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Migration of epidermal keratinocytes: mechanisms, regulation, and biological significance

Review article

G. Kirfel* and **V. Herzog**

Institute of Cell Biology, University of Bonn, Bonn

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Summary. Keratinocytes are the prevalent cell type of the epidermis, a multilayered cornified epithelium which provides the cellular basis of the outermost barrier between the organism and its environment. By this barrier function the epidermis protects the organism against a variety of environmental hazards such as dehydration and mechanical stress. Under normal conditions, keratinocytes of all layers are interconnected by desmosomes and anchored by hemidesmosomes to a specialised type of extracellular matrix, the basement membrane. When the epidermis is injured, a vitally important response is initiated with the aim to restore the protective function of the epithelium. A fast but provisional sealing is achieved by the deposition of the fibrin clot before within 24 h after wounding keratinocytes from the wound margins begin to migrate into the wound bed, where they start to proliferate and to form the new epithelium. The development of new high-resolution assays for the study of cell migration and motility has potentiated major progress in our understanding of keratinocyte migration in vitro and in situ. The data reviewed here point to a sophisticated cooperation between soluble motogenic growth factors, cell–matrix interactions, and cell-to-cell communications as major parts of the machinery regulating keratinocyte migration.

Keywords: Motogenic growth factor; Reepithelialisation; Integrin; Matrix metalloproteinase.

Abbreviations: APP Alzheimer amyloid precursor protein; ECM extracellular matrix; EGF epidermal growth factor; FAK focal adhesion kinase; IF intermediate filament; MF microfilament.

Introduction

Keratinocytes are the prevalent cell type of epithelial tissues which comprise skin and mucosa, including oral, oesophageal, corneal, conjunctival, and a variety of genital epithelia. Keratinocytes of the epidermis provide the cellular basis of the outermost barrier between the organism and its environment. By this barrier function the epidermis is able to protect the organism against a variety of environmental hazards such as UV irradiation, dehydration, toxic substances, and mechanical stress. This multilayered cornified epithelium is formed by several layers of keratinocytes in which a variety of other cell types are embedded that are engaged with specialised functions such as the melanin pigment-producing melanocytes, the immunocompetent Langerhans cells, and the neuroendocrine Merkel cells. The basal cell layer of the epidermis is firmly anchored by hemidesmosomes (Borradori and Sonnenberg 1999) to the basement membrane (Fig. 1A), a thin layer of specialised extracellular matrix (ECM) rich in laminin and collagen IV that separates the epithelium from the dermal connective tissue (Burgeson and Christiano 1997). The basal cell layer contains the epidermal stem cells, which are surrounded by transit amplifying cells that are stem cell daughters and that undergo a small number (estimated 3–5) of cell divisions (Potten 1981). The transit amplifying cell daughters, the committed cells, are destined to move upwards from the basal layer to finally undergo terminal differentiation (Adams and Watt 1990). This terminal differentiation is followed by a form of programmed cell death which differs from apoptosis, e.g., by distinct stimuli (Haake and Palakowska 1993, Maruoka et al. 1997, Gandarillas et al. 1999). Under normal conditions, keratinocytes of all layers are interconnected by desmosomes (Green and Gaudry 2000) linking

* Correspondence and reprints: Institut für Zellbiologie, Universität Bonn, Ulrich-Haberland-Strasse 61a, 53121 Bonn, Federal Republic of Germany.

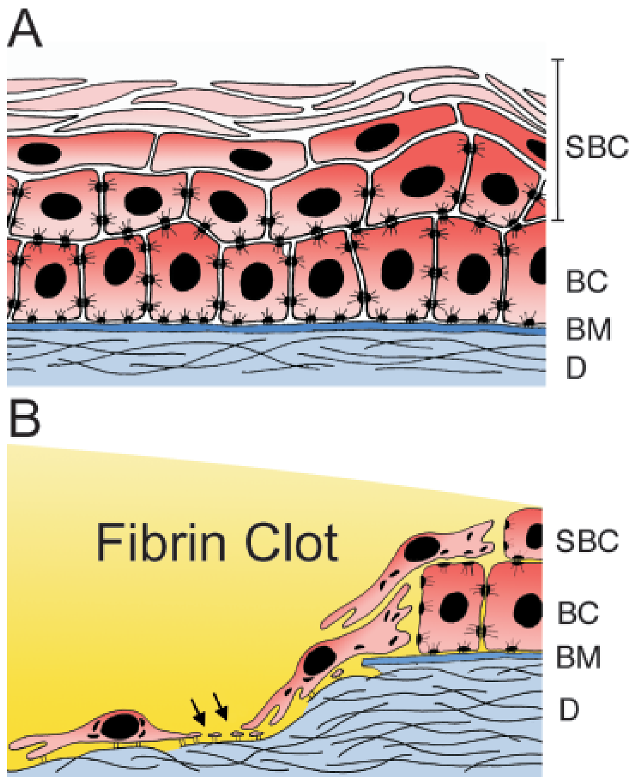

Fig. 1 A, B. Process of keratinocyte migration during epidermal wound healing. **A** In the unwounded state, the basal keratinocyte layer is anchored by hemidesmosomes to the basement membrane (*BM*) which separates the epidermis from the dermal connective tissue (*D*). **B** After wounding, a provisional wound sealing is formed by the fibrin clot before keratinocytes from the wound edges start to invade the wound bed along the border between the fibrin clot and the dermal ECM to form a neo-epithelium. Migrating cells derive from basal (*BC*) and suprabasal cell layers (*SBC*). During migration, keratinocytes leave behind migration tracks consisting of integrin macroaggregates (arrows) which result from membrane ripping at the cell rear and which might act as a guiding structure for trailing cells

the keratin intermediate filaments (IF) of neighbouring cells to a network of mechanically stabilised keratinocytes (Fig. 1A).

