, où l'activité cytotoxique est une activité CDC.

6. Anticorps selon l'une quelconque des revendications précédentes, où l'anticorps est un anticorps recombinant.

7. Anticorps selon la revendication 6, où l'anticorps a été produit dans une cellule de mammifère.

8. Anticorps selon la revendication 7, où la cellule de mammifère est sélectionnée parmi une cellule CHO, COS, de myélome, BHK, vero et Hela.

9. Anticorps selon la revendication 7 ou 8, où la cellule de mammifère est transformée avec un vecteur d'expression comprenant un gène codant pour l'anticorps.

10. Anticorps selon la revendication 9, où la cellule de mammifère comprend :

(a) un vecteur d'expression comprenant un gène codant pour la chaîne lourde (H) de l'anticorps et un vecteur d'expression distinct comprenant un gène codant pour la chaîne légère (L) de l'anticorps ; ou
(b) un seul vecteur d'expression codant pour à la fois la chaîne H et L.

11. Anticorps selon la revendication 9 ou 10, dans lequel :

(a) le gène codant pour la chaîne H de l'anticorps comprend la séquence de SEQ ID NO: 9 ; et/ou
(b) le gène codant pour la chaîne L de l'anticorps comprend la séquence de SEQ ID NO: 17.

12. Anticorps selon la revendication 9 ou 10, dans lequel :

(a) le gène codant pour la chaîne H de l'anticorps comprend la séquence de SEQ ID NO: 11 ; et/ou
(b) le gène codant pour la chaîne L de l'anticorps comprend la séquence de SEQ ID NO: 19.

13. Anticorps selon l'une quelconque des revendications précédentes, qui est un anticorps humanisé.

14. Anticorps tel que défini selon l'une quelconque des revendications précédentes, destiné à être utilisé dans le traitement du cancer, où le cancer exprime GPC 3.

15. Anticorps tel que défini selon l'une quelconque des revendications 1 à 13 destiné à être utilisé dans une lyse cellulaire, où les cellules devant être lysées expriment GPC 3.

16. Anticorps selon la revendication 15, où la cellule est une cellule cancéreuse.

17. Anticorps selon la revendication 14 ou 16, où le cancer est un hépatome, le cancer du pancréas, le cancer du poumon, le cancer du côlon, le cancer du sein, le cancer de la prostate, une leucémie ou un lymphome.

18. Formulation pharmaceutique comprenant l'anticorps tel que défini selon l'une quelconque des revendications 1 à 13, et un support et/ou additif pharmaceutiquement acceptable.

