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Conditional Inactivation of TACE by a Sox9 Promoter Leads to Osteoporosis and Increased Granulopoiesis via Dysregulation of IL-17 and G-CSF

Keisuke Horiuchi,‡ Tokuhiro Kimura,‡ Takeshi Miyamoto,‡ Kana Miyamoto,‡ Haruhiko Akiyama,§ Hironari Takaishi,‡ Hideo Morioka,‡ Takashi Nakamura,§ Yasunori Okada,‡ Carl P. Blobel,‡ and Yoshiaki Toyama‡

The TNF-α converting enzyme (TACE/ADAM17) is involved in the proteolytic release of the ectodomain of diverse cell surface proteins with critical roles in development, immunity, and hematopoiesis. As the perinatal lethality of TACE-deficient mice has prevented an analysis of the roles of TACE in adult animals, we generated mice in which floxed Tace alleles were deleted by Cre recombinase driven by a Sox9 promoter. These mutant mice survived up to 9–10 mo, but exhibited severe growth retardation as well as skin defects and infertility. The analysis of the skeletal system revealed shorter long bones and prominent bone loss, probably due to Sox9 expression from the promoter of c-Kit1/Sca-1" lineage cells, and a decrease in lymphopoiesis. Moreover, we found that serum levels of IL-17 and G-CSF were significantly elevated compared with control littermates. These findings indicate that TACE is associated with a regulation of IL-17 and G-CSF expression in vivo, and that the dysregulation in G-CSF production is causally related to both the osteoporosis-like phenotype and the defects in the hematopoietic system. The Journal of Immunology, 2009, 182: 2093–2101.

TACE is essential for membrane-bound pro-TNF-α and membrane-bound EGFR ligands to become fully active in vivo, molecular targeting of TACE is deemed beneficial for the treatment of these disorders. In addition to TNF-α and the ligands for EGFR, TACE has also been implicated in the processing of various membrane-bound molecules involved in the regulation of the immune system and hematopoietic system. These include the receptors for TNF-α, IL-6 receptor, Notch, ICAM-1, VCAM-1, c-Kit ligand, CD44, and cell surface CSF-1, to name a few examples. Because of the perinatal lethality of TACE-deficient mice, radiation-chimeric mice reconstituted with TACE-deficient bone marrow cells were used in several studies to evaluate the functions of TACE in the hematopoietic cells in vivo. However, the consequence of inactivation of TACE in nonhematopoietic cells in adult animals and its potential effects on the hematopoietic system remained poorly understood. In the current study, we took advantage of the Cre-LoxP system and generated conditional TACE-deficient mice, in which a Cre recombinase gene is expressed under the control of a Sox9 promoter (Tacefox; Sox9-Cre), henceforth referred as Tace/Sox9 (10, 23). Sox9 is an essential transcription factor for skeletal development and is expressed in all osteochondroprogenitor cells as membrane protein and is expressed virtually in all organs in vivo, with high levels of expression in the heart, skeletal muscle, lung, placenta, testis, and ovary (5, 7). Intriguingly, subsequent studies have revealed TACE as the key regulator for two clinically important signaling pathways, namely, the TNF-α-TNF receptor and the epidermal growth factor receptor (EGFR) ligands-EGFR signaling pathways. TACE is one of the most crucial modulators of the host defense and of the pathogenesis of various inflammatory disorders, such as endotoxin shock, Crohn’s disease, asthma, and rheumatoid arthritis (8–11), while EGFR signaling is dysregulated in many cancers and related to their etiology (12–14). Because TACE is essential for membrane-bound pro-TNF-α and membrane-bound EGFR ligands to become fully active in vivo, molecular targeting of TACE is deemed beneficial for the treatment of these disorders.
well as in many other organs, including the pancreas, heart, lung, brain, and skin, but not in hematopoietic cells (23, 24).