When the skin is injured, a vitally important response is initiated with the aim to restore the protective function of the epithelium. Efficient repair of cutaneous wounds demands a series of precisely controlled events in the epidermis and the dermis (Clark 1996, Woodley 1996, Martin 1997). A fast but provisional sealing is achieved by the deposition of the fibrin clot that consists of a cross-linked matrix of fibrin (Fig. 1B) (Clark et al. 1982, 1985) containing small amounts of the cell adhesion proteins fibronectin, vitronectin, and thrombospondin (Bornstein and Sage 2002) and numerous embedded blood platelets. Within 24 h after wounding, keratinocytes from the wound margins begin to migrate, to leave the tissue and to invade the wound bed, where they proliferate to form the new epithelium (Fig. 1B). During the last decade, there has been major progress in our understanding of keratinocyte migration in situ and in vitro. Nevertheless, numerous open questions concerning the signals inducing keratinocyte migration and the dynamics of cell–matrix interaction require new experimental approaches.

Keratinocyte morphology

After a wounding of the skin, basal epidermal keratinocytes undergo a dramatic change in cell shape due to the transition from stationary tissue residents to migrating cells (Ortonne et al. 1981). Thus, in the unwounded epidermis the basal keratinocytes exhibit a columnar phenotype with a characteristic baso-apical polarity (Fig. 1A). As the cells start to migrate across the wound bed, this polarity changes in that the cells become flat and elongated (Fig. 1B) with long cytoplasmic projections, called lamellipodia, and membrane ruffles at the cell front. The transition of cells from a columnar and interconnected to a flat and singular state requires a dramatic reorganization of the cytoskeleton and the junctional complexes. In the unwounded epidermis the basal keratinocytes are joined to neighbouring keratinocytes by desmosomes (Green and Jones 1996, Green and Gaudry 2000) and to the basement membrane by hemidesmosomes (Borradori and Sonnenberg 1999). By electron microscopy, these cell–cell and cell–matrix junctions appear as electron-dense studlike structures on the plasma membrane. When cells become migratory, these structures detach and become internalised from the membrane towards a perinuclear localisation (Krawczyk and Wilgram 1973). IF bundles which are indirectly connected to those of neighbouring cells by desmosomes and to the ECM by hemidesmosomes are also withdrawn from the membrane and reorganised when movement begins. At the same time the microfilament (MF) system is rearranged (Gabbiani et al. 1978) and numerous stress fibres containing the motorprotein myosin and the MF-bundling protein α-actinin are formed. Migrating epidermal cells do not terminally differentiate as keratinocytes of the normal epidermis do. For example, wound epidermal cells do not contain cytokeratin proteins normally found in mature stratified epidermis (Mansbridge and Knapp 1987) nor do they synthesise filaggrin, a cross-linker of keratin filaments. In contrast, migrating cells contain keratins which are typical for basal epidermis cells, such as cytokeratin 5 and 14 (Hertle et al. 1992). Nevertheless, the phenotype of migrating keratinocytes is not iden-

tical to basal cells, as migrating cells contain involucrin and transglutaminase, which usually are expressed in the stratum granulosum and spinosum of intact epidermis (Candi et al. 2002). It is still unclear whether keratinocyte migration during reepithelialisation is restricted to basal cells. Recent evidence from an organotypic model of wound healing in which keratinocytes were genetically labelled with retroviruses suggests that migration is not limited to basal cells but that suprabasal cells may also participate in that they "leapfrog" over basal cells (Fig. 1B) (Garlick and Taichman 1994). It is also not known in detail which signals contribute to the changes in morphology and which are the switches for migration. The following three mechanisms are currently discussed: signals from the contacts between matrix receptors of wound edge keratinocytes and the dermal collagens I and III; the binding of soluble motogenic mediators such as cytokines and growth factors to the corresponding receptors on the surface of keratinocytes at the wound margin; and the loss of neighbouring cells and the disruption of cell–cell contacts. In vitro, however, the addition of motogenic growth factors to isolated keratinocytes on appropriate ECM surfaces has been shown to be sufficient to turn a stationary into to a migratory cell (Fig. 2) (Kirfel et al. 2002). Furthermore, low Ca^{2+} levels can impart cultured keratinocytes with a migratory phenotype, while normal Ca^{2+} concentrations drive terminal differentiation (Hennings et al. 1980).

