DESCRIPTION

Title of Invention: ANTI-CDH3 HUMANIZED ANTIBODY AND USE THEREOF

Technical Field

[0001]

The present invention relates to an anti-CDH3 humanized antibody, an immunoconjugate thereof, and a medicament.

Background Art

[0002]

Cancers are major diseases and leading causes of death, yet the need for effective treatments remains unmet. In recent years, to address the issue of conventional chemotherapy damaging normal cells, there has been active research into cancer treatment using molecularly targeted drugs, i.e., agents designed to target specific molecules expressed exclusively on cancer cells.

[0003]

P-cadherin (CDH3), a cell membrane surface antigen, was identified as one of the targets. CDH3 is a membrane protein discovered as a molecule involved in calcium-dependent homophilic cell adhesion. Proteins having cadherin repeats consisting of approximately 110 amino acid residues with high mutual homology are referred to as the cadherin superfamily, and CDH3 is a major member of this group.

[0004]

Examples of elevated CDH3 expression in certain types of cancer cells have been reported, and antibody-based cancer therapies are being investigated for cancers where CDH3 expression is higher in tumor tissue than in normal tissue.

[0005]

One effective means of enhancing the anti-cancer activity of an antibody is to conjugate it with a substance (drug) that has potent toxicity. Administering a highly toxic substance singularly to a patient causes damage to normal tissue as well and thus cannot serve as an effective therapeutic means. However, when a drug is linked to an antibody that binds to a cancer cell-specific antigen, it leads to the acquisition of the ability to kill only cancer cells without adversely affecting normal tissue. Such drugs are called antibody-drug conjugates (ADCs). In other words, drugs exhibit no toxicity when conjugated to antibodies. However, once certain types of antibodies are conjugated to cells expressing their target antigens, they are internalized into the cells and degraded within lysosomes. Therefore, after a certain type of antibody conjugated to a drug is internalized into a cell, the antibody is intracellularly degraded, the drug is released, and toxicity is manifested only within specific cells, which is effective for killing these cells.

[0006]

Patent Documents 1 and 2 describe anti-CDH3 antibodies and antibody-drug conjugates thereof.

Prior Art Documents

Patent Documents

[0007]

Patent Document 1: WO2013/150623

Patent Document 2: WO2014/126198

Summary of Invention

Objects to be Solved by the Invention

[0008]

It is an object of the present invention to provide an anti-CDH3 humanized antibody suitable for producing a novel anti-CDH3 antibody and further producing an antibody-drug conjugate that more efficiently kills cancer cells expressing CDH3.

Means for Solving the Objects

[0009]

As a result of intensive studies directed towards achieving the above-described objects, the present inventors have succeeded in combining a complementarity-determining region (CDR) sequence defined by an antibody that specifically recognizes CDH3 with various human-derived framework region (FR) sequences, and introducing an appropriate amino acid mutation to improve affinity so as to generate an anti-CDH3 humanized antibody with low immunogenicity, and using the anti-CDH3 humanized antibody to generate an anti-CDH3 humanized antibody-drug conjugate as an immunoconjugate that more efficiently kills cancer cells expressing CDH3; thus, the present invention has been completed. According to the present invention, the following inventions are provided.

[0010]

<1> An antibody of any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 2; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4.

<2> The antibody according to <1>, which is any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 6; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 8.

<3> A fragment of the anti-CDH3 humanized antibody according to <1> or <2>, which has ability to bind to CDH3.

<4> The fragment of the anti-CDH3 humanized antibody according to <3>, which is Fab, $F(ab')^2$, or scFv.

<5> A partial sequence of the antibody according to <1> or <2>, which has ability to bind to CDH3.

<6> An immunoconjugate in which the anti-CDH3 humanized antibody according to any one of <1> to <5>, a fragment thereof, or a partial sequence thereof is linked to a chemotherapeutic agent or a radioactive substance.

<7> The immunoconjugate according to <6>, wherein the chemotherapeutic agent is a cytotoxic substance.

<8> The immunoconjugate according to <6> or <7>, wherein the anti-CDH3 humanized antibody, a fragment thereof, or a partial sequence thereof is linked to a chemotherapeutic agent via a linker.

<9> A medicament for treating a disease characterized by CDH3 overexpression, comprising the immunoconjugate according to any one of <6> to <8>.

<10> The medicament according to <9>, wherein the disease characterized by overexpression of CDH3 is a cancer.

[0011]

The present invention further provides a method for treating a disease characterized by CDH3 overexpression, comprising administering the aforementioned immunoconjugate to a patient.

The present invention further provides the use of the aforementioned immunoconjugate for producing a medicament for treating a disease characterized by CDH3 overexpression.

The present invention further provides the aforementioned immunoconjugate for use in treating a disease characterized by CDH3 overexpression.

Advantageous Effects of Invention

[0012]

The humanized anti-CDH3 antibody is expected to exhibit reduced immunogenicity compared to its original antibody. A humanized antibody is composed of an appropriate combination of the CDR sequences of the original antibody and human-derived FR sequences. In a case in which the antibody fails to demonstrate affinity for the antigen, attempts can be made to restore affinity by introducing further amino acid mutations into the antibody variable regions. An anti-CDH3 humanized antibody that specifically binds to CDH3 can be obtained by combining CDR sequences with appropriate FR sequences and introducing amino acid mutations, as necessary. An

immunoconjugate formed by linking the anti-CDH3 humanized antibody of the present invention obtained in this manner with a chemotherapeutic agent exhibits potent cytotoxic activity against CDH3-expressing cancer cells, compared to an antibody unbound to a chemotherapeutic agent.

By administering the immunoconjugate of the present invention to a patient having CDH3-expressing cancer cells, it is possible to exert a potent anti-cancer effect while also achieving reduced immunogenicity of the immunoconjugate itself. The immunoconjugate of the present invention is useful as an anticancer agent.

