The Roles of Growth Factors in Keratinocyte Migration

Mark A. Seeger and Amy S. Paller*

Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois.

Significance: The re-epithelialization of wounded skin requires the rapid and coordinated migration of keratinocytes (KC) into the wound bed. Almost immediately after wounding, cells present at or attracted to the wound site begin to secrete a complex milieu of growth factors. These growth factors exert mitogenic and motogenic effects on KCs, inducing the rapid proliferation and migration of KCs at the wound edge.

Recent Advances: New roles for growth factors in KC biology are currently being discovered and investigated. This review will highlight the growth factors, particularly transforming growth factor- α (TGF- α), heparin-binding epidermal growth factor (HB-EGF), insulin-like growth factor 1 (IGF-1), fibroblast growth factor 7 (FGF-7), FGF-10, and hepatocyte growth factor (HGF), which have conclusively been shown to be the most motogenic for KCs.

Critical Issues: The cellular and molecular heterogeneity of wounded tissue makes establishing direct relationships between specific growth factors and KC migration difficult *in situ*. The absence of this complexity in simplified *in vitro* experimental models of migration makes the clinical relevance of the results obtained from these *in vitro* studies ambiguous.

Future Directions: Deciphering the relationship between growth factors and KC migration is critical for understanding the process of wound healing in normal and disease states. Insights into the basic science of the effects of growth factors on KC migration will hopefully lead to the development of new therapies to treat acute and chronic wounds.

Amy S. Paller, MS, MD

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*Correspondence: Department of Dermatology, Northwestern University Feinberg School of Medicine, 676 N. St. Clair, Suite 1600, Chicago, IL 60611 (e-mail: apaller@northwestern.edu).

SCOPE AND SIGNIFICANCE

THIS REVIEW WILL SURVEY the wealth of literature that has been published regarding the *in vitro* and *in vivo* roles of growth factors in keratinocyte (KC) motility. The activities of these growth factors are regulated both temporally and spatially in the wound site in order to establish the well-choreographed process of wound healing. Among the complex milieu of molecules that are found in a wound site, growth factors have been shown to exert multiple effects on KCs, particularly on proliferation and migration.

TRANSLATIONAL RELEVANCE

Various growth factor therapies that stimulate KC migration, including epidermal growth factor (EGF), macrophage colony-stimulating factor, and fibroblast growth factor 2 (FGF-2), have been developed for treating acute and chronic wounds, although none are currently in use in the clinic.¹⁻³ Identifying and determining the exact functions of the numerous growth factors and molecules that regulate or modulate growth factor-induced signaling in wounds, particularly those which contribute to the re-epithelialization

process, may lead to the identification of potential new molecular targets for small-molecule or genetics-based therapies for wound healing.

CLINICAL RELEVANCE

Chronic wounds are a major medical problem, the treatment of which costs the U.S. healthcare system more than \$25 billion dollars annually.⁴ The rise in metabolic diseases, such as type-2 diabetes, has led to a similar increase in comorbidities such as chronic leg and foot ulcers, which together affect more than 2.5 million Americans.⁵ Becaplermin (recombinant platelet-derived growth factor BB [PDGF-BB]) is the only topical growth factor therapy for the treatment of lower extremity diabetic neuropathic wounds approved by the U.S. Food and Drug Administration,⁶ which highlights the need for additional growth factor-based therapies that are capable of inducing re-epithelialization for the treatment of chronic wounds.

BACKGROUND

The directed movement of cells within or between tissues is critical for the development and sustained viability of an organism. There are a variety of modes of cell migration, and the particular mode employed by a cell is determined by the integration of intrinsic cues from the migrating cell itself as well as extrinsic cues from the substratum, extracellular space, and neighboring cells.⁷ Interestingly, KCs are able to incorporate elements of both single cell and collective movement, resulting in efficient migration across a two-dimensional substratum as either single cells or collectively as a sheet (Fig. 1).^{8–11} KC migration is a critical step in the carefully orchestrated process of wound healing, which is necessary for maintaining the barrier function of the skin.¹² On wounding, KCs along the wound edge begin a dramatic rearrangement of their cytoplasmic and membrane structures. These changes include the disassembly of most hemidesmosomes (cell-extracellular matrix [ECM] contacts) and many desmosomes (cell-cell contacts), retraction of cytoplasmic keratin intermediate filaments from the periphery of the cell, and rearrangements of the actin cytoskeleton that facilitate the formation and retraction of lamellipodia and focal adhesions. The net effect of all of these cytoplasmic changes is to cause the overall morphology of the KC to become polarized and fan shaped *in vitro*,⁹ or flat and elongated with processes extended toward the direction of movement *in vivo*.^{13,14} Many of the cellular events associated

