Keratinocyte Migration and a Hypothetical New Role for Extracellular Heat Shock Protein 90 Alpha in Orchestrating Skin Wound Healing

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Significance: The treatment and care of patients with skin wounds are a major healthcare expenditure. Burn wounds, iatrogenic surgical wounds, venous stasis dermatitis ulcers, diabetic lower limb ulcers, pressure ulcers, and skin wounds from peripheral neuropathies are largely treated with only supportive care. Despite a great deal of research into using growth factors as therapeutic agents, to date, the field has been disappointing. The only biologic agent that is Federal Drug Administration (FDA) approved for promoting skin wound healing is recombinant platelet-derived growth factor (PDGF-BB), but its modest efficacy and expense limit its use clinically.

Recent Advances: Acute hypoxia induced by the clotting of dermal blood vessels during the wounding of skin is a major stress factor that leads to the re-programming of basal keratinocytes to initiate re-epithelialization. The laterally migrating keratinocytes secrete extracellular heat shock protein 90 alpha. Heat shock protein 90 alpha (hsp90 α) engages low-density lipoprotein receptor-related protein-1 (LRP-1) cellular receptors and works as an auto-crine factor to stimulate keratinocyte migration (re-epithelialization) and as a paracrine factor to stimulate the migration of dermal fibroblasts (fibroplasia) and microvascular endothelial cells (neo-vascularization). Hypoxia-triggered extracellular heat shock protein 90 alpha acts as the master regulator of initial skin wound healing.

Critical Issues: It is not yet known how the engagement of hsp90 α with the LRP-1 receptor leads to increased motility of keratinocytes, fibroblasts, or microvascular endothelial cells. Understanding the sequence of how an acute skin wound via hypoxic stress leads to cellular events that ultimately induce accelerated wound closure provides numerous targets for new wound-healing therapeutic agents.

Future Directions: Developing data for an investigational new drug (IND) application to the FDA for a Phase I study using hsp90 α in human skin wounds. Identifying the cellular signaling mechanisms by which hsp90 α enhances skin cell migration, leading to accelerated wound closure.

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Submitted for publication June 6, 2014. Accepted in revised form August 9, 2014.

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SCOPE AND SIGNIFICANCE

THIS REVIEW FOCUSES on the importance of cell migration in the main processes of skin wound healing. Epidermal keratinocytes migration is linked to the wound-healing pro-

cess of re-epithelialization, which closes the wound. Dermal fibroblasts (DFs) migration into the wound bed is linked to the process of fibroplasia and the re-building of a neo-dermis. Peri-wound endothelial cells migrat-

ing into the wound bed are linked to the process of neoangiogenesis and vascularization of the neodermis.

TRANSLATIONAL RELEVANCE

Naturally occurring agents such as heat shock protein 90 alpha (hsp90 α) can jump start the cell migration of three major cells in human skin—keratinocytes, DFs, and microvascular endothelial cells—and when added to full-thickness wounds made in animals (mice and pigs), induces accelerated wound closure. Therefore, this agent could be developed as a wound-healing agent for humans who have difficulty in healing skin wounds such as diabetic lower limb ulcers, pressure ulcers, and lower limb ulcers due to venous stasis dermatitis.

CLINICAL RELEVANCE

These concepts have great clinical relevance, because there is a great paucity of consistently efficacious biologic agents that can be added to non-healing wounds and jump start wound closure. The problem of nonhealing wounds in human beings is an enormous clinical problem with a very high burden on the healthcare costs of the United States.

BACKGROUND

The healing of human skin wounds is a complex and highly coordinated biological process.^{1,2} Of these processes, re-epithelialization, fibroplasia, and neo-angiogenesis are three critical processes for healing the wound. What they have in common is the requirement for cell migration. “Re-epithelialization” is the lateral migration of basal keratinocytes within the epidermis across the wound bed and, when successful, closes the wound. The wound bed is initially an amorphous mass of clotted serum within the rent in the skin. DFs from around the wound should migrate into the wound bed and synthesize and secrete new extracellular matrix (ECM) molecules, especially type I and type III collagen, to begin building a new dermis. This process has been called “fibroplasia.”

