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(54) **USAG-1 MOLECULE-TARGETING NEUTRALIZING ANTIBODY FOR TOOTH REGENERATION TREATMENT**

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(57) **ABSTRACT**

Provided are: an antibody which specifically binds to and neutralizes USAG-1 or an antigen-binding fragment thereof; and a pharmaceutical composition containing the antibody or the antigen-binding fragment.
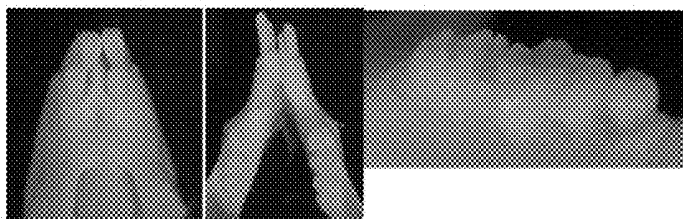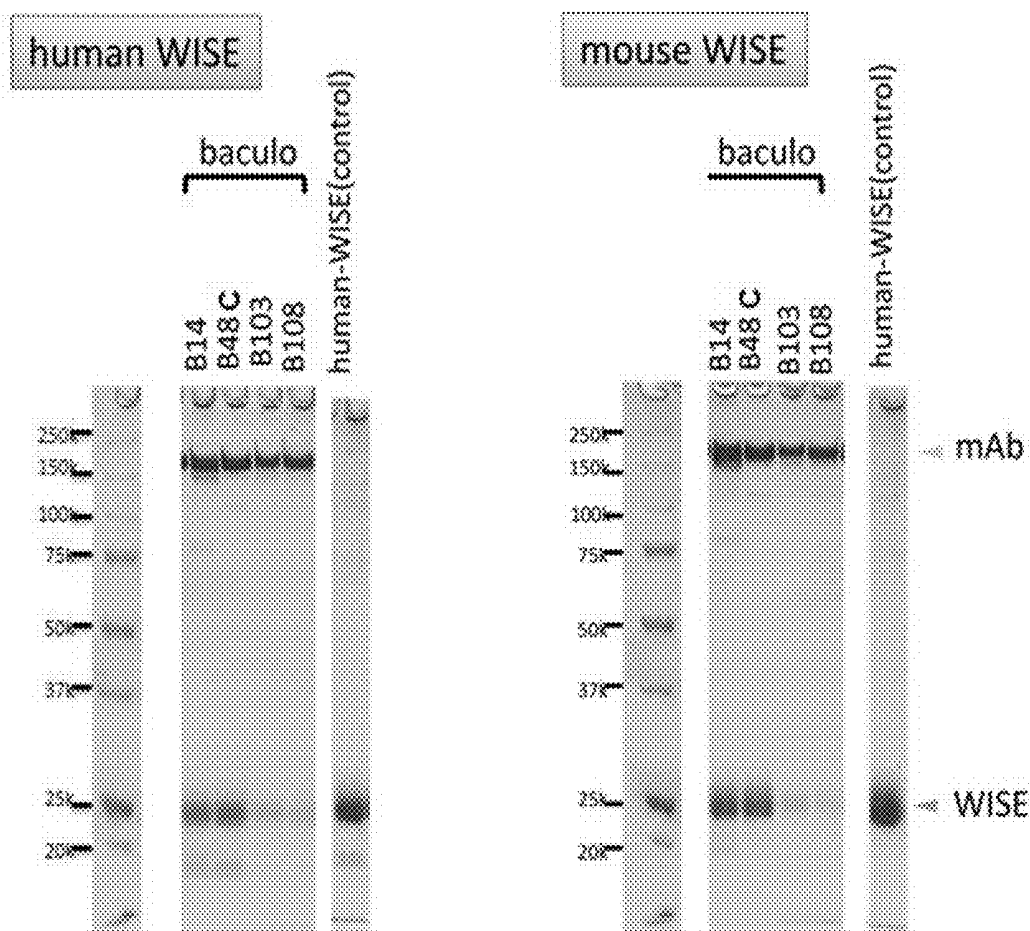
Specification includes a Sequence Listing.

[FIG. 1]

USAG-1 KO mouse #116
106bp del on exon 1
C57Bl/6J

[FIG. 2]

[FIG. 3]

VH (IgG1) (Underline: CDR 1,2,3 in order from 1 to 3; Italic: D region; Bold: J region;
            Other: V region)

EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYMHWVKQRPEQGLEWIGWIDPENGDTE
YASKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCITPYYYGSSFSYWYFDVWGTGTTVT
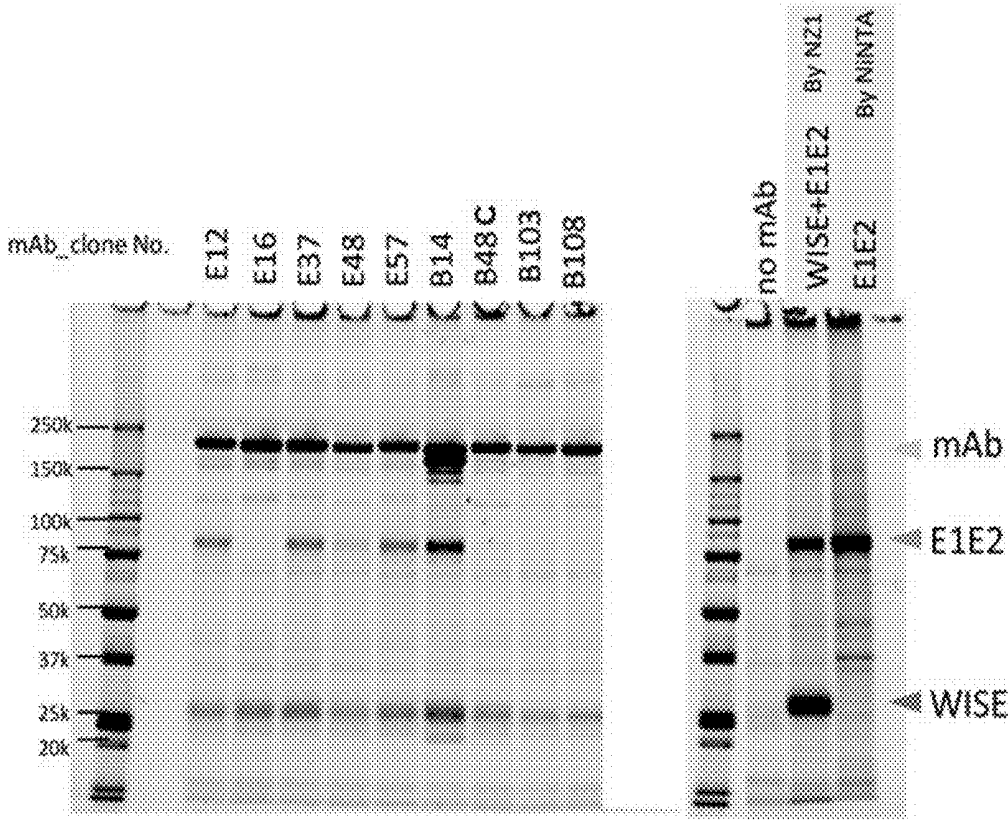VSS

