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Eph/ephrin signaling in epidermal differentiation and disease

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Abstract

Eph receptor tyrosine kinases mediate cell–cell communication by interacting with ephrin ligands residing on adjacent cell surfaces. In doing so, these juxtamembrane signaling complexes provide important contextual information about the cellular microenvironment that helps orchestrate tissue morphogenesis and maintain homeostasis. Eph/ephrin signaling has been implicated in various aspects of mammalian skin physiology, with several members of this large family of receptor tyrosine kinases and their ligands present in the epidermis, hair follicles, sebaceous glands, and underlying dermis. This review focuses on the emerging role of Eph receptors and ephrins in epidermal keratinocytes where they can modulate proliferation, migration, differentiation, and death. The activation of Eph receptors by ephrins at sites of cell–cell contact also appears to play a key role in the maturation of intercellular junctional complexes as keratinocytes move out of the basal layer and differentiate in the suprabasal layers of this stratified, squamous epithelium. Furthermore, alterations in the epidermal Eph/ephrin axis have been associated with cutaneous malignancy, wound healing defects and inflammatory skin conditions. These collective observations suggest that the Eph/ephrin cell–cell communication pathway may be amenable to therapeutic intervention for the purpose of restoring epidermal tissue homeostasis and integrity in dermatological disorders.

Keywords

Cell–cell communication; Cell adhesion; Desmosome; EphA2; Epithelial; Cadherin

1. Introduction

The epidermis is a self-renewing, stratified squamous epithelium that is constructed to resist mechanical trauma, maintain a protective barrier against environmental factors and limit excessive water loss [1–3]. Paramount to these essential functions is the remarkable life cycle of the epidermal keratinocyte. This begins with keratinocytes in the basal layer, which are anchored to the basement membrane and maintain their ability to proliferate. Basal keratinocytes give rise to post-mitotic cells that enter a program of terminal differentiation in the suprabasal layers. After elaborating skin-specific products and organelles in the

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intermediate layers, keratinocytes eventually undergo a specialized program of cell death in the superficial, cornified layer. Epidermal homeostasis is maintained by the balance between keratinocyte proliferation in the basal layer and death in the cornified layer; yet, there is still much to learn about the signaling pathways that regulate this process of keratinization.

The cellular microenvironment within the epidermis is enriched with surface proteins that have the potential to guide keratinocytes along a pathway of terminal differentiation. For example, cadherin family members that mediate calcium-dependent cell–cell adhesion not only support epidermal tissue integrity but also regulate keratinocyte proliferation, differentiation and barrier function [4–10]. Notch receptors are activated by their ligands present on adjacent cells thereby eliciting signals that transition proliferating keratinocytes into a more differentiated state [11–13]. Recently, Eph receptors and their membrane-associated ephrin ligands have been identified as cell–cell communication complexes that impact a wide-range of keratinocyte behaviors [14–17]. Although Eph receptors are implicated in the regulation of other cell types resident in the skin (e.g., melanocytes, Langerhans cells, dermal fibroblasts, lymphocytes, neural and vascular cells) [18–23], this review primarily focuses on their emerging roles in keratinocytes.

2. Eph receptor and ephrin ligand signaling complexes

Eph receptors represent the largest family of receptor tyrosine kinases (RTKs) in mammals and are activated by membrane-linked ephrin ligands residing on adjacent cell surfaces (Fig. 1) [24–26]. Eph receptors are subdivided into A and B subfamilies depending on their sequence homology and binding preferences for glycosylphosphatidylinositol (GPI)-linked ephrin-A or transmembrane ephrin-B ligands. In humans, there are nine EphA (1–8/10) and five EphB (1–4/6) receptor subtypes, which interact with five ephrin-A (1–5) and three ephrin-B (1–3) ligands. Although the binding of an Eph receptor to an ephrin ligand is generally limited to subtypes of the same class, the relative affinity for individual ligands varies among receptor subtypes and promiscuous interactions between subfamilies also exist [27–29].

