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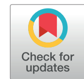
Mex3B inhibits DC-STAMP mRNA level and osteoclastogenesis



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ABSTRACT

Bone homeostasis is maintained through continuous remodeling by osteoclast-driven bone resorption and osteoblast-mediated bone formation. Osteoclasts are multinucleated giant cells (MNCs) differentiated from myeloid progenitors of the monocytic lineage. During osteoclast maturation, DC-STAMP (dendritic cell specific transmembrane protein) has been shown as a master determinant of osteoclast cell fusion. In this study, we demonstrate that Mex3B inhibits osteoclast fusion protein DCSTAMP expression and osteoclastogenesis. During differentiation of osteoclasts, the expression of Mex3B is down-regulated by cytokines such as RANKL and TNFa, resulting in relief of Mex3B-mediated down-regulation of DC-STAMP mRNA level. Our findings not only reveal critical mechanisms on regulation of DC-STAMP-mediated osteoclastogenesis, but also point to Mex3B as a potential therapeutic target for the treatment of human bone diseases.

Dear Editor,

Bone homeostasis is maintained through continuous remodeling by osteoclast-driven bone resorption and osteoblast-mediated bone formation. De-regulation of bone homeostasis causes diseases of adults such as periodontitis, rheumatoid arthritis, osteoporosis and osteopetrosis (Kim et al., 2020). Osteoclasts are multinucleated giant cells (MNCs) differentiated from myeloid progenitors of the monocytic lineage which is triggered by macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). During the osteoclast maturation, single-nucleated monocytes differentiate into multinucleated osteoclasts through several rounds of cell fusion. DC-STAMP (dendritic cell specific transmembrane protein) has been shown as a master determinant of osteoclast cell fusion. DC-STAMP-knockout mice exhibits a complete loss of cell fusion in osteoclasts and severely decreased bone resorption. The expression level of DC-STAMP determines the frequency of cell fusion and bone resorption activity (Yagi et al., 2005). However, how the level of DC-STAMP is regulated during osteoclast differentiation remains enigmatic.

We previously identified the RNA-binding protein Mex3B as a coreceptor of TLR3 in innate immunity (Yang et al., 2016). In this study, we identified an unexpected role of Mex3B in inhibition of osteoclast formation and bone resorption by negatively regulating DC-STAMP expression. We initially compared the transcription profiles between *Mex3b*$^{+/+}$ and *Mex3b*$^{-/-}$ bone marrow–derived macrophages (BMMs) upon stimulation with TNFα. Gene ontology analysis showed that the mRNA levels of genes involved in immune processes, extracellular matrix secretion, metabolism, and osteoclast differentiation were markedly altered (Figure S1A). Among them, the mRNA level of DC-STAMP was dramatically enhanced in *Mex3b*$^{-/-}$ BMMs (Fig. S1A). To confirm these results, we treated BMMs with RANKL or TNFα in the presence of M-CSF for various times. Consistent with previous studies, the mRNA levels of osteoclast-specific genes such as DC-STAMP, OC-STAMP and TRAP were dramatically induced at early time points after RANKL or TNFα stimulation and returned to normal levels at late time points in wild-type BMMs (Fig. 1A). In contrast, the mRNA level of Mex3B was high in unstimulated BMMs, and down-regulated at the early time points after RANKL or TNFα treatment and increased at the late time points (Fig. 1A). In addition, the mRNA level of DC-STAMP was markedly increased in un-stimulated or RANKL or TNFα-stimulated *Mex3b*$^{-/-}$ BMMs, whereas the mRNA levels of OC-STAMP and TRAP were comparable in *Mex3b*$^{+/+}$ and *Mex3b*$^{-/-}$ BMMs (Fig. 1A). In *Mex3b*$^{-/-}$ BMMs, DC-STAMP mRNA level showed time point-dependent increase and later dropped down to a level similar to WT control, indicating that other regulatory molecules, such as transcription factors IRF8 and Tal1, are also involved in down-regulating the expression of DC-STAMP. We further investigated how Mex3B regulates the mRNA level of DC-STAMP. qPCR assays indicated that nascent transcription of DC-STAMP mRNA was markedly enhanced in *Mex3b*$^{-/-}$ BMMs, indicating that Mex3B regulates transcription of the DC-STAMP gene or stability of nascent transcription RNA (Fig. 1B). These results suggest that Mex3B negatively regulates DC-STAMP expression under physiological and inflammatory conditions by down-regulating its mRNA level.

Since inflammatory cytokines play an essential role in pathological bone resorption (Tsukasaki & Takayanagi, 2019), we next examined whether Mex3B is involved in inflammatory response triggered by microbial components and certain inflammatory cytokines. We found that the mRNA level of Mex3B was markedly down-regulated after LPS and Pam3CSK4 but not IFNβ or IL-6 treatment. Transcription of their respective downstream genes induced by all these stimulants was comparable between *Mex3b*$^{+/+}$ and *Mex3b*$^{-/-}$ cells (Fig. S1B). These results suggest that Mex3B is specifically involved in signaling triggered by certain inflammatory stimulant or cytokines.