Likewise, the neodermis should become vascularized if the wound is going to heal and transform into functional human skin. Similar to the peri-wound fibroblasts, peri-wound human dermal microvascular endothelial cells (HDMECs) should migrate into the wound bed and establish new blood vessels, a process called “angiogenesis” or “neo-vascularization.” For these three core wound healing processes to be accomplished, there needs to be the migration of keratinocytes (“re-epithelialization”),

the migration of DFs (“fibroplasia”), and the migration of HDMECs (“angiogenesis/neo-vascularization”).

DISCUSSION

Acute low oxygen and stress re-programs basal keratinocytes

Keratinocytes in the unwounded state differentiate from the basal keratinocyte layer (*Stratum basale*) in which the cells are juxtaposed to the basement membrane zone (BMZ) located at the dermal–epidermal junction (DEJ). These basal keratinocytes differentiate and migrate upward toward the surface of the skin where they eventually form the *Stratum corneum*, the most outer horny layer of the epidermis and the main protective barrier of the skin. In this process, the basal keratinocytes, which are capable of proliferating, lose this ability and become nonproliferating *Stratum spinulosum* cells (the squamous cell layer), then *Stratum granulosum* cells, and, finally, *Stratum corneum* cells. This process is called “terminal differentiation,” as the cells lose their nuclei and become dead bags of cross-linked keratin fibers complexed with filaggrin. When wounded, however, basal keratinocytes become re-programmed from cells destined toward terminal differentiation into cells that are capable of lateral migration and continued proliferative potential. What causes this dramatic switch from a differentiation program to a migratory re-epithelialization program? We believe that a critical switch is the acute change in oxygen tension that the basal cell keratinocytes experience when the skin is wounded. That is, when the skin is wounded and the dermal blood vessels are clotted and no longer able to deliver oxygenated hemoglobin via the red blood cells to the skin, the basal keratinocytes experience the stress of acute hypoxia.

Varghese *et al.* directly measured the oxygen tension in human skin wounds under wound dressings and found that it was so low as to be almost immeasurable.³ O’Toole *et al.*,⁴ using *in vitro* keratinocyte migration assays, demonstrated that under hypoxic conditions, the keratinocytes exhibited increased cellular migration compared with cells migrating under normoxic conditions.⁴

Hypoxia-driven keratinocyte migration is mediated by hypoxia-inducible factor-1 and extracellular heat shock protein alpha

Woodley *et al.*⁵ showed that hypoxia-induced keratinocyte migration was mediated by hypoxia-inducible factor-1 (HIF-1), the master regulator of cellular responses to environmental hypoxia. *In vitro* experi-

ments showed that HIF-1 up-regulation led to the extracellular secretion of hsp90 α and increased cellular migration of keratinocytes, DFs and HDMECs. *In vivo* experiments with murine and porcine wound-healing models showed that topically applied hsp90 α dramatically accelerated wound closure by promoting re-epithelialization.^{6,7} When human keratinocytes (HKs) are stimulated to migrate by hypoxia, they exhibit enhanced lamellipodia-building proteins (ezrin, moesin, and radixin) and increased matrix metalloproteinases (MMP1, MMP2, and MMP9).⁴ These observations fit nicely into the earlier electron microscopy studies of Odland and Ross,⁸ who showed that when basal keratinocytes migrate over a wound bed, their morphology is transformed from cuboidal tombstone-like cells into flattened cells with lamellipodia and filopodia at their advancing plasma membrane edge.