Fig. 1

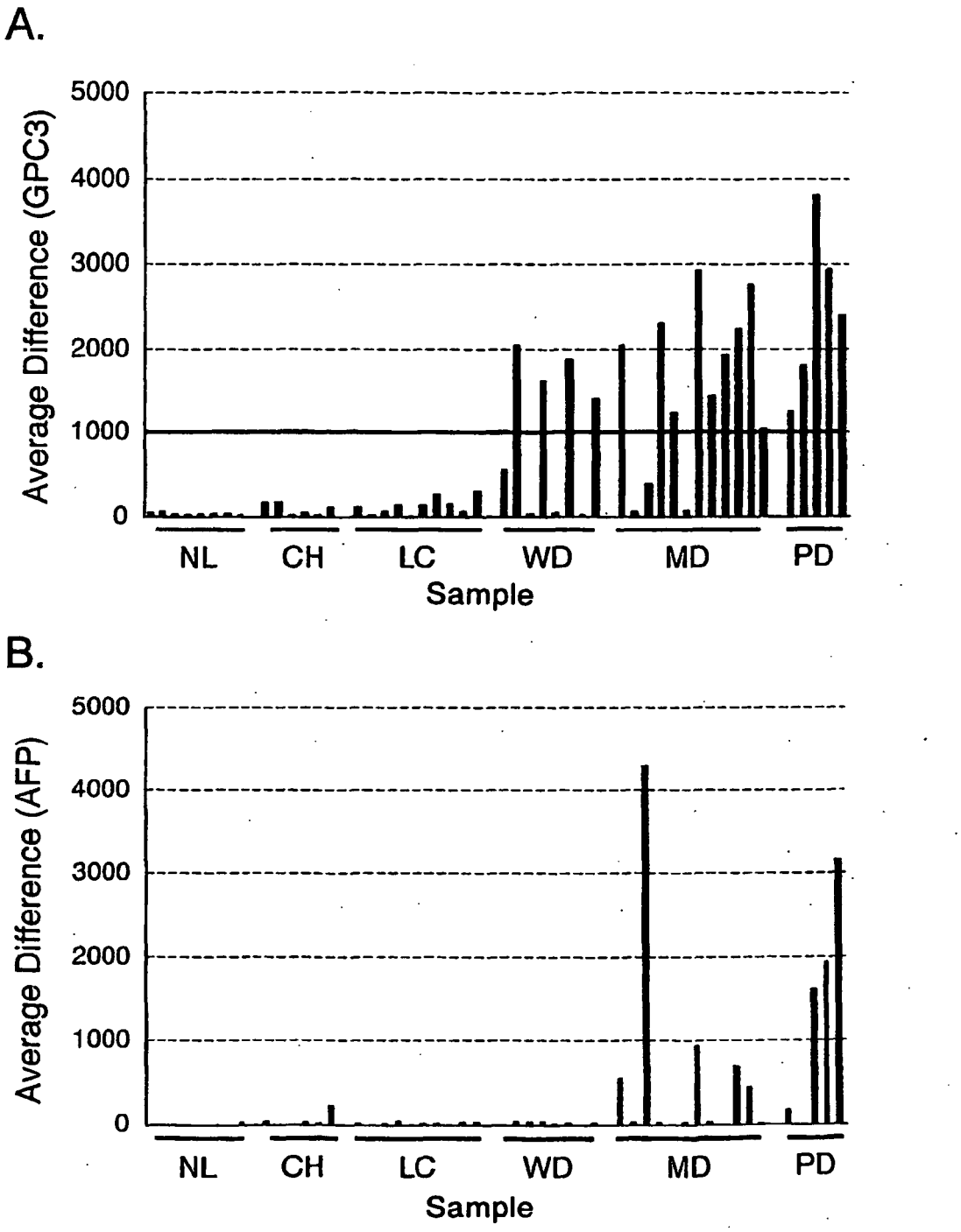

A.
Average Difference (GPC3)
5000
4000
3000
2000
1000
0
NL
CH
LC
WD
MD
PD
Sample
B.
Average Difference (AFP)
5000
4000
3000
2000
1000
0
NL
CH
LC
WD
MD
PD
Sample