We next examine the role of Mex3B in osteoclastogenesis. RANKL-induced osteoclastogenesis of the *Mex3b*$^{-/-}$ precursor BMMs was markedly increased in comparison with *Mex3b*$^{+/+}$ cells as shown by increased

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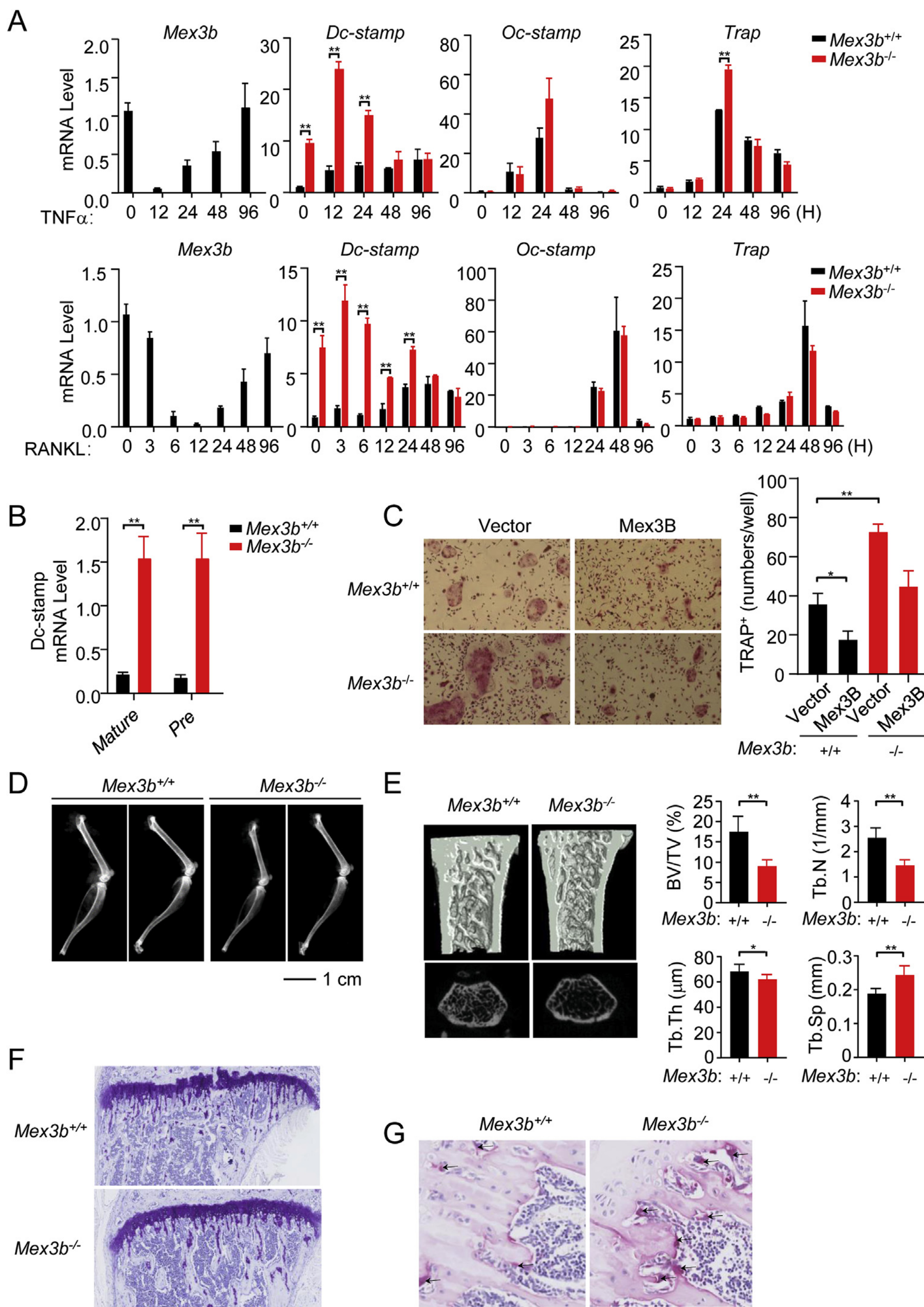

