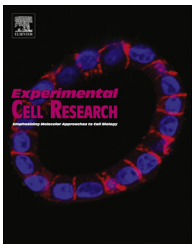
EXPERIMENTAL CELL RESEARCH 328 (2014) 217–227

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr



Research Article

Matrix metalloproteinase-8 regulates transforming growth factor-β1 levels in mouse tongue wounds and fibroblasts in vitro

Pirjo Åström[a,g], *Emma Pirilä*[a,g], *Riitta Lithovius*[a], *Heidi Heikkola*[b], *Jarkko T. Korpi*[c], *Marcela Hernández*[d], *Timo Sorsa*[b,h], *Tuula Salo*[a,e,f,g,*]

[a]*Department of Diagnostics and Oral Medicine, Institute of Dentistry, University of Oulu, PO Box 5281, Oulu 90014, Finland*
[b]*Department of Oral and Maxillofacial Diseases, Institute of Dentistry, University of Helsinki, Helsinki University Central Hospital, Helsinki, Finland*
[c]*Department of Oral and Maxillofacial Surgery, Institute of Dentistry, University of Oulu, Oulu University Hospital, Oulu, Finland*
[d]*Department of Pathology and Laboratory of Periodontal Biology, Faculty of Dentistry, University of Chile, Santiago, Chile*
[e]*Oulu University Hospital, Oulu, Finland, Institute of Dentistry, University of Helsinki, Helsinki, Finland*
[f]*Graduate Program in Estomatopatologia, Piracicaba Dental School, University of Campinas, Piracicaba-São Paulo, Brazil*
[g]*Medical Research Center (MRC), Oulu, Finland*
[h]*Division of Periodontology, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden*

ARTICLE INFORMATION

Article Chronology:
Received 3 May 2014
Received in revised form
4 July 2014
Accepted 8 July 2014
Available online 15 July 2014

Keywords:
Matrix metalloproteinase-8
Mouse
Fibroblasts
Transforming growth factor beta1

ABSTRACT

Matrix metalloproteinase-8 (MMP-8)-deficient mice (*Mmp8-/-*) exhibit delayed dermal wound healing, but also partly contradicting results have been reported. Using the *Mmp8-/-* mice we investigated the role of MMP-8 in acute wound healing of the mobile tongue, and analyzed the function of tongue fibroblasts *in vitro*. Interestingly, in the early phase the tongue wounds of *Mmp8-/-* mice healed faster than those of wild type (wt) mice resulting in significant difference in wound widths ($P=0.001$, 6–24 h). The *Mmp8-/-* wounds showed no change in myeloperoxidase positive myeloid cell count, but the level of transforming growth factor (TGF)-β1 was significantly increased ($P=0.007$) compared to the wt tongues. Fibroblasts cultured from wt tongues expressed MMP-8 and TGF-β1. However, higher TGF-β1 levels were detected in *Mmp8-/-* fibroblasts, and MMP-8 treatment decreased phosphorylated Smad-2 levels and α-smooth muscle actin expression in these fibroblasts suggesting reduced TGF-β1 signaling. Consistently, a degradation of recombinant TGF-β1 by MMP-8 decreased its ability to activate the signaling cascade in fibroblasts. Moreover, collagen gels with *Mmp8-/-* fibroblasts reduced more in size. We conclude that MMP-8 regulates tongue wound contraction rate and TGF-β1 levels. *In vitro* analyses suggest that MMP-8 may also play a role in regulating TGF-β1 signaling of stromal fibroblasts.

*Corresponding author.
E-mail addresses: pirjo.astrom@oulu.fi (P. Åström), emma.pirila@oulu.fi (E. Pirilä), riitta.lithovius@gmail.com (R. Lithovius), heidi.holappa@helsinki.fi (H. Heikkola), jarkko.korpi@helsinki.fi (J.T. Korpi), mhernandezrios@gmail.com (M. Hernández), timo.sorsa@helsinki.fi (T. Sorsa), tuula.salo@oulu.fi (T. Salo).

http://dx.doi.org/10.1016/j.yexcr.2014.07.010
0014-4827/

Introduction

The principal mechanisms of wound healing in vertebrate tissues are uniform, but also clear differences between various tissues exist [1]. The fluid of the oral cavity contains various growth factors, such as transforming growth factor-β1 (TGF-β1) that plays a protective role in mucosal immunity by enhancing the production of immunoglobulin A [2]. The skin wounds are characterized by scarring in which TGF-β1 and fibroblasts play crucial roles, whereas mucosal wounds heal faster with lower inflammatory response and minimal scar formation [1,3]. In addition, skin and oral buccal non-keratinized mucosal fibroblasts differ in their MMP expressions, collagen contraction and matrix reorganization ability as well as gene profile [4–6]. However, the exact molecular mechanisms and factors that drive the differences leading to enhanced healing of oral mucosal wounds are not completely understood.