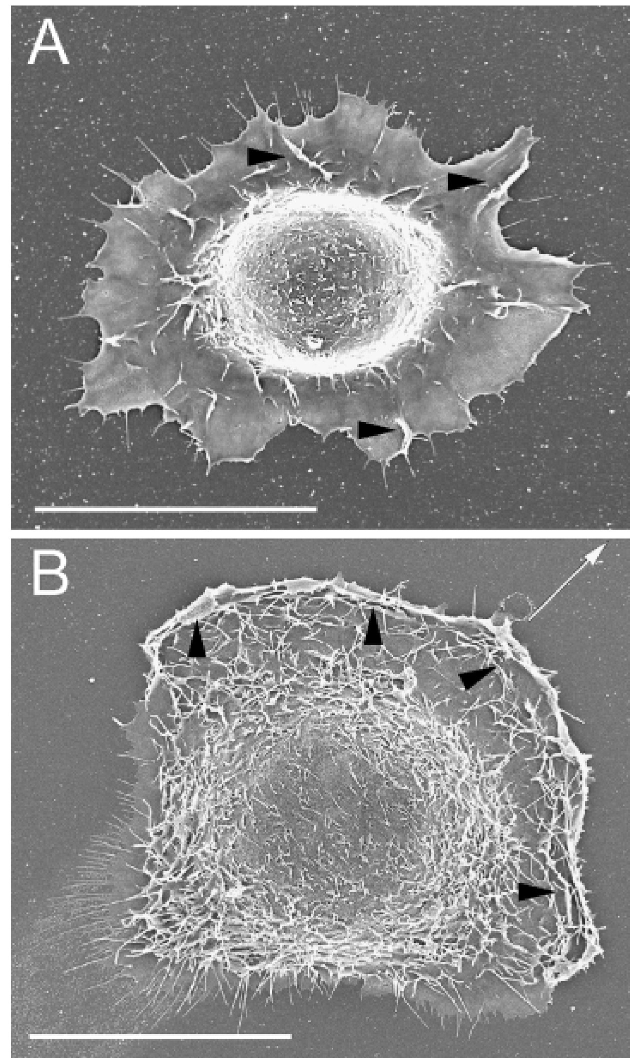

Fig. 2 A, B. Induction of keratinocyte polarity by the motogenic growth factor sAPP. Scanning electron microscopy showed significant changes in the morphology of isolated epidermal keratinocyte upon addition of sAPP. **A** Untreated cells were nearly isodiametric with the cell lamella visible at the entire circumference and only very few ruffles (arrowheads). **B** sAPP treatment induced a polarized morphology, with the cell lamella pointing towards the direction of migration (arrow) and carrying numerous ruffles (arrowheads). Bars: 20 μm

Extracellular matrix and integrins

In the unwounded state, basal keratinocytes are tethered by integrins (Hynes 1992, Sonnenberg 1993) to the basement membrane, which constitutes a thin layer of specialised extracellular matrix (Timpl 1996) and which is rich in collagen type IV and laminin 5. Laminin 5 is an adhesion protein and a member of the family of basement membrane glycoproteins, each of which is composed of three subunits (Tryggvason 1993). The laminin 5 heterotrimer is synthesised by keratinocytes as a precursor that undergoes specific proteolytic processing after secretion (Zhang and Kramer 1996). Recent studies indicate that unprocessed laminin 5 is capable of inducing cell migration while impeding the assembly of hemidesmosomes (Nguyen et al. 2000). After proteolytic processing, laminin 5 does no longer support migration but is involved in the construction of hemidesmosomal cell-anchoring structures (O'Toole et al. 1997, Borradori and Sonnenberg 1999). These data indicate that laminin 5 has a dual function in promoting either keratinocyte adhesion or migration, depending on the extent of processing.

Integrins are heterodimeric transmembrane proteins (Yamada et al. 1996) consisting of an α and a β subunit which bind with low affinity but high specificity to different ECM components including collagen IV, fibronectin, vitronectin, and laminin 5. The cytosolic domains of integrins are linked by complexes of bridging molecules to the cytoskeleton (Gumbiner 1993, Miyamoto et al. 1995, Hemler 1998, Geiger et al. 2001), i.e., the MF or the IF. The bridge to the MF is formed by tensin, the focal adhesion kinase (FAK) (Sieg et al. 2000), talin, vinculin, and α-actinin (Liu et al. 2000), whereas plectin (Hieda et al.

1992) and the bullous pemphigus antigen 230 (Mueller et al. 1989) are prominent linkers of integrins and IF. This transmembrane organisation facilitates integrins to bind to extracellular ligands and to transmit signals into the cytoplasm and the nucleus resulting in the reorganisation of the cytoskeleton ("outside-in" signalling) (Schlaepfer and Hunter 1998, Calderwood et al. 2000, Turner 2000). Conversely, cytoplasmic components can interact with the cytosolic domain of integrins to modulate the integrin–ECM binding affinity ("inside-out" signalling) with consequences for cell migration and ECM reorganisation (Ginsberg et al. 1992, Sastry and Horwitz 1993, Damsky and Ilic 2002).