Since both receptor and ligand are membrane-bound, the primary mode of Eph/ephrin signaling is thought to be focused at regions of cell–cell contact. Eph/ephrin complexes exist at the interface between two cells and elicit signals in a bidirectional manner, with ‘forward’ signaling mediated by Eph receptor activation and ‘reverse’ signaling occurring through ephrin ligands (Fig. 1). However, ligand- and kinase-independent functions have also been demonstrated for Eph receptors, indicating these cell surface proteins have the potential to signal in a contact-independent manner [30–35].

Attributing specific functions to individual Eph receptor sub-types is challenging given the large number of highly homologous receptor subtypes with promiscuous ligand binding characteristics and frequently overlapping expression patterns in tissues. Despite these hurdles, tremendous strides have been made in our understanding of Eph/ephrin signaling complexes in regulating developmental patterning events in the embryo as well as the consequences of their misexpression in cancer, a topic that has been reviewed in great detail elsewhere [25,36–39]. It is now evident that Eph receptors and ephrin ligands play prominent roles in the organization and maintenance of adult tissues, including various epithelial linings [37,40–42]. Among regenerating epithelial tissues such as those found in the gastrointestinal tract, cornea or skin, cell-type specific roles for Eph receptors are likely to exist depending on the character of receptor subtypes and signaling effectors expressed within the cell as well as the relative abundance of ligands present on its tightly associated neighbors.

3. The epidermal Eph/ephrin axis

Members of the entire family of Eph receptors and ephrin ligands can be detected in human skin, at least at the level of mRNA transcripts [43]. However a more limited repertoire is most likely expressed in the epidermis, which has been better characterized for the EphA/ephrin-A subfamily (Table 1). In particular, human epidermal keratinocytes contain EphA1, EphA2 and EphA4 along with three of their corresponding ligands, ephrin-A1, -A3 and -A4 [17]. Interestingly, the genes encoding these ephrin-A ligands are clustered on Ch1q21-22 within the epidermal differentiation complex, which is proximal to a series of genes associated with the keratinization process. In general, ephrin-A ligands appear to be concentrated in basal keratinocytes whereas EphA receptors can be found in all viable layers of the epidermis (Fig. 2). This suggests that there are two major biological interfaces within the interfollicular epidermis where ephrins can engage Eph receptors to elicit ligand-dependent signaling events: (1) between adjacent keratinocytes in the basal layer where cells retain the ability to proliferate and (2) at the basal–suprabasal interface where keratinocytes commit to a terminal differentiation pathway.

EphA receptors present in the more superficial layers of the epidermis may exist in an inactive state or could potentially signal via ligand-independent mechanisms. GPI-linked ephrin-A ligands can be released from the cell surface via proteases, phospholipases or as a consequence of alternative splicing and are further cross-linked to form higher-order signaling clusters by tissue-type transglutaminases [44–46]. It is therefore possible that EphA receptors become activated by soluble ephrin-A clusters or as of yet to be identified ligands present in the uppermost layers of the epidermis.

Both A and B class Eph receptors and ephrin ligands are present in hair follicles, including the slow cycling, stem cells of the bulge region (Table 2). The hair follicle undergoes a continuous cycle of growth (anagen), death (catagen) and rest (telogen) [1,47,48]. Under normal conditions, bulge stem cells are recruited to enter a pathway of terminal differentiation toward the base of the hair follicle in a process that is tightly coordinated by signals from dermal papilla cells present in the underlying stroma. During wound healing, bulge stem cells also migrate up toward the epidermis and contribute to its regeneration. Interestingly, microarray analysis demonstrated a higher abundance of Eph receptor mRNA transcript levels in bulge keratinocytes as compared to stem cells derived from other tissues [49]. Moreover, EphA4, EphB4 and ephrin-B1 co-localized with stem cells residing in the bulge [49]. Ephrin-A3 and ephrin-B2 are also found in dermal papilla cells [18,50], indicating a potential role for Eph receptors and ephrin ligands in determining cell fate and positioning during hair follicle regeneration and epidermal wound healing responses.