Brief Description of Drawings

[0013]

[Figure 1] Figure 1 shows the results of imaged capillary isoelectric focusing: A: ch2012; B: hu2012c; C: hu2012d.

[Figure 2] Figure 2 shows the results of thermal stability measurements by thermal shift assay: A: ch2012; B: hu2012c; C: hu2012d.

[Figure 3] Figure 3 shows the results of flow cytometry in which a humanized anti-CDH3 antibody was reacted with the lung cancer-derived cell line NCI-H358. The peak on the left indicates the negative control.

[Figure 4] Figure 4 shows a graph of the results from Table 1, in which internalization ability was measured.

[Figure 5] Figure 5 shows the results of the animal study (HCC1954 breast cancer model) using CDH3-chimerized and humanized antibody-drug conjugates.

Embodiments of Carrying out the Invention

[0014]

Next, the present invention will be described in more detail.

The antibody of the present invention is any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 2; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4.

[0015]

Preferably, the antibody of the present invention is any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 6; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 8.

[0016]

The anti-CDH3 humanized antibody of the present invention is provided by combining a CDR sequence defined by an antibody that specifically recognizes CDH3 (preferably human CDH3) with various suitable human-derived FR sequences, and further by introducing an appropriate amino acid mutation to improve affinity.

[0017]

In one embodiment, the antibody of the present invention binds to CDH3 expressed on the cell surface. In one embodiment, the antibody of the present invention binds to an epitope within the CDH3 region. Preferably, the antibody of the present invention binds to CDH3 expressed on the surfaces of human cells, and particularly preferably binds to CDH3 expressed on the surfaces of cancer cells.

[0018]

CDH3 (preferably human CDH3) or a partial peptide thereof can be used as an antigen for producing the antibody of the present invention. One example is a soluble CDH3 protein corresponding to the CDH3 extracellular region (corresponding to amino acids 108 to 654 of SEQ ID NO: 2 in WO2014/126198), although the invention is not limited thereto.

[0019]

The antibody of the present invention is a humanized monoclonal antibody. In the present invention, hybridomas are obtained through immunization of mice as a source material for generating a humanized monoclonal antibody. Such materials can be obtained by various methods well known in the art. For example, they can be obtained using the method described below, but are not limited to this method.

[0020]

To establish hybridomas producing an antibody that specifically binds to CDH3, CDH3 or a partial peptide thereof is first administered as an antigen to a mouse. The dosage per animal is 0.1 to 100 mg when no adjuvant is used, and 1 to 100 μg when an adjuvant is used. Examples of adjuvants include Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), and aluminum hydroxide adjuvant. Immunization is primarily performed by intravenous, subcutaneous, or intraperitoneal injection. Furthermore, the interval between immunizations is not particularly limited; immunization is performed 1 to 10 times, preferably 2 to 5 times, at intervals of several days to several weeks, preferably 2 to 5 weeks. Then, antibody-producing cells are harvested for 1 to 60 days, preferably 1 to 14 days, after the final immunization. Examples of antibody-producing cells

include spleen cells, lymph node cells, and peripheral blood cells, with spleen cells or local lymph node cells being preferred.

[0021]

To obtain a hybridoma, cell fusion is performed between antibody-producing cells and myeloma cells. For myeloma cells, generally available mouse-derived cell lines possessing drug selectivity (such as sensitivity to HAT medium) can be used. Examples thereof include P3X63-Ag.8.U1 (P3U1) and NS-1.

[0022]

Cell fusion can be performed by mixing antibody-producing cells at a concentration of 1×10^6 to 1×10^7 cells/mL and myeloma cells at a concentration of 2×10^5 to 2×10^6 cells/mL in an animal cell culture medium, such as serum-free DMEM or RPMI-1640, and conducting the fusion reaction in the presence of a cell fusion promoter. Polyethylene glycol or a similar compound with an average molecular weight of 1,000 to 6,000 Daltons can be used as a cell fusion promoter. Alternatively, antibody-producing cells and myeloma cells can be fused using a commercially available cell fusion apparatus that utilizes electrical stimulation.

[0023]

Hybridomas can be obtained through culture procedures using a selective medium. After appropriately diluting a cell suspension (e.g., with RPMI-1640 medium containing fetal bovine serum), cells are seeded onto a microtiter plate at a density of approximately 3×10^5 cells/well, selective medium is added to each well, and the cells are cultured while appropriately replacing the selective medium. As a result, cells that begin to grow approximately 14 days after the start of culture in a selective medium can be obtained as hybridomas.

[0024]

Next, the culture supernatant of the proliferating hybridomas is screened for the presence of an antibody of interest. Screening of hybridomas may be performed according to a standard method, which is not particularly limited. For example, a portion of the culture supernatant from wells containing growing hybridomas can be collected, and hybridomas producing antibodies that bind to CDH3 can be screened for using a method such as enzyme immunoassay or radioimmunoassay. Cloning of fused cells is performed using a method such as limiting dilution, thereby enabling the establishment of hybridomas as the final product, i.e., monoclonal-antibody-producing cells.

[0025]

Using the established hybridomas as a starting material, humanization of the antibody derived from the hybridomas can be achieved using a known method. Specifically, a DNA sequence designed to link the CDR sequences of a mouse antibody with the FR sequences of a human antibody is synthesized via PCR from several oligonucleotides prepared to have overlapping terminal regions.

A humanized antibody is obtained by ligating the obtained DNA with DNA encoding a human antibody constant region, subsequently incorporating the resulting construct into an expression vector, and introducing the vector into a host for production (such as EP239400 or WO96/02576).

[0026]

CDR sequences represent regions within the variable regions of antibodies that exhibit particularly marked differences and play a crucial role in determining antibody specificity. The amino acid residues belonging to this region are considered to include many residues directly involved in binding to the antigen and in specificity; three such regions exist in each of the light-chain and heavy-chain variable regions.