In this study, we describe the phenotypic analysis of Tace/Sox9 mice, which includes defects in bone metabolism and hematopoiesis, as well as impaired skin development, growth retardation, and infertility. Moreover, we found that the serum levels of G-CSF and its major upstream regulator, IL-17, were both significantly increased in Tace/Sox9 mice compared with control littermates. Because G-CSF is a potent stimulator of both osteoclastogenesis and granulopoiesis, these observations indicate that the dysregulated G-CSF production could be responsible for the osteoporosis-like phenotype and the defects in the hematopoietic system. Therefore, generation of Taceflox/flox, Sox9-Cre knock-in mice to generate Taceflox/flox/Sox9-Cre mice were viable and fertile, and were further crossed with Taceflox/flox mice to generate Taceflox/flox/Sox9-Cre mice. The genotype of the offspring from Taceflox/flox and Taceflox/flox/Sox9-Cre matings at 2 wk after birth followed a Mendelian distribution pattern (total = 166; Taceflox/Sox9, 40; Taceflox/Sox9-Cre, 43; Taceflox/Sox9-Cre, 41; Taceflox/Sox9-Cre, 42). CAG-CAT-Z reporter mice were generously provided by Dr. J.-I. Miyazaki (Osaka University School of Medicine, Osaka, Japan) (25). All mice were maintained under 12 h light-dark cycle with ad libitum access to regular feed and water. The mice were housed in a specific-pathogen-free environment and fed with sterile water and feed. All mice were used in the current study were of mixed genetic background (129Sv, C57BL/6), and all comparisons described in this study were between littermates. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Keio University School of Medicine.

Histology

Tissues were fixed in 4% paraformaldehyde/PBS, sectioned, and then stained with H&E. For 5-BrdU labeling, BrdU (30 μg/g body weight) was injected i.p. 3 h before sacrificing the animal. Immunostaining was performed using the following Abs; myeloperoxidase (MPO; DakoCytomation), CD3 (Serotec), B220 (C57BL/6), and all comparisons described in this study were between littermates. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Keio University School of Medicine.

Materials and Methods

Mice

Generation of Taceflox/flox and Sox9-Cre knock-in mice was previously described (10, 23). Taceflox/flox mice were mated with Sox9-Cre knock-in mice to generate Taceflox/flox/Sox9-Cre mice. Taceflox/flox/Sox9-Cre mice were viable and fertile, and were further crossed with Taceflox/flox mice to generate Taceflox/flox/Sox9-Cre mice. The genotype of the offspring from Taceflox/flox and Taceflox/flox/Sox9-Cre matings at 2 wk after birth followed a Mendelian distribution pattern (total = 166; Taceflox/Sox9, 40; Taceflox/Sox9-Cre, 43; Taceflox/Sox9-Cre, 41; Taceflox/Sox9-Cre, 42). CAG-CAT-Z reporter mice were generously provided by Dr. J.-I. Miyazaki (Osaka University School of Medicine, Osaka, Japan) (25). All mice were maintained under 12 h light-dark cycle with ad libitum access to regular feed and water. The mice were housed in a specific-pathogen-free environment and fed with sterile water and feed. All mice used in the current study were of mixed genetic background (129Sv, C57BL/6), and all comparisons described in this study were between littermates. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Keio University School of Medicine.

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Histomorphometric analysis

All histomorphometric analysis was conducted using 8-wk-old control and Tace/Sox9 mice littermates. Femurs were excised and fixed with 75% ethanol. Bone mineral density of the femurs was measured by Photon for Bone Mineral Quantity (Kyoto Kagaku). Two-dimensional images of the distal femurs were obtained by microcomputed tomography scan, Scan Xmate (Comscantecno) and the three-dimensional images were reconstructed by three-dimensional software, TRI/3D-BON (Ratoc System Engineering). Four mice for each group were analyzed.

Western blot analysis

Primary osteoblasts were harvested from the calvaria of newborn control or Tace/Sox9 mice. Bone marrow macrophages were prepared as described previously (10). In short, bone marrow was collected from the tibiae and femurs of 6-wk-old Tace/Sox9 or littermate control mice. RBCs were removed with RBC lysis buffer (Roche), and the remaining cells were pelleted on petri dishes. Adherent cells were grown in αMEM with 10% FCS, antibiotics, and 50 ng/ml recombinant mouse macrophage CSF (Wako Biochemicals) for 4 days and then used as bone marrow macrophages. The cells were lysed with 1% Triton-X/PBS containing protease inhibitor mixture (Sigma-Aldrich). The sample proteins were separated by SDS-PAGE and Western blots were performed as previously described, using anti-sera against the cytoplasmic domain of TACE (26).