VL (Igκ) (Underline: CDR 1,2,3 in oderder from 1 to 3; Bold: J region; Other: V region)

DVLMTQTPLSLPVSLGDQASISCRSSQ
SIVHSNGNTYLEWYLQKPGQSPKLLIY
KVSNRFSGVPDRFSGSGSGTDFTLKIS
RVEAEDLGVYYCFQGSHVPWTFGGGT
KLEIK

[FIG. 4]

Wnt

BMP
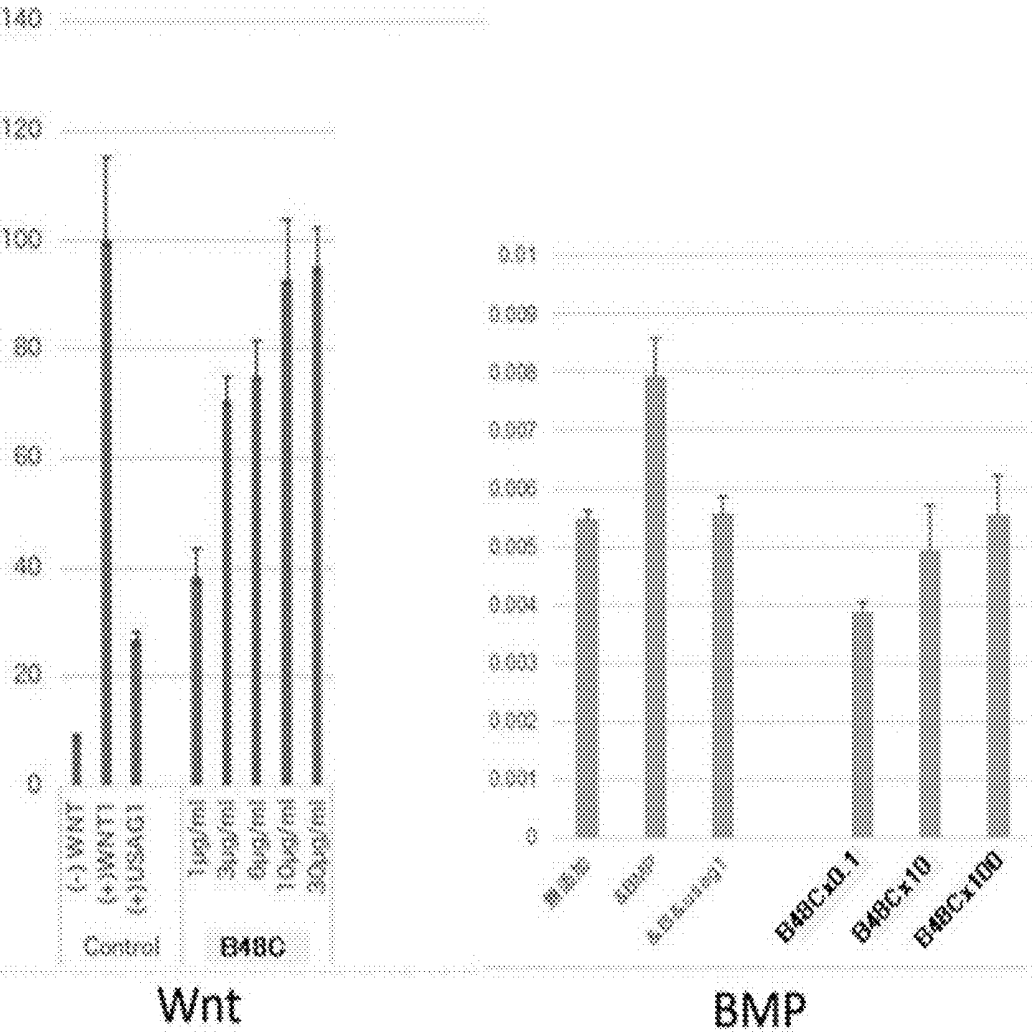
[FIG. 5]

USAG-1 MOLECULE-TARGETING NEUTRALIZING ANTIBODY FOR TOOTH REGENERATION TREATMENT

TECHNICAL FIELD

[0001] The present invention relates to a neutralizing antibody targeting USAG-1 for the treatment of tooth agenesis or tooth regeneration.

BACKGROUND ART

[0002] In the majority of patients, acquired diseases such as dental caries and periodontal disease result in tooth agenesis (patients with loss of teeth). As high as 1% incidence rate of congenital tooth agenesis is also reported. Currently, the only treatment method for missing teeth is prosthetic treatment which includes dental implants and dentures, and there is no fundamental treatment method. Numerous studies of tooth regeneration using tissue engineering approaches have been reported. Various cells such as stem cells (Non-Patent Literature 1) are used as cell sources. In addition, in order to allow teeth made in vitro to function in the oral cavity, an "organ primordium method" (Non-Patent Literature 2), a cell manipulation technology for regenerating a dental organ primordium (the rudiment of the organ) in a collagen gel, has been reported. However, owing to cost and safety problems for securing cell sources, tissue engineering approaches have not reached clinical application.

[0003] A large number of causative genes for congenital tooth agenesis have been identified, and many of them are common to both human and mouse. For example, RUNX2, MSX1, EDA, WNT10A, PAX9, AXIN2 etc. are known. Among the listed genes, WNT10A gene has been reported to cause congenital tooth agenesis in the largest number of patients. EDA gene is a causative gene for anhidrotic ectodermal dysplasia, which is a representative disease of syndromic congenital tooth agenesis. Congenital tooth agenesis is caused by tooth development stopped prematurely due to defect in the causative gene and suppression of the function of the causative gene.

CITATION LIST

Patent Literature

[0004] Patent Literature 1: Ohazama, J Denr Res, 2004
[0005] Patent Literature 2: Nakao, Nat Methods, 2007

SUMMARY OF INVENTION

Problem to be Solved by Invention

[0006] From a therapeutic viewpoint, the inventors conceived of a novel treatment method to treat congenital tooth agenesis. The new approach promotes differentiation induction from the state of tooth development that has been stopped prematurely to form a complete tooth. Object of the present invention is to provide a technique for treating tooth agenesis which comprises utilizing the differentiation induction inherent in a tooth organ, instead of utilizing surgical tissue transplantation.

Solution for Problem

[0007] As a result of diligent research, the present inventors succeeded in developing a neutralizing antibody targeting USAG-1. Furthermore, they found that administration of the antibody regenerated missing teeth in congenital tooth agenesis model mice and formed supernumerary teeth in congenital tooth agenesis model mice or wild-type mice. Thus the present invention was completed.