The major integrins in the intact epidermis (Table 1) are the collagen-binding α2β1 (Emsley et al. 2000), the laminin 5-binding α3β1 (Kreidberg 2000), and α6β4 (Mercurio et al. 2001), an integral component of hemidesmosomes that also binds laminin 5. After wounding, the wound edge keratinocytes come into contact with dermal collagens I and III and the fibrin clot constituents fibrin, fibronectin, and vitronectin. Simultaneously, the expression profile of integrins on wound margin keratinocytes changes (Grinnell 1992), characterized by the induction of specific integrins that bind proteins of the dermal matrix and specific components of the fibrin clot that act as a provisional matrix during wound repair (Table 1). The integrins include the fibronectin receptor α5β1 and the fibronectin and vitronectin receptors αvβ5 and αvβ6 (Table 1) (Martin 1997). Due to their lack in the fibrin-specific integrin αvβ3, migrating keratinocytes do not invade the fibrin clot but use the dermal ECM as provisional matrix for their movement towards the wound bed. Hence, keratinocyte migration dissects the fibrin clot from the wound bed and, thereby, contributes to the process of eschar slough during wound repair (Kubo et al. 2001).

During their migration over the dermal ECM, keratinocytes synthesise and deposit a variety of ECM components such as laminin, laminin 5, fibronectin, and collagen IV and, thereby, form a provisional basement membrane (Clark 1990, Caviani et al. 1993, Larjava et al. 1993, Gailit and Clark 1994). Hence, migrating or wound margin keratinocytes are capable of forming and modulating their own migration and adhesion substrates (Kirfel et al. 2003). This becomes evident when keratinocytes are grown in vitro on mere glass or plastic surfaces. Although these materials do not support cell migration, keratinocytes can efficiently move, albeit with a lower velocity as compared with fibronectin- or vitronectin-coated surfaces. Immunofluorescence reveals that keratinocytes on glass and plastic materials produce collagen IV, laminin, laminin 5, and fibronectin, which are deposited during migration and thereby form a clearly visible track that marks the paths the cells have used (O'Toole 2001, Kirfel et al. 2002). There is strong evidence from numerous complementary experiments that the interaction of keratinocytes with the freshly deposited, unprocessed laminin 5, the first matrix component expressed by keratinocytes during wound healing, is crucial for migration in situ and in vitro (Decline and Rousselle 2001). Accordingly, in an in vitro wound assay, keratinocyte migration can be inhibited by the application of blocking antibodies directed against laminin 5 and against its receptor, the α3β1 integrin or enzymes inducing the proteolytic processing of laminin 5. Furthermore, in β1 knockout mice keratinocyte migration is impaired, leading to a retarded cutaneous wound repair and abnormal epithelial architecture (Grose et al. 2002).

During cell migration, new integrin–substrate adhesions are formed at the tips of lamellipodia, whereas adhesion sites at the cell rear must be disrupted for a cell to move. It can be concluded that this dynamic behaviour of integrins is important in governing cell migration.

Table 1. Keratinocyte integrins and their ECM ligands

Integrin	ECM ligand	Cytoskeletal association	Expression in keratinocytes[a]		Involvement in migration	Reference
			Resting	Migrating		
α2β1	collagen	MF	+	+	+	Emsley et al. 2000
α3β1	laminin 5	MF	+	+	+	Kreidberg 2000
α5β1	fibronectin	MF	−	+	+	Martin 1997
α6β4	laminin 5	IF	+	−	−	Mercurio et al. 2001
αvβ5	vitronectin	MF	−	+	−	Klemke et al. 1994
αvβ6	vitronectin	MF	−	+	−	Martin 1997

[a] The transition from the resting to the migratory state of keratinocytes coincides with an altered expression pattern of integrins that allows dynamic interactions with the dermal ECM and with components of the provisional basement membrane

The breakage of the cell–substratum attachment needed to allow locomotion can, in principle, occur either by intracellular disruption of the cytoskeleton–integrin linkage or by extracellular release of the integrin–matrix linkage. Detachment of the cell from the substratum may involve pericellular proteolysis in which serine and matrix metalloproteinases play a crucial role (Werb 1997, Murphy and Gavrilovic 1999). Calpain, a Ca^{2+}-dependent cytosolic protease which localises to focal adhesions (Beckerle et al. 1987), can regulate cell locomotion and rear retraction in CHO cells by destabilising cytoskeletal linkages (Huttenlocher et al. 1997); in vitro experiments suggest that calpain cleaves the cytoplasmic domain of the β subunit of integrins and cytoskeletal molecules such as talin (Du et al. 1995, Cooray et al. 1996, Perrin and Huttenlocher 2002), and calpain inhibition hinders rear release by strengthening cytoskeletal linkages (Palecek et al. 1998). Disassembly of focal adhesions is followed by increased cell locomotion; it has been shown that the gradual loss of tyrosine phosphorylation of p125 FAK and c-Met coincides with the disruption of focal adhesions and the conversion to a motile phenotype (Matsumoto et al. 1994). In permeabilized fibroblasts a rapid breakdown of focal adhesions has been observed upon the addition of ATP leading to tyrosine phosphorylation of cytoskeletal components (Crowley and Horwitz 1995).