Fig. 1. Mex3B inhibits DC-STAMP mRNA level and osteoclastogenesis.
(A) Effects of Mex3b-deficiency on the mRNA levels of osteoclast-related genes in BMMs. *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ BMMs (2×10^5) cultured in M-CSF (50 ng/ml) were treated with TNFα (10 ng/ml) or RANKL (50 ng/ml) for the indicated times before qPCR analysis. Data shown are mean ± SEM, n = 2. $*P < 0.05$, $**P < 0.01$ (Student's *t*-test). Data are from one representative of three independent experiments with similar results.
(B) Levels of DC-STAMP pre-mRNA and mature mRNA in *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ BMMs. qPCR analysis was performed with primers specifically for pre-mRNA or mature mRNA of DC-STAMP. Data shown are mean ± SEM, n = 2. $*P < 0.05$, $**P < 0.01$ (Student's *t*-test). Data are from one representative of three independent experiments with similar results.
(C) *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ BMMs cultured in M-CSF (50 ng/ml) were transduced with Mex3B by retroviral-mediated gene transfer. Twenty-four hours after transduction, cells were treated with RANKL (50 ng/ml) for 5 days. Osteoclastogenesis was evaluated by TRAP staining (left) and the numbers of multinuclear TRAP-positive cells were shown in the right histograph. Data shown are mean ± SEM, n = 3. $*P < 0.05$, $**P < 0.01$ (Student's *t*-test). Data are from one representative of three independent experiments with similar results.
(D) Soft-X-ray photographs of the femur and tibia in *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ mice. n = 6. Scale bar, 1 cm.
(E) Microcomputed tomography of the femurs of *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ mice (left), and bone morphometric analysis of femurs isolated from *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ mice (right). BV/TV, bone volume per tissue volume; Tb.Th, trabecular bone thickness; Tb.N, trabecular number; Tb. Sp, trabecular separation. n = 5. $*P < 0.05$, $**P < 0.01$ (Student's t-test).
(F) Toluidine blue staining of tibial sections of *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ mice. n = 6.
(G) TRAP staining of tibial sections of *Mex3b*$^{+/+}$ or *Mex3b*$^{-/-}$ mice. n = 6.

number of cells with >3 nuclei and larger TRAP-stained osteoclasts (Fig. 1C). Reconstitution of Mex3B in *Mex3b*$^{-/-}$ BMMs inhibited RANKL-induced multinucleation and cell fusion (Fig. 1C). These results were further confirmed in mouse mononuclear macrophage cell line RAW264.7, which can differentiate to osteoclasts upon RANKL stimulation. Overexpression of wild-type Mex3B but not RNA binding-deficient mutant Mex3B$^{G83/177D}$ in RAW264.7 cells resulted in a decrease in the mRNA level of DC-STAMP (Fig. S1C). Consistently, overexpression of wild-type Mex3B but not Mex3B$^{G83/177D}$ inhibited osteoclastogenesis in RAW264.7 cells upon RANKL stimulation as characterized with a lower number and smaller size of mature osteoclasts (Fig. S1D). The expression level of wild type Mex3B was lower than Mex3B$^{G83/177D}$ mutant in RAW264.7 cells, which was consistent with a previous report that Mex3B destabilizes its own mRNA by binding to the 3' long conserved untranslated region (Takada et al., 2009). These results suggest that Mex3B is a negative determinant of osteoclast fusion by down-regulating DC-STAMP expression. It is plausible that Mex3B may down-regulate DC-STAMP mRNA level also by binding to and destabilizing it.

Finally, we analyzed the bone phenotypes of *Mex3b*$^{+/+}$ and *Mex3b*$^{-/-}$ mice. Soft X-ray analysis of the femurs and tibia showed *Mex3b*$^{-/-}$ mice had reduced radiopacity, implying a decrease in bone density (Fig. 1D). Microcomputed tomographic analysis of the femurs and toluidine blue staining of tibial sections showed that the trabecular bone volume, number, and thickness were markedly lower in *Mex3b*$^{-/-}$ mice,

suggesting that the *Mex3b*$^{-/-}$ mice have reduced bone mass and enhanced bone resorption activity (Fig. 1E&F). TRAP staining of proximal tibias sections showed that *Mex3b*$^{-/-}$ mice had increased accumulation of osteoclasts and larger osteoclast relative surface area (Fig. 1G). Moreover, we observed that *Mex3b*$^{-/-}$ mice of advanced age (>6 months) showed obviously smaller skeleton and reduced body weight compared to *Mex3b*$^{+/+}$ mice (Fig. S1E). These results suggest that Mex3B inhibits osteoclastogenesis and bone remodeling both *in vitro* and *in vivo*.

Based on our data, we propose a simplified model on how Mex3B functions in osteoclast formation and bone resorption. During differentiation of osteoclasts, the expression of Mex3B is down-regulated by cytokines such as RANKL and TNFα, resulting in relief of Mex3B-mediated down-regulation of DC-STAMP mRNA level. The increased expression of DC-STAMP promotes osteoclastogenesis. Our findings not only reveal critical mechanisms on regulation of DC-STAMP-mediated osteoclastogenesis, but also point to Mex3B as a potential therapeutic target for the treatment of human bone diseases.

Author contribution

Y.Y. designed research; Y.Y., S.-Y.W., Z.-Q.L., and H.-N.W. performed research; Y.Y., and S.-Y.W. analyzed data; Y.Y. wrote the manuscript.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellin.2021.100002.

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