Flow cytometry

Bone marrow cells were isolated by flushing femurs with 5% FCS/PBS, and splenocytes were collected by mechanical disruption of the spleen. The cells were filtered through a cell strainer (BD Falcon) to remove debris. We used the following mAbs for flow cytometric analysis: c-KIt (2B8), Sca-1 (E13-161.7), B220 (RA3-6B2), CD3 (SK-7), Gr-1 (RB6-8C5), IL-17, and Mac-1 (M1/70). All the Abs were from BD Biosciences. The percentages of B220+ CD3− (B lymphocytes) and B220+ CD3+ (T lymphocytes) in the bone marrow and spleen were determined by direct immunofluorescence using a laser flow cytometer (FACSCalibur system, BD Biosciences). A mixture of mAbs against CD4, CD8, B220, TER-119, Mac-1, and Gr-1 was used as a lineage marker (Lineage). For the detection of intracellular IL-17, the spleen cells were fixed and permeabilized using Cytofix/Cytoperm (BD Pharmingen) and were subjected to flow cytometric analysis.

Colonoy forming assay

Colon forming assays were performed using methylcellulose-based medium (Methocult no. 03534, Stem Cell Technologies) following the manufacturer’s instructions. Bone marrow cells (1 × 10^5/35 mm dish) and spleen cells (1 × 10^5/35 mm dish) isolated from 10-wk-old mice were incubated for 7 days and the number of the colonies was counted under a light microscope.

Dual x-ray absorptiometry and microcomputed tomography analysis

All microcomputed tomography analysis was conducted using 8-wk-old control and Tace/Sox9 mice littermates. Femurs were excised and fixed with 75% ethanol, embedded in glycolmethacrylate resin, and sectioned in 3-μm slices. For double labeling, mice were injected i.c. with calcein (8 mg/kg body weight) at 4 and 1 day before sacrifice. The sections were stained with toluidine blue and were subjected to histomorphometric analyses under a light microscope with a micrometer, using a semiautomatic image analyzer (Osteoplan II, Carl Zeiss). Parameters for the trabecular bone were measured in an area 1.62–2.34 mm^2 in size from 1.2 mm above the growth plate at the distal metaphysis. Four mice for each group were analyzed.

Determination of cytokine levels

The serum levels of IL-17, IL-23, G-CSF, soluble c-KIt ligand (sKitL) and SDF-1 were measured by sandwich ELISA (QuantiKine, R&D Systems) following the manufacturer’s instructions. Preparation of bone marrow extracellular extracts was performed as previously described (27). In short, the bone marrow was directly flushed into 500 μl of PBS containing protease inhibitors. The cells were pelleted at 400 × g, 4°C for 5 min. Supernatants containing bone marrow fluids were taken and stored at −80°C until further analysis.

Statistical analysis

All data are presented as mean ± SD. Student’s t test for two samples assuming equal variances was used to calculate the p values. Values of p < 0.05 were considered statistically significant.

Results

Tace/Sox9 mice exhibit skin defects and growth retardation but survive into adulthood

To analyze the functions of TACE in nonhematopoietic cells, we crossed Taceflox/flox mice with Sox9-Cre knock-in mice, in which a Cre recombinase gene preceded by an internal ribosome entry site was inserted into the 3’ untranslated region of the Sox9 gene (23). Sox9 is expressed in a relatively wide range of tissues, including bone, cartilage, skin, lung, pancreas, digestive tract, brain, and kidney as previously described (Fig. 1A) (23, 28, 29). In contrast, very limited expression was seen in the spleen and thymus, indicating that hematopoietic cells do not detectably express Sox9 at any stage of differentiation. Western blot analysis of primary osteoblasts from calvaria showed a nearly complete lack of TACE in the osteoblasts, whereas no significant change in the expression of TACE.
TACE was seen in the bone-marrow derived macrophages (Fig. 1B). *Tace/Sox9* mice were born with open eyes and were indistinguishable from the previously described *Taceαβ/αβ* mice and *Tace−/−* mice (data not shown) (10, 20). However, in contrast to *Taceαβ/αβ* mice and *Tace−/−* mice, which have thickened and misshapen aortic, pulmonic, and tricuspid valves (10, 30), there was no evident defects in these heart valves in *Tace/Sox9* mice (data not shown), even though SOX9 is expressed in the endocardial cushion and the valves (Fig. 1A) (28). None of the other genotypes (*Tacelox/lox*/*Sox9-Cre, *Tacelox−/−*, or *Tacelox+/lox*, henceforth referred to as Control) showed any evident phenotype or histological defects (data not shown). *Tace/Sox9* mice showed growth retardation with skin and hair defects similar to those seen in *Egfr−/−* mice (Fig. 1C) (31–33), but unlike *Egfr−/−* or *Tace−/−* mice, the majority of *Tace/Sox9* mice survived longer than 5 mo (Fig. 1D). The reason(s) for their death after 5 mo is not clear at present. A difference in the body weight became evident after 12 wk of age (Fig. 1E); whereas the average weight of *Tace/Sox9* mice was only up to 15% below that of Control mice until 8 wk of age. Additionally, female *Tace/Sox9* mice were sterile and the fertility of male *Tace/Sox9* mice was severely impaired (data not shown).