Labeling tissues with recombinant peptides that mimic the extracellular domains of the ligands and receptors can reveal where presumably unoccupied Eph receptors and ephrins are present within the skin. In mice, where the interfollicular epidermis is composed of fewer viable cell layers (2–3 compared to 8–10 in humans) and hair follicles are more prevalent, EphA/B receptors and ephrin-A/B ligands are found throughout the epidermis, hair follicles, sebaceous glands, and dermal papilla (Tables 1 and 2). However, the character of the receptor/ligand subtypes and how they relate to human skin remains less clear. For example, EphA1 has been found in all viable layers of the human epidermis but its expression is restricted to keratinocytes in the basal layer of mice [43,51]. Instead, EphA4 appears to be a major subtype present in the suprabasal layers of the mouse epidermis [14,18,49,52]. Interestingly, EphA4 not only has a high affinity for ephrin-A ligands but can also signal in response to ephrin-B2 [27,53,54]. In addition, EphA2 has been found in an increasing gradient of expression from the basal to suprabasal layers in mouse epidermis whereas other reports failed to detect significant levels of this RTK at various stages of

postnatal epidermal and hair follicle development [15,52]. Part of the reason for these differences may have to do with the developmental regulation of receptor subtypes; this has been shown to be the case for the corresponding epidermal ephrins whose levels vary after birth [18,52]. Much less is known about the distribution of EphB receptors in the human epidermis, although mRNA transcripts for EphB3 and EphB4 appear to be highly concentrated in the skin [17,43].

4. Eph/ephrin signaling complexes in keratinocytes

Keratinocytes purified from mouse and human skin provide a useful model to study the formation, organization and activation of native Eph/ephrin signaling complexes in untransformed epithelial cells ex vivo, since these primary cultures express both receptor and ligand. Along with other EphA receptors and ephrin-A ligands, EphA2 is prominently expressed in isolated keratinocyte cultures [15,16]. A chimeric ephrin-A1 ectodomain peptide fused to the Fc portion of human IgG (i.e., ephrin-A1-Fc) is capable of targeting EphA2, leading to its phosphorylation and triggering downstream signaling events that ultimately reduce the activity of Erk1/2-mitogen-activated protein kinase (MAPK) [15,16,55–57]. Interestingly, *epha2*–/– keratinocytes fail to respond to these soluble peptides or inhibit Erk1/2 activity even though these cells express EphA1 and EphA4 [15]. These findings suggest that EphA2 is the major receptor involved in early responses to ephrin ligand stimulation in undifferentiated keratinocytes.

To a certain extent, the high levels of EphA2 observed in primary keratinocytes may reflect the ‘activated’ state of these largely undifferentiated cells, which are further grown in the presence of mitogens (e.g., EGF) known to regulate the expression of this RTK [57,58]. Keratinocytes maintained in culture exhibit characteristics of cells in a wounded environment, such as a highly motile phenotype, increased rate of proliferation and limited differentiation. These factors might, at least in part, explain why it is difficult to detect EphA2 under homeostatic conditions in mouse skin. In support of this notion, EphA2 expression is increased in response to wound healing [22] and markedly down-regulated when keratinocytes are grown in a three-dimensional, organotypic raft model of the human epidermis (Lin and Getsios, unpublished observations), which is more mature and closely reflects the in vivo state [59].

Since EphA2 and ephrin-A1 are both expressed in primary keratinocyte cultures, ligands from adjacent cells probably activate EphA2 in *trans* (Fig. 1). Although ephrins expressed on the same cell surface are present in distinct membrane domains from EphA receptors, *cis* interactions between ligands and receptors impair downstream signaling events in neurons [60–64]. The relative organization of *cis* and *trans* complexes between ephrin-A1 and EphA2 in keratinocytes is unknown but this RTK does undergo contact-dependent activation when intercellular junctions are stabilized by switching cultures from low to high calcium [16]. As in other epithelial cell cultures [33,55,65–69], ligand-dependent activation of EphA2 relies upon the function of the calcium-dependent cell-cell adhesion molecule, E-cadherin. In cancer cell lines where E-cadherin function or expression is lost, EphA2-dependent signaling is also disrupted [66,68]. Consequently, pathological conditions that impair keratinocyte adhesion might limit EphA2-mediated signaling events.