Figure 1. Representative images of migrating single keratinocytes (KCs) and sheets of KCs. **(A)** KCs with the typical morphology of an individual migrating KC: fan-shape morphology and extension of lamellipodia in the direction of migration (arrow). **(B)** A sheet of KCs in a scratch wound assay exhibits the typical morphology of collectively migrating KCs: maintenance of cell-cell contacts, and extension of lamellipodia into the wound site by cells along the leading edge in the direction of migration (arrow). (A. Paller, Department of Dermatology, Northwestern University, unpublished data).

with KC migration are induced or regulated by individual growth factors or a combination of growth factors that are present in the milieu of molecules found in a typical wound site.

Growth factors are molecules that stimulate cellular growth, proliferation, differentiation, and/or migration. Growth factors can be small molecules such as hormones or macromolecules such as proteins, and can be secreted as fully functional molecules or as molecules that require further post-translational processing in order to be activated. The growth factors that induce KC migration have been shown to exert their effects via autocrine (*e.g.*, tumor necrosis factor- α [TNF- α] synthesized by KCs), paracrine (*e.g.*, EGF or insulin-like growth factor 1 [IGF-1] synthesized by fibroblasts [FBs]), or endocrine (*e.g.*, insulin synthesized by pancreatic β -cells) mechanisms, thereby producing biological effects that are either localized or dis-

tant from the tissue in which the growth factor was synthesized (Fig. 3). Growth factors have been shown to induce the migration of single cells or sheets of cells in monolayer cultures of KCs, or sheets of cells in the three-dimensional matrix of the wounded skin of animal models.^{15,16} Of course, growth factors are not the sole influence

on KC migration; the composition and remodeling of the ECM and extracellular space are also important for KC migration.¹⁷ The roles of growth factors in KC biology have been extensively reviewed.^{15,16,18,19} Our discussion will focus on growth factors that primarily induce KC migration (Table 1).

Table 1. Growth factors that induce KC proliferation and/or migration

Growth Factor	Endogenous Source of Factor	Growth Factor Receptor	In Vitro/Vivo Proliferation Reference	In Vitro Migration Reference	In Vivo Migration Reference
EGF family					
EGF	FB, MP, PL	EGFR	Bhora ¹⁰⁶	Haase ⁴¹	
TGF- α	KC, FB, MP, PL, LK	EGFR	Takahashi ¹⁰⁷	Li ³¹	Kim ¹⁰⁸
HB-EGF	KC	EGFR	Wang ¹⁰⁹	Stoll ³²	Johnson ¹¹⁰
Amphiregulin	KC	EGFR, HER2,3	Schelfhout ³³		
Betacellulin	KC	EGFR	Shirakata ¹¹¹		
Epiregulin	KC, FB	EGFR, HER2,3	Yoshikawa ¹¹²	Draper ³⁵	Draper ¹¹³
Neuregulin	FB, PNS	HER2,3		Kim ³⁴	Schelfhout ³³
Insulin family					
Insulin	PB	IR, IGF1R	Schupel ³⁹	Liu ⁴⁰	Liu ⁴⁰
IGF-1	FB, ML, HP	IGF1R, IR	Shen ³⁸	Haase ⁴¹	Semenova ⁴²
FGF family					
FGF-1	FB	FGFR2IIIb, 1IIIb	Shiple ⁴⁸	Tsuboi ⁴⁹	Mellin ⁵⁰
FGF-2	FB	FGFR2IIIb	Shiple ⁴⁸	Sogabe ⁵¹	Kibe ⁵²
FGF-7	FB, MS	FGFR2IIIb	Yang ⁵³	Tsuboi ⁵⁴	Marti ⁵⁵
FGF-10	FB, MS	FGFR2IIIb, 1IIIb	Radek ⁵⁶	Radek ⁵⁶	Soler ⁵⁷
VEGF family					
VEGF-A	KC, MP	VEGFR-1, -2	Wilgus ⁶⁷	Wilgus ⁶⁷	Roth ⁶⁶
Scatter factor family					
HGF	MS, KC	c-Met	Sato ⁷¹	Chmielowiec ⁶⁹	Chmielowiec ⁶⁹
MSP	HP	Ron	Wang ⁷⁴	Wang ⁷⁴	Santoro ⁷⁵
GM-CSF					
GM-CSF	KC, MS, FB, MP, LK	CD116	Kawada ⁷⁸		Fang ⁸⁰
AGF					
AGF	HP, PL, Mast cell	α_v integrin	Oike ⁸³	Zhang ⁸²	
HMGB1					
HMGB1	MP, LK	NA	Ranzato ⁸⁴	Straino ⁸⁵	Straino ⁸⁵
HSP90					
HSP90	KC	LRP-1		Woodley ⁸⁶	Tsen ⁸⁸
Chemokines					
CXCL1	KC	CXCR2	Kroeze ⁸⁹	Kroeze ⁸⁹	Devalaraja ⁹³
CXCL8	KC	CXCR1,2	Kroeze ⁸⁹	Kroeze ⁸⁹	Devalaraja ⁹³
CXCL10	KC	CXCR3		Kroeze ⁸⁹	Yates ⁹⁴
CXCL11	KC	CXCR3		Satish ⁹⁰	Yates ⁹⁴
CXCL12	MP	CXCR4	Kroeze ⁸⁹	Kroeze ⁸⁹	
CCL14	KC	CCR1		Kroeze ⁸⁹	
CCL17	KC	CCR4		Fujimoto ⁹¹	
CCL22	MP	CCR4		Kroeze ⁸⁹	
CCL27	KC	CCR10		Kroeze ⁸⁹	
Neuropeptides					
VIP	PNS	NA	Sung ⁹⁶	Wollina ⁹⁷	
SP	PNS	NK1R	Shi ⁹⁸		Gibran ¹⁰⁰
Cholinergic receptor agonists					
Ach	PNS, KC	nAChR		Chernyavsky ¹⁰³	Chernyavsky ¹⁰⁴
Ach	PNS, KC	mAChR		Chernyavsky ¹¹⁴	Chernyavsky ¹⁰⁵