*Tace/Sox9* mice exhibit shorter long bones and an osteoporosis-like phenotype

Histological analysis revealed an elongated hypertrophic zone and a shorter proliferating zone in the bones of *Tace/Sox9* mice compared with those of Control mice (Fig. 2A). The elongation of the growth plates was prominent at 2–3 wk of age, and became less evident in older mice. Unexpectedly, we found that the bone marrow of *Tace/Sox9* mice was not replaced with adipose tissue but remained filled with bone marrow cells (Fig. 2A). The bone marrow of metatarsi in Control mice became replaced with adipocytes at around 3 wk of age and was almost completely filled with fat cells by 8 wk after birth; however, the bone marrow of *Tace/Sox9* mice remained filled with bone marrow cells at least up to 4 mo after birth (data not shown). These observations indicated a possible defect in the hematopoietic system in *Tace/Sox9* mice. When DNA synthesis of the growth plate cells was evaluated by BrdU incorporation, there was a significant decrease in the number of BrdU-incorporated cells in *Tace/Sox9* mice (Fig. 2B). X-ray analysis revealed no overt abnormalities in the skeletal system; however, the femur length of *Tace/Sox9* mice was 10–15% shorter and the bone mass was decreased compared with that of Control (Fig. 2C). In contrast, there was no change in the growth rate of primary chondroblasts from rib cartilage or calvarial osteoblasts in vitro, indicating that impaired growth of chondroblasts in vivo was caused by a cell-extrinsic mechanism (data not shown). To further examine the bone defects found in X-ray analysis, we prepared sections from 8-, 16-, and 24-wk-old mice for histological evaluation. Van Gieson-stained sections of distal femurs of *Tace/Sox9* mice revealed prominent bone loss, which was manifested by less trabecular bone and thinner cortical bone (Fig. 3A). This phenotype was already evident at 8 wk and then progressively worsened. Microcomputed tomography analysis confirmed these observations (Fig. 3, B and C). Histomorphometric analysis revealed that both osteoclasts-related parameters (eroded surfaces (ES/BS), osteoclast numbers (N.Oc/B.Pm), and osteoclast surfaces
and osteoblasts-related parameters ((osteoid volume (OV/BV), osteoid surfaces (OS/BS), and osteoblast surfaces (Ob.S/BS)) were increased in Tace/Sox9 mice compared with Control mice. In contrast, the mineral apposition rate (MAR) and bone formation rate (BFR/BS) were not altered. These data show that inactivation of TACE under the control of a Sox9 promoter results in osteoporosis-like phenotype in Tace/Sox9 mice. A, Van Gieson-stained sections of the tibias from 8-, 16-, and 24-wk-old Control and Tace/Sox9 mice. Bars, 250 μm. B, Reconstructed three-dimensional images of microcomputed tomography of the distal femur of 8-wk-old mice. Bars, 1 mm. C, Microcomputed tomography analysis of the femur from 8-wk-old Control and Tace/Sox9 mice. BV/TV, bone volume/total volume; Tb.N, Tb.Sp, trabecular separation; BMD, bone mineral density; Cv, cortical volume; Ct, cortical thickness; *, p < 0.05; **, p < 0.005; n.s., not significant; n = 4 for each genotype. D, Histomorphometric analysis of the femur from 8-wk-old Control and Tace/Sox9 mice. ES/BS, eroded surface/bone surface; N.Oc/B.Pm, osteoclast numbers/osteoclast perimeter; Oc.S/BS, osteoclast surface/bone surface; OV/BV, osteoid volume/bone volume; OS/BS, osteoid surface/bone surface; Ob.S/BS, osteoblast surface/bone surface; MAR, mineral apposition rate; BFR/BS, bone formation rate, surface referent; *, p < 0.05; n.s., not significant; n = 4 for each genotype.
in two different bone defects: 1) elongation in growth plate and shorter long bones (0–8 wk after birth), and 2) high-turnover type osteoporosis characterized by increased activities in both osteoblasts and osteoclasts (8 wk after birth).