CDRs are defined by sequence comparison according to Kabat et al. (Sequences of proteins of immunological interest, 5th Ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) and also by three-dimensional structure according to Chothia et al. (J. Mol. Biol.; 196, p901 (1987)).

[0027]

Regarding the CDR sequences defined by Kabat, the light chain variable region CDRs are generally considered to be located around residues 24 to 34, 50 to 56, and 89 to 97, and the heavy chain variable region CDRs around residues 31 to 35, 50 to 65, and 95 to 102; however, not all residues in these regions necessarily participate directly in antigen binding, and it is not always that they correspond exactly to CDR sequences defined based on three-dimensional structures.

[0028]

In the present invention, FR sequences derived from human germlines, selected based on optimal alignment, were used.

[0029]

Specifically, the variable region sequences of the mouse antibody are screened against a library of known, publicly available human variable region sequences. Among them, the human variable region sequence with the highest sequence similarity can be used as the human germline-derived human FR sequence for the humanized antibody (Sims et al., J. Immunol. 151:2296 (1993); Chothia et al., J. Mol. Biol. 196:901 (1987); Tempest et al., Biotechnology 9:266 (1991)).

[0030]

The germline sequence with the highest sequence similarity can be identified by aligning the sequence of the original antibody against databases containing many such registered libraries (e.g., IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg)).

[0031]

The suitability of a selected FR sequence for humanization can be determined based on whether the combination with the respective antibody clone's CDR sequences is appropriate and

whether the sequence was able to maintain a suitable three-dimensional structure for the binding-target antigen.

[0032]

A humanized antibody that is expressed by grafting CDR sequences sandwiched between human-derived FR sequences often exhibits reduced affinity when the sequence selection is inappropriate. This indicates that there are residues within the FR sequences that also play an important role in maintaining the structure. It is possible to perform amino acid residue substitutions in response to this phenomenon. For example, based on alignment results, the sequence can be reshaped by substituting residues with those found in a mouse antibody at positions homologous to the human-derived sequence. This may make it possible to improve the reduction in affinity caused by humanization.

[0033]

The position of the amino acid residue to be substituted varies depending on the antibody of interest and, in many cases, is not identified until actual expression takes place. In the following Examples, it was intended to improve affinity by substituting amino acid residues at one or more positions selected from position 55 of the light chain variable region and positions 49, 71, and 78 of the heavy chain variable region, while positions where substitutions are performed in a relatively large number of documents (e.g., Proc. Natl. Acad. Sci. USA; 89, p. 4285 (1992)); however, the substitution positions, combinations, and types of substituted amino acid residues are arbitrary and not limited to these examples.

[0034]

Hosts for antibody production are often of mammalian origin. Meanwhile, those skilled in the art can appropriately select the specific host cell system best suited for the gene product to be expressed. Examples of common host cell systems include, but are not limited to, CHO-derived cell lines (Chinese hamster ovary cell lines), CV1 (monkey kidney line), COS (a derivative of CV1 expressing SV40 T-antigen), SP2/0 (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), 293 (human kidney), and 293T (a derivative of 293 expressing SV40 T-antigen). Host cell lines can be obtained from various manufacturers, the American Tissue Culture Collection (ATCC), or organizations that publish papers described in the literature.

[0035]

As the host cell system, a CHO-derived cell line deficient in the dgfr gene expression or SP2/0 can preferably be used (Urlaub, G. et al., Somat. Cell. Mol. Genet. 12, 555 (1986) [Non-Patent Document 4]; and Schulman, M. et al., Nature 276, 269 (1978) [Non-Patent Document 5]). Most preferably, the host cell system is a DHFR-deficient CHO cell line.

[0036]

Transfection of the plasmid into host cells can be performed using any technique. Specific methods include, but are not limited to, transfection (including the calcium phosphate method, DEAE method, lipofection, and electroporation), methods for introducing DNA utilizing envelopes such as that of Sendai virus, microinjection, and infection using viral vectors such as retroviruses or adenoviruses (Current Protocols in Molecular Biology, Chapter 9: Introduction of DNA into Mammalian Cells, John Wiley and Sons, Inc.). The most preferable technique is the introduction of the plasmid into a host by electroporation.

[0037]

These antibodies may be monovalent, bivalent, or multivalent antibodies, provided that they have the ability to bind to CDH3. Furthermore, as long as they retain the ability to bind to CDH3, these antibodies may be low-molecular-weight antibodies such as antibody fragments, modified antibodies, or partial sequences of antibodies. Examples of low-molecular-weight antibodies, such as antibody fragments, include Fab, Fab', F(ab')2, Fv, scFv (single-chain Fv), and diabodies. Furthermore, they may be derived from the fusion of an Fc region with an antibody fragment or a low-molecular-weight antibody, such as Fab, Fab', F(ab')2, Fv, scFv (single-chain Fv), or a diabody. To obtain such antibodies, genes encoding them may be constructed, introduced into expression vectors, and then expressed in suitable host cells.

[0038]

< Immunoconjugate >

According to the present invention, an immunoconjugate, in which the anti-CDH3 humanized antibody of the present invention, a fragment thereof, or a partial sequence thereof is linked to a chemotherapeutic agent or a radioactive substance, is provided. In other words, a preferred aspect of use for the antibody of the present invention is an immunoconjugate, i.e., an antibody-drug conjugate (ADC), in which the antibody is conjugated to a chemotherapeutic agent (such as a cytotoxic substance) or a radioactive substance. The immunoconjugate of the present invention can damage cancer cells, for example, by coming into contact with cancer cells expressing CDH3.