Originally, these biochemically regulated processes were thought to facilitate rear detachment of migrating cells by a process which does not necessarily induce any loss of cell material during migration. However, it has been shown that "membrane ripping" of cells occurs during migration (Bard and Hay 1975, W. Chen 1981). By this process, a major fraction of integrins, which have been shown to form macroaggregates on migrating chick fibroblasts, is left behind on the substratum, forming migration tracks clearly visible with high-resolution electron microscopy (Regen and Horwitz 1992). Detailed studies have shown that cells such as epidermal keratinocytes which move more often and over larger distances than fibroblasts (Lee et al. 1993), move rapidly at constant velocity, and it has been speculated that such cells do not expend the vast quantities of integrins observed in fibroblast migration (Palecek et al. 1996) and, therefore, do not form migration tracks. Indeed, the release of integrins during the migration of keratinocytes has not been reported until recently when the development of new light and electron microscopy techniques made it possible to show for the first time that migrating keratinocytes leave behind migration tracks consisting of membrane remnants which have been identified as integrin macroaggregates (Fig. 3) (Kirfel et al. 2003). These macroaggregates seem to derive from the fragmentation of retracting fibres at the cell rear and are attached to a meshwork of ECM proteins. Whereas a substantial fraction of integrins is lost during keratinocyte migration, actin and actin-associated proteins as well as most other cytosolic components remain associated with the cell and appear to be retained during membrane ripping. Because migration track proteins and membrane remnants provide multiple adhesion sites for other cells, our observations suggest that keratinocyte migration tracks fulfil a central biological role, e.g., as a provisional basement membrane which might be utilised during reepithelialisation processes. Indeed, in vitro migrating keratinocytes appear to recognise the tracks of other keratinocytes and to migrate along this provisional basement membrane (Kirfel et al. unpubl.).

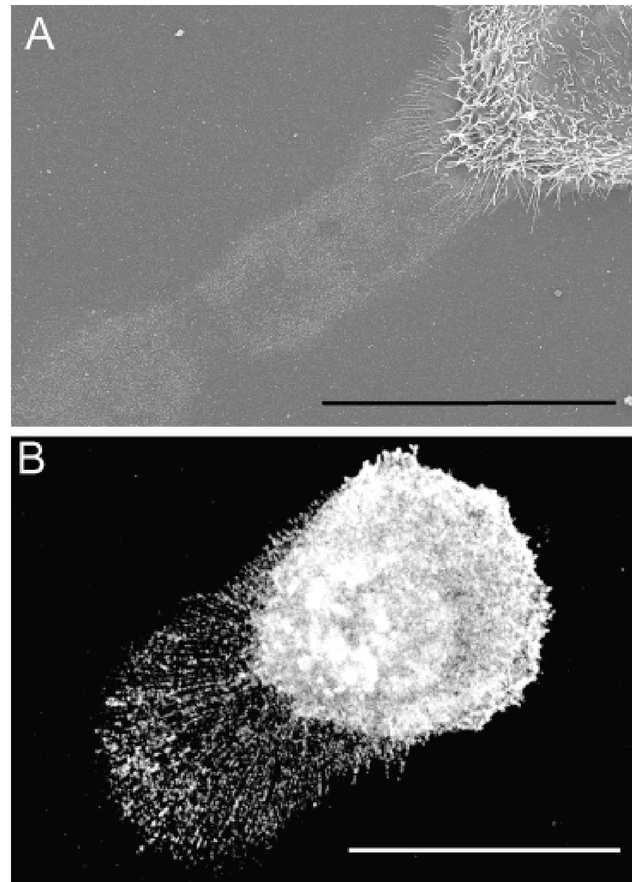

Fig. 3 A, B. Keratinocyte migration tracks contain β1-integrin macroaggregates. During migration isolated keratinocytes leave behind tracks of cellular material which become clearly visible by scanning electron microscopy (**A**). These migration tracks consist of spherical and tubular structures (macroaggregates) arranged like pearls on a string and carry high amounts of β1-integrin as visualized by immunofluorescence microscopy (**B**). Bars: 20 μm

Growth factors as motogens for keratinocytes

Growth factors are usually defined as soluble polypeptides that act as paracrine or autocrine modulators of cell proliferation and differentiation (Deuel 1989), which are produced by various cell types, as opposed to hormones, which are produced by specialised endocrine glands. Numerous growth factors have been shown to stimulate also the migration of different cell types and are accordingly called motogenic growth factors or motogens (Nickoloff et al. 1988, Manske and Bade 1994). Motogens can either induce chemokinesis, i.e., migratory reactions with random orientation, or chemotaxis, i.e., directional migrations following a gradient of growth factor concentrations. Motogens exert their influence on cells by binding to transmembranous protein receptors on the cell surface which convey signals via tyrosine kinases and other second-messenger systems such as cyclic AMP-dependent kinase and protein kinase C. A key event in stimulating motility is the activation of the small guanosine triphosphatase (GTPase) Rac, which mediates the actin polymerisation-driven lamellipodia extension and the assembly of focal adhesion complexes as part of the crawling response of tissue culture fibroblasts and epithelial cells (Felsenfeld et al. 1999).