Ach, acetylcholine; AGF, angiopoietin-related growth factor; FB, fibroblast; FGF, fibroblast growth factor; GM-CSF, granulocyte macrophage-colony stimulating factor; HB-EGF, heparin binding-epidermal growth factor; HGF, hepatocyte growth factor; HMGB1, high mobility group protein β 1; HP, hepatocytes; IGF-1, insulin-like growth factor 1; IGF1R, IGF-1 receptor; IR, insulin receptor; KC, keratinocyte; LK, leukocyte; LRP, LDL receptor-related protein 1; ML, melanocyte; MP, macrophage; MS, mesenchymal cells; MSP, macrophage-stimulating protein; NA, not available; NK1R, neurokinin 1 receptor; PB, pancreatic β -cells; PL, platelet; PNS, peripheral nervous system cells; SP, substance P; TGF, transforming growth factor- α ; VEGFR, VEGF RTK receptor; VIP, vasoactive intestinal peptide.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Methods for investigating KC migration

Several methods are routinely utilized to investigate cell migration both *in vitro* and *in vivo*.^{20,21} *In vitro* techniques can be employed to study the migration process in single cells (e.g., time-lapse single cell tracking, colloidal gold single cell migration assay, and chemotaxis chambers, such as the Boyden chamber) or sheets of cells (e.g., scratch wound assay), and while these assays are relatively simple to implement, it is difficult to establish the clinical relevance of these assays as they lack the complexity of *in vivo* KC migration.²⁰ *In vivo* KC migration is assessed in live animals by measuring the rate of wound closure through clinical observation and by measurement of the epidermal gap, dermal gap, and granulation tissue area in histological sections, but re-epithelialization in animal models is an approximation of the extent of KC migration, as the effects of cell proliferation and/or wound contraction cannot be excluded.²¹

Receptor tyrosine kinase growth factors that stimulate KC migration

EGF family. Receptor tyrosine kinases (RTK) are a diverse family of membrane-associated receptors that transmit signals from many of the growth factors which are critical for inducing KC migration (Fig. 2). The EGF family consists of several members, including EGF, transforming growth factor- α (TGF- α), heparin-binding EGF (HB-EGF), amphiregulin, betacellulin, biregulin, epiregulin, epigen, and several neuregulin isoforms.^{15,22,23} Human KCs express TGF- α , HB-EGF, amphiregulin, betacellulin, and epiregulin.^{15,24} These factors are synthesized as type I transmembrane proteins that are post-translationally processed into soluble factors by proteases, such as the disintegrin and metalloproteases (ADAMs), in a process known as ectodomain shedding, and they are then free to act as autocrine or paracrine growth factors.²⁵ The EGF-family ligands bind to a group of four homo- or heterodimeric RTKs: HER1 (commonly known as EGFR, ErbB-1), HER2 (Neu, ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4).

