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CXCR4 in Epidermal Keratinocytes: Crosstalk within the Skin*

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SUMMARY

Takekoshi et al. investigated the role of CXCR4 in IL-23-induced keratinocyte hyperproliferation using an epidermal-specific knockout mouse model and found that CXCR4 limited keratinocyte proliferation. Some reports in the literature support this idea while others contradict it; this disparity may be related to differential roles of CXCR4 in various cell types or to a recently identified second receptor (CXCR7). Nevertheless, CXCR4, and its ligand SDF-1, have been implicated in skin wound healing, systemic lupus erythematosus and basal cell carcinoma tumor angiogenesis, and further study is clearly merited.

Interleukin-23 (IL-23) is a cytokine recently found to be involved in the immune-pathogenesis of the skin disease psoriasis (Di Meglio and Nestle, 2010), and intradermal injection of IL-23 in mice produces a psoriasiform dermatitis, with erythema, epidermal hyperplasia and immune cell infiltration ((Takekoshi et al., 2013) and reviewed in (Di Meglio and Nestle, 2010)). Elevated IL-23 levels have also been observed in psoriatic lesions in patients, and a new biologic agent (a human monoclonal antibody) targeting a subunit of IL-23, ustekinumab, has been used successfully to treat the disease (Di Meglio and Nestle, 2010).

The chemokine receptor CXCR4 and its ligand, CXCL12 or stromal-derived factor-1 (SDF-1), are well-known for their roles in hematopoietic stem and immune cell function. SDF-1 acts as a signal for retention of hematopoietic stem cells in the bone marrow, and the granulocyte colony stimulating factor (G-CSF) used clinically to mobilize stem cells from the bone marrow into the peripheral circulation (brand name Neupogen) is thought to work by triggering degradation of SDF-1 (Petit et al., 2002). However, the possible role of this axis in skin diseases like psoriasis has received minimal attention to date.

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On the other hand, the involvement of SDF-1 and CXCR4 in skin wound healing has been investigated by various laboratories. SDF-1 and/or CXCR4 have been demonstrated to be up-regulated upon excisional or burn wounding of skin (Avniel et al., 2006). SDF-1 levels have been found to increase in numerous skin wound models in the epidermis [e.g., (Toksoy et al., 2007)], and in the blister fluid in burn wounds (Avniel et al., 2006). In the case of burn wounds, similar results were observed in human, pig and rat skin, indicating the conservation of the role of this axis (Avniel et al., 2006). The wounding-induced production of SDF-1 appears to arise from the dermal fibroblasts rather than the epidermal keratinocytes, which instead express the receptor CXCR4 (Avniel et al., 2006). However, numerous other cells found in the wound environment also express CXCR4, including infiltrating immune cells and the endothelial cells of the vasculature (Avniel et al., 2006).

In terms of effects on wound healing, some studies have determined that SDF-1/CXCR4 signaling delays healing of excisional and burn wounds, such that inhibition of the axis using SDF-1 neutralizing antibodies or chemical inhibitors of CXCR4 accelerates their healing (Avniel et al., 2006; Nishimura et al., 2012). In contrast, other studies have determined that topical application of SDF-1 itself to wound sites enhances healing (Gallagher et al., 2007; Rabbany et al., 2010; Henderson et al., 2011; Sarkar et al., 2011). These disparate findings are summarized in Table 1. One confounding factor in determining the role of this axis in wound healing is that elements of the signaling system are expressed by multiple cell types involved in wound repair, including keratinocytes, fibroblasts and immune and endothelial cells. Indeed, the ability to recruit endothelial cells to sites of injury has implicated the axis in angiogenesis (Chu et al., 2009; Nishimura et al., 2012). In addition, the CXCR4/SDF-1 axis is known to be critical in mobilization of stem cells from the bone marrow to sites of injury (Xu et al., 2013). Therefore, it is not clear whether the observed effects of SDF-1/CXCR4 signaling on wound healing are related to the resident cells in the skin (i.e., keratinocytes and fibroblasts) or to infiltrating immune cells, recruited stem cells or effects on blood supply to the wound area.