The finding that migrating HKs express increased MMPs fits nicely into the concept that keratinocyte migration is a "ratchet-like" process. That is, migrating keratinocytes secrete ECM macromolecules at their advancing plasma membrane edge, attach to the deposited ECM, and then secrete MMPs to detach from this ECM and continue migrating via repeated steps of matrix attachment and detachment.⁹ For many years, the dogma was that only fibroblasts, and not keratinocytes, could synthesize and secrete collagenase. It was then shown that HKs synthesized and secreted MMP1, MMP2, and MMP9. This was missed in earlier studies using functional assays, because the keratinocytes also make large quantities of tissue inhibitor of metalloproteinases (TIMPs) that nullified the collagenases and made them undetectable in functional collagenase assays.^{10,11} Petersen *et al.*,¹² in fact, demonstrated that when HKs are stimulated to migrate, they increase their synthesis and secretion of MMPs, again in accordance with the ratchet theory of keratinocyte motility.¹²

Growth factors can be mitogens or "motogens" (i.e., agents that make cells migrate)

Other biological processes, in addition to the acute hypoxia signal, also influence HK migration. Certain growth factors are not only "mitogens" that drive cell division but also "motogens" which drive cell migration by a different mechanism. Both epidermal growth factor (EGF) and transforming growth factor alpha (TGF α) enhance keratinocyte migration. These two factors share the same receptor on the keratinocyte, the EGF receptor, but TGF α is a more powerful keratinocyte "motogen" than EGF despite the fact that by Scatchard plots, EGF and TGF α bind equally well to the plasma

membranes of keratinocytes and have the same affinity.¹³ Why TGF α increases keratinocyte migration to a greater degree than EGF is not known. Recent evidence in our lab suggests that when TGF α is added to keratinocyte cultures, it induces the secretion of keratinocyte-derived hsp90 α , whereas EGF does not (unpublished observation). This may be an explanation of why TGF α is a superior motogen compared with EGF. To date, TGF α has not been tested in wounds in human beings. EGF, however, has been used in one trial in which acute split thickness wounds were made in 12 individuals. EGF or the vehicle control was topically applied to two identical wounds in the same patient. In all 12 individuals, wound closure was advanced in the wound receiving EGF compared with vehicle.¹⁴ This study was the first "proof of principle" study that a growth factor could enhance the closure of a human wound. The closure of human wounds is largely mediated by re-epithelialization. Therefore, since EGF enhances keratinocyte migration, it is likely that the mechanism by which exogenously administered EGF demonstrated enhanced closure of human wounds in this study was by its ability to drive keratinocyte migration *in vivo* and promote re-epithelialization. Nevertheless, it is possible that besides enhancing re-epithelialization, topical EGF could have had influences on other processes which are necessary for wound healing, but this was not addressed in the paper.

When an acute wound is made, the skin at the wound site suddenly experiences being bathed in serum rather than a filtrate of plasma. This sudden exposure to serum, similar to hypoxia, may present another signal that the basal keratinocytes should abandon the mode to differentiate and embrace a migratory mode. We noted in our keratinocyte migration assays that the presence of serum, but not plasma, would induce increased keratinocyte migration.¹⁵ Serum, compared with plasma, has much higher levels of TGF α , which is the major ingredient in human serum that is responsible for enhancing keratinocyte migration.¹⁶ The notion that fresh serum with its high concentration of TGF α can promote keratinocyte migration and re-epithelialization may explain a clinical maneuver which has been used for years in dermatology to "jump start" a nonhealing skin wound—namely to excise the wound, create a new fresh wound, and flood the area with fresh serum.