Figure 2. Receptors and ligands that have been shown to induce KC migration. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 3. Histologic (left panel) and schematic (right panel) representations of a healing full-thickness excisional wound in mouse skin outlining the distribution of motogenic growth factors during wound healing. Growth factors are secreted by a variety of cell types that are found distant from, adjacent to, and within the wound site. Growth factors originate from fibroblasts (FBs) and KCs, as well as from macrophages (MP), platelets (PL), leukocytes (LK), peripheral nervous system cells (PNS), pancreatic β -cells (PB), hepatocytes (HP), melanocytes (ML), and mesenchymal cells (MS). E, epidermis; HF, hair follicle; D, dermis; A, adipose tissue; M, muscle; HE, hyperproliferative epidermis; G, granulation tissue; Es, eschar (A. Paller, Department of Dermatology, Northwestern University, unpublished data). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

These receptors can be broadly subdivided into two groups based on their ligand specificities: HER1 binds all EGF-family ligands except the neuregulins, whereas HER2, 3, and 4 bind the neuregulins in addition to other select EGF-family ligands.¹⁵ HER1, 2, and 3, but not 4, are expressed by human KCs.²⁴

EGF, HB-EGF, TGF- α , epiregulin, and neuregulin signaling fosters KC migration. Although other HER receptors participate in signaling, EGFR is the dominant EGF-family receptor in KCs, as evidenced by the severe impairment of wound re-epithelialization observed in EGFR knockout mice.²⁶ EGFR is expressed throughout the epidermis, with its most prominent expression in the basal layer.²⁷ EGFR is activated in an autocrine manner by ligands synthesized by KCs, activated in a paracrine manner by ligands synthesized by platelets, lymphocytes, macrophages, or FBs, or trans-activated by angiotensin II- or antimicrobial peptide hCAP18/LL-37-induced HB-EGF autocrine signaling.^{15,16,28,29} While EGF itself induces KC migration in *in vitro* models¹⁸ and is detected in wound fluid,³⁰ the importance of EGF in KC migration *in vivo*, in contrast to its recognized role in proliferation, has not been established. On the other hand, TGF- α and HB-EGF strongly promote migration, and HB-EGF downregulates the proliferative phenotype of KCs both *in vitro* and *in vivo*.^{31,32}

Other EGF-family growth factors that have been shown to stimulate KC migration via EGFR or HER2 or 3 include neuregulin and epiregulin.^{33–35} Interestingly, the mode in which the EGF ligand is presented to KCs also appears to be important for determining whether the cells migrate or proliferate; EGF on a stationary substrate induces KC migration, while soluble EGF induces KC prolif-

eration, suggesting that soluble and membrane- or ECM-bound EGF-family ligands play distinct roles in directing KC activities.³⁶

Insulin growth factor family. Both insulin and IGF-1 bind to the insulin receptor (IR) and IGF-1 receptor (IGF1R) RTKs, with insulin binding with a higher affinity to IR than IGF1R and IGF-1 binding with a higher affinity to IGF1R than IR.³⁷ The insulin and IGF-1 signaling pathways diverge at protein kinase C δ (PKC δ), as insulin but not IGF-1 was able to induce PKC δ -mediated KC proliferation, suggesting that insulin and IGF-1 have non-overlapping mitogenic and motogenic functions in KCs.³⁸ Insulin is synthesized by β -cells in the pancreas, and it circulates hematogenously to the skin, where it has been shown to have mitogenic as well as motogenic effects on KCs both *in vitro* and *in vivo*.^{39,40} The combination of insulin and TGF- α was shown to stimulate KC migration to the same extent as whole human blood serum, suggesting that these two growth factors act synergistically in promoting KC migration.³¹

IGF-1 is synthesized by hepatocytes and in the dermis by FBs, and it has been shown to have both mitogenic and motogenic effects on KCs *in vitro* and *in vivo*.^{38,41,42} IGF-1 and IGF1R are found throughout the epidermis, particularly in the basal layer, and IGF-1 and IGF1R expression is significantly down-regulated in diabetic epidermis, indicating that IGF1R signaling is important for normal wound healing and is suppressed in diabetes-induced impaired wound healing.³⁰ The administration of IGF-1 and EGF simultaneously to KCs has a synergistic effect on KC migration, due to EGFR ligand-mediated stimulation of the mitogen-activated protein kinase