[0039]

The chemotherapeutic agent is preferably a cytotoxic substance. Examples of the chemotherapeutic agent can include, but are not limited to, duocarmycin, duocarmycin analogs and derivatives, CC-1065, duocarmycin analogs based on CBI, duocarmycin analogs based on MCBI, duocarmycin analogs based on CCBI, doxorubicin, doxorubicin conjugate, morpholino-doxorubicin, cyanomorpholino-doxorubicin, dolastatin, dolastatin-10, combretastatin, calicheamicin, maytansinoids or their derivatives (e.g., maytansine, maytansine analogs, DM1, DM4), auristatin or a derivative thereof (e.g., auristatin E, auristatin EB (AEB), auristatin EFP (AEFP), monomethyl

auristatin E (MMAE), monomethyl auristatin F (MMAF), 5-benzoylvaleric acid AE ester (AEVB)), tubulysin, disorazol, epothilone, paclitaxel, docetaxel, SN-38, topotecan, exatecan, deruxtecan, rhizoxin, echinomycin, colchicine, vinblastine, vindesine, estramustine, cemadotin, amanitin, eleutherobin, methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, melphalan, leurosine, leurosidine, actinomycin, daunorubicin, daunorubicin conjugate, mitomycin C, mitomycin A, carminomycin, aminopterin, tallysomycin, podophyllotoxin, podophyllotoxin derivatives, etoposide, etoposide phosphate, vincristine, Taxol, Taxotere, retinoic acid, butyric acid, N8-acetylspermidine, and camptothecin.

[0040]

The number of chemotherapeutic agent molecules bound per molecule of the humanized anti-CDH3 antibody, a fragment thereof, or a partial sequence thereof is not particularly limited, but preferably an average of 1 to 10 molecules, and more preferably an average of 3 to 5 molecules of the chemotherapeutic agent are bound. As an example, an average of 1 to 10 DM1 molecules may be bound per molecule of the anti-CDH3 humanized antibody, a fragment thereof, or a partial sequence thereof.

[0041]

The immunoconjugate of the present invention can be prepared by conjugating the chemotherapeutic agent and the antibody described above using a known method. The antibody and the chemotherapeutic agent may be directly linked via linking groups or the like possessed by themselves, or they may be indirectly linked via a linker or another substance.

[0042]

Examples of linking groups used when the drug is directly conjugated include disulfide bonds formed via SH groups and linkages mediated by maleimide. For example, the intramolecular disulfide bond of the Fc region of the antibody and the disulfide bond of the drug are reduced, and the two are bound via a disulfide bond. There is also a method involving maleimide. Alternatively, the Fc region of the antibody can be genetically engineered and introduced. For example, there is a method for introducing cysteineinto an antibody using genetic engineering.

[0043]

It is also possible to indirectly link the antibody and the chemotherapeutic agent via another substance (a linker). It is desirable for the linker to have one or more types of functional groups that react with the antibody, the drug, or both. Examples of functional groups include amino group, carboxyl group, mercapto group, maleimide group, and pyridinyl group. The linker is preferably a divalent reactive cross-linking reagent.

[0044]

Examples of the linker can include, but are not limited to, N-succinimidyl 4-

(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (Sulfo-SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), κ-maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ-maleimidobutyric acid N-succinimidyl ester (GMBS), ε-maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(α-maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(β-maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), N-(p-maleimidophenyl)isocyanate (PMPI), N-succinimidyl 4-(2-pyridylthio)pentanoate (SPP), N-succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), p-aminobenzyloxycarbonyl (PAB), and N-succinimidyl 4-(2-pyridylthio)butanoate (SPDB). For example, this linker may be formed by combining para-aminobenzoic acid (PABA) with a peptide linker such as valine-citrulline (Val-Cit) or alanine-phenylalanine (Ala-Phe), or by using appropriate combinations of the aforementioned linkers.

[0045]

In one example, the linker may be cleaved by a protease.

Furthermore, the linker may contain at least one of valine-citrulline (Val-Cit), alanine-phenylalanine (Ala-Phe), and para-aminobenzoic acid (PABA).

[0046]

Regarding the method for conjugating the drug and the antibody, the conjugation can be performed in accordance with methods described in, for example, Cancer Res. 52, p. 127 (1992); Cancer Res. 68(22), p. 9280 (2008); Nature Biotechnology 26(8), p. 925 (2008); Bioconjugate Chemistry 19, p. 1673 (2008); Cancer Res. 68(15), p. 6300 (2008); or JP 2008-516896 A.

[0047]

Another embodiment of the present invention is a so-called immunotoxin, in which a drug is conjugated to an antibody either chemically or via genetic engineering. Examples of drugs that may be used include, but are not limited to, diphtheria toxin A-chain, Pseudomonas endotoxin, ricin A-chain, abrin A-chain, modeccin A-chain, gelonin, and saporin.

[0048]

Furthermore, in another embodiment of the present invention, a radioactive substance can be conjugated to an antibody. As the radioactive substance, a cytotoxic radioactive metal is preferred when used as a cancer therapeutic agent, whereas a non-cytotoxic radioactive metal is preferred when used as a cancer diagnostic agent.

[0049]

Examples of such cytotoxic radioactive metals include yttrium-90 (90Y), rhenium-186 (186Re), rhenium-188 (188Re), copper-67 (67Cu), iron-59 (59Fe), strontium-89 (89Sr), gold-198

(198Au), mercury-203 (203Hg), lead-212 (212Pb), dysprosium-165 (165Dy), ruthenium-103 (103Ru), bismuth-212 (212Bi), bismuth-213 (213Bi), holmium-166 (166Ho), samarium-153 (153Sm), lutetium-177 (177Lu), astatine-211 (211At), and actinium-225 (225Ac). Among these radioactive metals, 90Y, 153Sm, and 177Lu are preferred in terms of half-life, radiation energy, the ease of labeling reaction, labeling efficiency, complex stability, and the like, although they are not limited thereto.

