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Fibrillin-1 insufficiency alters periodontal wound healing failure in a mouse model of Marfan syndrome

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ABSTRACT

Objective: Marfan syndrome (MFS) is a systemic connective tissue disorder caused by insufficient fibrillin-1 (FBN-1), a major component of microfibrils that controls the elasticity and integrity of connective tissues. FBN-1 insufficiency in MFS leads to structural weakness, which causes various tissue disorders, including cardiovascular and periodontal disease. However, the role of FBN-1 insufficiency in the destruction and regeneration of connective tissue has not yet been clarified. To investigate the role of FBN-1 insufficiency in tissue destruction and regeneration.

Design: We used a ligature-induced (LI) periodontal disease model in fbn-1-deficient mice (fbn-1$^{c1039G/+}$ mice) with MFS and investigated the regeneration level of periodontal tissue and as an inflamatic marker, the expression of the matrix metalloproteinase (mmp)-9 and tumor necrosis factor (tnf)-α.

Results: Interestingly, fbn-1$^{c1039G/+}$ mice exhibited slowed wound healing compared with wild type mice, but periodontal tissue destruction did not differ between these mice. Moreover, fbn-1$^{c1039G/+}$ mice exhibited delayed bone healing in association with continuous mmp-9 and tnf-α expression. Furthermore, inflammatory cells were obvious even after the removal of ligatures.

Conclusion: These data suggest that fibrillin-1 insufficiency in fbn-1$^{c1039G/+}$ mice interfered with wound healing in connective tissue damaged by inflammatory diseases such as periodontal disease.

1. Introduction

Marfan syndrome (MFS) is an autosomal dominant disorder of connective tissue that affects approximately 1 in 5000 people (Judge & Dietz, 2005). MFS is caused by missense mutations of FIBRILLIN-1 (FBN-1) (Dietz et al., 1991), a component of extracellular microfibrils, leading to a systemic disorder of connective tissues, including aortic aneurysms and dissection, ocular lens dislocation, emphysema, bone overgrowth and severe periodontal disease (Judge & Dietz, 2005; Straub, Grahame, Scully, & Tonetti, 2002). FBN-1 is a 350-kDa glycoprotein (Sakai, Keene, & Engvall, 1986) that consists of three functional FBN-1-1 genes (FBN-1, -2, and -3) and exhibits superimposable modular structures consisting of 46/47 epidermal growth factor (EGF)-like domains (Kielty, Sherratt, & Shuttleworth, 2002). FBN-1 is a major insoluble extracellular matrix component in connective tissue microfibrils and limits tissue elasticity via fibrillin-1 microfibril formation (Noda et al., 2013). Fibrillin-rich microfibrils contribute to the extracellular regulation of endogenous transforming growth factor-β (TGF-β) activity by providing a structural platform, i.e., latent TGF-β-binding proteins (LTBPs) (Ramirez & Sakai, 2010). FBN-1 haploinsufficiency impairs tissue integrity and dysregulates TGF-β activation and signaling, resulting in the up-regulation of tissue destruction-related genes such as mmp-9 (Chung et al., 2007; Neptune et al., 2003). Anti-TGF-β therapy is being studied as a general therapy to delay or prevent tissue destruction in MFS patients. Treatment with losartan, an angiotensin II type I receptor blocker that can attenuate TGF-β signaling, in a mouse model of MFS prevented aortic root growth by suppressing elastic fiber fragmentation. Losartan treatment also decreased the rate of aortic root dilation in children with MFS (Brooke et al., 2008).

The activation of IL-6-STAT3 signaling has also been shown to

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contribute to aneurysmal dilation in the mgR/mgR hypomorphic fibrillin-deficient mouse model via increased mmp-9 activity, which results in collagen fibril degradation (Ju et al., 2014). These findings suggested that long-term disease progression in MFS is caused by a pathogenic immune response that interferes with tissue remodeling and repair after injury. Administration of β-adrenergic receptor or angiotensin II receptor blocker type 1 antagonist and precise surgical treatment are important for the treatment of MFS (Milewicz, Dietz, & Miller, 2005; Shores, Berger, Murphy, & Pyeritz, 1994), but wound healing in destroyed connective tissue and damaged tissue is not improved. Thus, clarifying the role of FBN-1 insufficiency in the inflammatory destruction and regeneration of connective tissue is important (Saito & Tsuji, 2012).

In untreated patients, periodontal disease results in soft tissue destruction and progressive bone destruction, which lead to tooth mobility and subsequent tooth loss (Kinney, Ramseier, & Giannobile, 2007; Suda, Moriyama, & Ganburged, 2013). Periodontal disease is caused by a bacterial infection that activates the innate immune response via Toll-like receptors, resulting in the up-regulation of innate immunity cytokines such as tnf-α, IL-1, and IL-6 to ultimately result in progressive tissue destruction (Garlet, 2010). MFS has been shown to increase the susceptibility to severe periodontal disease in association with periodontal ligament dysfunction due to microfibril insufficiency, suggesting that FBN-1 microfibril formation plays a central role in periodontal ligament formation (Shiga et al., 2008; Straub et al., 2002). Notably, the elastic fibers of the periodontal ligament, known as oxytalan fibers, primarily consist of FBN-1 microfibrils and do not contain significant amounts of elastin. Therefore, the periodontal ligament is likely more susceptible than other connective tissues to breakdown in the MFS mouse model. Thus, periodontal disease is a useful model to assess the effect of MFS on inflammatory tissue destruction.