(MAPK/ERK) pathway, leading to cell contraction and proliferation, and IGF-1 stimulation of the phosphatidylinositol-3-kinase (PI3K) pathway, leading to membrane protrusion and cell spreading.⁴¹ The insulin/IGF-1 signaling axis in KCs has recently been shown to be regulated by GM3 ganglioside, which is increased in diabetic epidermis, suggesting a direct link between KC membrane lipid components and insulin/IGF-1-induced KC migration.⁴³ IGF-1 signaling is also regulated extracellularly by IGF binding proteins (IGFBP), which bind and restrict IGF-1 from interacting with IGF1R. The IGFBPs, in turn, are regulated by the activity of matrix metalloproteinases (MMP), indicating multiple levels of regulation for IGF-1 signaling in KCs.⁴⁴

FGF family. The FGF family consists of at least 22 members, of which FGF-1 (acidic FGF), FGF-2 (basic FGF), FGF-7 (keratinocyte growth factor [KGF-1]), FGF-10 (KGF-2), and possibly FGF-22 have been shown to be important for KC migration.^{18,45} These ligands bind to at least two FGFRs with somewhat overlapping functions expressed by KCs, FGFR2IIIb (keratinocyte growth factor receptor), and FGFR1IIIb.⁴⁶ FGFR2IIIb binds FGF-1, -2, -7, and -10, and FGFR1IIIb binds FGF-1 and 10.¹⁵ The FGFRs are RTKs that require the binding of both FGFs and their coreceptors, heparin, or heparan sulfate proteoglycans, in order for signal transduction to occur.⁴⁷ FGF-1, -2, -7, and -10 have been shown to have both mitogenic and motogenic effects on KCs both *in vitro* and *in vivo*.^{48–57} In addition to binding to heparin and heparan sulfate proteoglycans in the ECM, FGFs bind to FGF binding proteins (FGFBP), which release FGFs from the ECM, thereby increasing the pool of free FGFs available for binding to FGFRs.⁵⁸ FGF-2 stimulates lamellipodia formation and Rac activation, leading to KC migration, and has a synergistic effect with EGF on inducing KC proliferation.^{51,59} FGF-7 and 10 appear to be the most potent motogenic FGFs, and are highly expressed during the early stages of wound healing, but blocking FGF-7 expression does not affect the overall rate of wound healing, suggesting overlapping or compensatory mechanisms for FGF signaling in KCs.^{60,61} KCs are also able to indirectly stimulate the production of FGF-7 by secreting interleukin-1 α and β (IL-1 α and β) and TNF- α , which stimulate FBs to produce the pro-motogenic FGF-7.^{62–64} Interestingly, it has been suggested that the elevated levels of IL-1 β and TNF- α observed in chronic wounds lead to increased expression of MMPs that degrade ECM components and secreted growth factors such as the FGFs. The result is impaired KC migration in chronic wounds

due to a disruption of the ECM substratum and a decreased availability of functional growth factors.¹⁶

Vascular endothelial growth factor family. The vascular endothelial growth factor (VEGF) family consists of 6 members, which bind to a set of 3 VEGF RTK receptors (VEGFR). KCs and macrophages produce VEGF-A, which induces angiogenesis and granulation tissue formation during wound healing, and decreased VEGF-A expression in the skin has been linked to impaired wound healing.^{65,66} In addition to synthesizing VEGF-A, KCs also express VEGFR-1, -2, and -3. VEGFR-1 and -2 induce KC proliferation and migration and decrease cell adhesion on VEGF-A binding.^{67,68} Therefore, VEGF-A functions as an autocrine mitogenic and motogenic growth factor for KCs, and as a paracrine growth factor for angiogenesis and granulation tissue formation.