[0050]

Meanwhile, suitable examples of non-cytotoxic radioactive metals used in diagnostic agents include technetium-99m (99mTc), indium-111 (111In), indium-113m (113mIn), gallium-67 (67Ga), gallium-68 (68Ga), thallium-201 (201Tl), chromium-51 (51Cr), cobalt-57 (57Co), cobalt-58 (58Co), cobalt-60 (60Co), strontium-85 (85Sr), mercury-197 (197Hg), and copper-64 (64Cu), although the metals are not limited thereto.

[0051]

To bind these radioactive metal elements to an anti-CDH3 antibody, it is preferable to react the antibody with a metal chelating reagent and then react the resulting product with a radioactive metal element to form a complex. In the modified antibody obtained in this manner, a radioactive metal element is bound via a metal chelating reagent.

[0052]

Examples of metal chelating reagents used for such complex formation include: (1) quinoline derivatives such as 8-hydroxyquinoline, 8-acetoxyquinoline, 8-hydroxyquinaldine, oxyquinoline sulfate, O-acetyloxine, O-benzoyloxine, and O-p-nitrobenzoyloxine; and quinolone-based compounds having a quinoline skeleton, such as norfloxacin, ofloxacin, enoxacin, ciprofloxacin, lomefloxacin, tosufloxacin, fleroxacin, and sparfloxacin; (2) compounds such as chloranilic acid, aluminon, thiourea, pyrogallol, cupferron, bismuthiol (II), galloyl gallic acid, thioride, 2-mercaptobenzothiazole, and tetraphenylarsonium chloride; (3) ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and compounds having a backbone similar to these, such as dihydroxyethylglycine, diaminopropanoltetraacetic acid, ethylenediaminediacetic acid, ethylenediaminedipropionate, hydroxyethylethylenediaminetriacetic acid, ethylenediaminetetrakis(methylenephosphonic acid), glycol ether diaminetetraacetic acid, hexamethylenediaminetetraacetic acid, hydroxyethyliminodiacetic acid, iminodiacetic acid, diaminopropanetetraacetic acid, nitrilotriacetic acid, nitrilotripropionic acid, nitrilotris(methylenesulfonic acid) trisodium salt, triethylenetetraminehexaacetic acid, methyl-DTPA, cyclohexyl-DTPA, aminobenzyl-EDTA, isothiocyanatobenzyl-EDTA, isothiocyanatobenzyl-DTPA, methylisothiocyanatobenzyl-DTPA, cyclohexyl isothiocyanobenzyl-DTPA, maleimidopropylamidobenzyl-EDTA, maleimidopentylamidobenzyl-EDTA, maleimidedecylamidobenzyl-EDTA, maleimidopentylamidobenzyl -DTPA,

maleimidedecylamidobenzyl-EDTA, maleimidedecylamidobenzyl DTPA; (4) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), 1,4,7,10-tetraazacyclododecane (Cyclen), 1,4,8,11-tetraazacyclotetradecane (Cyclam), isothiocyanatobenzyl-DOTA, and isothiocyanatobenzyl-NOTA

[0053]

Among these metal-chelating reagents, isothiocyanobenzyl-DOTA, methylisothiocyanobenzyl-DTPA, and cyclohexylisothiocyanobenzyl-DTPA are preferred in terms of the ease of the reaction for introducing a metal chelate into an antibody, the labeling efficiency, the stability of the complex, and the like.

[0054]

The binding of a radioactive metal element to an antibody can be performed according to a standard method. For example, the binding can be carried out by reacting an antibody with a metal-chelating reagent to prepare a labeling precursor in advance, and subsequently reacting the labeling precursor with a radioactive metal element.

[0055]

< Medicament >

According to the present invention, a medicament for treating a disease characterized by CDH3 overexpression, comprising the immunoconjugate of the present invention is provided.

The immunoconjugate provided by the present invention may appropriately contain a pharmaceutically acceptable carrier, an excipient, a diluent, or the like, in order to maintain the drug in a stable state. The immunoconjugate of the present invention can be formulated, for example, as an injection. The dosage of the immunoconjugate of the present invention depends on factors such as the severity of the patient's symptoms, age, body weight, and the method of administration. The weight of the antibody (as the active ingredient) typically falls within the range of approximately 10 ng to 100 mg per kg of body weight.

[0056]

Diseases that can be treated with the immunoconjugate of the present invention are not particularly limited, provided that CDH3 is expressed on the cells involved. Examples thereof include cancers. Examples of cancers can include, but are not limited to, colorectal cancer, non-small cell lung cancer, breast cancer, head and neck cancer, ovarian cancer, lung cancer, invasive bladder cancer, pancreatic cancer, metastatic brain cancer, thyroid cancer, head and neck squamous cell carcinoma, esophageal squamous cell carcinoma, lung squamous cell carcinoma, cutaneous squamous cell carcinoma, melanoma, mammary carcinoma, lung adenocarcinoma, cervical squamous cell carcinoma, pancreatic squamous cell carcinoma, colonic squamous cell carcinoma, gastric squamous

cell carcinoma, prostate cancer, osteosarcoma, and soft tissue sarcoma. The medicament of the present invention can be used as an antitumor agent.

[0057]

The present invention will be described in further detail by the following examples; however, these examples are intended for illustrative purposes only, and the scope of the invention is not limited by them. All prior art documents cited herein are incorporated herein by reference.

Examples

[0058]

Example 1: Method for Producing Humanized Antibody

(1) Production of Anti-CDH3 Mouse Antibody

An anti-CDH3 mouse antibody was prepared as described in Examples 1 to 9 of Japanese Patent No. 6377601, and the sequences of the variable regions were obtained through sequence analysis of the anti-CDH3 mouse antibody.

[0059]

Sequence analysis was performed using ApE and GENETYX (GENETYX CORPORATION). CLUSTALW was used for gene alignment. HPRD, NCBI BLAST, UCSC (University of California, Santa Cruz) Blat, and UniProt were used to obtain and verify gene and protein information.