Contact-dependent activation of EphA2 is required for the transient suppression of Erk1/2-MAPK signaling in human keratinocytes, similar to what was found with cells stimulated by soluble ephrin-A peptide mimetics [15,16]. However, striking differences in protein phosphorylation events downstream of Eph receptors can occur depending on whether these RTKs are activated by membrane-bound or soluble ephrins [70]. In addition, ephrin-A1-Fc peptide treatment not only leads to receptor phosphorylation but also the rapid loss of

EphA2 from the cell surface and its subsequent down-regulation [16,58,71–75]. In contrast, abundant EphA2 remains concentrated at borders and continues to signal following contact-dependent activation in keratinocytes. The recruitment, organization and stabilization of EphA2 following activation by membrane-bound ephrins on adjacent cells is therefore likely to be somewhat distinct from what occurs in response to soluble peptide mimetics and may influence the amplitude and nature of downstream signaling events.

Although the relationship between Eph receptor autophosphorylation, endocytosis and recycling in keratinocytes remains unclear, it appears to be distinct from the scenario that plays out during axon guidance where the rapid termination of Eph/ephrin signaling mediates cell retraction and growth cone collapse [39]. The trans-endocytosis of Eph–ephrin signaling complexes found in neurons and other cell types undergoing cell repulsion events is associated with changes in the activation of small Rho family GTPases, particularly Rac1 [39,76]. Following receptor internalization, post-translational modification by the E3 ubiquitin ligase, c-Cbl, targets these proteins for degradation [71,77–80]. Similar endocytic and degradatory mechanisms occur following exposure to soluble ephrin peptide but perhaps not when EphA2 responds to membrane-bound ligands on adjacent epithelial cells. Under these circumstances, EphA2 may be more stable at the cell surface by virtue of its ability to interact with the lipid phosphatase, SHIP-2, which decreases the levels of PIP3 and interferes with Rac1-mediated endocytosis [72,81]. Alternatively, protein tyrosine phosphatases (e.g., LMW-PTP, PTP1B) that bind these receptors may control the stability and signaling of EphA2 at cell borders [82–84].

Proteolytic cleavage of ephrin-A ligands represents yet another mechanism by which cells can dampen Eph/ephrin signaling complexes, although the extent to which this occurs in keratinocytes is not known. EphA3 constitutively interacts with ADAM10 [85,86]. This protease is capable of cleaving ephrin-A proteins on adjacent cells upon ligand binding after a conformational change is induced within the EphA3 cytoplasmic tail. As a consequence, the cleavage and subsequent internalization of EphA3/ephrin-A signaling complexes occurs in a tightly regulated manner. The ability of EphA2 to interact with ADAM10 depends on the size of the signaling cluster present in the plasma membrane [87] but could presumably work in a similar fashion. Interestingly, Notch receptors serve as a major substrate for ADAM10 in the epidermis [88], suggesting that the distribution of EphA2 on the cell surface may also impinge on the activation of this other cell–cell communication network implicated in keratinocyte differentiation.

The ability of GPI-linked ephrin ligands to trap EphA2 helps stabilize this RTK at sites of cell–cell communication [70,87,89–91]. The size and organization of EphA2 signaling clusters can be quite distinct between relatively normal and highly metastatic breast cancer cell lines. This ultimately influences downstream signaling events that alter the actin cytoskeleton and gene expression [87]. These observations highlight the importance of analyzing native Eph/ephrin complexes in an appropriate cellular context. In the case of the epidermis, keratinocytes may signal via EphA2 in a distinct manner from what has been described in other transformed cell lines. Although primary cultures provide useful insight into EphA2 signaling in relatively normal epithelial cells, keratinocytes are situated within the context of a complex, stratified epithelium. The use of three-dimensional organotypic models that mimic the appropriate tissue architecture of the epidermis [59] and/or animal models will likely be critical for understanding how EphA2 and related receptors are organized and signal in keratinocytes.