Scatter factor family. The scatter family of growth factors consists of hepatocyte growth factor (HGF, scatter factor 1) and macrophage-stimulating protein (MSP, scatter factor 2). Both are synthesized as a single chain that is proteolytically cleaved into two chains. The RTK c-Met is expressed by KCs and binds HGF. HGF is synthesized primarily by FBs and, after skin injury, is also expressed by KCs to promote re-epithelialization.⁶⁹ The strong motogenic effect of HGF is mediated by signal transducer and activator of transcription 3 (STAT3) signaling, and is inhibited by suppressor of cytokine signaling 3 (SOCS3).⁷⁰ HGF is also mitogenic for KCs and induces expression of VEGF-A.^{71,72} MSP is produced and secreted into serum by hepatocytes, and activates the RTK receptor Ron. Ron then activates Akt, which further phosphorylates Ron and $\alpha_6\beta_4$ integrin to create a binding site for the 14-3-3 scaffold protein. Binding of 14-3-3 forms a complex between Ron and $\alpha_6\beta_4$ integrin, displacing $\alpha_6\beta_4$ integrin from hemidesmosomes and relocating it to lamellipodia. Simultaneously, $\alpha_3\beta_1$ integrin-mediated cell spreading on laminin-332 is induced and p38 and NF- κ B signaling is activated, all of which culminates in KC migration.⁷³ As such, MSP is both a mitogenic and motogenic factor for KCs.^{74,75}

Other growth factors that stimulate KC migration

Granulocyte macrophage-colony stimulating factor. Granulocyte macrophage-colony stimulating factor (GM-CSF) is synthesized by a variety of cells, including KCs on injury or exposure to ultraviolet light, and binds to the CD116 receptor. GM-CSF binding to CD116 induces activation of the JAK/STAT signaling pathway.⁷⁶ GM-CSF is a potent

autocrine mitogen for KCs.^{77,78} GM-CSF activity results in faster wound closure, but it has not been established whether this effect is due to increased KC proliferation resulting from increased KC production of the mitogenic factors IL-6 and interferon- γ , increased migration of KCs into the wound bed, or a combination of the two.^{79,80} Clinically, the application of recombinant GM-CSF has been shown to be efficacious for the treatment of chronic wounds and burns but has no effect on wound healing in healthy individuals; these effects are most likely mediated through increased recruitment of macrophages and neutrophils and increased vascularization at the wound site rather than via a direct effect on KC migration.⁸¹

Angiopoietin-related growth factor. Angiopoietin-related growth factor (AGF, angiopoietin-like protein 6) is a member of the angiopoietin-like protein (Angptls) family. Angptls regulate metabolism and angiogenesis, and are active primarily in the liver, although AGF has been detected in the skin. There is no known KC receptor for Angptls, but AGF may bind to α_v integrin via a conserved RGD-motif.⁸² Mice that overexpress AGP exhibited a thickened epidermis, indicative of increased KC proliferation, as well as more rapid wound healing relative to control mice.⁸³ AGF induced KC adhesion, cell spreading, and migration at AGF concentrations that are lower than those required to induce KC proliferation.⁸² AGF-induced KC migration is blocked by a synthetic RGD peptide, suggesting that AGF interacts directly with the extracellular portion of α_v integrin on KCs to induce migration.⁸²

High mobility group protein B1. High mobility group protein $\beta 1$ (HMGB1, amphoterin) is a nuclear protein that binds to the minor groove of double-stranded kinked DNA, and is secreted by macrophages and monocytes. Secreted HMGB1 binds to the receptor for advanced glycation end products, and helps maintain the inflammatory response. HMGB1 induces KC proliferation and migration via an unknown receptor.^{84,85} HMGB1 activates the ERK1/2 pathway in KCs, and MEK inhibitors block the proliferative effect of HMGB1.⁸⁴ HMGB1 treatment increases the rate of wound closure in diabetic mice and retards the rate of wound closure in normal mice.⁸⁵ Therefore, HMGB1 functions as both a mitogenic and motogenic factor for KCs.

Heat shock protein 90. Hypoxic conditions often occur during the course of wound healing. It has been shown that *in vitro* hypoxic conditions activate hypoxia-inducible factor 1, which induces KCs to express heat shock protein 90 (HSP90),

which then acts in an autocrine fashion on KCs via the LDL receptor-related protein 1 (LRP-1) to activate KC migration but not proliferation.⁸⁶ It has also been shown that TGF- α upregulates HSP90 expression, thereby inducing KC migration.⁸⁷ Activation of LRP-1 by HSP90 leads to the activation of Akt1 and 2, which induces faster wound healing *in vivo*.⁸⁸