Growth factors of the wound region are derived from different sources (Table 2) including blood platelets within the fibrin clot (Raines et al. 1990, Sporn and Roberts 1992), inflammatory cells such as granulocytes and monocytes, and dermal fibroblasts (Derynck 1988, Werner 1998). In addition, keratinocytes themselves produce a variety of growth factors relevant for wound healing (Coffey et al. 1987, Brachmann et al. 1989).

For many years the epidermal growth factor (EGF) family of growth factors, comprising EGF itself (Cohen 1987), transforming growth factor alpha (TGF-α) (Barrandon and Green 1987), and more recently the heparin-binding epidermal growth factor (HB-EGF) (Higashiyama et al. 1991, Iwamoto and Mekeda 2000), all acting as ligands for the EGF receptor (Carpenter 1993), were considered the key regulators of keratinocytes at the wound margin. Studies on the responsiveness of cultured keratinocytes to EGF suggest that these growth factors act on the epidermis as motogens as well as mitogens to drive wound closure. Over the last decades, numerous growth factors not belonging to the EGF family, such as transforming growth factor beta (TGF-β) (Brandes et al. 1991), have been identified as keratinocyte motogens (Decline et al. 2003). TGF-β is known to significantly stimulate the migration of keratinocytes in vitro, while it inhibits their proliferation (Shipley et al. 1986, Hebeda 1988, Yue and Mulder 2001). TGF-β has been reported to localise to the region of keratinocyte migration at the wound edges (Schmid et al. 1993). Moreover, in the presence of TGF-β the synthesis of laminin 5 has been shown to be up-regulated in migrating cells (Korang et al. 1995, Kainulainen et al. 1998). One decade ago, the keratinocyte growth factor (KGF) (Finch et al. 1989; Rubin et al. 1989, 1995), a member of the fibroblast growth factor family, which is up-regulated more than 100-fold within 24 h by dermal fibroblasts at the wound region, was identified as key regulator of keratinocyte migration and proliferation (Werner et al. 1992). KGF, exogenously applied to skin wounds, had mitogenic and motogenic effects on keratinocytes during the healing process (Staiano-Coico et al. 1993). More recently, sAPP, the secretory form of

Table 2. Motogenic growth factors for keratinocytes and their sources[a]

Growth factor	Source(s)	Involvement in keratinocyte behaviour			Reference(s)
		Proliferation[b]	Migration[b]	Chemotaxis[c]	
EGF	platelets	+	+	+	Carpenter 1993
HB-EGF	macrophages	+	+	−	Iwamoto and Mekeda 2000
KGF(FGF 7)	dermal fibroblasts	+	+	+	Werner et al. 1992, Werner 1998
TGF-α	macrophages, keratinocytes	+	+	−	Derynck 1988
TGF-β	platelets, macrophages	−	+	+	Yue and Mulder 2001
sAPP	platelets, keratinocytes	+	+	+	Hoffmann et al. 2000, Kirfel et al. 2002

[a] With the exception of TGF-β, all listed growth factors are motogenic and mitogenic for keratinocytes
[b] +, stimulation by growth factor; −, inhibition by growth factor
[c] +, induction of chemotactic response; −, induction of chemokinetic response

the Alzheimer amyloid precursor protein (APP), has been shown to act as a growth factor for epithelial cells including epidermal keratinocytes (Pietrzik et al. 1998, Hoffmann et al. 2000, Schmitz et al. 2002). APP, the precursor of sAPP, is expressed predominantly in the basal keratinocyte layer of the unwounded epidermis with the highest levels found in proliferation-competent cells. After wounding, all cell layers of the hyperproliferative epithelium at the wound margin show an increased APP expression (Kummer et al. 2002). By culture models of keratinocyte differentiation, the release of sAPP was found to be significantly higher in proliferating than in quiescent, partially differentiated cells. In vitro, sAPP stimulates the proliferation of keratinocytes about fourfold and acts, therefore, as a potent mitogen, i.e., a mitosis-promoting agent. The motility-promoting (motogenic) effect of sAPP has been demonstrated by a new stroboscopic cell motility assay that allows the quantification of different motility parameters with high resolution in time and space (Hinz et al. 1999, Kirfel et al. 2002). These parameters include the migration velocity and the velocity and frequency of lamellipodia protrusion and ruffle retraction. All parameters were stimulated by sAPP and EGF about twofold. Most recently, the insulin-like growth factor IGF-1 (Stracke et al. 1988), which is produced by dermal fibroblasts and macrophages, has been shown to stimulate membrane protrusion and migration in keratinocytes (Haase et al. 2003) and, therefore, might be added to the growing list of keratinocyte motogens with possible implications in reepithelialisation. Numerous motogenic growth factors such as TGF-β, EGF, and KGF have been shown to induce not a randomly orientated migration, i.e., chemokinesis, but a properly directed, chemotactic movement along a motogen gradient. Recently, by a modified Boyden chamber assay, evidence has been presented that sAPP also exerts a chemotactic effect on keratinocytes (Kirfel et al. 2002) and thus might be involved in guiding keratinocytes towards the wound bed during epidermal wound healing. As sAPP is also released by blood platelets (Bush et al. 1990), fibrin clots represent an additional source of sAPP derived directly from the wound bed and supporting keratinocytes in their directionality of migration for reepithelialisation.