Fig. 2

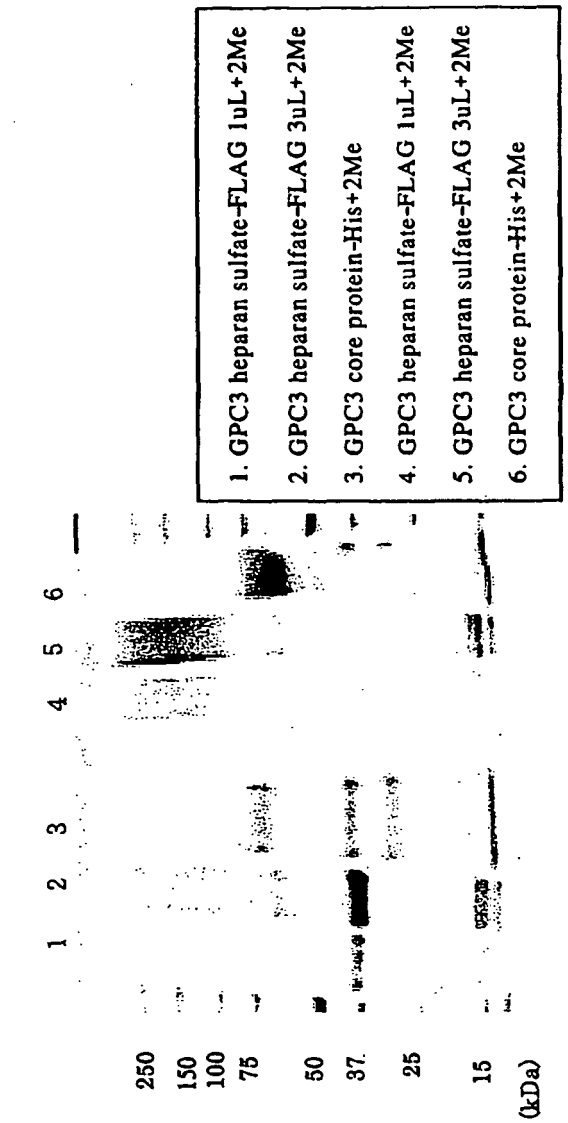

1. GPC3 heparan sulfate-FLAG 1uL+2Me
2. GPC3 heparan sulfate-FLAG 3uL+2Me
3. GPC3 core protein-His+2Me
4. GPC3 heparan sulfate-FLAG 1uL+2Me
5. GPC3 heparan sulfate-FLAG 3uL+2Me
6. GPC3 core protein-His+2Me
1 2 3 4 5 6
250
150
100
75
50
37
25
15
(kDa)