[0060]

Among the variable regions of the mouse-hybridoma-derived anti-CDH3 mouse antibody sequenced as described above, the amino acid sequences corresponding to the CDRs are shown below.

GYSFTAYN: SEQ ID NO: 9 (CDR-H1)

IDPYSGII: SEQ ID NO: 10 (CDR-H2)

ARRGYYDGGFDY: SEQ ID NO: 11 (CDR-H3)

QDITNY: SEQ ID NO: 12 (CDR-L1)

YTS: SEQ ID NO: 13 (CDR-L2)

QQDSKHPRT: SEQ ID NO: 14 (CDR-L3)

Note that CDR-H1, CDR-H2, and CDR-H3 represent the CDR sequences constituting the heavy chain, and CDR-L1, CDR-L2, and CDR-L3 represent the CDR sequences constituting the light chain. The indicated CDR amino acid sequences were obtained by searching the nucleotide sequences of the anti-CDH3 mouse antibody variable regions using IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg).

[0061]

(2) Construction of Transient Expression Vector for Anti-CDH3 Antibody

For chimerization, regarding the light chain, a gene was designed by linking the gene for the

light chain variable region of the anti-CDH3 mouse antibody to a chimeric light chain expression vector encoding human Ck. Regarding the heavy chain, the design involved linking the gene encoding the heavy chain variable region of the anti-CDH3 mouse antibody to a vector encoding the human Cg1 region.

[0062]

For humanization, mouse-derived CDR sequences were combined with human-derived FR sequences, and a human Fc region was further linked, thereby constructing a stable production cell line using a humanized antibody expression vector. More specifically, the procedure was as follows.

[0063]

The regions corresponding to the mouse FR sequences were replaced with human-derived FR sequences, and the full-length sequence was artificially synthesized by GenScript. Human germline framework sequences were used for the amino acid sequences of the regions corresponding to the FR sequences. The germline framework sequences were designed by inputting the nucleotide sequences of the cloned anti-CDH3 mouse antibody into IMGT/V-QUEST (https://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanIg) and selecting the sequences with the highest similarity. In addition, amino acid sequences were substituted (reshaped) to address the reduced affinity.

[0064]

The amino acid sequences of the heavy-chain or light-chain variable regions of the humanized anti-CDH3 antibody used in this study are shown below.

Antibodies hu2012c and hu2012d have the same CDR sequences. In addition, hu2012d incorporated the amino acid sequence substitutions as indicated.

[0065]

Antibody No. hu2012c-VH

EVQLVQSGAEVKKPGATVKISCKVSGYSFTAYNMHWVQQAPGKGLEWMGFIDPYSGIIT
YAEKFQGRVTITADTSTDTAYMELSSLRSEDTAVYYCARRGYYDGGFDYWGQGTLVTVSS
(SEQ ID NO: 1)

[0066]

Antibody No. hu2012c-VL

DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYYTSRLETGVPSR
FSGSGSGTDFTFTISSLQPEDIATYYCQQDSKHPRTFGQGTKVEIK
(SEQ ID NO: 2)

[0067]

Antibody No. hu2012d-VH

EVQLVQSGAEVKKPGATVKISCKVSGYSFTAYNMHWVQQAPGKGLEWMGFIDPYSGIIT

YAEKFQGRVTITVDKSTDTAYMELSSLRSEDTAVYYCARRGYYDGGFDYWGQGTLVTVS
S

(SEQ ID NO: 3)

(A72V and T74K substitutions are present relative to hu2012c-VH)

[0068]

Antibody No. hu2012d-VL

DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSR
FSGSGSGTDFTFTISSLQPEDIATYYCQQDSKHPRTFGQGTKVEIK

(SEQ ID NO: 4)

(E55H substitution is present relative to hu2012c-VL)

[0069]

The artificially synthesized genes designed to possess these sequences were inserted into pCXN vectors: the heavy-chain variable region gene was inserted into a pCXN vector containing a human IgG1-derived constant region gene, and the light-chain variable region gene was inserted into a pCXN vector containing a human κ-chain-derived constant region gene.

[0070]

The full-length amino acid sequence of the anti-CDH3 humanized antibody obtained by allowing the genes to be expressed is shown below. Here, the anti-CDH3 humanized antibody hu2012c is defined as consisting of a heavy chain of hu2012c-H and a light chain of hu2012c-L, and the anti-CDH3 humanized antibody hu2012d is defined as consisting of a heavy chain of hu2012d-H and a light chain of hu2012d-L; however, the respective heavy and light chains may be interchanged for expression.

Antibody No. hu2012c-H

EVQLVQSGAEVKKPGATVKISCKVSGYSFTAYNMHWVQQAPGKGLEWMGFIDPYSGIIT
YAEKFQGRVTITADTSTDTAYMELSSLRSEDTAVYYCARRGYYDGGFDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPPKSCDKTHTCPPCPAPELLGGP
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK(SEQ ID NO: 5)

[0071]

Antibody No. hu2012c-L

DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYYTSRLETGVPSR
FSGSGSGTDFTFTISSLQPEDIATYYCQQDSKHPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQ

LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC(SEQ ID NO: 6)

[0072]

Antibody No. hu2012d-H

EVQLVQSGAEVKKPGATVKISCKVSGYSFTAYNMHWVQQAPGKGLEWMGFIDPYSGIIT
YAEKFQGRVTITVDKSTDTAYMELSSLRSEDTAVYYCARRGYYDGGFDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 7)

[0073]

Antibody No. hu2012d-L

DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSR
FSGSGSGTDFTFTISSLQPEDIATYYCQQDSKHPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA
DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 8)

[0074]

Example 2: Imaged Capillary Isoelectric Focusing (icIEF)

Imaging capillary isoelectric focusing (icIEF) of ch2012 and hu2012 was performed using an iCE280 analyzer (ProteinSimple).

