Pyk2 contributes to reepithelialization by promoting MMP expression. Focus on “Delayed skin wound repair in proline-rich protein tyrosine kinase 2 knockout mice”

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MATRIX METALLOPROTEINASES (MMPs) play an important role in wound healing (reviewed in Ref. 5). In the skin, the hemidesmosome attachment between keratinocytes and basement membrane is disrupted by MMP activity, so that keratinocytes are released to migrate under the wound matrix and contact the underlying dermis (Fig. 1A). A number of MMPs have been shown to enhance migration. Keratinocyte migration on type I collagen depends on MMP-1 activity in humans (and MMP-13 activity in mice) by proteolysis of cell-matrix adhesion sites.

In this issue of American Journal of Physiology-Cell Physiology, Koppel and colleagues (3) examine the role of proline-rich protein tyrosine kinase 2 (Pyk2) in modulating dermal wound healing through the regulation of MMP expression and keratinocyte migra-

Fig. 1. A: matrix metalloproteinases (MMPs) are produced by several cell types. MMP production by keratinocytes facilitates keratinocyte migration from the wound edge. B: wounding activates proline-rich protein tyrosine kinase 2 (Pyk2), which stimulates PKCδ and MMP expression.
tion. Pyk2, a member of the focal adhesion kinase family, regulates diverse cellular functions, including proliferation, differentiation, apoptosis, cytoskeletal remodeling, and cell motility. Its activity is stimulated by G protein-coupled receptors, growth factor receptors, integrins, and environmental stress and is regulated by tyrosine phosphorylation, which enhances binding of the Src homology 2 domains of Src family kinases to further increases its activity. Intracellular calcium mobilization and PKC are required for optimal phosphorylation.

Koppel et al. (3) showed normal epidermal architecture in adult Pyk2 knockout mice, thereby demonstrating that Pyk2 is not essential for skin formation. However, in Pyk2-knockout mice, wound healing in vivo and wound closure were delayed, indicating impaired keratinocyte migration in an in vitro “scratch wound” in epidermal keratinocytes from these mice. In contrast, in keratinocytes engineered to overexpress Pyk2, in vitro reepithelialization was accelerated, apparently through enhanced migration. Pyk2 overexpression induced several MMPs, including MMP-1, MMP-9, and MMP-10; this response was blocked by a dominant-negative PKCδ. Pyk2-stimulated MMP expression was required for keratinocyte migration, since an MMP inhibitor blocked migration enhanced by Pyk2 overexpression. Taken together, these results indicate that wound healing activates Pyk2, which stimulates PKCδ, which, in turn, stimulates MMP expression to facilitate migration (Fig. 1B). Although Koppel et al. focused on keratinocyte migration, they left open the possibility that Pyk2 affects other cellular functions, such as proliferation.

The central role of MMPs in facilitating keratinocyte migration and wound repair has been shown in many different studies. Deletion of the tetraspanin CD9, which downregulates MMP-9 expression, enhances keratinocyte migration in vitro and accelerates dermal healing in vivo (2). In mice that express TIMP-1 under control of the MMP-9 promoter in keratinocytes, healing of skin wounds is impaired and migration of keratinocytes is reduced (8). Moreover, deletion of MMP-9 in vivo interferes with reepithelialization of dermal wounds, and blocking of MMP-9 in vitro inhibits keratinocyte migration (4). Thus, Pyk2 activation is another important piece in the puzzle in regulating MMP expression to promote keratinocyte migration and facilitate dermal healing. However, too much of a good thing is detrimental, and excessive and prolonged MMP activity is associated with diabetic and chronic wounds. Upregulation of MMPs is needed to initiate healing, but downregulation of MMP activity is needed at later stages (5). In chronic wounds, MMP-1, MMP-2, MMP-8, and MMP-9 levels are increased and TIMP-1 and TIMP-2 levels are abnormally low.

Although MMPs are critical in keratinocyte migration, other studies examining genetically modified mice have provided insight into migration and wound-healing behavior of keratinocytes. Activation of the transcription factor FOXO1 in keratinocytes is needed for normal wound closure, and FOXO1 regulates keratinocyte migration through a mechanism that involves transcriptional regulation of transforming growth factor-β1 expression and expression of factors that protect cells from oxidative stress (7). Many growth factors and cytokines have been tested for their stimulation of keratinocyte migration; transforming growth factor-β1 is one of the most potent (6). PKD1 is another gene that is needed for reepithelialization, in part because it promotes keratinocyte migration. Mice lacking PKD1 expression in keratinocytes exhibit impaired healing, reduced keratinocyte migration, and deficient integrin recycling (9). Deletion of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a pattern recognition receptor involved in host defense, delays wound healing and reepithelialization (1). Keratinocytes from NOD2-deficient mice exhibit reduced keratinocyte migration in an in vitro scratch assay, suggesting that NOD2 is necessary for cell migration. The events downstream to NOD2 that facilitate keratinocyte migration are not known.

From the data cited above, it is clear that keratinocyte migration is a critical cellular event in reepithelialization of wound surfaces. A number of factors, including MMPs, contribute to this process. MMPs release keratinocytes from underlying basement membrane and facilitate attachment and detachment, which represent an integral aspect of cell migration. The study of Koppel et al. (3) indicates that Pyk2 activation plays an important role in stimulating MMP expression to facilitate migration. However, Pyk2 has been shown to stimulate a variety of cellular activities that are also important in wound healing, such as proliferation. Furthermore, Pyk2 may also stimulate other functions of keratinocytes downstream of PKCδ, such as differentiation, keratin expression, and formation of an epithelial barrier. In addition, the constellation of signals that lead to Pyk2 activation in wound healing is likely to be important, given the potential for Pyk2 to be a key node in integrating various upstream pathways.

REFERENCES