Fig. 3

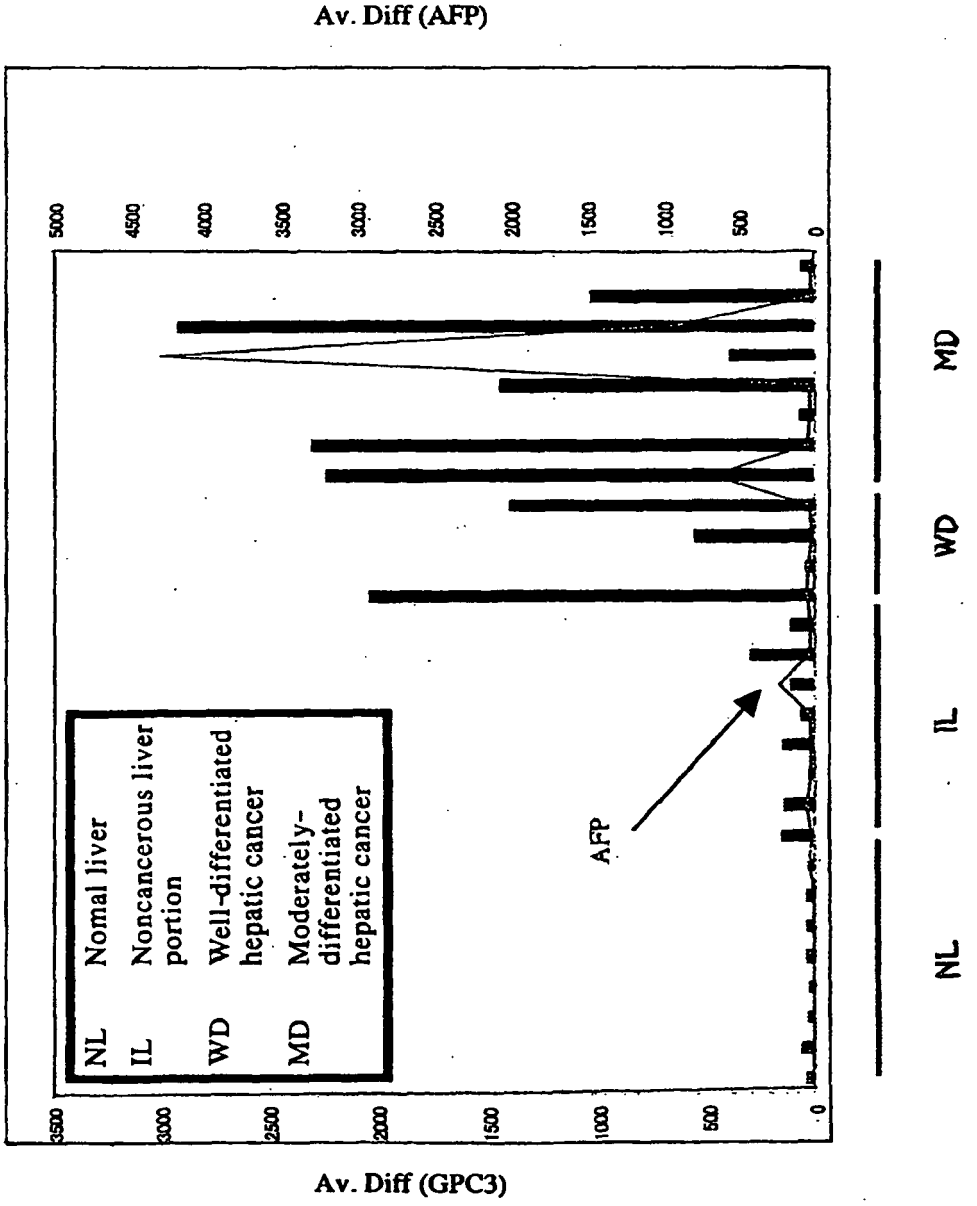

Av. Diff (AFP)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
NL Nomal liver
IL Noncancerous liver portion
WD Well-differentiated hepatic cancer
MD Moderately-differentiated hepatic cancer
AFP
3500
3000
2500
2000
1500
1000
500
0
Av. Diff (GPC3)
NL
IL
WD
MD

Fig. 4

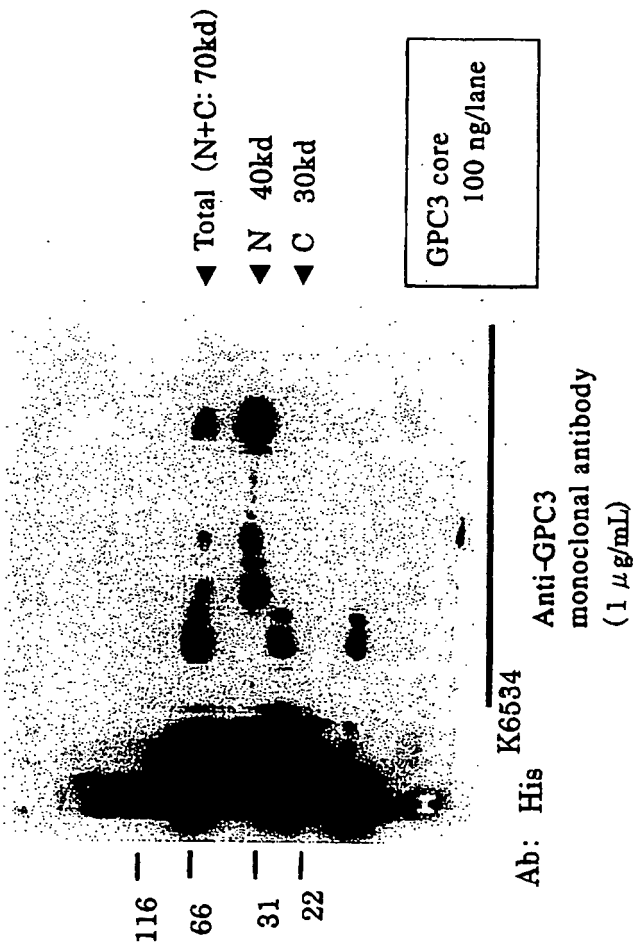

Total (N+C: 70kd)
N 40kd
C 30kd
GPC3 core
100 ng/lane
116
66
31
22
Ab: His
K6534
Anti-GPC3
monoclonal antibody
(1 μg/mL)