Data in the literature support modulation of all of these processes by the SDF-1/CXCR4 axis. Thus, Florin et al. (Florin et al., 2005) have demonstrated that SDF-1, which is produced by dermal fibroblasts through a Jun-dependent effect on its expression, results in keratinocyte proliferation. This effect can be observed upon treatment of keratinocytes in feeder-layer co-culture with irradiated fibroblasts and prevented by neutralizing antibodies to SDF-1 or CXCR4. Jun-deficient fibroblasts showed minimal SDF-1 production and in co-culture with keratinocytes induced less keratinocyte proliferation. This deficiency in keratinocyte proliferation could be rescued by administration of SDF-1 to the co-cultures (Florin et al., 2005).

In contrast, Avniel et al. (Avniel et al., 2006) have suggested that this axis is more involved in supporting fibrosis than epithelialization. In addition, these authors argued that the ability of the CXCR4 antagonist AMD3100 to accelerate burn wound healing is related to its ability to reduce infiltration of eosinophils into the wound bed, which they propose are inhibitory to epithelialization (Avniel et al., 2006). On the other hand, AMD3100 administration to skin wounds in diabetic mice increases macrophage mobilization and activation as well as circulating endothelial progenitor cells (Nishimura et al., 2012). The CXCR4 antagonist also accelerates diabetic wound healing and increases collagen formation and vascularity at the wound site. Thus, evidence from this report suggests effects of the SDF-1/CXCR4 pathway on fibroblasts, immune cells and endothelial cells/angiogenesis.

Still other groups suggest that SDF-1 enhances wound healing by recruiting bone marrow-derived mesenchymal stem cells to the wound bed (Xu et al., 2013). Xu et al. (Xu et al., 2013) also report that inhibition of SDF-1/CXCR4 signaling (with blocking/neutralizing

antibodies to CXCR4 and/or to SDF-1) inhibits neovascularization, a result seemingly in contrast to a report demonstrating an ability of the CXCR4 *antagonist* AMD3100 to enhance vascularity (Nishimura et al., 2012). Additionally, Hu et al. (Hu et al., 2013), report AMD3100 pretreatment blocks mesenchymal stem cell homing to the wound margin and reduces epithelialzation and wound closure. Also in support of a proangiogenic role of the axis, Chu et al. (Chu et al., 2009) have shown that CXCR4 overexpression in basal cell carcinoma-derived keratinocytes promotes tumor angiogenesis through a pathway involving extracellular signal-regulated kinase 1/2 (ERK1/2) and nuclear factor-kappa B (NF-κB). In summary, then, there are essentially opposite roles for this axis in skin wound healing and angiogenesis reported in the literature.

It is not clear how to reconcile these disparate results of the SDF-1/CXCR4 axis on wound healing and angiogenesis. One possibility is that inhibition of SDF-1 signaling locally at the injury site may affect local cell survival, proliferation or migration as well as the homing of infiltrating cells, or at a distance affect the mobilization of inflammatory and reparative cells, resulting in different, or overlapping effects. In addition, Nishimura et al. (Nishimura et al., 2012) suggest that some of the observed effects of AMD3100 are independent of CXCR4. Indeed, it has recently been discovered that in addition to CXCR4, SDF-1 can bind to a second receptor, CXCR7 (reviewed in (Duda et al., 2011)). To date there is little information about the expression patterns or signaling of this receptor, and it seems possible that some of the effects observed with SDF-1 and inhibitors of this pathway might be related to effects on CXCR7 rather than CXCR4. Indeed, AMD3100 appears to act as an agonist at the CXCR7 receptor (Kalatskaya et al., 2009). Therefore, upon blocking of CXCR4 receptors SDF-1 may act instead through CXCR7. CXCR7 has also been implicated in regulating cell proliferation, adhesion, and migration (reviewed in (Maksym et al., 2009)). Furthermore, in the presence of high levels of SDF-1, the CXCR4 receptor becomes desensitized, probably through interaction with β-arrestin, which is also important in CXCR4 (and possibly CXCR7) internalization and turnover (Duda et al., 2011). Therefore, continuous exposure of cells to SDF-1, for example, perhaps when topically applied in matrices that protect the cytokine from degradation, may result in down-regulation of CXCR4 and/or CXCR7. Thus, it will be critical to distinguish between effects mediated by SDF-1-activated processes versus effects mediated by inhibition of SDF-1 signaling by down-regulation of its receptor(s).

