multinucleated osteoclasts by cell-cell fusion [see FIG. 2C]. These mature and multinucleated osteoclasts cause bone resorption [Int J Mol Sci. 2020 Aug 8;21(16):5685.].

[0008] Obesity is a biological phenomenon caused by the interaction of a complex of genetic, metabolic, environmental, and behavioral factors, defined as abnormal or excessive accumulation of fat, and can have a bad impact on health. Particularly, obesity is known as an important risk factor for causing various adult diseases such as high blood pressure, type 2 diabetes, cancer, liver disease, hyperlipidemia, arteriosclerosis, etc. Obesity results from a chronic imbalance between caloric intake and energy expenditure, is accompanied by loss of metabolism, endocrine and immune function of adipose tissue, and causes metabolic abnormalities and decreased responsiveness (resistance) to insulin, a major fat storage signaling hormone, which causes fat accumulation in metabolic organs other than adipose tissue, resulting in several types of lipotoxicity. The most representative pathology of lipotoxicity is an inflammatory response, which may be characterized as chronic low-degrade inflammation. In obesity, before metabolic abnormalities and insulin resistance appear, the activation of macrophages occurs in adipose tissue and leads to a pre-inflammatory step. As such, obesity induces a low-intensity chronic inflammatory state in the body, causing various metabolic diseases. As described above, adipose tissue macrophages play an important role in the occurrence of chronic inflammation and metabolic complications in the body due to obesity.

[0009] Fat stored in adipocytes is used as an important energy source in the body. Obesity is caused by excessive differentiation of adipocytes and unbalanced oversupply of energy. In adipose tissue, there are various cells such as adipose-

[00030] FIG. 3 shows the expression of osteoclast (OC) differentiation-related target genes, Ctsk, Acp5, c-Fos, and Nfatc1, confirmed by qPCR, at 4 days after treatment with M-CSF or M-CSF + RANKL (M-CSF: 25 ng/ml, RANKL: 100 ng/ml) to bone marrow cells of wild type (WT) and TM4SF19KO mice.

[00031] FIG. 4A shows whether an actin belt is formed in the osteoclast multinucleation, confirmed by F-actin staining, after differentiating the BMMs of wild-type and TM4SF19KO mice.

[00032] FIG. 4B shows the pit formation, confirmed by 1% toluidine blue staining, after plating BMMs on a Dentin disc and differentiating them.

[00033] FIG. 5A shows micro-CT analysis results for 8-week-old wild-type (WT) and TM4SF19KO female mice, which have been dissected without ovary removal (sham) or ovariectomized (OVX) and then have femurs fixed on day 31.

[00034] FIG. 5B shows 3D micro-CT parameters (bone microstructure parameters) including a total mineral density (BMD), a bone volume (BV), a ratio of bone volume to tissue volume (% BV/TV), an average trabecular number (Tb.N), a trabecular separation (Tb.Sp), and a trabecular thickness (Tb.Th), obtained by analyzing micro-CT images using a 3D imaging program.

[00035] FIG. 6 is a TRAP staining result for the femurs of 8-week-old wild-type (WT) and TM4SF19KO mice, which have been fixed and decalcified.

[00036] FIG. 7A shows a TRAP staining result which is obtained after cells isolated from the bone marrow of wild-type (WT) mice and TM4SF19EC2Δ mice in which TM4SF19 extracellular domain 2 (116-165) is knocked out by CRISPR, are treated with M-CSF (25 ng/ml) or M-CSF + RANKL (100 ng/ml), and differentiated. FIG. 7B shows a TRAP staining result after cells isolated from the bone marrow of wild-

type (WT) mice and TM4SF19EC2Δ mice are treated by fixing the RANKL concentration to 100 ng/ml and the M-CSF concentration is changed to 25 ng/ml or 100 ng/ml and differentiated. FIG. 7C shows the formation of an actin belt during the differentiation of multinucleated osteoclasts, confirmed by F-actin staining, after differentiating the bone marrow of wild-type (WT) and TM4SF19EC2Δ mice. FIG. 7D shows pit formation, confirmed by 1% toluidine blue staining, after plating wild-type (WT) and TM4SF19EC2Δ BMMs on a Dentin disc and differentiating them.