In this study, inflammatory tissue destruction and wound healing were investigated in a periodontal disease model, fbn-1$^{C1039G/+}$ mice, to elucidate the effect of FBN-1 insufficiency on the progression of periodontal disease.

2. Materials and methods

2.1. Animals

C57BL/6NCrSlc mice were purchased from Sankyo Labo Service Corporation (Tokyo, Japan), and fbn-1$^{C1039G/+}$ mice were generously provided by Dr. Harry C. Dietz (Johns Hopkins University School of Medicine, USA). All experimental protocols were approved by Tohoku University and Tokyo University of Science Animal Care and Committee.

2.2. Experimental periodontal disease model

The ligature-induced (LI) periodontal disease mouse model was generated by inserting silk ligatures (5–0) (Johnson and Johnson, New Brunswick, NJ, USA) into the lower and upper second molars of 6-week-old C57BL/6NcrSlC mice (WT) and fbn-1$^{C1039G/+}$ mice (C1039G/+) under Nembutal-induced deep anesthesia. This process activated inflammatory responses against the increased presence of microbial biofilm and destruction of alveolar bone (Jin et al., 2007). Non-ligated molars served as controls (W/O). To investigate wound healing in the periodontal disease model, the mandibles were scanned using μCT (Morita, Kyoto, Japan).

2.3. Quantification of alveolar bone loss

For a quantitative three-dimensional (3-D) analysis of alveolar bone loss, the mandibles and maxillae were scanned using CT (Hitachi Aloka Medical). The 3-D views were constructed with the AVIZO imaging software program (Visualization Sciences Group, Burlington MA, USA). Alveolar bone loss was analyzed by measuring the distance from the lingual cemento-enamel junction (CEJ) of the second molar to the lingual-mesial root to the lingual alveolar bone crest (ABC) parallel to the sagittal plane. The distance from the CEJ to the ABC (ABC-CEJ) was used to quantify bone resorption.

2.4. Histological and histochemical analysis

Harvested tissues were fixed with 4% paraformaldehyde and decalcified with 20% formic acid and 10% citric acid for 3 days. Then, 5 μm sections were stained with hematoxylin and eosin. For the immunohistochemical analysis, the sections underwent antigen retrieval with 0.1% pepsin in 0.01 N hydrochloric acid before being incubated with primary antibodies at 4 °C overnight and secondary antibodies at RT for 1 h. The following primary antibodies were used: rabbit anti-Fibrillin-1 (gifted by Nakamura Tomoyuki, Kansai Medical University, Japan), goat anti-Collagen I (SouthernBiotech, Birmingham, AL, USA), rat anti-Ly6G (Abcam, Cambridge, UK), goat anti-MMP-9 (R&D, Minneapolis, MN, USA) and rabbit anti-TNF alpha (Abcam). The following secondary antibodies were used: Alexa Fluor 555 donkey anti-rabbit IgG (Life Technologies, Grand Island, NY, USA), donkey anti-rat IgG (Life Technologies) and donkey anti-goat IgG (Life Technologies). After antibody incubation, the nuclei were stained with Hoechst (Life Technologies). The samples were observed under a confocal laser scanning microscope (LSM510; Carl Zeiss, Oberkochen, Germany), and the fluorescence intensity was quantified by ImageJ.

2.5. Cell culture

A dental mesenchymal cell line (m3cl-60) was maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's nutrient F-12 medium (DMEM/F12; Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (Life Technologies), 31 μg/ml of penicillin, 50 μg/ml of streptomycin, 10 μg/ml of insulin and 10 μg/ml of transferrin (Sigma).

2.6. Gene expression

RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan), and cDNA was synthesized from 1 μg of RNA using 5 × RT Master Mix (TOYOBO, Osaka, JAPAN). Real-time PCR was performed using KOD SYBR qPCR Mix (TOYOBO) according to the manufacturer's protocol. The following cycling conditions were used: 40 cycles of 95 °C for 15 s and 60 °C for 45 s. The expression of the target gene was normalized to that of the internal standard gene β-actin. The specific primer pairs used were as follows: fbn-1 (forward, 5′AAGGGGTTAATGTCATGATGT CAC-3′ reverse, 5′-CCACACAAGAACATAAAACCAAGG-3′), β-actin (forward, 5′-TCACAGGATGCAGAAGGAGA-3′ reverse, 5′-GCTGGAAGG TGGACAGTGAG -3′), collagen I (colI) (forward, 5′-ACGCCATCAAGGT CTACTGC-3′ reverse, 5′-GAATCCATCGGTCATGCTCT-3′), type 12 collagen (col XII) (forward, 5′-CTATTGTGGTGCCAGGGAAT-3′ reverse, 5′-CCTT-GGTCCACTTCTTGGAA-3′), and mmp-9 (forward, 5′-TGAATCA GCTGGCTTTTGTG-3′ reverse, 5′-ACCTTCCAGTAGGGGCAACT-3′).

3. Results

3.1. Spatiotemporal changes of LI

We hypothesized that this LI model recapitulated not only the tissue destruction process but also the wound healing process (Eskan et al., 2012) 14 days after the placement of the ligature in WT (Fig. 1A). To analyze spatiotemporal changes in alveolar bone loss and regeneration in mandibles, periodontal disease model mice were scanned by μCT. The resultant μCT images showed that model mice exhibited progressive bone resorption after 3–14 days of ligation compared with control mice. By contrast, periodontal disease model mice exhibited