[0008] That is, the present invention relates to:

[0009] [1] An antibody or antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprising three heavy chain complementarity determining regions that comprise amino acid sequences having at least 90% sequence identity with amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, and/or three light chain complementarity determining regions that comprise amino acid sequences having at least 90% sequence identity with amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively;

[0010] [2] The antibody or antigen fragment thereof according to [1], which specifically binds to USAG-1 and neutralizes BMP signaling inhibitory activity of USAG-1;

[0011] [3] The antibody or antigen fragment thereof according to [1] or [2], which specifically binds to USAG-1 and neutralizes WNT signaling inhibitory activity of USAG-1;

[0012] [4] The antibody or antigen-binding fragment thereof according to any one of [1] to [3], which comprises a heavy chain variable region that comprises an amino acid sequence having at least 90% sequence identity with an amino acid sequence set forth in SEQ ID NO: 3, and/or a light chain variable region that comprises an amino acid sequence having at least 90% sequence identity with an amino acid sequence set forth in SEQ ID NO: 4;

[0013] [5] An antibody or antigen-binding fragment thereof that competes with the antibody or antigen-binding fragment thereof according to any one of [1] to [4] for binding to USAG-1;

[0014] [6] The antibody or antigen-binding fragment thereof according to any one of [1] to [5], wherein the antibody is a humanized antibody or a chimeric antibody; and

[0015] [7] A pharmaceutical composition for dental regenerative therapy, which comprises the antibody or antigen-binding fragment thereof according to any one of [1] to [6].

Effects of the Invention

[0016] In the present invention, we succeeded in regenerating teeth in vivo by using an antibody preparation. Treatment with the antibody preparation of the present invention can be clinically applied as a tooth regenerative therapy in a general dental and oral surgical approach such as conventional tooth extraction, orthodontics, and tooth transplantation.

BRIEF DESCRIPTION OF DRAWINGS

[0017] FIG. 1 shows USAG-1KO mice established using CRISPR-CAS9.

[0018] FIG. 2 shows experimental results of binding of mouse anti-USAG-1 antibodies to mouse/human USAG-1 proteins.

[0019] FIG. 3 shows sequences of heavy chain and light chain variable regions of antibody.

[0020] FIG. 4 shows the neutralizing activity of the antibody of the present invention on the WNT signaling inhibitory activity and the BMP signaling inhibitory activity of mouse USAG-1.

[0021] FIG. 5 shows results of pull-down assay, showing the interaction between a complex of the mouse anti-USAG-1 antibody with mouse USAG-1 protein and an LRP6-E1E2 domain.

MODE FOR CARRYING OUT THE INVENTION

[0022] USAG-1 (Uterine Sensitization Associated Gene-1) is a bone morphogenetic protein (BMP) antagonist and a Wnt antagonist, and is also called Sostdc-1, Ectodin, or Wise. It is known that in USAG-1 deficient model mice, an increase in BMP signaling is observed, leading to the formation of supernumerary teeth. The present inventors crossed a Runx2-deficient mouse, which is a model mouse for congenital tooth agenesis, with a USAG-1 gene-deficient mouse, which is a model mouse for supernumerary teeth (teeth exceeding the normal number of teeth), to produce a double-knockout mouse. As a result of analysis of the double-knockout mouse, it was found that tooth formation was recovered. Thus it was suggested that inhibition of USAG-1 could treat tooth agenesis.

[0023] This time, the present inventors crossed congenital tooth agenesis model mice lacking causative genes Msx1, Eda and Wnt10a other than Runx2 with an USAG-1 gene-deficient mouse that was newly created by CRISPER-CAS9 system as a model mouse for supernumerary teeth, to produce double-knockout mice. As a result of analysis of the double-knockout mice, it was found that tooth formation was recovered in all the tooth agenesis model mice. Thus it was shown that the treatment by inhibition of USAG-1 can be applied to patients with congenital tooth agenesis caused by various gene mutations.

[0024] Then, in the present invention, a human USAG-1 recombinant protein whose activity was confirmed was used as an antigen to produce antibodies. Thus antibodies that specifically bind to USAG-1 were obtained. These antibodies were found to increase BMP signaling and/or Wnt signaling.

[0025] Therefore, an aspect of the present invention provides an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, that is, an anti-USAG-1 neutralizing antibody and an antigen-binding fragment thereof. As used herein unless otherwise specified, USAG-1 means mammalian USAG-1. Examples of the mammal include, but not limited to, a human, a dog, a cat, a horse, a mouse, a ferret, a suncus, a pig, and a monkey, and a human is preferable.

[0026] As used herein, neutralizing refers to inhibiting the function of USAG-1. The functions of USAG-1 include, for example, BMP signaling inhibitory activity (also referred to as "BMP antagonist activity") and Wnt signaling inhibitory activity (also referred to as "Wnt antagonist activity"). The antibody or antigen-binding fragment thereof of the present disclosure inhibits the BMP signaling inhibitory activity and/or the Wnt signaling inhibitory activity of USAG-1. Therefore, the antibody or antigen-binding fragment thereof of the present disclosure neutralizes either or both of the BMP signaling inhibitory activity of USAG-1 and the Wnt signaling inhibitory activity of USAG-1. For example, the

antibody or antigen-binding fragment thereof of the present disclosure includes, but not limited to, an antibody or an antigen-binding fragment thereof that specifically binds to USAG-1 and neutralizes the BMP signaling inhibitory activity of USAG-1 and does not neutralize the Wnt signaling inhibitory activity of USAG-1, and an antibody or an antigen-binding fragment thereof that specifically binds to USAG-1 to neutralize the Wnt signaling inhibitory activity of USAG-1 and does not neutralize the BMP signaling inhibitory activity of USAG-1. As used herein, "inhibition" includes suppression and reduction.

[0027] The neutralizing activity of an antibody or an antigen-binding fragment may be determined by a conventional method. The activity of neutralizing the BMP antagonist activity of USAG-1 (also referred to as "BMP antagonist neutralizing activity") can be measured in vitro by, for example, an ALP (alkaline phosphatase) assay or a reporter assay. In the ALP assay, for example, osteoblast progenitor cells and the like are cultured in the presence of BMP with addition of USAG-1 protein and an antibody or an antigen-binding fragment thereof, and ALP generated when differentiation into osteoblasts is induced is measured. The activity of neutralizing the Wnt antagonist activity of USAG-1 (also referred to as "Wnt antagonist neutralizing activity") can be determined in vitro, for example, by a reporter assay. In the reporter assay, for example, a vector containing a promoter region that reacts with BMP or Wnt, ligated to a reporter gene such as luciferase is introduced into a cell, the cell is cultured in the presence of BMP or Wnt with addition of USAG-1 protein and an antibody or an antigen-binding fragment thereof, and expressed luciferase activity is measured. The BMP antagonist activity to be neutralized by the anti-USAG-1 antibody or antigen-binding fragment thereof of the present disclosure may be an antagonist activity against any BMP family. For example, the anti-USAG-1 antibody or antigen-binding fragment thereof of the present disclosure may neutralize the antagonist activity against BMP2, BMP4, BMP6, BMP7, etc. The Wnt antagonist activity to be neutralized by the anti-USAG-1 antibody or antigen-binding fragment thereof of the present disclosure may be an antagonist activity against any Wnt family. For example, the anti-USAG-1 antibody or antigen-binding fragment thereof of the present disclosure may neutralize the antagonist activity against Wnt-1, Wnt-3, etc.