Matrix metalloproteinases (MMPs) constitute a family of 23 human enzymes able to cleave numerous extracellular matrix (ECM) components and modify various bioactive non-matrix molecules. In wound healing, individual MMPs have been shown to possess either destructive or constructive properties, depending on spatial and temporal distribution as well as the phase and nature (acute *versus* chronic) of the healing process [7].

MMP-8 (collagenase-2) effectively degrades type I collagen, but it also modulates other ECM and non-matrix molecules. Polymorphonuclear neutrophils are the main source of MMP-8, but also *e.g.* fibroblasts have been shown to produce it [8,9]. MMP-8 has been shown to be the predominant collagenase in healing skin wounds, with a 100-fold increase of MMP-8 in chronic wounds compared with acute wounds [10]. A previous study with MMP-8-deficient (*Mmp8-/-*) mice reported delayed skin wound healing with altered neutrophil influx and increase in TGF-β1 signaling molecule phosphorylated Smad (PSmad)-2 levels [11]. However, another study reported impaired rat skin wound healing in an excess of MMP-8 expression [12]. The partly contradicting findings demonstrate the complex role of MMP-8 in tissue repair. To further examine the role of MMP-8 in the tissue repair process we now examined oral mucosal wound healing in the keratinized masticatory mucosa of the tongue. Because of the unique features of inflammation and behavior of the cells of the oral cavity, we hypothesized that examining wound healing in this site may reveal previously unknown functions of MMP-8. We showed, for the first time, the distinct effect of MMP-8 in skin and tongue wound healings. We also demonstrated a significant effect of MMP-8 on tongue wound TGF-β1 levels and the ability of MMP-8 to regulate TGF-β1 activity and fibroblast behavior *in vitro*.

Materials and methods

Animals

Mmp8-/- mice [13] in C57BL/6J/129/SvJ background were used, with equal number of female and male mice (3–4 months old). Age-matched wild type (wt) C57BL/6J mice were used as controls. The experiments were approved by the Animal Care and Use Committee at the University of Oulu or the National Animal Care and Use Committee of Finland and carried out in accordance with the principles of the Helsinki Declaration.

Experimental wounds and sample preparation

Tongue wounds

Mmp8-/- and wt mice were anesthetized by isoflurane inhalation and full thickness wounds were created by penetrating from the dorsal right side through to the ventral side of the anterior tongue with a 1-mm biopsy punch at an equal distance from the midline. The mice were given buprenorfine (0.05 mg/kg) as an analgesic 30 min before wounding and for 24 h after the wounding. Healing was followed at 6 h, 24 h, 48 h, 4 d, and 11 d, after which animals were sacrificed and their tongues were collected (anterior part with the wound). The harvested tongues were fixed for histological and immunohistochemical analyses in 4% (w/v) formalin for 20 h or snap-frozen in liquid nitrogen for protein extraction (indicated separately below in parentheses for each time point). Equal numbers of wt and *Mmp8-/-* mice were used at all time points. The 6-h wound experiments were implemented with 58 mice (38 tongues fixed and 20 tongues frozen), 24-h wound experiments with 60 mice (40 tongues fixed and 20 frozen), 48-h wound experiments with 40 mice (all fixed), 4-d wound experiments with 21 mice, and 11-d wound experiments with 21 mice (all fixed).

Serum samples

For blood collection from the hind limb (~200 μl venous blood), the mice were anesthetized intraperitoneally with a mixture of Hypnorm (0.315 mg phentanylsitrate+10 mg fluanisone/ml H_20), Dormicum (midazolam 5 mg/ml) and aqua (1:1:2) 0.1 ml/10 g. The sera were separated by centrifugation (10 min, 5000 rpm) and the samples were immediately stored in −70 °C without any preservatives.

Tongue sample processing

The fixed tongue samples were embedded in paraffin and cut into 6-μm tissue sections. In order to detect the wounds uniformly aside, the samples were oriented in paraffine so that the cutting of the tongue always occurred vertically from dorsal to ventral side. Frozen tongues were minced in liquid nitrogen, and incubated at +4 °C for 2 h in a protein extraction (PE)[1] buffer [50 mM Tris–HCl, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% (v/v) Brij-35 (Sigma-Aldrich, St. Louis, MO, USA), a protease inhibitory cocktail without EDTA (Roche Diagnostics, IN, USA) and a phosphatase inhibitor (Sigma-Aldrich) as instructed, pH 7.4]. The samples were centrifuged at 14,900*g* for 10 min and filtered with a Millex®-GV 0.22-μm filter (Millipore, Carrigtwohill, County Cork, Ireland). Protein concentrations were determined with a DC Protein Assay kit (Bio-Rad Laboratories Inc., Hercules, CA).

Skin wounds

Skin wounds were performed as previously described [11] with a total of 12 mice (*Mmp8-/-* and wt males). Shortly, the mice were anesthetized with isoflurane inhalation, the dorsal hair was shaved and the skin cleaned with 70% (v/v) ethanol. 8 mm skin wounds were created with the biopsy punch to either sides of the back skin of the mice and the wounds were photographed. After

[1] PE: protein extraction.