[0075]

(1) Preparation of loading mixture

A loading mixture was prepared by mixing Pharmalyte 3-10, a pI marker, and a 0.5% methylcellulose solution. The preparation volume was adjusted as appropriate according to the number of samples.

Pharmalyte 3-10: 2.0 μL/sample
Acidic pI marker (4.22): 0.25 μL/sample
Alkaline-side pI marker (9.46): 0.25 μL/sample
0.5% Methylcellulose: 17.5 μL/sample
Total: 20.0 μL/sample

[0076]

(2) Sample preparation

In a case in which the sample contained 20 mM or more of a salt, it was exchanged with 20

mM phosphate buffer (pH 6.0). Urea was dissolved in 20 mM phosphate buffer (pH 6.0), thereby preparing a 10 M urea solution immediately before use.

Each sample was prepared at the following proportions. The sample concentration was set at 140 to 200 μg/mL for an antibody and 400 μg/mL for a drug-labeled antibody.

[0077]

For measurement at n=1

10M Urea:	10 μL
Antibody (140-200 μg/mL):	20 μL
Loading mixture:	20 μL
Total:	50 μL

[0078]

(3) Marker preparation

Each marker was prepared at the following proportions.

10M Urea:	20 μL
20 mM phosphate buffer (pH 6.0):	40 μL
Loading mixture:	40 μL
Total:	100 μL

[0079]

The prepared sample and marker were thoroughly mixed using a vortex mixer and centrifuged for 3 to 5 minutes in a benchtop centrifuge. The sample and marker were dispensed into a dedicated glass vial; the vial was briefly centrifuged in a benchtop centrifuge to remove air bubbles, placed in the vial holder of the iCE280 analyzer, and subjected to measurement.

[0080]

(4) Results

Figure 1 shows the electrophoretograms obtained by imaged capillary isoelectric focusing of the chimeric antibody ch2012 and the humanized antibodies hu2012c and hu2012d. For ch2012, the acidic and basic peaks were heterogeneous and exhibited a plurality of peaks. On the other hand, the isoelectric points (pI) of the major peaks for the humanized antibodies hu2012c and hu2012d were 7.99 and 8.60, respectively, and virtually no peaks other than the major peaks were detected. By humanizing ch2012, an antibody exhibiting a nearly single peak, which facilitates equivalence assessment, was obtained.

[0081]

Example 3: Thermal Shift Assay (TSA)

(1) Sample preparation

Dye (1000x) from the Protein Thermal Shift Dye Kit (1 μL) was diluted with 124 μL of

Milli-Q water, thereby preparing Dye (8x) (125 μL, for 50 samples). The preparation volume was adjusted as appropriate based on the number of samples.

The sample antibodies were exchanged with DPBS, and their concentration was adjusted to 0.25 mg/mL.

The designated 8-strip PCR tubes or 96-well plates were placed on ice, and the reaction mixture was prepared using the following proportions for measurement at n=1.

[0082]

Dye (8x): 2.5 μL

0.25 mg/mL Ab: 17.5 μL

(Antibody concentration in reaction mixture: 5.0 μg)

Total: 20.0 μL

[0083]

The prepared samples were measured using a StepOnePlus real-time PCR system (Thermo Fisher Scientific; StepOnePlus (registered trademark)).

[0084]

(2) Measurement conditions

Temperature range: 20°C to 90°C

Heating rate: 0.2°C

Interval: 10 sec

Number of times: 350 times

Analysis was performed using Protein Thermal Shift Software (registered trademark).

[0085]

(3) Results

The results of the thermal shift assays for ch2012, hu2012c, and hu2012d are shown in Figure 2.

Analysis yielded Tm values of 59.0°C for ch2012 and 68.8°C for hu2012d, revealing that the humanized hu2012d exhibited a higher Tm value. Humanizing ch2012 improved thermal stability.

[0086]

Example 4: Antibody Binding Activity

Antibodies having the sequences shown in Example 1 were produced, and their binding activities were evaluated by flow cytometry.

Each cell line (NCI-H358 cells, in which high CDH3 expression has been confirmed) for the reaction was detached from a culture plate by treatment with 2 mM EDTA-PBS and then suspended in FACS solution at a concentration of 1×10^6 cells/mL. This cell suspension was seeded into a 96-well plate at 50 μL/well. Purified hu2012c and hu2012d antibodies were added to the mixture to

result in a concentration of 0.3 μg/mL, and the mixture was incubated at 4°C for 60 minutes. After washing twice with FACS solution (PBS containing 1% BSA, 2 mM EDTA, and 0.1% NaN_3; 150 μL/well), Alexa Fluor 488-labeled goat F(ab')2 anti-human IgG (Invitrogen) was added at 4 μg/mL, and the mixture was incubated at 4°C for 30 minutes. Subsequently, the cells were washed twice with FACS solution, and flow cytometry was performed. As a result, strong reactivity with the CDH3-expressing cell line was observed (Figure 3). The humanized antibody hu2012d exhibited stronger binding activity than hu2012c.

[0087]

Example 5: Measurement of Internalization Capacity

The measurement was performed using a saporin-labeled anti-human IgG antibody (Hum-ZAP; Advanced Targeting Systems). Since cell killing requires internalization mediated by saporin, the internalization capacity can be evaluated by measuring the extent of cell killing. Relative activity was expressed with the cell viability in the absence of the saporin-labeled anti-human IgG antibody set to 100%. More specifically, the measurement was as follows.

[0088]

(1) Measurement method

The human breast cancer-derived cell line HCC1954 and the human lung cancer-derived cell line NCI-H358 were used as human CDH3-expressing cells. A mixture was prepared to contain 5,000 cells, 100 ng (or 0 ng) of Hum-ZAP, and 100 ng of an anti-human CDH3 chimeric antibody or anti-human CDH3 humanized antibody per well. The mixture was added to a 96-well microplate and incubated at 37°C in a 5% CO_2 incubator for 3 days.

