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A SECRETED MMP IS REQUIRED FOR RE-EPITHELIALIZATION DURING WOUND HEALING

Abstract

Matrix metalloproteinases (MMPs) are a class of enzymes that play an important role in wound healing by degrading extracellular matrix components. However, the specific function of MMPs in re-epithelialization has been difficult to elucidate in mammalian models due to the redundancy among the 24 mammalian MMPs. To address this, the role of MMPs was examined in vivo using *Drosophila melanogaster*, which has only two MMPs.

The study found that the secreted MMP, Mmp1, is crucial for re-epithelialization in *Drosophila* by remodeling the basement membrane, promoting cell elongation and actin cytoskeletal reorganization, and activating extracellular signal-regulated kinase signaling. The study also demonstrated that Mmp1 expression is upregulated via the jun N-terminal kinase (JNK) pathway following wounding but is expressed independently of the JNK pathway in unwounded epidermis. Overexpressing Mmp1 in a JNK-dependent manner enhances healing and promotes basement membrane repair, which may facilitate tissue regeneration.

Overall, the study provides important insights into the role and control of MMPs in wound healing, highlighting the specific function of Mmp1 in promoting re-epithelialization. These findings may have important clinical implications for the development of new therapies to enhance wound healing in patients with impaired healing capabilities.

Introduction

The healing of a skin wound in humans is a complex process that involves the interplay between cells and the extracellular matrix (ECM), which is further complicated by tissue design that restricts molecular interactions. The process of wound healing consists of multiple phases, including hemostasis, inflammation, re-epithelialization, and resolution. During the hemostasis phase, platelets form a clot that is later replaced by a temporary fibrin matrix. White blood cells

play a crucial role in fighting infections and clearing away cellular and ECM debris through phagocytosis in the inflammatory phase. The migration of epidermal epithelial cells into the wound site follows, and the fibrin matrix is replaced by collagen-based scar tissue during the re-epithelialization stage. Finally, the temporary ECM is replaced, and white blood cells are eliminated during the resolution phase (Singer and Clark 1999; Shaw and Martin 2009; Goldberg and Diegelmann 2010). There are two main categories of pathologies that are associated with wound healing: excessive wound healing, which leads to fibrotic scarring, and incomplete wound healing, which results in ulcers and persistent inflammation (Goldberg and Diegelmann 2010).

The MMPs are a group of extracellular proteases that play a significant role in wound healing. During this process, at least ten MMPs have been reported to be up-regulated (Gill and Parks 2008). These proteases can be divided into two categories: membrane-anchored and secreted. The MMPs' inhibitors are tissue inhibitors of metalloproteinases (TIMPs) (Gomis-R¨th et al. 1997). MMPs have the ability to break down most ECM components, as well as modify many signaling molecules that are critical to wound healing through proteolytic activity. Despite being considered proinflammatory during the wound-healing process, MMPs are situated at the intersection of inflammation, ECM remodeling, and cell signaling. The functional significance of MMPs in wound healing is demonstrated by the modest delay in excisional wound closure in mice with knockouts of secreted MMPs (Bullard et al. 1999). However, due to genetic redundancy, it is challenging to fully comprehend the actions of the MMP family in vivo in mice (Page-McCaw et al. 2007).

Drosophila serves as an established model system for studying wound healing, with only two MMPs present in this organism: one secreted (DmMmp1) and one membrane anchored (DmMmp2) (Wood et al. 2002; Page-McCaw et al. 2003). Unlike humans, *Drosophila* possesses only one endogenous TIMP that inhibits its MMPs, as opposed to the four TIMPs found in humans. The simplicity of the *Drosophila* system, along with the tissue origins and regulatory mechanisms of its secreted and membrane anchored MMPs, allows for the differentiation of their functions as

a class in vivo. *Drosophila* wound healing comprises four phases: hemostasis, inflammation, re-epithelialization, and resolution, which are similar to those in humans, including the migratory re-epithelialization phase (Galko and Krasnow 2004). Therefore, the *Drosophila melanogaster* model organism presents a suitable platform for investigating the requirement of MMPs during the re-epithelization phase of wound healing. Additionally, this model organism allows for the examination of how MMPs are regulated during the wound healing process. Such insights could have significant implications for the development of therapeutic interventions for wound healing, which could be of great benefit to society.