Chemokines. Chemokines are small proteins secreted by a variety of cell types that induce chemotaxis in an autocrine or paracrine manner. There are several subfamilies of chemokines, including the CXC and CC chemokines, which have either a CXC or CC amino-acid motif at their N-termini. Several chemokines, including CXCL1, 8, 10, 11 and CCR14, 17, 27 are synthesized by KCs; while CXCL12 and CCL22 are synthesized by macrophages. All of these chemokines stimulate KC migration, and CXCL1, 8, and 12 stimulate KC proliferation *in vitro* in an autocrine or paracrine manner.⁸⁹⁻⁹¹ Interestingly, high concentrations of CXCL8, similar to levels observed in burn wounds, inhibit KC migration, suggesting that the local titer of chemokines is important for defining their effects on KCs.⁹² Chemokines are bound by CXC or CC receptors (CXCR or CCR). The chemokines CXCL1 (growth-related oncogene- α) and CXCL8 (IL-8) bind to CXCR2, and wound closure was significantly impaired in a CXCR2 knockout mouse, suggesting that CXCL1 and/or 8 signaling is required for normal KC migration *in vivo*.⁹³ The chemokines CXCL10 and CXCL11 bind to CXCR3, and wound closure was significantly impaired in a CXCR3 knockout mouse, suggesting that CXCL10 and/or 11 signaling is required for normal KC migration *in vivo*.⁹⁴ Chemokine-mediated signaling, therefore, makes essential contributions to inducing and regulating KC migration.

Neuropeptides. Neuropeptides are small peptides that function as paracrine signaling molecules among nervous system cells, and on cutaneous injury, they are released by sensory neurons to interact with KCs. Neuropeptides generally not only help facilitate the inflammatory response at the wound site, but are also mitogenic and/or motogenic for KCs.⁹⁵ The neuropeptide vasoactive intestinal peptide (VIP) induces KC proliferation indirectly by stimulating the synthesis of TGF- α ; VIP also stimulates KC migration *in vitro*.^{96,97} Substance P (SP) is a neuropeptide that acts via the neurokinin 1 receptor (NK1R). In addition to being secreted by sensory neurons, both SP and the neuropeptide calcitonin gene-related peptide are expressed by KCs, and they stimulate the production of pro-inflammatory cyto-

kines and KC proliferation.^{98,99} Treatment with SP was shown to stimulate re-epithelialization in wounded diabetic mouse skin.¹⁰⁰ Interestingly, neutral endopeptidase (NEP), a metallopeptidase that degrades SP and other neuropeptides, was shown to be upregulated in diabetic mouse wounds. Inhibition of NEP accelerates re-epithelialization, suggesting that impaired KC migration in diabetic wounds may be due, in part, to decreased motogenic signaling from neuropeptides such as SP.¹⁰¹

Cholinergic receptor agonists. KCs express both nicotinic and muscarinic acetylcholine (ACh) receptors (nAChR and mAChR respectively), and synthesize ACh. The $\alpha 3$, $\alpha 5$, $\alpha 7$, $\alpha 9$, $\alpha 10$, $\beta 1$, $\beta 2$, and $\beta 4$ nACh receptor subunits have been detected in KCs, and homo- or heteropentamer combinations of these subunits form ACh-gated membrane channels that mediate the influx of Na^+ and Ca^{2+} and the efflux of K^+ .¹⁰² The M1, 2, 3, 4, and 5 mACh receptors have been detected in KCs, and these receptors are coupled to G-proteins that transmit signals on ACh binding.¹⁰² Activation of the $\alpha 3$ nAChR induces KC migration via PKC δ and RhoA, activation of $\alpha 7$ nAChR induces KC migration via PI3K and Rac/Cdc42, and activation of $\alpha 9$ nAChR induces KC migration both *in vitro* and *in vivo* by modulating cell–cell and cell–ECM adhesion.^{103,104} The $\alpha 7$ nAChR and M1 mAChR enable KCs to sense ACh gradients, which initiates polarization of the cell and expression of α_2 and α_3 integrins at the leading edge of the cell via activation of the Ras/Raf-1/MEK1/ERK pathway, resulting in the stabilization of lamellipodia at the leading edge during the initial steps of ACh-mediated chemotaxis.¹⁰³ Lastly, activation of the mAChR M4 induces KC migration both *in vitro* and *in vivo* by upregulating the “migratory” integrins $\alpha_5\beta_1$, $\alpha_v\beta_5$, and $\alpha_v\beta_6$, and activation of the mAChR M3 inhibits KC migration both *in vitro* and *in vivo* by upregulating the “sedentary” integrins $\alpha_2\beta_1$ and $\alpha_3\beta_1$.¹⁰⁵ Therefore, autocrine and paracrine activation of the KC cholinergic receptors plays an active role in inducing and regulating KC migration.