[00037] FIG. 8 shows the expression of target genes (Ctsk, Acp5, c-Fos, and Nfatc1) associated with osteoclast differentiation in wild-type (WT) mice and TM4SF19EC2Δ mice, analyzed by qPCR.

[00038] FIG. 9A shows a micro-CT analysis result for 8-week-old wild-type (WT) and TM4SF19EC2Δ female mice, which have been dissected without ovary removal (sham) or ovariectomized (OVX), and then 31 days later, their femurs were fixed.

[00039] FIG. 9B shows 3D micro-CT parameters (bone microstructure parameters) including a total mineral density (BMD), a bone volume (BV), a ratio of bone volume to tissue volume (% BV/TV), an average trabecular number (Tb.N), a trabecular separation (Tb.Sp), and a trabecular thickness (Tb.Th), obtained by analyzing micro-CT images using a 3D imaging program.

[00040] FIG. 10 is a micro-CT analysis result showing bone microstructure parameters for 8-week-old wild-type (WT), TM4SF19KO, and TM4SF19EC2Δ female mice, which have been dissected without ovary removal (sham) or ovariectomized (OVX), and then 31 days later, their femurs were fixed.

[00041] FIG. 11 is a set of micro-CT images for 8-week-old wild-type (WT), TM4SF19KO, and TM4SF19EC2Δ female mice, which have been dissected without

osteoclastogenesis, and TM4SF19 in 293T cells or osteoclast precursor Raw 264.7 cells. It also shows the interaction between integrin αv or integrin β3 in which 3HA is tagged at the C-terminus, and TM4SF19 or a TM4SF19 EC2 (115-175)-deleted variant in which 3Flag is tagged at the N-terminus. FIG. 12I shows the interaction between TM4SF19, and DC-Stamp or siglec-15, which are membrane proteins involved in the regulation of osteoclast functions and cytoskeletal rearrangement during multinucleated osteoclast formation, confirmed by immunoprecipitation.

[00043] FIG. 13 is a result of confirming cell proliferation, colony formation, and cell migration after overexpressing TM4SF19 in murine breast cancer cells E0771 [LPCX: retroviral control vector].

[00044] FIG. 14 is a result confirming the inhibition of lung metastasis after injecting murine breast cancer cells E0771 into the tail vein of wild-type (WT), TM4SF19KO mice, and TM4SF19EC2Δ mice.

[00045] FIG. 15 shows the expression of target genes (fibronectin, vimentin, CDH2, SNAI1 and SNAI2) involved in cell migration, confirmed by qPCR (FIG. 15A), and the expression of metastasis-associated proteins (vimentin, slug, snail, E-cadherin, β-actin), confirmed by western blotting (FIG. 15B), after cells are isolated from the bone marrow of wild-type (WT) mice and TM4SF19KO mice and differentiate into bone marrow-derived macrophages, and co-cultured with breast cancer cells to allow cell migration [BMDM: bone marrow-derived macrophages].

[00046] FIG. 16 shows the expression of genes (C/EBPα, PPARγ, TM4SF19) confirmed by qPCR (top), and the expression of proteins (TM4SF19, PPARγ, and FABP4), confirmed by western blotting (bottom), after differentiation of human adipose-derived mesenchymal stem cells (hADMSCs).

[00079] FIG. 39B shows 3D micro-CT parameters including a total mineral density (BMD), a bone volume (BV), a ratio of bone volume to tissue volume (% BV/TV), an average trabecular number (Tb.N) and a trabecular separation (Tb.Sp), which are obtained by analyzing the micro-CT images of FIG. 39A using a 3D imaging program.

[00080] FIG. 39C shows the histopathological analysis result for mouse femur tissue through H&E staining.

[00081] FIG. 39D shows that bone loss is remarkably inhibited after the tail vein injection of hIgG1-Fc, mouse TM4SF19-Fc (116-165), and human TM4SF19-Fc (131-169) after an ovariectomy in 8-week-old female mice.

[00082] FIG. 40A is a set of graphs showing the mRNA expression of Ctsk and Acp5 in differentiated cells under conditions of M-CSF or M-CSF and RANKL treatment and treatment with 10 μg/ml of mouse TM4SF19-Fc.