Result

The Role of MMP1 in Tissue Remodeling During *Drosophila* Wound Healing

During tracheal growth and metamorphosis in *Drosophila*, Mmp1, a secreted MMP, plays an essential role in developmental tissue remodeling (Galko and Krasnow 2004). To understand the involvement of Mmp1 in larval epidermal tissue remodeling during wound healing in *Drosophila*. Both wild-type and Mmp1-null mutant larvae were punctured and given a healing window. The epidermis was subsequently dissected, fixed, and stained with anti-FasIII and 4′,6-diamidino-2-phenylindole (DAPI) to visualize cell borders and nuclei. The results of wound closure were then compared by locating nuclei and cell borders between the two groups. Also, the role of Mmp2 in wound healing was investigated and tested for redundant roles by administering puncture wounds to double-mutant Mmp2 Mmp1 animals.

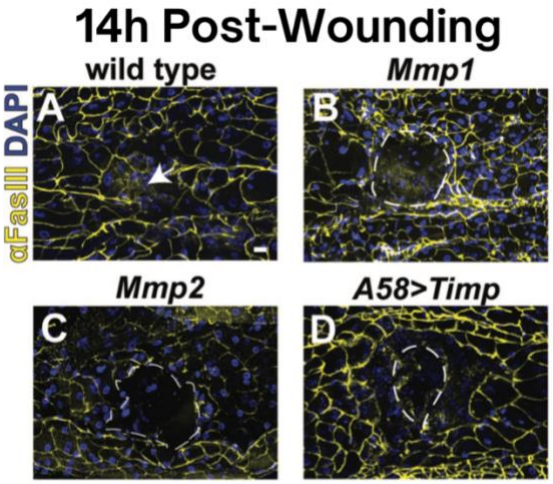
The study found that MMP1 is required for tissue remodeling during wound healing of the larval epidermis in *Drosophila*. In wild-type larvae, the epidermal cells at the wound edge elongated and began spreading into the wound, and 79% of the animals closed their wounds by 14 hours (Fig. 1A). However, MMP1-null animals showed a failure of re-epithelialization, and their wounds gapped open by 1 hour, remaining larger than the original puncture. The wounds of MMP1 mutants never closed, even after 50 hours post-wounding (Fig. 1B).

The study also revealed that MMP2 is necessary for wound closure in a non-redundant manner (Fig. 1C), and each *Drosophila* MMP is required for re-epithelialization. Furthermore, MMP1 and MMP2 act redundantly in the hemostasis phase of wound healing. When puncture was administered to double-mutant MMP2 MMP1 flies, the clotting defect was pronounced and fully penetrant, causing all the flies to bleed out and die shortly after wounding (Fig. 1D).

Re-epithelialization Requires Mmp1 Expression in The Epidermis

The coordination of various tissue types, such as epidermal epithelial cells and hemocytes (innate immune cells of the blood), is involved in the process of epidermal wound healing. To determine which tissue(s) required Mmp1 during re-epithelialization, an inducible RNA interference (RNAi) targeting construct (Uhlirova and Bohmann 2006) controlled by tissue-specific GAL4 drivers A58-GAL4 (epidermis) and He-GAL4 (hemocytes) to suppress Mmp1 was utilized.

The results showed that in wild-type animals, wounds closed in 86% of them by 18 hours post-wounding (Fig. 2A). However, when Mmp1 was knocked down specifically in the epidermis (A58>Mmp1- dsRNA), wounds remained open in 100% of the animals 18 hours post-wounding, with an average wound area significantly larger than that of the wild type (Fig. 2B). In contrast, knocking down Mmp1 in hemocytes (He>Mmp1-dsRNA) did not affect healing, indicating that Mmp1 expression in the epidermis is necessary for re-epithelialization (Fig. 2C). To confirm that the catalytic activity of Mmp1 was necessary for wound closure, a catalytically inactive Mmp1, UAS-Mmp1E225A, was expressed in the epidermis. The results showed that wounds remained open in 100% of the animals tested 18 hours post-wounding, with an average wound area like that of the RNAi-mediated knockdown of Mmp1 (Fig. 2D), indicating that Mmp1's catalytic activity is necessary for wound closure.