Transforming growth factor beta is a double-edged sword in wound healing

Transforming growth factor beta (TGF β) is rich in the wound bed of a healing skin wound. It is

known that $TGF\beta$ induces local fibroblasts to increase their synthesis and secretion of ECM molecules such as collagen I and III, while it also decreases the fibroblast's expression of MMPs. Together, the $TGF\beta$ influences are pro-wound matrix and are thought to help the immature wound bed transform into a neo-dermis. Sarret *et al.* showed that the presence of $TGF\beta$ markedly inhibits the proliferative potential of HKs, but that keratinocyte migration is not inhibited by $TGF\beta$.¹⁷

Among the three mammalian $TGF\beta$ family members, $TGF\beta 3$ appears to play a critical role in the initial phase of wound closure, by coordinating the time of epidermal and dermal cell migration.¹⁸ This finding is summarized in Figure 1, in which $TGF\beta 3$ plays a "traffic control" role to halt DF and endothelial cell migration until keratinocyte migration occurs and the re-epithelialization process is complete. How these observations translate *in vivo* into a healing skin wound is not clear and requires further study. Re-epithelialization is thought to be accomplished by both keratinocyte migration and keratinocyte proliferation, so in theory, the presence of high levels of $TGF\beta$ in the

wound might inhibit re-epithelialization at some level via its impact on keratinocyte proliferation. Once keratinocytes begin to migrate horizontally over the wound bed (within hours of wounding), the keratinocytes behind the migratory cells lose contact inhibition and begin to proliferate and subsequently add to the migratory leading edge of keratinocytes. Nevertheless, the proliferative cells are behind the migratory leading edge cells and may not even be in contact geographically with $TGF\beta$ in the wound bed.

Connective tissue macromolecules influence basal keratinocyte migration

Other major influences on HK migration are the ECM molecules to which the cells are juxtaposed. For example, a substratum of type I dermal collagen enhances keratinocyte migration, while a substratum of laminin 1 (aka laminin 111), a major matrix glycoprotein in the BMZ within the DEJ, inhibits keratinocyte migration.¹⁹ The two most predominant laminin isoforms within the DEJ of human skin are laminin 5 (aka laminin 332) and laminin 10 (aka 511). In the setting of wound healing, secreted laminin 332 is proteolytically processed into a pro-motility smaller molecule. This processing may expose a cryptic site that promotes cell migration or it may release an EGF-like fragment which can promote both cell proliferation and migration.^{20,21}

Keratinocytes engage ECM molecules via integrin receptors. When juxtaposed on type I collagen, their collagen-driven migration is mediated by the $\alpha 2\beta 1$ integrin.²² When juxtaposed to a matrix of fibronectin, their migration is mediated by the $\alpha 5\beta 1$ integrin.²³ When there is migration on vitronectin, an ECM expressed early in healing skin wounds, the keratinocytes use $\alpha v\beta 5$ integrin.²⁴

Migrating keratinocytes regardless of the substrate to which they are apposed build and dismantle sequentially focal adhesions along their journey. When they engage their ECM via the appropriate integrin, focal adhesion kinase (FAK¹²⁵) is phosphorylated and activated, which allows disassembly of focal adhesions to enable the ratchet like traction of the migrating cells to continue.²⁵ Taken together, the ECM-driven haptotaxis and the serum factor-driven chemotaxis determine the initial and optimal migration of keratinocytes in early wound healing.

Extracellular heat shock protein 90 alpha plays a central role in skin wound healing

As previously mentioned, within the last several years, our laboratory has discovered another factor that un-expectedly drives keratinocyte migration as an autocrine factor secreted from the basal kerati-

Figure 1. A schematic representation of how plasma $\rightarrow$ serum $\rightarrow$ plasma transitions coordinate the orderly skin cell migration during wound healing. Three major types of skin cells—HKs, DFs, and HDMECs—are shown here, as indicated by different colors. The dermal cells express higher levels of $T\beta RII$ (symbolized by Y) than the epidermal cells. Therefore, the dermal cells are sensitive to the anti-promotility effect of $TGF\beta 3$ (red stars), whose concentration increases after the transition from plasma to serum in the wound bed. Contributions by other cell types and matrix components to skin wound healing are omitted for the sake of simplicity. The relative numbers and proportions of the various types of cells do not quantitatively reflect those in real human skin. BM, basement membrane; DF, dermal fibroblast; HDMECs, human dermal microvascular endothelial cells; HK, human keratinocyte; $TGF\beta 3$, transforming growth factor beta 3. (Taken from Brandyopadhyay *et al.*, JCB, 172:1093–1105, 2006 with permission.) To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