Fig. 5

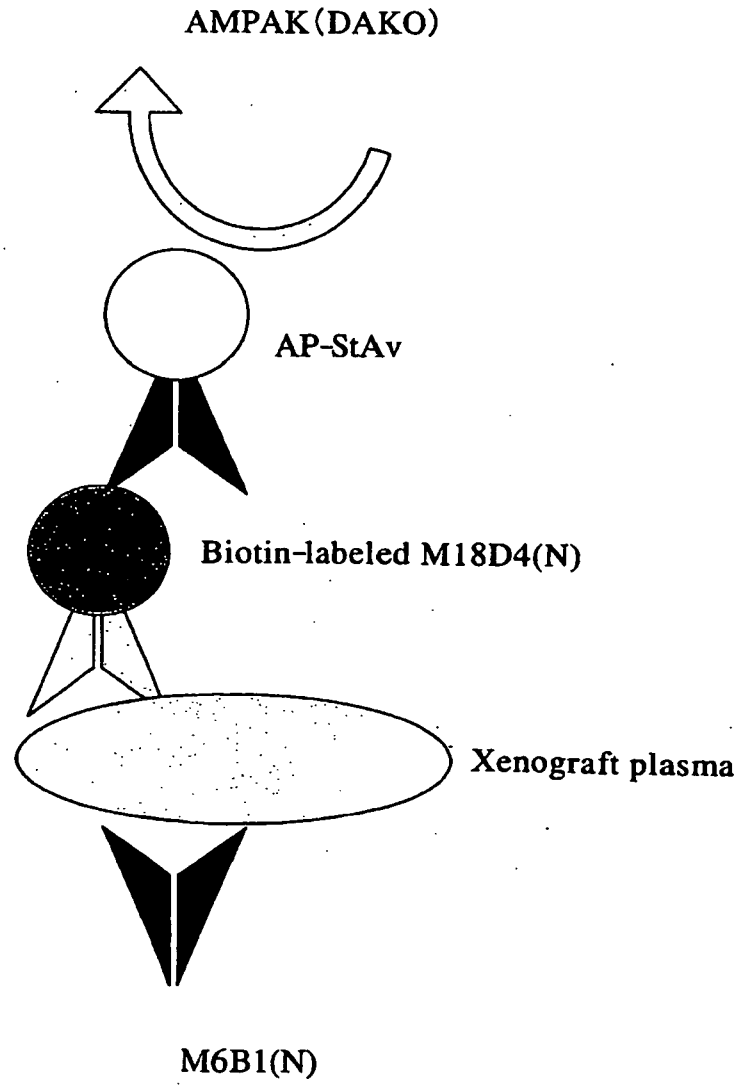

OD measurement
AMPAK(DAKO)
AP-StAv
Biotin-labeled M18D4(N)
Xenograft plasma
M6B1(N)