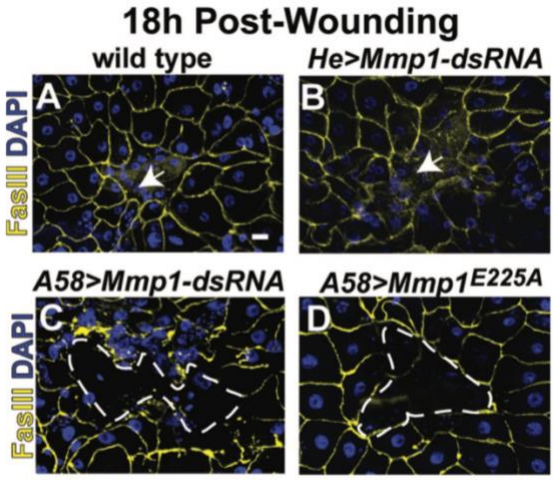


Fig. 1 Fig. 2

Fig. 1: Both *Drosophila* MMPs are needed for re-epithelialization. (A–D) The wounded larval epidermis from the specified genotypes was examined 14 hours post-wounding, and cell borders were labeled with anti-FasIII (in yellow), while nuclei were labeled with DAPI (in blue). The closed wound center is indicated by the white arrow (in A), and the white dashed lines outline open wounds (in B-D).

Fig. 2: Re-epithelialization requires Mmp1 expression in the epidermis, as demonstrated in wounded epidermis from the indicated genotypes 18 hours post-wounding. Closed wound regions are marked by white arrows (in A and B), while open wounds are outlined by white dashed lines (in C and D) (Stevens and Page-McCaw 2012).

Discussion

Using *Drosophila* as a model organism, the function of MMP was analyzed in wound healing. The results indicate that Mmp1 and Mmp2 are crucial for re-epithelialization and hemostasis. Mmp1 plays a specific role in the epidermis, promoting cell elongation, actin cytoskeleton reorganization, basement membrane repair, and ERK signaling. Due to page restrictions, it is not possible to present all the experiments conducted. Nevertheless, the study has overcome the complexities associated with the large and redundant set of MMPs expressed in mammalian wounds, thus providing a promising avenue for further investigation. These findings could prove valuable in developing more effective treatments for patients with complex wound healing processes, such as those with diabetes.

Reference

Bullard KM, Lund L, Mudgett JS, Mellin TN, Hunt TK, Murphy B, Ronan J, Werb Z and Banda MJ (1999) Impaired Wound Contraction in Stromelysin-1–Deficient Mice: Annals of Surgery 230:260.

Galko MJ and Krasnow MA (2004) Cellular and Genetic Analysis of Wound Healing in Drosophila Larvae. PLoS Biol 2:e239.

Gill S and Parks W (2008) Metalloproteinases and their inhibitors: Regulators of wound healing. The International Journal of Biochemistry & Cell Biology 40:1334–1347.

Goldberg SR and Diegelmann RF (2010) Wound Healing Primer. Surgical Clinics of North America 90:1133–1146.

Gomis-R¨th F-X, Maskos K, Betz M, Bergner A, Huber R, Suzuki K, Yoshida N, Nagase H, Brew K, Bourenkov GP et al. (1997) Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1. Nature 389:77–81.

Page-McCaw A, Ewald AJ and Werb Z (2007) Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 8:221–233.

Page-McCaw A, Serano J, Santé JM and Rubin GM (2003) Drosophila Matrix Metalloproteinases Are Required for Tissue Remodeling, but Not Embryonic Development. Developmental Cell 4:95–106.

Shaw TJ and Martin P (2009) Wound repair at a glance. Journal of Cell Science 122:3209–3213.

Singer AJ and Clark RAF (1999) Cutaneous Wound Healing. N Engl J Med 341:738–746.

Srivastava A, Pastor-Pareja JC, Igaki T, Pagliarini R and Xu T (2007) Basement membrane remodeling is essential for *Drosophila* disc eversion and tumor invasion. Proc Natl Acad Sci USA 104:2721–2726.

Stevens LJ and Page-McCaw A (2012) A secreted MMP is required for reepithelialization during wound healing. MBoC 23:1068–1079.

Uhlirova M and Bohmann D (2006) JNK- and Fos-regulated Mmp1 expression cooperates with Ras to induce invasive tumors in Drosophila. EMBO J 25:5294–5304.

Wood W, Jacinto A, Grose R, Woolner S, Gale J, Wilson C and Martin P (2002) Wound healing recapitulates morphogenesis in Drosophila embryos. Nat Cell Biol 4:907–912.