[0028] Furthermore, in the present invention, one of the obtained antibodies was sequenced and analyzed. Then, the variable regions and complementarity determining regions of the antibody were determined. The antibody comprises a heavy chain comprising an amino acid sequence set forth in SEQ ID NO: 1 and a light chain comprising an amino acid sequence set forth in SEQ ID NO: 2, and the heavy chain comprises a heavy chain variable region (SEQ ID NO: 3) comprising heavy chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7, and the light chain comprises a light chain variable regions (SEQ ID NO: 4) comprising light chain complementarity determining regions set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10. The antibody particularly has Wnt antagonist neutralizing activity.

[0029] Therefore, in an aspect of the present invention, the above-described antibody and a mutant thereof are provided as the antibody or antigen-binding fragment thereof of the present disclosure. An example of the antibody or a mutant

thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises three heavy chain complementarity determining regions comprising amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, or three light chain complementarity determining regions comprising amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises three heavy chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, or three light chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively. More preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises three heavy chain complementarity determining regions consisting of amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, or three light chain complementarity determining regions consisting of amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively. Still more preferably provides is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises three heavy chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, or three light chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively.

[0030] A further example of the antibody or a mutant thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises three heavy chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, or three light chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises three heavy chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, or three light chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively, and comprises

substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences.

[0031] A further example of the antibody or a mutant includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises a heavy chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 3 or a light chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 4. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 3 or a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 4. More preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises a heavy chain variable region consisting of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 3 or a light chain variable region consisting of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 4. Still more preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1 and comprises a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 or a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4.

[0032] A further example of the antibody or a mutant thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 3 or a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 4, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises a heavy chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 3 or a light chain variable region consisting of an amino acid sequence set forth in SEQ ID NO: 4, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences.

[0033] A further example of the antibody or a mutant thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises three heavy chain complementarity determining regions comprising amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively and three light chain complementarity determining regions comprising amino acid sequences having at

least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises three heavy chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively and three light chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively. More preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises three heavy chain complementarity determining regions consisting of amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively and three light chain complementarity determining regions consisting of amino acid sequences having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively. Still more preferably provides is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises three heavy chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively and three light chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively.

[0034] A further example of the antibody or a mutant thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises three heavy chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively and three light chain complementarity determining regions comprising amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises three heavy chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively and three light chain complementarity determining regions consisting of amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences.

[0035] A further example of the antibody or a mutant thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises a heavy chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID

NO: 3 and a light chain variable region comprising an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 4. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 4. More preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises a heavy chain variable region consisting of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region consisting of an amino acid sequence having at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with an amino acid sequence set forth in SEQ ID NO: 4. Still more preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, and comprises 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.

[0036] A further example of the antibody or a mutant thereof includes an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 4, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences. Preferably provided is an antibody or an antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprises 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, and comprises substitution, deletion, insertion or addition of one to several amino acid residues in at least one of the above-mentioned amino acid sequences.

[0037] As used herein, the term "several" means about 2 to 10, and preferably means, depending on the length of an amino acid sequence, about 2 to 7, for example, 3, 4, 5, or 6. As used herein, the "substitution" may be a conservative or non-conservative substitution, and preferably a conservative substitution. Conservative substitution is known to those skilled in the art, and refers to a substitution that does not affect the biological activity of the resulting molecule. Examples of conservative amino acid substitution include a substitution of alanine to glycine or serine, a substitution of arginine to lysine or histidine, a substitution of asparagine to glutamine or histidine, a substitution of aspartic acid to glutamic acid or asparagine, a substitution of cysteine to serine or alanine, a substitution of glutamine to asparagine, a substitution of glutamic acid to aspartic acid or glutamine, a substitution of glycine to alanine, a substitution of histidine to asparagine or glutamine, a substitution of isoleucine to leucine or valine, a substitution of leucine to isoleucine or valine, a substitution of lysine to arginine or histidine, a

substitution of methionine to leucine, isoleucine or tyrosine, a substitution of phenylalanine to tyrosine, methionine or leucine, a substitution of proline to alanine, a substitution of serine to threonine, a substitution of threonine to serine, a substitution of tryptophan to tyrosine or phenylalanine, a substitution of tyrosine to tryptophan or phenylalanine, and a substitution of valine to isoleucine or leucine.

[0038] As used herein, the sequence identity may be determined in optimal alignment of two sequences according to a conventional method. For example, the sequence identity may be determined using an algorithm known in the art, such as BLAST or FASTA. The antibody or antigen-binding fragment thereof of the present disclosure may comprise substitution, deletion, insertion or addition of an amino acid residue(s) within the above-mentioned range of sequence identity.

[0039] Further, in another aspect of the present invention, an antibody or an antigen-binding fragment thereof that binds to a whole extent or a part of the same epitope as an epitope on USAG-1 to which the antibody or a mutant thereof, or an antigen-binding fragment thereof binds is provided as the antibody or antigen-binding fragment thereof of the present disclosure. Furthermore, the present invention provides an antibody or an antigen-binding fragment thereof that competes with the antibody or a mutant thereof, or an antigen-binding fragment thereof for binding to USAG-1 or for binding to a whole extent or a part of an epitope on USAG-1.

[0040] As used herein, the term "competition" means that an antibody or an antigen-binding fragment thereof competes with a reference antibody (e.g., the above-mentioned antibody or a mutant thereof, or an antigen-binding fragment thereof) in a binding assay using an USAG-1 protein or polypeptide. For example, if a test antibody or an antigen-binding fragment thereof reduces the binding of a reference antibody to an USAG-1 protein or polypeptide in the binding assay, the test antibody "competes" with the reference antibody. An antibody that competes with a reference antibody, for example, reduces the binding of the reference antibody to an antigen protein or polypeptide by at least about 40%, preferably at least about 50%, more preferably at least about 60%, still more preferably at least about 80%, or still more preferably at least 90%. The competitive binding assay can be performed by known methods in the art including, but not limited to, ELISA, flow cytometry, SPR (surface plasmon resonance), and BLI (Bio-Layer Interferometry).