Splenomegaly and dysregulated lymphopoiesis and granulopoiesis in Tace/Sox9 mice

In addition to the hypercellularity found in the bone marrow (Fig. 2A), Tace/Sox9 mice had a proportionally larger spleen than in Control mice (Fig. 4A). These observations led us to assess potential defects in the hematopoietic system. Flow cytometric analysis of the bone marrow cells and spleen cells from 8-wk-old Tace/Sox9 mice revealed a relative decrease in the population of B lymphocytes (CD3<sup>-</sup> B220<sup>+</sup>) and T lymphocytes (CD3<sup>+</sup> B220<sup>-</sup>) and an increase in the population of Mac-1<sup>+</sup> Gr-1<sup>+</sup> cells (which includes monocytes/macrophages and neutrophils) compared with those of Control mice (Fig. 4, B and C). There was an absolute lymphopenia in the bone marrow; however the number of lymphocytes in the spleen was comparable in Tace/Sox9 and Control mice. In contrast, no apparent difference in these cell populations was observed in the thymus (data not shown).

Because the soluble form of TNF-α is essential for the development of the lymphoid pulps in the spleen (34), we examined whether this structure was affected in Tace/Sox9 mice. The spleen sections were immunostained with B220 (a B lymphocyte marker) and with CD3 (a T lymphocyte marker). The lymphocytes (B220-positive cells and CD3-positive cells) were fewer and the formation of lymphoid pulps was less mature at 3 wk of age compared with Control mice, indicating that the development of lymphoid follicles was retarded, but not disturbed, at this stage. In contrast, splenomegaly was not evident at this stage of splenic development (Fig. 4D). In 8-wk-old Tace/Sox9 mice, lymphoid follicles had developed to a comparable stage as in Control mice, but an expansion of the red pulp of the spleen led to splenomegaly (Fig. 4E). The thymus was transiently smaller in Tace/Sox9 mice at around 3 wk of age compared with Control; however, it developed to a comparable level by 8 wk after birth without any apparent histological abnormalities (data not shown). These results suggest that
a TACE-deficient environment results in the dysregulation of lymphoid development and granulopoiesis.

**Extramedullary hematopoiesis and increased KSL cell population are observed in adult Tace/Sox9 mice**

Consistent with the results of flow cytometric analysis, histology of the spleen in 8 wk-old Tace/Sox9 mice revealed an increase in the number of granulocytic cells of various differentiation stages, and also megakaryocytes in the red pulp (Fig. 5A). Immunostaining with MPO (a marker for myeloid cells) and vWF (a marker for megakaryocytes) confirmed these observations (Fig. 5B). Thus, the enlargement of the spleen in Tace/Sox9 mice was caused by the expansion of the red pulp filled primarily with proliferating granulocytic cells. Moreover, MPO-positive cells and vWF-positive cells were also present in the liver of Tace/Sox9 mice, indicating the presence of extramedullary hematopoiesis in this organ as well (Fig. 5C). The degree of extramedullary hematopoiesis in the liver became more prominent as these mutant mice became older (data not shown). To assess whether the population of hematopoietic stem cells was also affected in Tace/Sox9 mice, we analyzed the population of KSL cells in bone marrow, which is enriched in hematopoietic stem cells (35). As shown in Fig. 5D, the population of KSL cells was approximately three times higher in the bone marrow of Tace/Sox9 mice compared with that of Control mice. Consistent with these findings, the number of colony forming units (CFU-GM) in the bone marrow and spleen was significantly higher in Tace/Sox9 mice than in Control mice (Fig. 5E). Analysis of complete blood counts revealed a slight, yet statistically significant, increase in the number of white blood cells and platelets, and a decrease in hemoglobin levels and hematocrit (Fig. 5F). These observations suggest that cell-extrinsic inactivation of TACE led to a significant increase in granulopoiesis, which ultimately resulted in extramedullary hematopoiesis in the spleen and liver.