[00083] FIG. 40B shows the result of confirming the expression of osteoclast differentiation marker proteins after the treatment of 5 μg/ml of mTM4SF19-Fc while differentiating under a condition in which cells extracted from WT mouse bone marrow are treated with M-CSF or M-CSF and RANKL (M-CSF 25 ng/ml, RANKL 100 ng/ml).

[00084] FIG. 41 shows surface binding of mTM4SF19-Fc before and after the differentiation of Raw264.7 cells, confirmed by FACS analysis.

[00085] FIG. 42A schematically shows an experimental schedule for confirming the prevention and treatment of rheumatoid arthritis using TM4SF19-Fc. FIG. 42B shows a result of confirming the joint state of mice 42 days after the induction of collagen induced arthritis (CIA). FIG. 42C shows a result of analyzing CIA scores and the number of swollen joints, which was investigated during the

experimental period. FIG. 42D is a set of micro-CT images showing that inflammation and bone damage occurring in the paw of collagen-induced arthritis mouse models are dose-dependently inhibited by mTM4SF19-Fc treatment. FIG. 42E is a result showing that inflammation and bone damage occurring in the paw of collagen-induced arthritis mouse models are dose-dependently inhibited by mTM4SF19-Fc treatment. FIG. 42F shows 3D micro-CT parameters (bone microstructure parameters) including a total mineral density (BMD), a bone volume (BV), a ratio of bone volume to tissue volume (% BV/TV), an average trabecular number (Tb.N), and a trabecular separation (Tb.Sp) by analyzing the micro-CT images of the femurs of the collagen-induced arthritis mice using a 3D imaging program. FIG. 42G is a result of confirming cartilage damage in collagen-induced arthritis mice using toluidine blue. Compared to a hIgG1-Fc treated group, it is shown that the cartilage damage is inhibited in the mTM4SF19-Fc treated group. FIG. 42H shows the semi-therapeutic effects of mTM4SF19-Fc and hTM4SF19-Fc (120-169) in LPS-injected arthritis-induced mouse models three days after collagen antibody treatment, assessed by arthritis score. Compared to hIgG1-Fc, it was confirmed that the arthritis disease score was lowered by TM4SF19-Fc treatment and a paw thickness was reduced. FIG. 42I shows the therapeutic effect of mTM4SF19-Fc in arthritis-induced mouse models, which are subjected to LPS injection three days after collagen antibody treatment, 8 days after the LPS injection at which the arthritis disease score is the maximum. Compared to an untreated group, TM4SF19-Fc treatment leads to decreases in arthritis disease score and paw thickness, and inhibited cartilage damage, confirmed by toluidine blue.

[00086] FIG. 43A schematically shows an experimental schedule for confirming a bone metastasis inhibitory effect on breast cancer using hTM4SF19-Fc. FIG. 43B

shows a result of confirming a cancer progression status through bioluminescence image analysis. FIG. 43C shows micro-CT scans of the joint of mice with bone metastasis of breast cancer. FIG. 43D shows the result of histological analysis for the joint of mice in which breast cancer has metastasized to the bone, through H&E staining.

[00087] FIG. 44A schematically shows an experimental schedule for confirming a bone metastasis inhibitory effect on breast cancer using hTM4SF19-Fc (120-169). FIG. 44B shows the result of confirming the cancer progression status through bioluminescence imaging analysis. FIG. 44C shows the result of luminescence analysis on the joint of mice in which the metastasis of breast cancer to the bone occurs. FIG. 44D shows micro-CT scans of the joint of the mice in which the metastasis of breast cancer to the bone occurs.

[00088] FIG. 45 is a result confirming that lung metastasis is remarkably inhibited by mTM4SF19-Fc (116-165) and hTM4SF19-Fc (145-169) after lung metastasis is caused by injecting murine E0771 breast cancer cells into the tail vein of wild-type mice.

[00089] FIG. 46A shows that high-fat diet-induced obesity is inhibited by mTM4SF19-Fc treatment.

[00090] FIG. 46B shows that mTM4SF19-Fc treatment induces the effect of reducing the weight of white fat in an organ and inhibiting fatty liver by a high-fat diet, confirmed by an organ weight and a ratio of an organ weight to a body weight.

[00091] FIG. 47 shows the effect of reducing fatty liver and reducing triglycerides in plasma according to mTM4SF19-Fc treatment, confirmed by H&E staining and Masson & trichrome staining.