[0089]

Subsequently, 10 μL of Cell Counting Kit-8 (DOJINDO LABORATORIES) was added to each well. After mixing and incubation, the number of viable cells was determined by measuring A450/620 using a plate reader after 2 hours.

[0090]

(2) Calculation of internalization capacity

The comparison of antibody internalization capacities was performed according to the following formula.

Sample (ZAP+): Mean signal value of the sample in the presence of HumZAP

Sample (ZAP-): Mean signal value of the sample in the absence of HumZAP

NoAb(ZAP+): Mean signal value of wells without antibody in the presence of HumZAP

NoAb(ZAP-): Mean signal value of wells without antibody in the absence of HumZAP

BK: Mean signal value of the well (Medium only)

[0091]

(3) Results

The results are shown in Table 1 and Figure 4.

In both NCI-H358 and HCC1954 cells, hu2012c and hu2012d resulted in a greater reduction in the percentage of viable cells compared to ch2012. This indicates a higher level of internalization of the saponin-labeled anti-human IgG antibody, demonstrating that humanization enhances internalization capacity.

[0092]

[Table 1]

Table 1 Percentage of Viable Cells

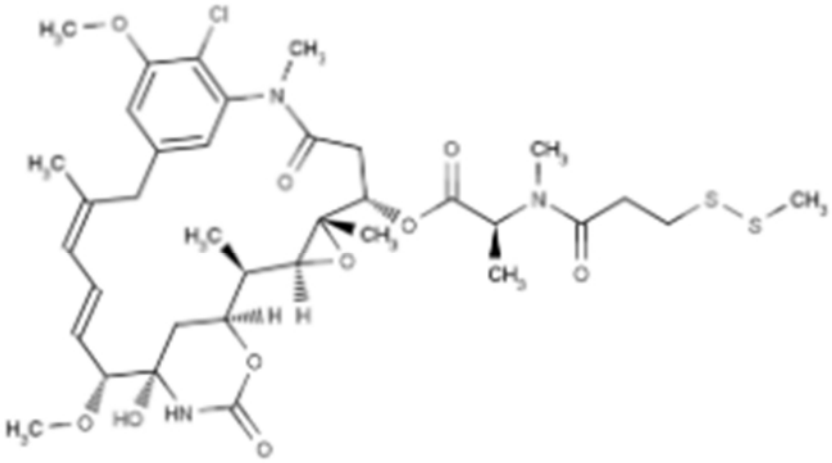
Antibody Name	Viable Cell % NCIH358	Viable Cell % HCC1954
ch2012	91.1	46.3
hu2012c	85.8	28.8
hu2012d	84.0	28.6

[0093]

Example 6: *In Vivo*

(1) Preparation of DM1-labeled antibody

When DM1-SMe (CAS 138148-68-2) was used as the starting material for the conjugated drug, DM1-SH was obtained by performing a reduction treatment with TCEP followed by HPLC purification using a C18 column. Separately, a DM1-labeled antibody was prepared by introducing a maleimide group into the antibody using sulfo-SMCC, followed by reaction with DM1-SH.



DM1SMe structural formula

[0094]

(2) Preparation of xenograft

HCC1954 cells were cultured and expanded until the number of cells reached approximately twice the amount required for transplantation. In addition, the right ventral region of each mouse was shaved on the day before cell transplantation.

[0095]

(3) Transplantation

After washing the cells with PBS(-), they were detached using TrypLE Express (Thermo Fisher Scientific) and centrifuged at 1,000 rpm for 5 minutes, thereby collecting the pellet. The pellet was suspended in antibiotic- and serum-free RPMI-1640 medium to a concentration of 5×10^7 cells/mL, and 100 μL of the suspension was transplanted subcutaneously into the right flank of each mouse.

For the *in vivo* study, five animals per group were used, and ch2012-DM1 and hu2012(c/d)-DM1 were administered via the tail vein at a dose of 5 mg/kg. The first dose was administered when the mean tumor volume reached 100 to 150 mm^3, followed by a second dose of the same amount one week later, for a total of two administrations.

The results are shown in Figure 5. It was shown that hu2012c and hu2012d exhibit higher pharmacological efficacy than ch2012. It was suggested that humanization enhanced thermal stability and internalization capacity, resulting in increased therapeutic efficacy.

CLAIMS

[Claim 1]

An antibody of any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 2; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4.

[Claim 2]

The antibody according to claim 1, which is any of the following antibodies:

(1) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 5 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 6; or

(2) an anti-CDH3 humanized antibody comprising a heavy chain consisting of an amino acid sequence set forth in SEQ ID NO: 7 and a light chain consisting of an amino acid sequence set forth in SEQ ID NO: 8.

[Claim 3]

A fragment of the anti-CDH3 humanized antibody according to claim 1 or 2, which has ability to bind to CDH3.

[Claim 4]

The fragment of the anti-CDH3 humanized antibody according to claim 3, which is Fab, $F(ab')^2$, or scFv.

[Claim 5]

A partial sequence of the antibody according to claim 1 or 2, which has ability to bind to CDH3.

[Claim 6]

An immunoconjugate in which the anti-CDH3 humanized antibody according to any one of claims 1 to 5, a fragment thereof, or a partial sequence thereof is linked to a chemotherapeutic agent or a radioactive substance.

[Claim 7]

The immunoconjugate according to claim 6, wherein the chemotherapeutic agent is a cytotoxic substance.

[Claim 8]

The immunoconjugate according to claim 6 or 7, wherein the anti-CDH3 humanized antibody, a fragment thereof, or a partial sequence thereof is linked to a chemotherapeutic agent via a linker.

[Claim 9]

A medicament for treating a disease characterized by CDH3 overexpression, comprising the immunoconjugate according to any one of claims 6 to 8.

[Claim 10]

The medicament according to claim 9, wherein the disease characterized by overexpression of CDH3 is a cancer.