[0041] Epitope binning is a technique for classifying two or more antibodies based on their epitopes, for example, by using SPR or BLI. In epitope binning, for example, an antigen protein (target) is added to a biosensor on which a reference antibody is immobilized to allow the reference antibody to bind the target, the biosensor holding a complex of the reference antibody and the target is reacted with a test antibody, and then, binding of the test antibody to the biosensor (i.e., binding of the test antibody to the target captured by the reference antibody immobilized on the biosensor) and dissociation of the binding are analyzed. If the test antibody shares the same epitope with the reference antibody, the test antibody cannot bind to the biosensor because the epitope on the target is already occupied by the binding of the reference antibody. Conversely, if the test antibody recognizes a different epitope from that recognized by the reference antibody, the test antibody can bind to the

biosensor. Furthermore, if the test antibody recognizes a region sterically close to an epitope recognized by the reference antibody, the test antibody cannot bind to the biosensor because the binding of the reference antibody to the target interferes with the binding of the test antibody to the epitope. Thus, use of epitope binning enables to determine whether two or more antibody clones compete for binding to a target protein.

[0042] The antibody or antigen-binding fragment thereof of the present disclosure binds to USAG-1 or a whole extent or a part of an epitope on USAG-1 at a KD of for example 1 μM or less, preferably 100 nM or less, more preferably 50 nM or less, still more preferably 30 nM or less, still more preferably 10 nM or less, still more preferably 8 nM or less, or still more preferably 5 nM or less.

[0043] In the present invention, the antibody is preferably an isolated antibody. In the present invention, the antibody may be a polyclonal antibody or a monoclonal antibody. In the present invention, the antibody may be a human antibody, a humanized antibody, a chimeric antibody, or a multispecific antibody (for example, a bispecific antibody). The humanized antibody includes a human immunoglobulin (recipient antibody) in which the complementarity determining regions (CDRs) of a recipient are replaced by residues from the CDRs of a non-human species (donor antibody) having desired specificity, affinity and binding ability. Optionally, the Fv framework region (FR) residues of the human immunoglobulin may be replaced by the corresponding non-human residues. In addition, the humanized antibody may comprise residues that are not found in the recipient antibody or the donor antibody. Generally, humanized antibodies comprise at least one variable region, typically two variable regions, in which all or substantially all CDRs are replaced by non-human immunoglobulin CDRs and all or substantially all of FR regions consist of human immunoglobulin sequences. The chimeric antibody includes an antibody produced by a genetic recombination technique in which variable regions derived from a donor antibody are linked to constant regions of a recipient antibody. The above antibodies can be produced by methods known in the art.

[0044] In the present invention, examples of the antigen-binding fragment include, but not limited to, $F(ab')_2$, Fab', Fab, Fv, rIgG, Fd, a linear antibody, ScFv, Fv-clasp, a minibody, a diabody, a triabody, a tetrabody, a single domain antibody (nanobody), and a multispecific antibody formed from antibody fragments. The antibody fragments can be prepared by methods known in the art.

[0045] An isolated nucleic acid encoding the antibody or antigen-binding fragment thereof of the present disclosure is also included in the present invention.

[0046] The antibody or antigen-binding fragment thereof of the present disclosure specifically binds to USAG-1 to inhibit the function of USAG-1 and then induce tooth formation. Accordingly, another aspect of the present invention provides a pharmaceutical composition for dental regeneration therapy comprising the antibody or antigen-binding fragment thereof of the present disclosure. The dental regeneration includes, for example, regeneration of a missing tooth (recovery of a missing tooth), and formation of a new tooth such as a third dentition.

[0047] The pharmaceutical composition of the present disclosure may contain a pharmaceutically acceptable carrier, and an additive such as a stabilizer or an excipient, in addition to the antibody or antigen-binding fragment thereof

of the present disclosure. Examples of the pharmaceutically acceptable carrier include, but not limited to, physiological saline, buffer, glycol, glycerol, gelatin, gelatin hydrogel, polylactic acid, collagen sponge, agarose, polyvinyl alcohol, alginic acid, fibrin gel, an ethylene-vinyl acetate copolymer, and a lactic acid-glycolic acid copolymer. Examples of the additive include, but not limited to, a carbohydrate such as glucose, sucrose or dextran, an antioxidant such as ascorbic acid or glutathione, a chelating agent, and a low molecular weight protein. Those skilled in the art can appropriately select the carrier and additive as mentioned above based on an administration form, administration route or the like of the pharmaceutical composition. Pharmaceutical composition of the present disclosure can be produced using the antibody or antigen-binding fragment thereof, and the additive as appropriate by a conventional method.

[0048] Examples of the form of the pharmaceutical composition of the present disclosure include a tablet, a powder, a capsule, a granule, a syrup, a sustained release tablet, a sustained release capsule, an enteric coated drug, an intercalating drug, an infusion, and an injection. A preferable example thereof is an injection. The pharmaceutical composition of the present disclosure is systemically or topically administered. The administration route may be appropriately selected by those skilled in the art, and examples thereof include, but not limited to, oral, nasal, subcutaneous, intravenous, intramuscular, and intraosseous administration. The pharmaceutical composition of the present disclosure may be locally administered, for example, to the site of tooth formation.

[0049] In the present invention, the dental regenerative therapy includes the treatment of congenital tooth agenesis and the treatment of acquired tooth loss. Congenital tooth agenesis that can be treated with the pharmaceutical composition of the present disclosure is not particularly limited, and may also include congenital tooth agenesis due to any causative gene. Examples of congenital tooth agenesis that can be treated with the pharmaceutical composition of the present disclosure include, but not limited to, congenital tooth agenesis whose causative gene is RUNX2, MSX1, EDA, WNT10A, PAX9, or AXIN2. Preferable examples thereof include congenital tooth agenesis whose causative gene is RUNX2, MSX1, EDA, or WNT10A. Further, the antibody of the present disclosure induced the formation of supernumerary teeth in wild-type mice. Therefore, the pharmaceutical composition of the present disclosure can induce tooth formation even in normal individuals in which the causative gene of tooth agenesis is not deficient and individuals losing teeth after birth.

[0050] Pharmaceutical composition of the present disclosure may be administered to a mammal. Examples of the mammal include a human, a dog, a cat, a horse, a mouse, a ferret, a suncus, a pig, and a monkey. A preferable example thereof is a human.

[0051] A dose of the pharmaceutical composition of the present disclosure is not particularly limited. The dose can be appropriately determined by those skilled in the art based on the amount of the antibody or antigen-binding fragment thereof of the present disclosure contained in the pharmaceutical composition, the body weight of a subject to be administered, etc. so that a desired dose of the antibody or antigen-binding fragment thereof of the present disclosure can be administered. For example, the antibody or antigen-binding fragment thereof of the present disclosure is admin-

istered in an amount that produces a neutralizing activity such that BMP signaling is increased by at least 30%, preferably at least 60% and/or a neutralizing activity such that Wnt signaling is increased by at least 30%, preferably at least 60%, as compared with the case where the antibody or antigen-binding fragment thereof of the present disclosure is not administered. The neutralizing activities may be determined based on the activity measured in vitro by, for example, an ALP assay or a reporter assay.