The motogenic effect of numerous growth factors such as EGF and sAPP appears to depend in a synergistic manner on the proper interaction of integrins with the ECM. Apparently, integrin and growth factor signalling pathways interact through several mechanisms from the coclustering of the two receptor types to the activation of common downstream signalling pathways (Schwartz and Ginsberg 2002). Recently, FAK, which localises to sites of integrin clustering, has been reported to act as a bridging molecule that links growth factor receptor and integrin signalling pathways (Sieg et al. 2000). Accordingly, cells lacking FAK appear to be refractory to motility signals from EGF. The response to EGF could be rescued by the stable reexpression of FAK. In fact, integrins seem to enable growth factor signalling in many cases, i.e., normal growth factor signalling does not occur unless cells are adherent to the ECM through integrins. The strong synergy between soluble and ECM-derived stimuli is manifested in the activation of mitogen-activated protein (MAP) kinase by the receptors of both signals, resulting in the direct induction of cell migration (Stoker and Gherardi 1991; P. Chen et al. 1994; Klemke et al. 1994, 1997).

Cell–cell adhesion and signalling

Adhesion between epithelial cells is generally mediated by three types of junctions, tight junctions, desmosomes, and adherens junctions, which together constitute the intercellular junctional complex (Perez-Moreno et al. 2003). These complexes contain transmembrane receptors, usually glycoproteins that mediate binding at the extracellular surface. At the cytosolic surface the receptors are connected by bridging molecules to the cytoskeleton, thereby establishing mechanical linkage and molecular lines of communication (Jamora and Fuchs 2002). This connection is necessary for the formation of stable cell-to-cell contacts and for the integration of cell-to-cell contacts with changes in morphology during epithelial differentiation and the transition from a tissue-resident to a migratory cell type during the process of epithelial wound repair.

The transmembrane core of adherens junctions consists of cadherin receptors which, in the presence of Ca^{2+}, interact in a homophilic manner with cadherins on the surface of adjacent cells and cluster at sites of cell-to-cell contacts in most solid tissues. E-cadherin, the best characterized member of the cadherin family, is primarily expressed in epithelia including the epidermis. The cytosolic domains of adherens junction cadherins are associated with the MF system via linker proteins known as catenins (Hinck et al. 1994, Braga 2002). Besides acting as mechanical linkers between cadherins and the MF system, catenins are compounds of the signalling pathways leading to the activation or inhibition of the small GTPases Rac, Rho, and Cdc42

(Fukata and Kaibuchi 2001). These GTPases are known as molecular switches for the reorganisation of the MF system which can initiate the activation of actin-associated proteins capable of regulating filament assembly or disassembly. In the intact epithelium, clustered cadherins seem to switch GTPases towards positions that favour the formation of stable MF bundles typical for epithelial cells (Braga et al. 2000). Upon epithelial wounding, cells lose their direct neighbours and the cadherins become detached from their binding opponents. This detachment seems to move the GTPase switches to positions that initiate the reconstruction of the MF system necessary for cell migration during reepithelialisation. As motogenic growth factors such as EGF and sAPP are also known to modulate the MF system by switching small GTPases on or off, cadherin/catenin-mediated signalling and soluble motogens might cooperate in regulating cell migration during epithelial wound healing.

Matrix proteases

To reach the wound bed, migrating keratinocytes must move along the dermal ECM at the interface between the fibrin clot and the dermal matrix, i.e., the leading keratinocytes have to proteolytically dissolve and to remodel the fibrin barrier ahead of them to create a path (Murphy and Gavrilovic 1999). The main enzyme for fibrinolysis is plasmin derived from plasminogen within the clot which can be activated either by tissue-type-specific plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) (Ossowski and Aguirre-Ghiso 2000). In migrating keratinocytes both activators and the receptor for uPA are up-regulated. In addition, various members of the matrix metalloproteinase (MMP) family (McCawley and Matrisian 2001, Seiki 2002), each of which cleaves a specific subset of matrix proteins, are also up-regulated by wound edge keratinocytes (Grondahl-Hansen et al. 1988, Romer et al. 1994). MMP-9 can cleave collagens type IV and VII in the basement membrane and is thought to be responsible for detaching keratinocytes from their substrate (Salo et al. 1994). MMP-1 specifically degrades collagens I and III, which are abundant within the dermal matrix (Saarialho-Kere et al. 1992). This proteinase is up-regulated in those basal keratinocytes that have migrated beyond the basement membrane, suggesting that cell–matrix interactions may control the expression of MMP-1. Mice deficient in MMP-3 and MMP-7 are defective in epidermal wound repair (Bullard et al. 1999). MMP-10 has a wider substrate specificity and is also up-regulated at the wound margin, but its expression is increased when wound healing is impaired (Saarialho-Kere et al. 1994). The expression of MMPs during wound healing is up-regulated simultaneously with the expression of distinct matrix receptors of the integrin family. In addition, certain MMPs can bind to integrins, thereby providing a mechanism for localised matrix degradation (Chapman et al. 1999). For example, the collagen-cleaving MMP-1 was shown to bind to $\alpha 2$-integrins and to be colocalised with the collagen-binding $\alpha 2\beta 1$-integrin at the leading edge of migrating keratinocytes. Since MMP-1 cleavage of collagen results in the exposure of integrin binding sites, there might be a cooperation between matrix receptors and proteinases to perform an efficient migration on collagens. More recently, the uPA receptor (Blasi 1999, Mondino et al. 1999), a glycosyl phosphatidylinositol-anchored membrane protein (Chapman et al. 1999), was recognised as a multifunctional protein that interacts with integrins and, thereby, regulates integrin function and initiates signalling events that alter cell adhesion, migration, and proliferation (Wei et al. 1996, Ossowski and Aguirre-Ghiso 2000, Preissner et al. 2000, Simon et al. 2000).