Fig. 6

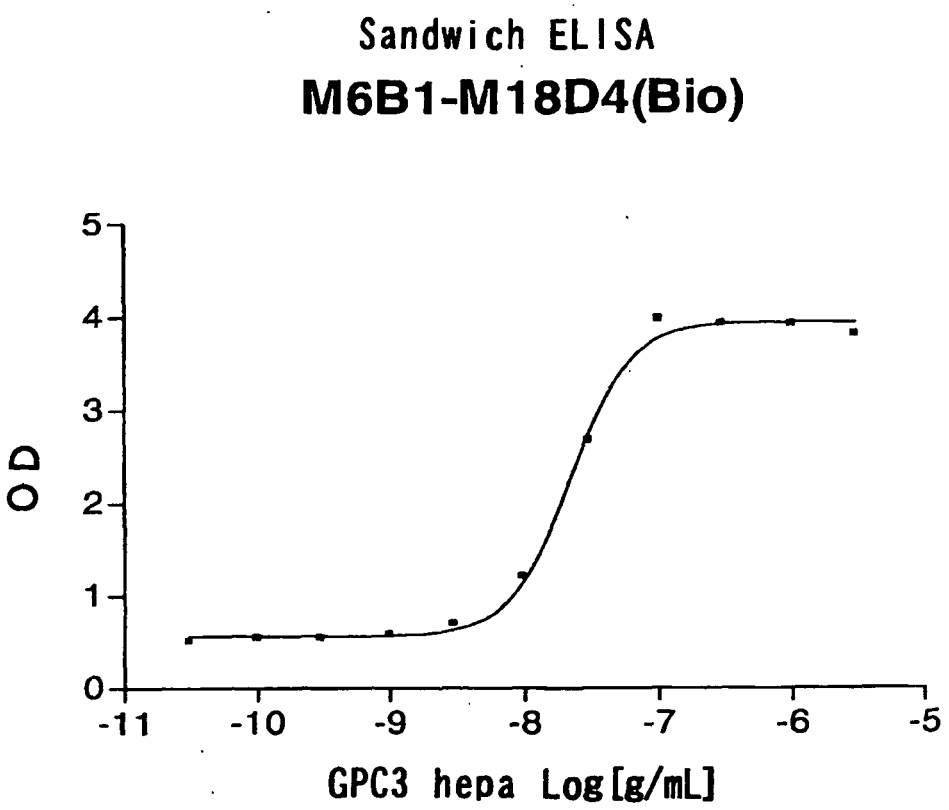

Sandwich ELISA
M6B1-M18D4(Bio)
OD
5
4
3
2
1
0
-11
-10
-9
-8
-7
-6
-5
GPC3 hepa Log[g/mL]