[000330] Lung metastasis phenotypes of the breast cancer cells of the wild-type (WT), TM4SF19KO, and TM4SF19EC2Δ mice were confirmed, and the number of nodules generated in the lungs was counted

[000331] As a result, it was confirmed that the TM4SF19KO and TM4SF19EC2Δ mice significantly inhibit lung metastasis of breast cancer cells, compared to the wild-type (WT) mice (FIG. 14).

[000332] (2) Confirmation of inhibition of expression of breast cancer cell metastasis-associated genes and proteins by TM4SF19 deficiency

[000333] Environments around cancer cells act as important factors for cancer metastasis, and among these factors, the degree of metastasis may change due to an anti-tumor factor released from macrophages. For co-culture, bone marrow was obtained from the TM4SF19KO mouse (TMKO) and the wild-type (WT) to differentiate into bone marrow-derived macrophages (BMDM), and put into the lower part of a migration chamber, and breast cancer cells were put into the upper part of the chamber. The expression of target genes (fibronectin, vimentin, CDH2, SNAI1, and SNAI2) involved in cell migration in the migrated cells was confirmed by qPCR, and western blotting was carried out to confirm the expression of metastasis-associated proteins (vimentin, slug, snail, E-cadherin, and β-actin).

[000334] As a result, it was confirmed that, by the co-culture with TM4SF19KO mouse macrophages, the expression of target genes (fibronectin, vimentin, CDH2, SNAI1, and SNAI2) involved in cell migration was inhibited (FIG. 15A), and the expression of metastasis-related proteins (vimentin, slug, snail, E-cadherin, and β-actin) was

measured after sacrificing and fixing the femur a week after the last injection. The concentrations of the mouse TM4SF19-Fc used herein were 2.5 mg/kg and 5 mg/kg.

[000421] FIG. 39A is a set of micro-CT images showing bone loss rescued by mouse TM4SF19-Fc.

[000422] 3D micro-CT parameters (bone microstructure parameters) including a total mineral density (BMD), a bone volume (BV), a ratio of bone volume to tissue volume (% BV/TV), an average trabecular number (Tb.N) and a trabecular separation (Tb.Sp) were obtained by analyzing the micro-CT images using a 3D imaging program of FIG. 39A (FIG. 39B).

[000423] As shown in FIG. 39B, it was confirmed that the total BMD (bone mineral density), % BV/TV (bone volume per tissue volume), and Tb.N (trabecular number) are decreased by the ovariectomy and dose-dependently increased by mTM4SF19-Fc. On the other hand, it was confirmed that the Tb. Sp (trabecular separation) was reduced by mTM4SF19-Fc. From the above results, it can be confirmed that mouse TM4SF19-Fc rescued ovariectomy-induced bone loss.

[000424] In addition, mouse femur tissues were histopathologically analyzed through H&E staining. As a result, as shown in FGI. 39C, it can be confirmed that Tb.N (trabecular number) was reduced by the ovariectomy and the reduction in Tb.N was rescued by TM4SF19-Fc treatment in a dose-dependent manner.

[000425] In addition, after an ovariectomy on 8-week-old female mice, mouse TM4SF19-Fc (116-165) and human TM4SF19-Fc (131-169) were intravenously injected. Compared to the untreated group, it was confirmed that bone loss is significantly inhibited by human and mouse TM4SF19-Fc (FIG. 39D). The mouse

[000444] FIG. 42D is a set of micro-CT images showing that inflammation and bone damage occurring in the paw of collagen-induced arthritis mouse models are dose-dependently inhibited by mTM4SF19-Fc treatment. The toes were bent by collagen-induced arthritis, and bone damage occurred due to inflammation in toe joints and ankles. However, compared to a control, a hIgG1-Fc-treated mouse, the joint inflammation and bone damage in the mTM4SF19-Fc-treated mouse were dose-dependently inhibited.

[000445] Referring to FIG. 42E, inflammation and bone damage occurring in the paw of collagen-induced arthritis mouse models are dose-dependently inhibited by mTM4SF19-Fc treatment, and it can be confirmed that, compared to the control hIgG1-Fc-treated mouse, bone damage was inhibited in the mice having the same RA scores.