Assays for studying cell migration in vitro

To study the impact of motogens and matrix composition on cell migration and motility in vitro, a great variety of assays have been developed which are usually based on light microscopy and time lapse analysis. To assay the locomotion of cells, various migration track assays are currently used (Manske and Bade 1994). The phagokinetic track assay (Albrecht-Buehler 1977) is based on the clearing of the culture substratum from protein-coated colloidal gold particles by migrating cells. This assay has the advantage that the migratory behaviour can directly be observed during microscopic inspection. However, it cannot be excluded that the large number of protein-coated gold particles internalised by many cells affects their migration and viability. The ECM track assay (Bade and Nitzgen 1985) relies on the immunocytochemical demonstration of the ECM proteins deposited by the migrating cells onto the culture substratum and, therefore, allows no direct observation and involves time-consuming preparations. A newly developed migration track assay is based on improved light and electron microscopy techniques which make possible the visualisation of tracks without labelling procedure by visualising the integrin macroaggregates left behind by migrating cells after their release from adhesion sites (Kirfel et al. 2003).

The stroboscopic cell migration assay (Hinz et al. 1999) is based on video-microscopic techniques in combination

with a specialised software application and allows to quantify simultaneously a multitude of migration and motility parameters with highest resolution in space and time (Hinz et al. 1999, Kirfel et al. 2002). This assay is extremely sensitive and even minimal alterations in lamellipodia dynamics and migration velocity in response to external stimuli can be recorded.

To evaluate chemotactic effects of soluble mediators on the migration of cells, a variety of experimental approaches have been developed, including the orientation chamber assay (Zigmond 1988) and the under agarose assay (Stokes et al. 1990). The filter membrane assays, as originally introduced by Boyden (1962), are based on a chamber of two medium-filled compartments separated by a microporous membrane. Generally, cells are placed in the upper compartment and allowed to migrate through the pores into the lower chamber, which usually contains the potential chemoattractant. After an appropriate incubation time, the cells on both sides of the filters are counted either light microscopically after staining of the cells or electron microscopically (Kirfel et al. 2002). The availability of membranes with different pore sizes makes the Boyden chamber assay suitable for a great variety of cell types and the possibility to coat such membranes with various ECM substrates allows to study keratinocyte migration under conditions simulating the wound bed or invasive processes under chemotactic points of view.

Concluding remarks

Due to the development of new techniques and concepts, research on cell migration has become a field of central interest in cell biology. Consequently, our understanding of keratinocyte migration during wound healing has progressed considerably in recent years. Part of the difficulty in unravelling the regulatory mechanisms that control keratinocyte migration is the redundancy of signals and the complexity of the cross talk between the systems such as soluble motogens, ECM components, and matrix proteases. It is almost certain that growth factor and matrix molecules are not the only relevant signals inducing keratinocyte migration during wound healing. Changes of gap-junctional connections at the wound margin may help to coordinate proliferative and migratory activities (Goliger and Paul 1995). Mechanical signals in the form of cell stretching and even ripping of the plasma membrane may also prove to be important activators of keratinocyte migration (Matin 1997). Finally, the regulation of keratinocyte migration might also involve inhibitors which are able to reduce the migratory rate in morphogenetic processes. It has recently been shown that inhibition of keratinocyte migration occurs by activation of the β-adrenergic receptor of keratinocytes (J. Chen et al. 2002). Specific inhibitors might be necessary to reduce the action of motogens or their release. Blocking the release of sAPP with metalloprotease inhibitors resulted in strongly reduced keratinocyte proliferation (C. Siemes et al., University of Bonn, Bonn, Federal Republic of Germany, unpubl.) and might also lead to the inhibition of keratinocyte migration. Animal models of wound healing combined with knockout systems and the development of new in vitro assays to study keratinocyte migration will considerably improve our knowledge on keratinocyte migration and epidermal wound healing during the next years.

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