5. Eph/Ephrin regulation of keratinocyte proliferation

Several lines of evidence are consistent with the idea that Eph/ephrin signaling complexes negatively regulate keratinocyte proliferation. For example, the intravenous injection of ephrin-A/B or EphA/B recombinant peptides in mice increased epidermal and follicular keratinocyte proliferation [14]. These findings suggested that Eph/ephrins normally limit keratinocyte proliferation in the basal layer where they are co-expressed, since the peptides likely act as antagonists when systemically administered into mice by interfering with native signaling complexes. It remains possible that other cell types targeted by these recombinant proteins in the skin could alter the proliferative state of keratinocytes by less direct means. For example, the systemic loss of ephrin-B2, which is primarily expressed by dermal fibroblasts in the perinatal period, leads to epidermal hyperproliferation but the targeted deletion of this ligand from keratinocytes has no effect on their growth characteristics [18]. These null mutant mice instead have elevated levels of IL-1 family members, which could signal from the dermis to increase keratinocyte proliferation. Alternatively, recombinant peptides that target the vasculature or resident immune cells in the skin might trigger inflammatory responses that indirectly lead to epidermal hyperproliferation.

EphA2 is a good candidate for directly participating in the regulation of keratinocyte proliferation but perhaps only under certain physiological conditions. In particular, *epha2* null mutant mice exhibit increased tumor cell proliferation when subjected to two-stage chemical carcinogenesis regimens whereas keratinocyte proliferation was unchanged under normal conditions [15]. Moreover, *epha2*–/– keratinocytes proliferate similar to their wild-type counterparts but only the latter can be growth-restricted by the addition of ephrin-A1-Fc peptide in vitro. The ligand-mediated inhibition of growth likely involves the ability of EphA2 to inhibit Erk1/2-MAPK signaling, which is mediated by EphA2's effects on p120RasGAP in other epithelial cell types [56,83]. It should be noted that ephrin-A-Fc peptide delivery does not necessarily reduce proliferation in all keratinocytes and has actually been shown to marginally increase the growth of cells cultured from the outer root sheath of hair follicles [50].

EphA2 appears to be dispensable for human keratinocytes exiting the cell cycle, at least in response to stabilization of cell–cell contacts and calcium-induced differentiation in vitro [16]. Yet, this RTK is required for efficient restriction in the size of epidermal cell colonies by ephrin peptide mimetic. Part of the reason that these keratinocyte colonies are smaller may have to do with a reduction in proliferation, since a recent microarray study identified several cell cycle regulatory genes as potential targets of ephrin-A-Fc peptide stimulation in human keratinocytes [17]. However, a sub-population of keratinocytes continues to proliferate at a rate similar to controls even in these compacted cell colonies [16]. Importantly, proliferation was unaltered by ephrin-A1-Fc peptide when keratinocytes were grown in low calcium conditions where the EphA2 receptor could still be activated but was not concentrated at cell–cell contacts. As in other epithelial cell types, ligand-mediated targeting of EphA2 inhibits keratinocyte migration, which might also limit the lateral expansion of cells at the edges of these colonies [17]. Finally, ephrin-A1-Fc peptide increases cell compaction and triggers stratification [16]. Hence, the targeting of EphA2 by these peptide mimetics acts not only to limit the lateral growth of keratinocyte colonies but also leads to their vertical expansion. These findings provided the first clue that Eph/ephrin signaling could be potentially harnessed to alter the morphology and differentiated state of keratinocytes.