[0052] A further aspect of the present invention provides a method of regenerating a tooth which comprises administering the antibody or antigen-binding fragment thereof of the present disclosure to a subject in need. The above-mentioned pharmaceutical composition can be used as the antibody or antigen-binding fragment thereof of the present disclosure. The subject in need is a subject having a missing tooth, and examples of the subject include mammals as mentioned above. A route of administration and a dose of the antibody or antigen-binding fragment thereof of the present disclosure, and the treatment for tooth regeneration are as described above for the pharmaceutical composition of the present disclosure.

[0053] Hereinafter, the present invention will be described in more detail with reference to Examples which the present invention is not limited to.

Example 1

Preparation of Antibody

[0054] For preparation of mouse USAG-1 neutralizing antibodies, a rat USAG-1 protein derived from a baculovirus expression system was used as an antigen. The neutralizing antibodies were prepared by the iliac lymph node method using USAG-1 KO (#116) mice that was established using CRISPR-CAS9 (FIG. 1). In a Wnt reporter assay using HEK293 cells, the Wnt inhibitory activity of the baculovirus expression system-derived rat USAG-1 protein was confirmed. In an ALP assay with addition of BMP7 using C2C12 cells, the BMP inhibitory activity of the baculovirus expression system-derived rat USAG-1 protein was confirmed. Primary screening of the obtained antibody clones was performed by ELISA using the immunizing antigen (the baculovirus expression system-derived rat USAG-1 protein) and the *Escherichia coli* expression system-derived human USAG-1 protein, and a large number of positive wells were found. Based on a cut-off value of 1.0, 111 clones were selected. After expansion, the clones were subjected to sandwich ELISA using the immunizing antigen. When a cut-off value of absorbance was set to 0.5 or more (His tag) and 1.4 or more (Myc tag), positive wells were found in about half of the clones. As a result of measurement of antibody subclasses, they were found to be IgG1, 2a, 2b, and G3.

[0055] The neutralizing activity of each antibody was confirmed as follows. In an experimental system in which an ALP activity in C2C12 cells increased by addition of 300 ng/ml of BMP7 (manufactured by R & D systems) was suppressed by addition of 300 ng/ml of a mammalian cell expression system-derived rat USAG-1 protein (manufactured by MyBiosource), each antibody was added to confirm neutralization of the suppressed ALP activity. The antibodies were classified into a group of those having mild neutralizing activity (*: 60-100% neutralizing activity), a group of those having moderate neutralizing activity (**: 100-140%

neutralizing activity), and a group of those having high neutralizing activity (***: 140% or more neutralizing activity). For a BMP reporter assay, a commercially available cell line incorporating BRE-Luc, BMP Responsive Reporter Osteoblast Cell Line (Briter cell) (manufactured by Kerafast, Inc.) was used. In an experimental system in which a luciferase activity expressed by addition of 300 ng/ml of BMP7 was suppressed by addition of 300 ng/ml of the mammalian cell expression system-derived rat USAG-1 protein, each antibody was added to confirm neutralization of the suppressed luciferase activity. The antibodies were classified into a group of those having mild neutralizing activity (*: 40-60% neutralizing activity) and a group of those having moderate neutralizing activity (**: 60% or more neutralizing activity). For the WNT reporter assay, an expression plasmid for expressing a TOP-Flash reporter gene having a DNA sequence to bind transcription factor TCF that activates downstream of the WNT signaling, a WNT1 gene, and a reporter gene under the control of an HSV-thymidine kinase promoter for obtaining an internal standard value was introduced into HEK293 cells, and the cells were cultured with addition of the mammalian cell expression system-derived rat USAG-1 protein at a concentration (EC50) for producing 50% of the maximum inhibitory effect on Wnt signaling. An addition amount of the USAG-1 protein was determined in advance. A culture supernatant of an antibody-producing hybridoma was added to the cells so as to be 25%, 20% or 10% in a medium for screening. The experiment was performed three times for evaluation. When added at 25%, the antibodies were classified into a group of those having mild neutralizing activity (*: a luminescence correction value of 1.5-2.0) and a group of those having moderate neutralizing activity (**: a luminescence correction value of 2.0 or more) When added at 20%, the antibodies were classified into a group of those having mild neutralizing activity (*: a luminescence correction value of 1-1.1) and a group of those having moderate neutralizing activity (**: a luminescence correction value of 1.1 or more). When added at 10%, the antibodies were classified into a group of those having mild neutralizing activity (*: a luminescence correction value of 1-1.1) and a group of those having moderate neutralizing activity (**: a luminescence correction value of 1.1 or more) The % of neutralizing activity was calculated based on the activity (100%) under the condition of no addition of USAG-1 protein. Neutralizing activity based on the luminescence value was calculated as a value relative to the activity (1) when no antibody was added. Finally, a mouse anti-USAG-1 neutralizing antibody (B48C) was obtained.

[0056] Further, a binding assay was performed using moue/human N-terminal PA-tagged USAG-1 proteins to confirm that the obtained mouse anti-USAG-1 neutralizing antibody B48C recognizes the human USAG-1 protein in the same way as three antibodies (B14, B103, B108) obtained by the previous study (WO2021/010346). Each of the mouse anti-USAG-1 neutralizing antibodies [5 μg in 250 μl protein A/G IgG binding buffer (Pierce™)+250 μl PBS] was captured on Protein A sepharose (30 μl) (room temperature, 1.5 hours). A culture supernatant (0.75 mL) of Expi293F cells transiently expressing a human or mouse N-terminal PA-tagged USAG-1 (WISE) recombinant protein was added to the Protein A sepharose, and then incubated (room temperature, 2 hours). Washing with a PBS buffer was performed 3 times to remove unbound proteins.

All proteins bound to the sepharose were eluted, and bands were detected by SDS-PAGE electrophoresis and CBB (Coomassie Brilliant Blue) staining. As a result, all of the tested mouse anti-USAG-1 neutralizing antibodies bound to both the mouse USAG-1 and the human USAG-1 protein (FIG. 2).

[0057] Further, antibody B48C was sequenced. A full-length heavy chain sequence containing a signal sequence and a full-length light chain sequence containing a signal sequence of the antibody are shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. Variable regions of the antibody are shown in FIG. 3.

