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MFG-E8, a novel homeostatic regulator of osteoclastogenesis

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Abstract

Although the glycoprotein MFG-E8 (milk fat globule-epidermal growth factor-factor 8) has been investigated extensively as an anti-inflammatory and homeostatic molecule, a possible role in bone homeostasis and disease was not addressed until recently. Our group has now shown that MFG-E8 is expressed by human and mouse osteoclasts and regulates their differentiation and function (Abe et al., J Immunol 2014;193:1383–1391). Whereas genetic deficiency or antibody-mediated neutralization of MFG-E8 enhances osteoclastogenesis and promotes inflammation-induced bone loss in mice, local administration of recombinant MFG-E8 blocks bone loss. These findings establish MFG-E8 as a novel homeostatic regulator of osteoclastogenesis and suggest that MFG-E8 could be exploited therapeutically to treat disorders associated with inflammatory bone loss, such as periodontitis and rheumatoid arthritis.

Introduction

The milk fat globule-epidermal growth factor (EGF)-factor 8 (MFG-E8; also known as lactadherin) is a secreted glycoprotein expressed in a range of tissues by a variety of cells including macrophages, fibroblasts, dendritic and epithelial cells [1]. In addition to a *N*-terminal signal peptide required for secretion, the molecule consists of two *N*-terminal EGF-like domains and two *C*-terminal discoidin-like domains with sequence homology to blood coagulation factors V and VIII [2]. MFG-E8 was shown to promote the phagocytosis of apoptotic cells by acting as an opsonin that bridges phosphatidylserine on apoptotic cells (bound by the *C*-terminal discoidin-like domains of MFG-E8) to αvβ3 integrin on phagocytes (bound by an RGD motif in the *N*-terminal region of MFG-E8) [3]. Efficient apoptotic cell clearance can prevent secondary necrosis and unwarranted inflammation. Moreover, MFG-E8 was shown to have direct anti-inflammatory properties and to suppress inflammatory tissue damage in several disease models [4]. In both humans and animal models, the expression of MFG-E8 declines considerably in inflammatory conditions [1], suggesting the potential use of MFG-E8 as a biomarker. In this regard, a recent study has demonstrated a negative association between the serum levels of MFG-E8 and the severity of coronary artery stenosis [5]. The authors suggested that MFG-E8 could serve as a marker

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Conflict of interest

The author declares that there is no conflict of interest.

of vascular complications or even be considered as a new therapeutic approach for atherosclerosis.

MFG-E8 is structurally similar with developmental endothelial locus-1 (Del-1) [2], an endothelial cell-secreted glycoprotein that regulates inflammatory cell recruitment [6,7]. MFG-E8 and Del-1 share about 50% amino-acid identity and Del-1 contains an additional EGF domain (*i.e.*, it has three EGF and two discoidin-like domains). Our group has recently shown that Del-1 acts homeostatically to regulate local inflammation in periodontitis [8], a biofilm-induced inflammatory disease causing loss of bone support of the dentition [9]. Because of its documented anti-inflammatory effects and its structural similarity with Del-1, MFG-E8 attracted the attention of our research group and we thus set out to determine its role in periodontitis [10]. To this end, we used the ligature-induced periodontitis model in mice, where the placement of silk ligatures around molar teeth facilitates bacteria-mediated inflammation and bone loss [11].

Osteoclasts express and release MFG-E8

In the first experiment, we monitored the expression of MFG-E8 mRNA in the periodontal tissue. Consistent with the demonstrated downregulation of MFG-E8 expression in several models of inflammation [1], the periodontal MFG-E8 mRNA levels were drastically decreased within 24h following placement of the ligatures. Subsequently, and quite unexpectedly, the expression of MFG-E8 mRNA exhibited progressive elevation for several days (until day 8). The resurgence of MFG-E8 expression correlated with the appearance of osteoclasts (OCLs), giant multinucleated cells (MNCs) that resorb bone during physiological bone remodeling but also under pathologic inflammatory conditions (*e.g.*, rheumatoid arthritis and periodontitis) that greatly potentiate their resorptive activity [12,13]. When the numbers of OCLs in the periodontal tissue dropped (from day 8 to day 10), so did the expression of MFG-E8. Moreover, we detected MFG-E8 protein in regions coinciding with the expression of cathepsin K (the predominant osteoclastic protease) and the presence of tartrate-resistant acid phosphatase (TRAP) positive MNCs.

The stunning spatial and temporal correlation of MFG-E8 re-expression with osteoclastogenesis prompted us to examine the intriguing possibility that MFG-E8 may derive from OCLs in the course of periodontitis. Indeed, we showed for the first time that OCLs express and release MFG-E8 protein, as shown by cell immunofluorescence, immunoblotting of whole-cell lysates, and immunoprecipitation from culture supernatants. MFG-E8 protein was detected in three different systems of receptor activator of NF-κB ligand (RANKL)-induced osteoclastogenesis, including osteoclasts generated from RAW264.7 cells, mouse bone marrow-derived precursors, and human CD14+ monocytes.