nocytes in response to the stress of acute hypoxia induced by wounding the skin. We noted in our keratinocyte migration assays that the cells exhibited increased migration when the oxygen tension in the cell culture incubator was decreased. We then examined the conditioned medium under normoxic and hypoxic conditions and noted four new and different protein bands in the conditioned medium of hypoxic cells. Using mass spectrometry, we identified one of these bands as the secreted, extracellular form of heat shock protein 90 alpha (xhsp90 α).

The intracellular form of this protein has been known for many years as a chaperone protein with more than 100 client proteins that are shepherded through the cytosol, endoplasmic reticulum, and Golgi apparatus. In fact, a great deal of research has been done on the intracellular form of hsp90 α , a molecule considered a reasonable target for anti-cancer therapy, as it regulates both cell proliferation and inflammation. Adding the xhsp90 α to keratinocyte migration assays under normoxic conditions induced the same increased keratinocyte migration observed in assays done under hypoxic conditions. Further studies showed that hsp90 α secretion was mediated by HIF-1 α , which becomes rapidly up-regulated in HKs stressed by hypoxia (Fig. 1).²⁶

Under the conditions of skin wounding, all of the dermal blood vessels are clotted and the basal keratinocytes in the avascular epidermis experience acute hypoxia. This stress invokes an up-regulation of HIF-1 α and the subsequent secretion of xhsp90 α by the exosomal protein trafficking pathway rather than by conventional endoplasmic reticulum/Golgi pathway.^{5,26–28} Once secreted by the HK in response to hypoxia, xhsp90 α acts as an “autocrine motogen” to stimulate migration in the cell that secreted it, namely the keratinocyte. It does this by binding to the lipoprotein receptor low-density lipoprotein receptor-related protein-1 (LRP-1) on the cell surface of the keratinocyte.⁵ This mechanism is schematically shown in Figure 2. LRP-1 receptors are on many different types of cells, including HKs, DFs, and HDMECs. When it is secreted by hypoxic, migrating keratinocytes involved in the process of re-epithelialization into the extracellular spaces of the wound bed, it can engage keratinocytes, DFs, and HDMECs via the same receptor.⁵ What is not known is how the engagement of this receptor by xhsp90 α induces cellular signaling that leads to enhanced cellular motility of HKs, HFs, and HDMECs. In addition, there are at least four distinct subdomains of the LRP-1 receptor and various ligands have affinity for different LRP-1 subdomains, leading to different biologic behaviors of the cells. Hypothetically, the sig-

Figure 2. A model for hypoxia-driven keratinocyte migration and re-epithelialization. Hypoxia drives hsp90 α secretion. This is mediated by HIF-1 α . Secreted hsp90 α then binds to the LRP-1 receptor and promotes migration of HKs. Likewise, it can also bind to nearby, peri-wound DFs and HDMECs, which also have the LRP-1 receptor on their cell surface and induce cell motility and fibroplasia and neo-vascularization, respectively. HIF-1 α , hypoxia-inducible factor-1 alpha; hsp90 α , heat shock protein 90 alpha; LRP-1, low-density lipoprotein receptor-related protein-1. (Taken from Woodley *et al.*, JCS, 122:1495–1498, 2009 with permission.)

naling pathway or the network of pathways induced by xhsp90 α could be the same or completely different in HKs, DFs, and HDMECs.