Fig. 7

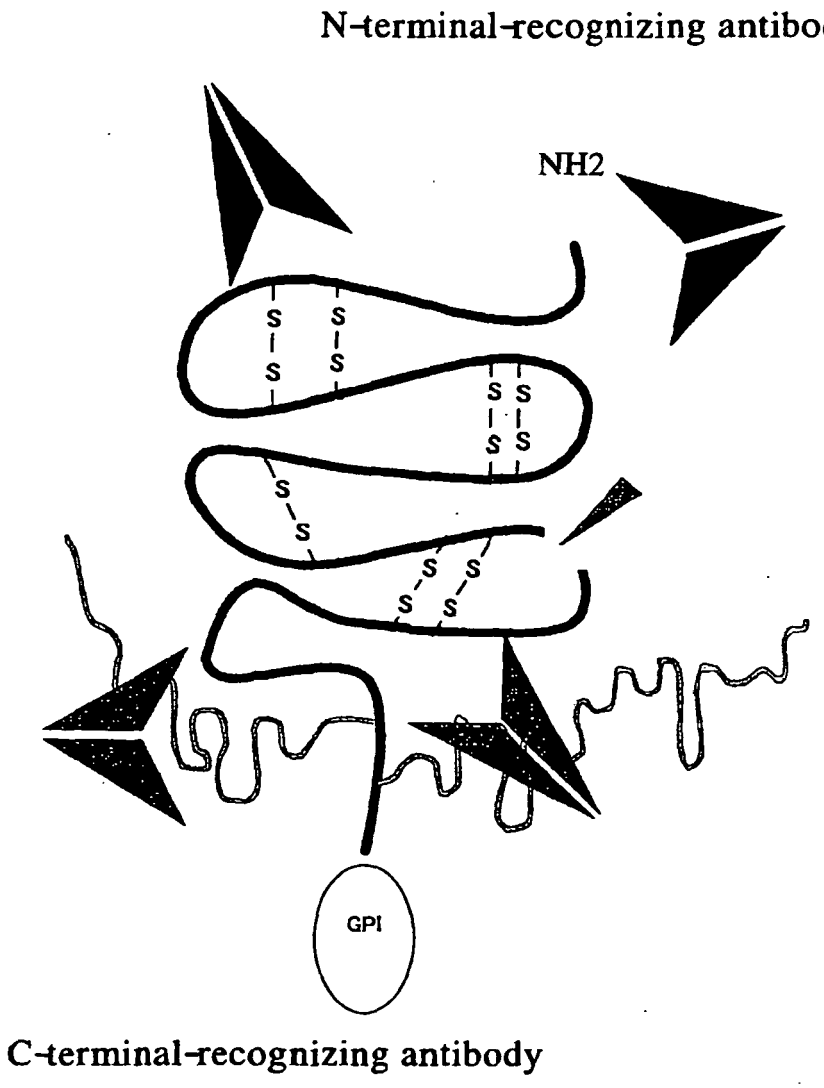

N-terminal-recognizing antibody
NH2
S
GPI
C-terminal-recognizing antibody

Fig. 8

	Form of soluble GPC3		
	N-terminus only	N+C	C-terminus only
N-N ELISA	+	+	−
N-C ELISA	−	+	−
C-C ELISA	−	+	+

Fig. 9

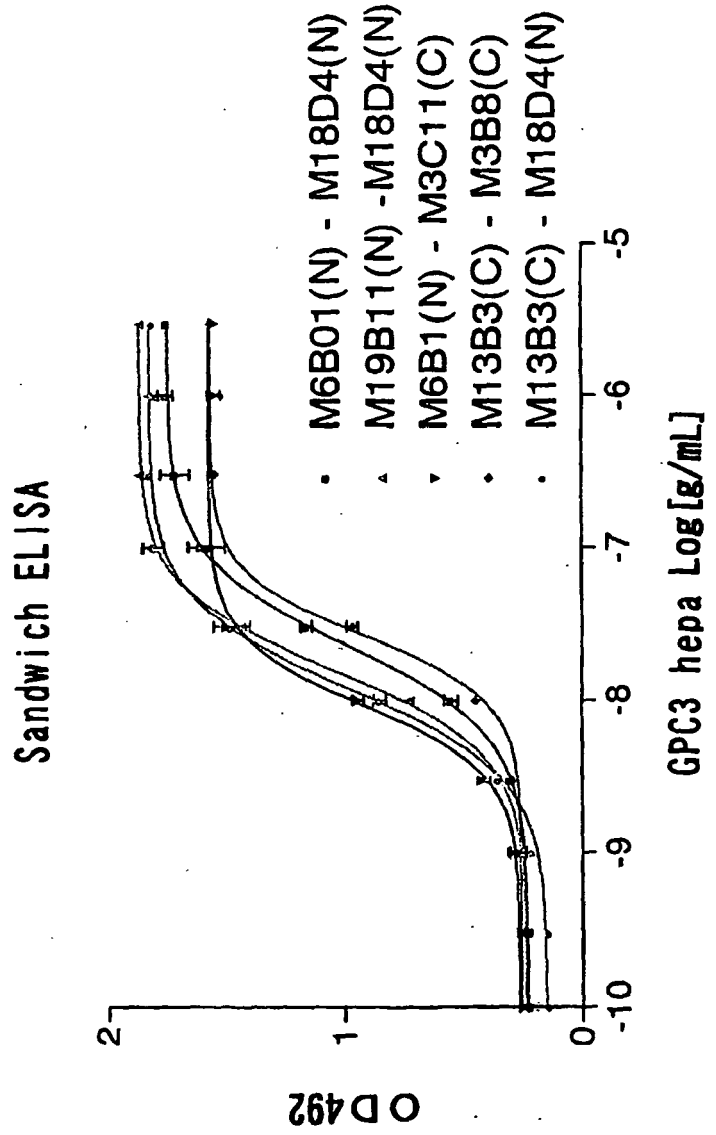

Sandwich ELISA
M6B01(N) - M18D4(N)
M19B11(N) -M18D4(N)
M6B1(N) - M3C11(C)
M13B3(C) - M3B8(C)
M13B3(C) - M18D4(N)
OD492
GPC3 hepa Log[g/mL]
2
1
0
-10
-9
-8
-7
-6
-5