6. Eph/Ephrin regulation of keratinocyte adhesion

As keratinocytes mature in the upper layers, their intercellular junctions are modified in a manner that is essential for the proper organization and function of the epidermis (Fig. 3). For example, basal keratinocytes express P-cadherin and E-cadherin; both of these adherens junction proteins are required to maintain epidermal integrity [5,6,8–10]. E-cadherin also plays a key role in the function of tight junctions in the more superficial granular layer [92]. Tight junctions work in concert with the lipid barrier in the overlying stratum corneum to limit water and ion loss and prevent the entry of noxious agents through the skin. Finally, desmosomes change in size and molecular composition in the suprabasal epidermis not only to strengthen cell–cell adhesion but also to regulate normal morphogenesis [4]. For example, desmoglein 1 (Dsg1) is critical for maintaining tissue integrity in the uppermost layers of the epidermis. This desmosomal cadherin also plays a key role in the negative regulation of EGFR–Erk1/2 signaling as keratinocytes transition into a terminal differentiation pathway in the suprabasal layers [93]. Eph/ephrin signaling complexes are integrated into this elaborate keratinocyte adhesion apparatus and are likely to be influenced by the adhesive state of the tissue (Fig. 3). In fact, EphA2 levels are increased in Dsg1-deficient epidermal cultures where EGFR–Erk1/2 signaling is abnormally high [93]. It has become increasingly clear that Eph RTKs themselves can act upon these junctional complexes to modulate epidermal adhesion.

The targeting of EphA2 by soluble ligands leads to the stabilization of E-cadherin at junctions, cell compaction and increased polarization in a canine kidney epithelial cell line (i.e., MDCK cells) in a manner that interferes with its recycling by the Arf6 GTPase [65]. Keratinocytes also undergo compaction following ligand stimulation, which is associated with a marked reorganization of the actin cytoskeleton [16]. Since Rho GTPase activating proteins (GAPs) and exchange factors (GEFs) are commonly involved in downstream Eph signaling and adherens junctions are regulated by the maturation of the cortical actin cytoskeleton [94–101], the ability of EphA2 to promote keratinocyte compaction probably involves changes in actin organization. A tight balance in the reorganization of actin by EphA2 might influence the outcome on adhesion since the overexpression of this RTK can also disrupt adherens junctions in breast cancer cell lines by increasing RhoA activity [102].

EphA2's effects on E-cadherin may also influence the tight junction barrier in the granular layer, a possibility that has yet to be formally tested. In MDCK cells, EphA2 interacts with and phosphorylates claudin-4 thereby increasing paracellular permeability [67]. It will be important to determine if there is a ligand-independent role of EphA2 on epidermal tight junctions since ephrins may not be concentrated in these uppermost layers (Fig. 3). One interesting possibility for why ephrins loosen the tight junction barrier may have to do with heterotypic interactions between keratinocytes and antigen presenting dendritic cells, termed Langerhans cells. Langerhans cells breach the epidermal tight junction barrier to probe for antigens in the stratum corneum [103]. Whether Langer-hans cells use ephrins to target EphA RTKs on keratinocytes in these uppermost layers in order to remodel tight junctions has potential clinical relevance since this barrier becomes leaky in inflammatory skin conditions, such as atopic dermatitis (commonly referred to as eczema) [104].

The maturation of desmosomes is largely responsible for the ligand-mediated increase in keratinocyte adhesion downstream of EphA2 signaling [16]. In particular, an increase in the expression of the differentiation-associated Dsg1 is required for EphA2-mediated strengthening of adhesion. Moreover, bacterial exfoliative toxins that cleave the adhesive ectodomain of Dsg1 abrogate the ability of soluble ephrin-A1-Fc peptide to enhance keratinocyte adhesion. However, Dsg1 is not the only target of this EphA/ephrin-A pathway as its partner desmosomal cadherin (i.e., desmocollin 1) and their associated keratin

intermediate filament protein pairs (i.e., keratin 1/10) present in the suprabasal layers also increase following exposure to soluble ephrin-A1-Fc [16,17]. It has become apparent that the differentiated state of keratinocytes is intimately entwined with the effects of the EphA/ephrin-A axis on desmosomal adhesion.