[000446] In addition, by analyzing the micro-CT images of femurs of the collagen-induced arthritis mouse models using a 3D imaging program, 3D micro-CT parameters (bone microstructure parameters) including BMD, a bone volume (BV), a ratio of bone volume to tissue volume (% BV/TV), an average trabecular number (Tb.N), and a trabecular separation (Tb.Sp) were obtained.

[000447] FIG. 42F shows that, compared to the Sham group, BMD and Tb.N are reduced by collagen-induced arthritis, but bone loss is inhibited by TM4SF19-Fc.

[000448] FIG. 42G is a result of confirming cartilage damage in collagen-induced arthritis mice using toluidine blue. Compared to a hIgG1-Fc treated group, it is shown that the cartilage damage is inhibited in the mTM4SF19-Fc treated group.

[000449] In addition, in collagen antibody-induced arthritis mouse models, the semi-therapeutic effect of mTM4SF19-Fc and hTM4SF19-Fc (120-169) was

confirmed by arthritis scores. Three days after the administration of a collagen antibody into the Balbc mouse, arthritis was induced by LPS injection. Compared to the control, an arthritis inhibitory effect was shown at 25 mg/kg of mTM4SF19-Fc and 25 mg/kg of hTM4SF19-Fc (120-169) (FIG. 42H). It was confirmed that the arthritis disease score is lowered and the foot thickness was reduced by TM4SF19-Fc treatment, compared to hIgG1-Fc.

[000450] In addition, after arthritis was induced by LPS injection three days after collagen antibody administration into the collagen-induced arthritis mouse models, the therapeutic effect of mTM4SF19-Fc was confirmed by an arthritis score.

[000451] To confirm the therapeutic effect of mTM4SF19-Fc after inducing arthritis by LPS injection three days after collagen antibody administration, from day 8 when the arthritis disease score was the highest, 50 mg/kg of mTM4SF19-Fc was treated, and from day 9 to day 19, arthritis disease scores were checked every 2 days.

[000452] Compared to the untreated group, it was confirmed that, by mTM4SF19-Fc treatment, the arthritis scores were lowered, the foot thicknesses were reduced, and cartilage damage confirmed by toluidine blue was inhibited (FIG. 42I). That is, the arthritis treatment effect of mTM4SF19-Fc was shown.

[000453] Example 14: Inhibition of bone metastasis of breast cancer using hTM4SF19-Fc

[000454] According to the experimental schedule of FIG. 43A, MDA-MB231-luc breast cancer cells (a cell line expressing luciferase in MDA-MB231 for imaging) were injected into the tail of a 6-week-old female NOD-SCID mouse via a caudal artery (CA) route to induce bone metastasis. After the induction of bone

metastasis, hIgG1 or hTM4SF19-Fc was intravenously injected. 10 mpk or 25 mpk of hTM4SF19-Fc began to be intravenously injected two days after the cancer cell injection and administered twice a week. A cancer progression state was confirmed through bioluminescence imaging analysis.

[000455] At 45 days after cancer cell injection, bone metastasis was detected through bioluminescence imaging analysis. As shown in FIG. 43B (left), compared to the control, which is the hIgG1-administered group, it was confirmed that bone metastasis is inhibited in the hTM4SF19-Fc-administered group. As a result of luminescence analysis on a leg excised from a mouse sacrificed following luciferin injection on day 45, as shown in FIG. 43B (right), it was confirmed that bone metastasis is inhibited by hTM4SF19-Fc treatment.

[000456] FIG. 43C shows the result of micro-CT scans for joints of a mouse in which bone metastasis of breast cancer occurs. According to the micro-CT analysis result, it was confirmed that bone damage occurred due to the bone metastasis of breast cancer, and bone damage was dose-independently rescued by hTM4SF19-Fc treatment.

[000457] In addition, histological analysis was performed through H&E staining on joints of the mice in which the bone metastasis of breast cancer occurs. As a result, as shown in FIG. 43D, it can be confirmed that bone metastasis of breast cancer and tumor growth are dose-dependently inhibited by hTM4SF19-Fc treatment.

[000458] According to the experimental schedule of 44A, MDA-MB231-luc breast cancer cells (a cell line expressing luciferase in MDA-MB231 through imaging) were injected into the caudal artery (CA) of a 6-week-old female NOD-

SCID mouse to induce bone metastasis. From 30 days after the induction, 50 mg/kg of the control hIgG1 or hTM4SF19-F began to be intravenously administered.