Perhaps even more interesting than xhsp90 α 's role as an “autocrine motogen” for HKs is that it also likely plays a role in both wound-healing fibroplasia and neo-angiogenesis by its ability also to promote the cell migration of DFs and HDMECs once it is secreted into the extracellular compartment. In the extracellular space, the keratinocyte-derived xhsp90 α also acts as a “paracrine motogen” for DFs and HDMECs in the peri-wound environment. Similar to keratinocytes, fibroblasts and HDMECs also have the same LRP-1 receptor on their cell surfaces. Within the wound bed, keratinocyte-derived xhsp90 α binds to the LRP-1 receptors on both fibroblasts and HDMECs and induces cellular motility. The increased motility of perilesional DFs likely leads to the ingress of these cells into the wound clot/wound bed, where they can then synthesize and secrete new dermal matrices, particularly type III collagen and type I collagen. Likewise, the xhsp90 α -induced increased migration of peri-lesional HDMECs likely leads to the ingress of these cells into the wound clot/wound bed, where they can form new dermal vascular tubes that eventually vascularize the neo-dermis.²⁹

Growth factors, such as platelet-derived growth factor (PDGF), EGF, and TGF α , tend to be relatively small molecules. When exogenously added to skin wounds, these small molecules face a wound bed that is rich in proteolytic enzymes and capable of degrading them. That is likely one reason why PDGF-BB (Becaplermin), the only Federal Drug Administration (FDA)-approved growth factor in-

dedicated for human skin wounds, needs to be added to the wounds every day in order to have efficacy. In addition to proteolytic enzymes in the wound bed, the wound bed is also rich in TGF β isoforms. TGF β isoforms serve to induce ECM in the neodermis by regulating fibroblast synthesis and secretion of collagens and glycoproteins, as well as MMPs and TIMPs. Nevertheless, these TGF β isoforms also nullify the activities of growth factors in the wound bed.¹⁸ Interestingly, xhsp90 α in the wound bed is not nullified or even inhibited by the presence of TGF β .⁷ Therefore, it has the ability to continue to be biologically active and stimulate keratinocyte migration (re-epithelialization), DF migration (fibroplasia), and HDMEC migration (neo-vascularization). These three salient wound-healing skin cells under the regulation of xhsp90 α secreted by the hypoxic, migrating keratinocytes is a clear instance in which there are coordinated epidermal–mesenchymal interactions leading to the complex process of closing skin wounds.

A summary of the actions of the secreted form of hsp90 α action in wound healing is shown in Figure 3. Briefly, before injury, the keratinocytes, fibroblasts, and HMECs in intact, un-wounded skin are

nonmigratory (Step 1). Within hours after skin injury, basal keratinocytes begin to migrate laterally across the wound bed, which is basically a serum clot. This initial keratinocyte migration is likely induced by hypoxia-driven hsp90 α autocrine signaling plus serum-derived TGF α . As the keratinocytes migrate over the wound bed and become engaged in the process of re-epithelialization, they secrete hsp90 α into the extracellular space of the wound bed. During this initial early postwound period, human dermal fibroblasts and HDMECs at the wound edge are unable to immediately move into the wound bed due to the presence of TGF β 3 (Step 2). Once the secreted hsp90 α reaches a threshold concentration of 10–30 μ M, it triggers the DFs and HDMECs to migrate into the wound bed from the surrounding wound edge even in the presence of TGF β 3 (Step 3) and initiate the processes of fibroplasia and neo-vascularization. When the migrating keratinocytes have completely re-surfaced the wound, the process of re-epithelialization is finished. The peri-wound fibroblasts that have migrated into the wound bed begin to lay down new ECM and create a neo-dermis and re-model the wound. Peri-wound HDMECs that have migrated