Example 2

Epitope Binning

[0058] The five mouse USAG-1 neutralizing antibodies (E12, E16, E37, E48, E57) that were obtained using the *Escherichia coli* expression system-derived human USAG-1 protein as an antigen in the previous study (WO2021/010346), the three mouse USAG-1 neutralizing antibodies (B14, B103, B108) that were obtained using the baculovirus expression system-derived rat USAG-1 protein as an antigen in the previous study (WO2021/010346), and the neutralizing antibody (B48C) prepared in Example 1 were subjected to epitope binning. Epitope binning was performed using Octet (registered trademark) Red (manufactured by Pall ForteBio). Briefly, each of the nine antibodies was immobilized on a biosensor as a capture antibody, a target comprising a purified full-length recombinant mouse USAG-1 was added to bind the capture antibody, and then the biosensor was reacted with a test antibody to detect a binding signal. This cycle was repeated in succession using 9 test antibodies including the same antibody as used for capture. An antibody that does not compete with the capture antibody immobilized on the biosensor for the recognition site can bind to the captured USAG-1 protein, and thus the signal increases. In contrast, a competing antibody weakly binds to the captured USAG-1 protein, and thus no increase or little increase in the signal was observed. Based on the signal data obtained, the 9 antibodies were grouped based on their epitopes. Results are shown in Table 1.

TABLE 1

Group	Antibody
1	E12
	E37
2	E16
	E48
	E57
	B14
3	B48C
4	B103
	B108

Example 3

In Vitro Test of Antibody—1

[0059] For the antibody obtained in Example 1, affinity (KD values) was determined by using a BLI method based on Octet (registered trademark) Red (manufactured by Pall ForteBio). Specifically, each antibody was immobilized on the biosensor, the purified recombinant mouse USAG-1

protein was added at three different concentrations (10 nM, 30 nM, 100 nM), and binding and dissociation curves were obtained. The affinities were calculated by subjecting the obtained curves to global fitting with an analysis program attached to the Octet device. As a result, a KD value of 3.26 nM was shown.

Example 4

In Vitro Test of Antibody—2

[0060] The BMP and Wnt signaling inhibition neutralizing activities of the antibody B48C that was sequenced in Example 1 were determined. Experiments were carried out using mouse USAG-1 recombinant protein. A mouse USAG-1 (WISE) recombinant protein with a PA tag added to the N-terminal was transiently expressed in Expi293F cells, and a stable expression line was established. Affinity purification was performed using a PA tag system to obtain 0.2 mg of PA-mUSAG-1 (WISE) from 150 mL of a culture supernatant. The purified PA-mUSAG-1 (WISE) protein was shown to have a molecular weight of about 28 kDa, which is close to the theoretical value (24 kDa), by electrophoresis under reduction (R) and non-reduction (NR). The N-terminal PA-tagged mouse USAG-1 (WISE) protein derived from the mammalian cell Expi293F cell expression system showed dose-dependent WNT signaling inhibitory activity in the WNT reporter assay and dose-dependent BMP signaling inhibitory activity in the BMP ALP assay. The mouse USAG-1 protein whose activity was confirmed was used to confirm the neutralizing activity of the above-mentioned antibody. In the WNT reporter assay, cells into which a vector containing a luciferase gene ligated to a promoter and a vector for expressing Wnt1 (1 µg) were introduced were cultured in a medium with addition of 1 µg of the mouse USAG-1 recombinant protein and the antibody in an amount of 1, 3, 6, 10 or 30 µg/ml medium, and luciferase activity was measured. In the BMP ALP assay, C2C12 cells were cultured with 30 ng/ml of the mouse USAG-1 recombinant protein and a 0.1-fold (3 ng/ml), 10-fold (300 ng/ml) or 100-fold (3 µg/ml) amount of the antibody in the presence of 30 ng/ml of BMP7, and ALP activity was measured. Results are shown in FIG. **4**. In the WNT reporter assay, the antibody was shown to neutralize the WNT signaling inhibitory activity of USAG-1 by almost 100%.

Example 5

In Vitro Test of Antibody—3 (Pull-Down Assay with Protein a Sepharose Using PA-Tagged USAG-1 and E1E2)

[0061] Wnt binds to Frizzled and its co-receptor, a low-density lipoprotein receptor-related protein (LRP) 5/6 receptor to transmit a signal in cells. LRP6 has four extracellular domains (E1 to E4). Among the domains, E1E2 is known to be involved in the binding of USAG-1. Specifically, USAG-1 binds to the E1E2 region of LRP6 to inhibit Wnt signaling. Thus, the mouse anti-USAG-1 neutralizing antibody B48C obtained in Example 1 as well as the neutralizing antibodies (E12, E16, E37, E48, E57, B14, B103, B108) that were obtained by the previous study (WO2021/010346) were used to determine whether the binding of USAG-1 to LRP6-E1E2 was inhibited.

[0062] Each mouse anti-USAG-1 neutralizing antibody (5 µg in 1 ml PBS) was captured on Protein A sepharose (30 µl) (room temperature, 1.5 hours). A culture supernatant (1 mL) of Expi293F cells transiently expressing a N-terminal PA-tagged mouse USAG-1 recombinant protein was added to the Protein A sepharose, and then incubated (room temperature, 2 hours). Then, a culture supernatant (1 mL) expressing

LRP6-E1E2 (a region of amino acid numbers 1 to 629 of human LRP6, fused to a His tag) was added, and incubated (room temperature, 2.5 hours). Washing with a PBS buffer (1 mL) was performed 3 times to remove unbound proteins. All proteins bound to the sepharose were eluted, and bands were detected by SDS-PAGE electrophoresis and Oriole gel fluorescence staining. Results are shown in FIG. **26**. In FIG. **26**, the right panel shows results obtained when the same experiment was performed except that the mouse anti-USAG-1 neutralizing antibody was not captured (no mAb) as a negative control, when a complex of the mouse USAG-1 recombinant protein and LRP6-E1E2 was immunoprecipitated with a PA-tagged antibody NZ-1 (WISE+E1E2 By NZ1) as a positive control, and when the expression level of LRP6-E1E2 only was precipitated with a Ni-NTA resin capable of adsorbing the His tag (E1E2 By NiNTA).

[0063] As a result, it was found that LRP6-E1E2 did not bind to a complex of antibody B48C with USAG-1 (FIG. **5**). Therefore, it was found that the epitope of the antibody overlaps or is in a region sterically close to the LRP6 binding site of USAG-1, and the antibody binds to USAG-1 to inhibit the binding of USAG-1 to LRP6, and thereby the inhibition of Wnt signaling by USAG-1 is neutralized.

Example 6

In Vivo Administration Test of Antibody—1 (Mouse)

[0064] The antibody obtained in Example 1 was intraperitoneally administered in a single dose to mother mice pregnant with congenital tooth agenesis model mouse due to EDA homozygous deficiency, congenital tooth agenesis model mouse due to EDA heterozygous deficiency, or wild-type mice (16 µg/g body weight). In born offspring mice, the presence of supernumerary teeth and fused teeth and the presence or absence of recovery of missing teeth were examined. Results are shown in Table 2. The antibody particularly recovered missing teeth in the EDA-deficient mice. Herein, the term "recovery" means that a born EDA-deficient mouse has a tooth at the site (does not lose M3) where a tooth is normally lost in an EDA-deficient mouse.