**Serum G-CSF and IL-17 levels are highly elevated in Tace/Sox9 mice**

The phenotypical analysis described above revealed that lack of TACE activity under the control of a Sox9 promoter in vivo resulted in high-turnover type osteoporosis, up-regulation in granulopoiesis and extramedullary hematopoiesis in adult animals. We next sought to answer whether there could be a common cell-extrinsic factor that could perhaps be responsible for the apparently unrelated hematopoietic and bone defects. Various cytokines...
FIGURE 6. Dysregulated production of G-CSF and IL-17 in Tace/Sox9 mice. Analysis of serum G-CSF (A), serum and bone marrow (BM) SDF-1 (B), serum sKitL (C), and serum IL-17 (D) levels in Tace/Sox9 (T/S9) or Control (Cont) mice by ELISA. E: An increase in IL-17 producing cells in Tace/Sox9 mice. n = 3. FACS analysis shown here is a representative result from three independent experiments. **, p < 0.005; n.s., not significant.

Recent studies have revealed IL-17 as an important upstream regulator of G-CSF in vivo (45–48). IL-17 is a proinflammatory cytokine produced by multiple cell types, including CD4⁺ αβ T cells, γδ T cells, NK cells, and neutrophils, and induces granulopoiesis via induction of G-CSF. Furthermore, IL-17 can also indirectly up-regulate osteoclastogenesis (49). To assess a possible involvement of IL-17, we examined the serum IL-17 levels and found that they were significantly elevated in Tace/Sox9 mice compared with Control mice (Fig. 6D, Control, <5 pg/ml; Tace/Sox9, 231 ± 123 pg/ml). Given these observations, we next examined whether the population of IL-17-producing cells was affected in Tace/Sox9 mice, and found that there was an increase in the number of IL-17-producing cells in Tace/Sox9 spleen compared with that in Control animals (Fig. 6E). Of note, most of these cells were negative for CD4, indicating that this cell population predominantly consisted of γδ T cells and/or NK cells. Taken together, these results indicate that a TACE-deficient environment led to a dysregulation of the IL-17/G-CSF axis and that the marked increase in the serum levels of these cytokines was most likely related to the hematopoietic defects and osteoporosis-like phenotype observed in Tace/Sox9 mice.

Discussion

Currently, there are few studies describing the functions of TACE in adult animals, primarily due to the perinatal lethality of Tace⁻/⁻ mice. The Tace/Sox9 mice presented in the current study are unique in that, even with various anomalies in a wide range of organs, most survived to adulthood with little variance in the phenotype among the individual mutant mice. Thus, Tace/Sox9 mice may be very highly useful tools for the analysis of the functions of TACE in vivo. Most importantly, because SOX9 is not expressed in hematopoietic cells and TACE should therefore not be inactivated in lymphocytes or monocytes in Tace/Sox9 mice, the hematopoietic defects are most likely not caused by a cell-autonomous lack of TACE activity in hematopoietic cells.

The defects in the growth plate and the decreased size of long bones observed in Tace/Sox9 mice highly resemble those seen in Egfr⁻/⁻ mice and the mice humanized for EGFR (which have almost complete loss of EGFR expression in the bone cells) (50). Therefore, impaired EGFR signaling in the developing bone appears to be causally related to these defects. Previous studies have shown that at least five EGFR ligands (heparin-binding EGF-like growth factor, amphiregulin, TGFα, epiregulin, and epigen) can be shed by TACE (7, 51, 52), and that loss of TACE in vivo usually results in loss of functional activation of these EGFR ligands. However, because bone defects resembling those in Egfr⁻/⁻ or Tace/Sox9 mice have not yet been reported in mice lacking any of the EGFR-ligands, multiple EGFR ligands, or other unidentified substrates of TACE may also be involved in this phenotype. In contrast, the current study suggests that dysregulation of IL-17 and G-CSF production in Tace/Sox9 mice is, at least in part, responsible for the osteoporosis-like phenotype and hematopoietic defects observed in Tace/Sox9 mice.