Figure 3. Secreted hsp90, but not conventional growth factors, promotes re-epithelialization and recruits DFs and HDMECs into the wound bed. *Step 1* is un-wounded, intact skin with relatively low levels of TGF β and minimal cell migration. In this state, the basal keratinocytes are programmed to move upward and differentiate. *Step 2* is during initial wounding of the skin, which releases TGF β from several sources and the presence of high levels of TGF β in the wound bed inhibits growth factor function while promoting extracellular matrix deposition into the wound bed. *Step 3* is during early wound healing when basal keratinocytes migrate over the wound bed and secrete hsp90 α . The secreted hsp90 α reaches a threshold concentration in the wound bed, which initiates the inward migration of DFs and HDMECs into the wound bed and begins development of a neodermis. *Step 4* is when the migrating HKs have completed the re-epithelialization process and the DFs and HDMECs, which are now resident cells of the neodermis, remodel the wound and build new blood vessels, respectively. HDF, human dermal fibroblast. (Taken from Cheng *et al.*, JCI, 121:4348–4361, 2011 with permission.) To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

into the wound bed/neo-dermis begin to re-form dermal blood vessels (Step 4). Implicit in the scenario just described is that the initiating event which jump starts the wound healing program is acute hypoxia, Mother Nature's signal to the skin cells that the programs of homeostasis and terminal differentiation should be turned off and a new wound-healing program with elements of a recapitulation of gestation should be immediately initiated. We believe that injury-induced secretion of hsp90 α is the predominant factor and driving force which leads this new hypoxia-driven wound-healing program rather than conventional growth factors. After initial wound closure, the dermal remodeling and neovascularization processes take many months to complete. Many other factors, including conventional growth factors, may play roles in the later events of wound healing, when the TGF β levels decrease

The sub-domain of xhsp90 α responsible for the enhanced motility of keratinocytes, fibroblasts, and HDMECs is called the F-5 fragment. As shown in Figure 4, the F-5 fragment is distinct from the sub-domain within the molecule that binds to ATP and causes hydrolysis of ATP and is involved in the intracellular chaperone functions of hsp90 α . The sub-domain of xhsp90 α that acts as an autocrine and paracrine factor within the wound bed to enhance skin cell motility resides within a small 115 amino acid distinct F-5 fragment of xhsp90 α . Moreover, xhsp90 α or F-5 fragment, when added *in vivo* to standardized skin wounds made on normal mice,

Figure 4. F-5 fragment carries out the extracellular functions of hsp90 α . A schematic representation of seven human hsp90 α protein/peptides (wild type and mutants). The 115-amino-acid fragment, called F-5, is sufficient for the extracellular functions of hsp90 α , specifically its function to drive the migration of HKs, DFs, and HDMECs. (Taken from Cheng *et al.*, JCI, 121:4348–4361, 2011 with permission.) To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

diabetic mice, pigs, or diabetic pigs, accelerates the closure of the wounds when compared with vehicle controls via enhanced re-epithelialization⁷ (Fig. 5). Unlike canonical growth factors that are functionally inhibited by elevated glucose, xhsp90 α maintains its biological activity in the presence of elevated glucose. Diabetic skin wounds are often slow to heal, but there are also nondiabetic slow-to-heal skin wounds such as decubitus pressure ulcers and chronic stasis dermatitis lower limb ulcers. Due to a paucity of animal models that reflect nondiabetic chronic wounds, we do not yet have data showing that xhsp90 α or its F-5 fragment would enhance the closure of chronic wounds due to these etiologies.

The exact timing of the various processes and events leading to the successful healing of a skin wound is not clear and often appears overlapping. Wound contraction contributes as well to the healing of skin wounds, particularly in loose skin animals such as mice and rats. Humans, similar to pigs, are tight skin animals, and human skin wounds also exhibit wound contraction, but to a lesser degree than rodents. We do not have data showing that xhsp90 α has an influence on wound contraction. We do have data showing that xhsp90 α promotes the migration of keratinocytes, DFs, and

Figure 5. Topical hsp90 α promotes wound closure. Topical recombinant hsp90 α (0.45 mM) accelerated skin wound closure in diabetic (db/db) mice from 35 to 18 days with a single application. Becaplermin (RegranexTM) also accelerated wound closure from 35 to 30 days with one application. (Taken with permission from Cheng *et al.*, JCI, 121:4348–4361, 2011.) To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