MFG-E8 regulates OCL differentiation and function in vitro and in vivo

To characterize the role of MFG-E8 in osteoclastogenesis, we generated OCLs from wild-type (WT) or MFG-E8-deficient (*Mfge8*$^{-/-}$) osteoclast precursors (OCPs) from the bone marrow of mice. *Mfge8*$^{-/-}$ OCPs underwent more efficient osteoclastogenesis than WT OCPs, consistent with higher expression of OCL differentiation and functional markers. Moreover, *Mfge8*$^{-/-}$ OCLs caused enhanced resorption pit formation in comparison to their

WT counterparts. Furthermore, exogenously added recombinant MFG-E8 inhibited RANKL-induced expression of OCL markers (NFATc1, β3 integrin, and cathepsin K), osteoclastogenesis from mouse or human osteoclast precursors, and resorption pit formation. These data indicate that MFG-E8 is a novel negative regulator of osteoclastogenesis, at least *in vitro*.

To test the relevance of MFG-E8 in *in vivo* osteoclastogenesis, we subjected WT and *Mfge8*$^{-/-}$ mice to ligature-induced periodontitis. *Mfge8*$^{-/-}$ mice exhibited more bone loss and increased osteoclastogenesis in the periodontal tissue than WT controls. Consistent with this, local gingival microinjection of an anti-MFG-E8 mAb (but not isotype control) exacerbated ligature-induced periodontal bone loss in WT mice. Moreover, in a model of aging-associated periodontitis, *Mfge8*$^{-/-}$ mice experienced >60% more naturally occurring chronic periodontal bone loss than age-matched WT controls. Taken together, these data support the importance of endogenous MFG-E8 in bone homeostasis [10]. In this regard, our preliminary (unpublished) experiments using micro-computed tomography (μCT) indicated a modest reduction in the tissue mineral density of tibiae of *Mfge8*$^{-/-}$ mice as compared to WT controls, suggesting that MFG-E8 might also regulate bone mass in the absence of an inflammatory condition.

MFG-E8 inhibits experimental periodontitis

The observed upregulation of MFG-E8 during osteoclastogenesis is in line with most biological systems where the expression of negative regulators is upregulated to control functional activity and prevent pathological states [14,15]. Whereas endogenously produced MFG-E8 acts homeostatically to restrain or mitigate unwarranted osteoclastogenesis, high concentrations of exogenously added MFG-E8 could inhibit further this process and provide a therapeutic effect. In this regard, we showed that local gingival microinjection of recombinant MFG-E8 inhibited bone loss in WT mice subjected to ligature-induced periodontitis. Similar to its effect in WT mice, recombinant MFG-E8 also protected *Mfge8*$^{-/-}$ mice against ligature-induced bone loss. We also observed decreased expression of proinflammatory cytokines and chemokines (*e.g.*, IL-6, IL-17, and CXCL2) in the periodontal tissue of MFG-E8 treated mice undergoing ligature-induced periodontitis (as compared with controls), which is consistent with the reported anti-inflammatory action of MFG-E8 [2,16,17]. The ability of MFG-E8 to inhibit the expression of proinflammatory molecules suggests an indirect way by which MFG-E8 can down-regulate osteoclastogenesis and bone loss. Taken together with the strong connection between inflammation and osteoclastogenesis [18], our findings suggest that the therapeutic application of MFG-E8 is capable of a two-pronged attack on periodontitis and perhaps other inflammatory bone disorders (*e.g.*, rheumatoid arthritis and ankylosing spondylitis).

Interestingly, MFG-E8 deficiency was associated with elevated total microbiota counts in the periodontal tissue and, accordingly, treatment of WT mice with rMFG-E8 significantly decreased the bacterial load. However, MFG-E8 failed to exert direct antimicrobial activity in disk inhibition zone assays. We concluded that the suppressive effect of MFG-E8 on the microbiota is likely mediated by its capacity to inhibit inflammation and thereby to limit growth of periodontal bacteria that utilize tissue breakdown products (*e.g.*, peptides from

collagen degradation and heme-containing compounds)[19]. In this regard, earlier work showed that local treatments with anti-inflammatory or pro-resolution agents causes a significant reduction in the total counts of periodontal bacteria in animal models of periodontitis [20,21].

Conclusion: MFG-E8 as a potential therapeutic in inflammatory bone loss

In contrast to inflammatory conditions, such as sepsis, colitis, acute lung injury, ischemia/reperfusion injury, atherosclerosis, and Alzheimer's disease, where MFG-E8 expression is downregulated [1,22, 23], there are certain pathological conditions (chronic pancreatitis, obesity, and tumorigenesis) in which MFG-E8 is expressed at high levels and is implicated in their pathogenesis [24–26]. Therefore, caution is required in future MFG-E8 based therapeutic strategies involving systemic administration, although the local administration of MFG-E8 in conditions with localized bone loss (*e.g.*, periodontitis and rheumatoid arthritis) is unlikely to involve undue risks. In conclusion, our study shows that endogenously produced MFG-E8 acts in an autocrine manner to regulate OCL homeostasis, and provides proof-of-principle that recombinant MFG-E8 can be considered as a new therapeutic platform for the treatment of inflammatory bone loss.

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