Because TACE is usually involved in the processing of membrane-bound molecules (1–4), the dysregulation of IL-17 and G-CSF production in Tace/Sox9 mice was an unexpected result. Macrophages and osteoclasts, but not osteoblasts, express the G-CSF receptor, and an administration of G-CSF leads to increased osteoclast numbers in vivo and in vitro culture system (40). The receptor for IL-17 is expressed on osteoblasts, and it has been shown that IL-17 indirectly stimulates bone resorption through the induction of receptor activator of NF-κB ligand, a membrane bound protein which is expressed on osteoblasts and essential for...
osteoclastogenesis (49). These observations suggest that the osteoporosis-like phenotype and the increase in osteoclast activity observed in Tace/Sox9 mice are likely caused by the increased serum levels of IL-17 and G-CSF. Consistent with this interpretation, human G-CSF transgenic mice, which produce high levels of human G-CSF (~1000 pg/ml) in sera, not only exhibit hematopoietic defects (including granulocytosis, extramedullary hematopoiesis, and increase in hematopoietic stem cell population), but also develop osteoporosis with increased osteoclast activity (41, 53, 54). Because the serum G-CSF levels were even higher in Tace/Sox9 mice (2639 ± 299 pg/ml) compared with that in human G-CSF transgenic mice, it would be reasonable to assume that the defects in both bone metabolism (increased osteoclast activity and loss of bone mass) and hematopoiesis were primarily caused by the elevated G-CSF production. Nevertheless, further studies will be required to confirm the relation between these defects and the dysregulation of IL-17 and G-CSF expression observed in Tace/Sox9 mice.

Recent studies have revealed a regulatory model involved in neutrophil homeostasis and cytokine production (45, 47). The proposed model suggests that sequestration of IL-23 from macrophages and dendritic cells is suppressed by phagocytosis of apoptotic neutrophils, which in turn reduces the production of IL-17 and G-CSF, and ultimately down-regulates granulopoiesis. In the case for leukocyte adhesion molecule-deficient animals, such as Cd18−/− mice (45, 47), the neutrophils are unable to efficiently transmigrate into critical peripheral tissues, and therefore macrophages and dendritic cells cannot phagocyte apoptotic neutrophils. Consequently, these cells continue to produce IL-23, which in turn results in an increased granulopoiesis via IL-17 and G-CSF. Along these lines, a concomitant increase in serum G-CSF and in IL-17 levels in Tace/Sox9 mice was found. However, any increase in serum IL-23 was minor compared with the substantial increase in IL-17 and G-CSF levels (the serum IL-23 levels were above the detection limit (≥2.28 pg/ml); according to the manufacturer’s instructions) only in one of nine Control mice (3.9 pg/ml), whereas they were detectable in six of seven Tace/Sox9 mice (average, 5.8 pg/ml)). Furthermore, a single injection of IL-17 neutralizing Ab into Tace/Sox9 mice did not significantly affect the serum G-CSF levels (data not shown). Therefore, although we cannot rule out possible involvements of insufficient neutrophil transmigration or defective phagocytosis by macrophages and dendritic cells in Tace/Sox9 mice, it is more likely that a TACE-deficient environment directly or indirectly leads to the increased production of IL-17 and G-CSF.

The link between the elevation of serum IL-17, G-CSF, and the lack of TACE in SOX9-expressing cells remains to be determined.

Processing of membrane-bound c-KIT ligand by MMP-9 and/or Cathepsin K and the increase in the plasma sKITL have been shown to increase mobilization of median bone marrow progenitors (43, 44). However, we found that the serum levels of sKITL were significantly lower in Tace/Sox9 mice, suggesting that release of sKITL may not be absolutely required for G-CSF-induced stem cell mobilization (55, 56). Because the membrane-bound c-KIT ligand has indispensable roles in hematopoiesis, as highlighted by the impaired development of hematopoietic cells in the mice with Steel-Dickie mutation, in which only a soluble truncated c-KIT ligand is encoded (57), it is possible that altered processing of c-KIT ligand in the hematopoietic niche in Tace/Sox9 mice may also have contributed to the dysregulation of KSL cells population. Nevertheless, the current study further corroborates TACE as the major sheddase for membrane-bound c-KIT ligand (17).

In conclusion, the current study revealed an unexpected role for TACE in the modulation of bone metabolism and hematopoiesis in vivo. Our data show that conditional inactivation of TACE under the control of a Sox9 promoter led to a dysregulation in IL-17 and G-CSF production, which ultimately resulted in diverse defects in both bone metabolism and hematopoiesis in adult animals, thereby providing the first evidence for a role of TACE in the regulation of IL-17/G-CSF. Because IL-17/G-CSF is apparently not a direct target molecule for TACE, further investigations will be necessary to elucidate the causal link between TACE-deficient environment and the increase in IL-17 production. Nevertheless, a wide variety of defects and the early death in Tace/Sox9 mice unequivocally demonstrate indispensable roles of TACE in normal bone growth and adult homeostasis.

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Disclosures

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References