HDMECs using *in vitro* cellular motility assays, and we have *in vivo* data showing that in our animal wound models the presence of exogenous excess of promotes re-epithelialization. We do know that when an acute skin wound is made, there is a delay of several hours before basal keratinocytes begin to move laterally across the wound bed. It is possible that the dual influences of flooding the keratinocytes with serum TGF α plus the acute stress of hypoxia jump start this process. Then, as the migrating neo-epithelium begins to march across the wound bed, it deposits xhsp90 α into the wound bed, which, in turns, initiates fibroplasia and neoangiogenesis by promoting the migration of fibroblasts and HDMECs. Although speculative, this scenario would, in broad strokes, account for the fact that re-epithelialization appears to begin slightly before fibroplasia and angiogenesis. There should be some built-in inefficiency in this process, however, possibly due to the inflammatory response and engulfment of the wound bed with proteolytic enzymes, because we can dramatically accelerate the closure of skin wounds in animals by providing an excessive amount of xhsp90 α to healing wounds with just one application. Our laboratory is now attempting to define the optimal doses and application frequency of xhsp90 α with the hope that we can bring this novel wound-healing agent from the laboratory bench to the bedside of patients with nonhealing skin wounds.

SUMMARY

For skin wounds to heal, the migration of the three main types of skin cells—keratinocytes, fibroblasts, and endothelial cells—should occur. Without the migration of these cells, the main processes of wound healing—re-epithelialization, fibroplasia, and neovascularization—will not occur and the wound will not heal. While canonical growth factors from the serum and cells in the wound bed play roles in wound healing, the master regulator of the critical processes of cell migration is likely xhsp90 α , which acts as an autocrine migration factor for keratinocytes and a paracrine migration factor for DFs and microvascular endothelial cells. Adding excessive xhsp90 α to healing wounds in normal mice, diabetic mice, and pigs dramatically accelerates wound closure, suggesting that this agent could be developed for clinical use in human beings for patients with difficult-to-heal skin wounds such as diabetic lower limb ulcers, stasis dermatitis ulcers, and pressure ulcers.

TAKE HOME MESSAGE

Keratinocyte-derived extracellular heat shock protein 90 alpha promotes the cellular migration of keratinocytes as an autocrine "motogen" and dermal fibroblasts and microvascular endothelial cells as a paracrine "motogen" in the wound bed under the conditions of acute hypoxia from wounding, and it drives three essential skin wound healing processes—namely, re-epithelialization, fibroplasia and neovascularization.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This work was supported by NIH RO1 AR47981 to Mei Chen; RC4AR060535 and RO1 AR33625 to Mei Chen and David T. Woodley; RO1 GM066193 and RO1 GM067100 to Wei Li; and VA Merit Award to David T. Woodley.

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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NIH RO1 grants, one Challenge Grant, and one VA Merit Review Grant support the USC Laboratory for Investigative Dermatology.

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Abbreviations and Acronyms

BMZ = basement membrane zone
 ECM = extracellular matrix
 DEJ = dermal–epidermal junction
 DF = dermal fibroblast
 EGF = epidermal growth factor
 FAK = focal adhesion kinase
 FDA = Federal Drug Administration
 HDF = human dermal fibroblast
 HDMECs = human dermal microvascular endothelial cells
 HIF-1 α = hypoxia-inducible factor-1 alpha
 HK = human keratinocyte
 hsp90 α = heat shock protein 90 alpha
 IND = investigational new drug
 LRP-1 = low density lipoprotein receptor-related protein-1
 MMP = matrix metalloproteinase
 PDGF = platelet-derived growth factor
 TGF α = transforming growth factor alpha
 TGF β = transforming growth factor beta
 TIMP = tissue inhibitor of metalloproteinase
 xhsp90 α = extracellular heat shock protein 90 alpha