[000459] Bioluminescence imaging was analyzed every 7 days until 21 days after injection, and it was confirmed that bone metastasis was inhibited by hTM4SF19-Fc (120-169) (FIG. 44B). After luciferin injection, as a result of luminescence analysis on legs excised from the mouse, which had been sacrificed after luciferin injection, it was confirmed that bone metastasis was inhibited by TM4SF19-Fc treatment (FIG. 44C). The micro-CT images showed that bone destruction occurred due to bone metastasis of breast cancer and was rescued by hTM4SF19-Fc treatment (FIG. 44D).

[000460] Example 15: Inhibition of lung metastasis of breast cancer using TM4SF19-Fc

[000461] Murine E0771 breast cancer cells were injected into the tail vein of a wild-type mouse to induce lung metastasis. After intravenous injection of 10 mg/kg each of hIgG1-Fc, mouse TM4SF19-Fc (116-165) and human TM4SF19-Fc (145-169), the inhibition of lung metastasis of the breast cancer cells was confirmed by staining the cells with Indian ink and counting the number of nodules in the lungs. As shown in FIG. 45, it was confirmed that lung metastasis was significantly inhibited by mouse TM4SF19-Fc (116-165) and human TM4SF19-Fc (145-169).

[000462] Example 16: Effect of inhibiting high-fat diet-induced obesity and fatty liver by mTM4SF19-Fc treatment

[000463] After six-week-old mice fed a high-fat diet for 7 weeks were purchased and acclimated by feeding them a high-fat diet for 1 week, they were administered mouse TM4SF19-Fc twice a week through subcutaneous or intravenous injection while the mice were fed a high-fat diet for 9 weeks. As a result, as can be seen in FIG. 46A, it can be confirmed that obesity caused by a high-fat diet was suppressed by the administration of 10 mg/kg of mTM4SF19-Fc.

[000464] As a result of sacrificing mice and analyzing the distribution of white fat in organs, as can be seen in FIG. 46B, it can be confirmed that the weight of white fat was reduced by mTM4SF19-Fc treatment, and an apparent fatty liver inhibitory effect was also confirmed.

[000465] In addition, as a result of observing liver tissues through H&E staining and Masson and trichrome staining, as can be seen from FIG. 47 (left), compared to the hIgG1-Fc-treated control, the effect of decreasing fatty liver was clearly shown. In addition, it was confirmed that triglycerides in plasma decreased (FIG. 47 (right)).

[000466] Example 17: Adipocyte differentiation inhibitory effect by TM4SF19-Fc treatment

[000467] C3H10T1/2 cells were treated with purified mouse TM4SF19-Fc (116-165) and it was confirmed that adipocyte differentiation was dose-dependently inhibited (FIG. 48).

[000468] For a control, a differentiation induction factor was not treated, and for an experimental group, mouse TM4SF19 115-165-Fc as well as the differentiation induction factor (treated with dexamethasone (1 μM), IBMX (500

and migration were examined. As a result, it was confirmed that osteosarcoma cell proliferation, migration and colony formation were increased by TM4SF19 overexpression (FIG. 51, FIG. 52).

[000483] In addition, after knock-out of TM4SF19 in a 143b osteosarcoma cell line through CRISPR, different clones (#4 and #6) were used to examine cell growth through MTT analysis, and examine colony formation.

[000484] As a result, it was confirmed that the proliferation and colony formation of the osteosarcoma cells (143b) are inhibited by the inhibition of TM4SF19 expression (FIG. 53).

[000485] TM4SF19 overexpression increases osteosarcoma cell proliferation and migration. The inhibition of TM4SF19 expression inhibits osteosarcoma cell proliferation and colony formation.

[000486] (2) TM4SF19-Fc treatment

[000487] By treating the 143b osteosarcoma cell line with hTM4SF19-Fc (120-169), cell growth was examined by MTT analysis and a cell count, and colonization ability was examined by colony formation, and cell migration was examined.

[000488] The inhibition of the 143b osteosarcoma cell growth by hTM4SF19-Fc treatment was confirmed by MTT analysis (FIG. 54A) and a cell count (FIG. 54C), the colonization ability was confirmed by colony formation (FIG. 54B), and the cell migration was confirmed (FIG. 54D).