Fig. 10

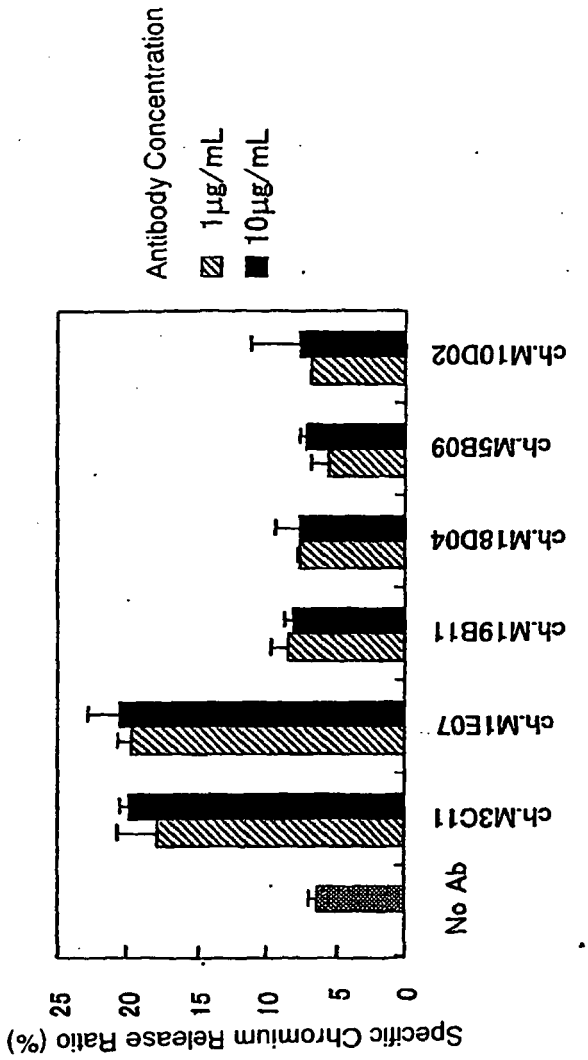
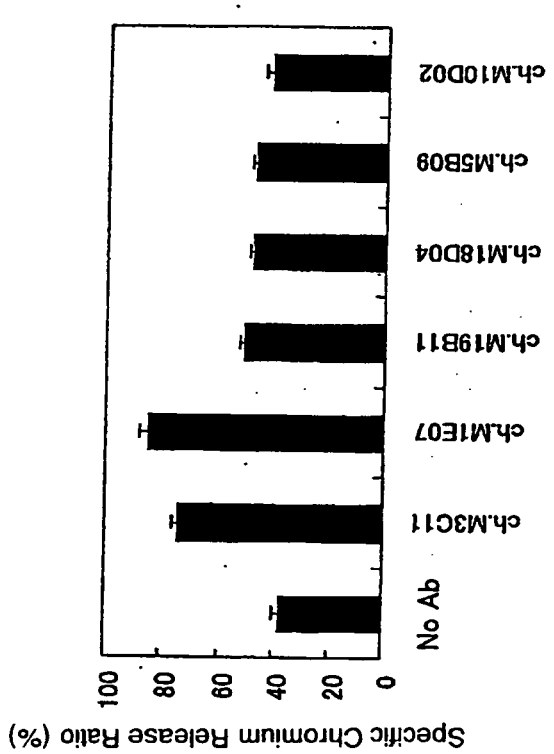

Antibody Concentration
1μg/mL
10μg/mL
Specific Chromium Release Ratio (%)
0
5
10
15
20
25
No Ab
ch.M3C11
ch.M1E07
ch.M19B11
ch.M18D04
ch.M5B09
ch.M10D02

Fig. 11


Specific Chromium Release Ratio (%)
0
20
40
60
80
100
No Ab
ch.M3C11
ch.M1E07
ch.M19B11
ch.M18D04
ch.M5B09
ch.M10D02

REFERENCES CITED IN THE DESCRIPTION

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