SUMMARY

The mechanism of KC migration is unique among migratory cells in that it incorporates elements of single cell motility and collective motility. The ability to migrate efficiently and rapidly is critical for KCs to fulfill one of their fundamental

TAKE-HOME MESSAGES

- Growth factors are critical mitogenic and motogenic inducers for KCs.
- TGF- α , HB-EGF, IGF-1, FGF-7, FGF-10, and HGF are the most potent motogenic growth factors for KCs.
- Molecules that are not traditionally considered growth factors have mitogenic and motogenic effects on KCs.
- The signaling inputs from many growth factors are integrated to coordinate KC migration.
- Growth factor signaling is regulated spatiotemporally in order to ensure proper KC migration during execution of the wound-healing program.
- Growth factor signaling is disrupted in disease states such as type-2 diabetes, resulting in impaired KC migration.

tasks—the re-epithelialization of wounded skin tissue in order to maintain the integrity of the skin barrier. The process of KC migration is coordinated by the integration of a diverse set of growth factor signals that are autocrine, paracrine, or hematologic in origin (Table 1 and Fig. 3). TGF- α , HB-EGF, IGF-1, FGF-7, FGF-10, and HGF are the growth factors that appear to have the strongest direct motogenic effect on KCs in experimental systems. The expression and presentation of these growth factors are tightly regulated spatiotemporally in order to ensure that the events which are required for proper wound healing occur in the correct sequence. Given the potent mitogenic and motogenic effects that many growth factors have on KCs in experimental systems, it is surprising that growth factor therapies have had limited success in the treatment of both normal and chronic wounds in the clinic. There is currently one FDA-approved growth factor treatment for wounds, Regranex[®] (recombinant PDGF-BB), prescribed specifically for diabetic foot ulcers, and clinical trials have been conducted for EGF, GM-CSF, and FGF-2 as well.⁴ The limited success of exogenous growth factor therapies could be due to (1) difficulties in delivering biologically active growth factors to the proper target sites in the wound; (2) altered expression (from decreased synthesis or degradation) of growth factors, their receptors, or secondary molecules; or (3) blockade of activation of receptors or secondary molecules necessary for growth factor-mediated signaling in the proteolytic and microbe-rich environment of chronic wounds. The continued identification and investigation of KC-specific motogenic growth factors and the secondary molecules that modulate growth factor-mediated signaling will, therefore, be important for gaining a better understanding of disease states in which proper KC migration is disrupted, and along

with improved topical delivery mechanisms, will be critical for the development of novel therapeutics that are capable of reversing these disease states.

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ABOUT THE AUTHORS

Amy S. Paller, MS, MD, is the Walter J. Hamlin Professor of Dermatology, Chair of the Department of Dermatology, and Director of the Skin Disease Research Center at Northwestern University. **Mark Seeger, PhD**, is a postdoctoral fellow in the laboratory of Dr. Paller in the Department of Dermatology at Northwestern University.

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Abbreviations and Acronyms

ACh = acetylcholine
 AGF = angiopoietin-related growth factor
 ECM = extracellular matrix
 EGF = epidermal growth factor
 FB = fibroblast
 FGF = fibroblast growth factor

FGFBP = FGF binding proteins
 GM-CSF = granulocyte macrophage-colony stimulating factor
 HB-EGF = heparin binding-epidermal growth factor
 HGF = hepatocyte growth factor
 HMGB1 = high mobility group protein β 1
 HP = hepatocytes
 HSP90 = heat shock protein 90
 IGF-1 = insulin-like growth factor 1
 IGF1R = IGF-1 receptor
 IGFBP = IGF binding protein
 IL = interleukin
 IR = insulin receptor
 KC = keratinocyte
 LK = leukocyte
 MAPK = mitogen-activated protein kinase
 ML = melanocytes
 MMP = matrix metalloproteinases

MSP = macrophage-stimulating protein
 NEP = neutral endopeptidase
 NK1R = neurokinin 1 receptor
 PB = pancreatic β -cells
 PDGF-BB = platelet-derived growth factor BB
 PI3K = phosphatidylinositol-3-kinase
 PKC δ = protein kinase C δ
 PNS = peripheral nervous system cells
 RTK = receptor tyrosine kinases
 SOCS3 = suppressor of cytokine signaling 3
 SP = substance P
 STAT3 = signal transducer and activator of transcription 3
 TGF- α = transforming growth factor- α
 TNF- α = tumor necrosis factor- α
 VEGF = vascular endothelial growth factor
 VEGFR = VEGF RTK receptor
 VIP = vasoactive intestinal peptide