In this issue of the Journal of Investigative Dermatology, Takekoshi et al. (Takekoshi et al., 2013) describe the generation of an epidermal keratinocyte-specific knockout of CXCR4 by the crossing of floxed CXCR4 transgenic mice with a keratin 14 promoter-driven Cre recombinase transgenic mouse model. Using this model the authors demonstrated an involvement of CXCR4 in keratinocytes themselves in regulating the epidermal keratinocyte hyperproliferative response to IL-23. Thus, intradermal injection of IL-23 in skin of epidermal-specific CXCR4-deficient mice resulted in a greater hyperproliferative response relative to the wild-type controls, suggesting that normally SDF-1/CXCR4 signaling limits keratinocyte proliferation *in vivo* (Takekoshi et al., 2013). *In vitro* IL-22 (the downstream mediator of IL-23) stimulates keratinocyte proliferation, and this effect can be inhibited by co-treatment with SDF-1. The mechanism appears to involve increased SOCS3 expression and Stat3 phosphorylation/activation. Importantly, these authors also showed reduced CXCR4 and SOCS3 levels in psoriatic lesions compared to the junctional zone around lesions (Takekoshi et al., 2013), also supporting the idea that this pathway normally limits keratinocyte (hyper)proliferation and suggesting a possible role in the etiology of psoriasis. Recently, Chow et al. (Chow et al., 2010) have shown that keratinocytes, which normally do not express SDF-1, demonstrate increased expression not only of SDF-1, but also of both of its receptors, CXCR4 and CXCR7, when infected by the human papilloma virus (HPV). This result suggests that up-regulation of SDF-1/CXCR4(CXCR7) signaling may be a

general response of keratinocytes to injury and other challenges including viral infection. Since epidermal injury can also trigger the development of psoriatic lesions, referred to as the Koebner phenomenon, it seems reasonable to propose that this axis indeed plays a role in psoriasis.

In addition to a likely involvement in wound healing and psoriasis, other autoimmune-associated skin diseases may also involve the SDF-1/CXCR4 axis. For example, in mouse models of systemic lupus erythematosus (SLE), CXCR4 antagonists have been demonstrated to decrease the severity of the disease as well as the accompanying nephritis (reviewed in (Chong and Mohan, 2009)). Thus, additional investigation of this important SDF-1/CXCR4/CXCR7) axis in skin biology is clearly warranted. The floxed CXCR4 mouse model used by Hwang and colleagues (Takekoshi et al., 2013) will be an important tool in defining the role of various skin cell types in effects of SDF-1, CXCR4 and CXCR4 antagonists on skin functions, such as epidermal proliferation and hyperplasia and wound healing.

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Table 1

Controversial Role of the SDF-1/CXCR4 Axis in Skin

Role of SDF-1/CXCR4 Axis*	Reagents Used	Method of Application	Type of Injury/Model (system)	Reference
Acceleration of wound healing	(1) SDF-1 in GAG scaffold (2) SDF-1 in Hydrogel (3) SDF-1 in Alginate (4) SDF-1 (5) SDF-1, SDF-1 & CXCR4 neutralizing Abs	(1) Topical (2) Topical (3) Topical (4) Injection into wound site (5) Exogenous application or co-culture	(1) Excisional wound (mice) (2) Excisional (mice) (3) Excisional (mice) (4) Excisional (diabetic mice) (5) *in vitro* (human keratinocytes, mouse fibroblasts)	(1) Sarkar et al. (2) Rabbany et al. (3) Henderson et al. (4) Gallagher et al. (5) Florin et al.
Inhibition of wound healing	(1) CXCR4 neutralizing Ab or peptide antagonist (TN14003) (2) CXCR4 antagonist AMD3100	(1) SQ (2) Topical	(1) Burn wound (mice) (2) Excisional (diabetic mice)	(1) Avniel et al. (2) Nishimura et al.
Increased angiogenesis	(1) CXCR4 overexpression (2) CXCR4 antagonist or SDF-1 neutralizing Ab	(1) Cell autonomous (2) Application to stem cells or injection into wound	(1) Human BCC-derived tumors (mice) (2) Topical (mice)	(1) Chu et al. (2) Xu et al.
Decreased angiogenesis	CXCR4 antagonist AMD3100	Topical	Excisional (diabetic mice)	Nishimura et al.

*Abbreviations used: Ab, antibody; BCC, basal cell carcinoma; GAG, glycosaminoglycan; SQ, subcutaneously