TABLE 2

	$EDA^{+/+}$ (wild-type) mouse	$EDA^{+/-}$ mouse		$EDA^{-/-}$ mouse
Antibody	Supernumerary tooth/Fused tooth	Supernumerary tooth/Fused tooth	Recovery	Supernumerary tooth/Fused tooth
B48C	0/1	0/1	3/4 (75%)	1/4

Example 7

In Vivo Administration Test of Antibody—2 (Mouse)

[0065] The antibody obtained in Example 1 was intraperitoneally administered in a single dose to mother mice pregnant with congenital tooth agenesis model mouse Wnt10a homozygous deficient mice, congenital tooth agenesis model mouse Wnt10a heterozygous deficient mice, or wild-type mice (16 µg/g body weight). In born offspring mice, the presence of supernumerary teeth and fused teeth and the presence or absence of recovery of missing teeth were examined. Results are shown in Table 3. The antibody

induced the formation of supernumerary teeth and fused teeth in the homozygous deficient mice.

TABLE 3

	Wnt10a+/+ (wild-type) mouse	Wnt10a+/− mouse	Wnt10a−/− mouse
Antibody	Supernumerary tooth/Fused tooth	Supernumerary tooth/Fused tooth	Supernumerary tooth/Fused tooth
B48C	0/2	0/9	2/5 (40%)

INDUSTRIAL APPLICABILITY

[0066] The antibody or antigen-binding fragment thereof of the present disclosure can be used for the treatment of congenital tooth agenesis and acquired tooth loss. In addition, the antibody or antigen-binding fragment thereof of the present disclosure is effective for the formation of the third dentition. Thus, the antibody or antigen-binding fragment thereof of the present disclosure leads to development of a molecular-targeted drug for tooth regeneration in the pharmaceutical field and establishment of a dental regenerative therapy based on formation of the third dentition.

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<213> ORGANISM: Mus musculus

<400> SEQUENCE: 1

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Val Asn Ser Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
            20                  25                  30

Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile
        35                  40                  45

Lys Asp Asp Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
    50                  55                  60

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala
65                  70                  75                  80

Ser Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn
                85                  90                  95

Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Tyr Cys Ile Thr Pro Tyr Tyr Tyr Gly Ser Ser Phe Ser Tyr Trp
            115                 120                 125

Tyr Phe Asp Val Trp Gly Thr Gly Thr Thr Val Thr Val Ser Ser Ala
    130                 135                 140

Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala
145                 150                 155                 160

Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe
                165                 170                 175

Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly
            180                 185                 190

Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser
        195                 200                 205

Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Thr
    210                 215                 220

Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile
225                 230                 235                 240

Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu
                245                 250                 255

Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr
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              260              265               270

Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys
        275              280               285

Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val
    290              295               300

His Thr Ala Gln Thr Lys Pro Arg Glu Glu Gln Ile Asn Ser Thr Phe
305              310               315               320

Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly
                325              330               335

Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile
            340              345               350

Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val
        355              360               365

Tyr Thr Ile Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser
    370              375               380

Leu Thr Cys Met Ile Thr Asn Phe Phe Pro Glu Asp Ile Thr Val Glu
385              390               395               400

Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro
                405              410               415

Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val
            420              425               430

Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu
        435              440               445

His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His Ser
    450              455               460

Pro Gly Lys
465


<210> SEQ ID NO 2
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Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
1               5               10              15

Ser Ser Ser Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val
            20              25              30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile
        35              40              45

Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro
    50              55              60

Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
65              70              75              80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly 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

Phe Gln Gly Ser His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
        115             120             125

Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro
    130             135             140
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Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu
145                 150                 155                 160

Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly
                165                 170                 175

Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser
            180                 185                 190

Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp
        195                 200                 205

Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr
    210                 215                 220

Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
225                 230                 235
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Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Asp
            20                  25                  30

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
        35                  40                  45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Ser Lys Phe
    50                  55                  60

Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
            85                  90                  95

Ile Thr Pro Tyr Tyr Tyr Gly Ser Ser Phe Ser Tyr Trp Tyr Phe Asp
            100                 105                 110

Val Trp Gly Thr Gly Thr Thr Val Thr Val Ser Ser
        115                 120
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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1               5                   10                  15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
            20                  25                  30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
            85                  90                  95
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-continued

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Ser His Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
        100                 105                 110


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<213> ORGANISM: Mus musculus

<400> SEQUENCE: 5

Gly Phe Asn Ile Lys Asp Asp Tyr
1               5


<210> SEQ ID NO 6
<211> LENGTH: 8
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<213> ORGANISM: Mus musculus

<400> SEQUENCE: 6

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1               5


<210> SEQ ID NO 7
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 7

Ile Thr Pro Tyr Tyr Tyr Gly Ser Ser Phe Ser Tyr Trp Tyr Phe Asp
1               5                   10                  15

Val


<210> SEQ ID NO 8
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<213> ORGANISM: Mus musculus

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Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
1               5                   10                  15


<210> SEQ ID NO 9
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 9

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1               5


<210> SEQ ID NO 10
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 10

Phe Gln Gly Ser His Val Pro Trp Thr
1               5
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1. An antibody or antigen-binding fragment thereof that specifically binds to and neutralizes USAG-1, comprising three heavy chain complementarity determining regions that comprise amino acid sequences having at least 90% sequence identity with amino acid sequences set forth in SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7 respectively, and/or three light chain complementarity determining regions that comprise amino acid sequences having at least 90% sequence identity with amino acid sequences set forth in SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively.

2. The antibody or antigen fragment thereof according to claim 1, which specifically binds to USAG-1 and neutralizes BMP signaling inhibitory activity of USAG-1.

3. The antibody or antigen fragment thereof according to claim 1, which specifically binds to USAG-1 and neutralizes WNT signaling inhibitory activity of USAG-1.

4. The antibody or antigen-binding fragment thereof according to claim 1, which comprises a heavy chain variable region that comprises an amino acid sequence having at least 90% sequence identity with an amino acid sequence set forth in SEQ ID NO: 3, and/or a light chain variable region that comprises an amino acid sequence having at least 90% sequence identity with an amino acid sequence set forth in SEQ ID NO: 4.

5. An antibody or antigen-binding fragment thereof that competes with the antibody or antigen-binding fragment thereof according to claim 1 for binding to USAG-1.

6. The antibody or antigen-binding fragment thereof according to claim 1, wherein the antibody is a humanized antibody or a chimeric antibody.

7. A pharmaceutical composition for dental regenerative therapy, which comprises the antibody or antigen-binding fragment thereof according to claim 1.

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