7. Eph/ephrin regulation of keratinocyte differentiation and survival

Targeting EphA2 with soluble ephrin-A1-Fc peptide can elicit a robust program of terminal differentiation that leads to the stratification of keratinocytes in vitro [16]. Similar enhancement of keratinocyte differentiation was found when the expression level of full-length ephrin-A1 was increased in these primary cell cultures, indicating that these effects were not an artifact of the soluble peptide delivery approach (Kaplan and Getsios, unpublished observations). In both cases, EphA2 levels were markedly reduced by ephrins coincident with their adopting a more differentiated phenotype. Although human epidermal keratinocytes also express EphA1 and EphA4, the contribution of these related RTKs during keratinocyte differentiation remains to be determined. It should be noted that mice lacking EphA1, EphA2 or EphA4 do not exhibit gross epidermal abnormalities, which may be due to functional redundancy within the EphA family, the use of alternative pathways that override the need for EphA/ephrins in differentiation or perhaps even species differences between rodents and humans.

There is reason to believe that not all epidermal EphA receptors are functionally redundant. In particular, the relative affinity for ephrin ligands differs among these receptors with EphA4 being the highest and EphA1 being the lowest affinity receptor for ephrin-A1 [27]. Although these RTKs share sequence homology, important differences may be relevant to skin biology. For example, EphA1 lacks a PDZ-protein binding motif [51] that could distinguish the effector molecules it interacts with compared to EphA2 and EphA4. Moreover, a non-conserved amino acid residue (Glu547) within the EphA1 transmembrane domain is regulated by its ionization state [105,106] and could modulate the dimerization and formation of lateral clusters in the plasma membrane of the upper layers of the epidermis where the pH levels are reduced and the composition of lipids changes [3,107]. The most compelling evidence for specific roles of EphA/ephrin-A signaling complexes comes from a recent microarray study showing that soluble ephrin-A peptides from all family members promote keratinocyte differentiation but differences in gene expression could be distinguished among ephrin subtypes [17]. Ephrin-A ligands collectively suppressed targets of AP-1 transcription factors, which have been previously implicated in keratinocyte differentiation. In contrast, ephrin-A1 exhibited a partially unique gene signature compared to other ephrins found in the epidermis (i.e., ephrin-A3 and -A4), including the reduction in ubiquitin-associated proteolysis genes. Preferential binding of ephrin-A1 to specific EphA subtypes could therefore have discrete effects on protein stability in different layers of the epidermis.

The ability of ephrins to facilitate the transition into a more differentiated phenotype does not appear to be restricted to the interfollicular epidermis. Specifically, ephrin-A3 is expressed in the bulge area of growing hair follicles along with EphA4 [52]. Furthermore, injection of recombinant ephrin-A3-Fc peptide increased hair follicle density in mice indicating that these signaling complexes recruit bulge stem cells toward a differentiation program during follicular regeneration. It will be important to delineate specific pathways downstream of EphA/ephrin-A signaling complexes that elicit these pro-differentiation programs in keratinocytes.

Finally, EphA2 may play a role in keratinocyte survival in the suprabasal layers of the epidermis, especially after exposure to UV radiation. The levels of this RTK have been

shown to increase in UV irradiated keratinocytes in a manner that depends on Erk1/2-MAPK signaling [108]. In mouse embryonic fibroblasts, EphA2 is required for efficient execution of apoptotic responses in response to UV and similar mechanisms have been proposed for keratinocytes. Interestingly, a serine residue (Ser897) within the EphA2 cytoplasmic domain can be phosphorylated by AKT and is involved in promoting ligand-independent migration of glioma cell lines [31]. Since the PI3K-AKT pathway is activated in the suprabasal layers of the epidermis to provide a survival signal for keratinocytes after they have detached from the basement membrane [109,110], it is possible that EphA2 serves as an important mediator of AKT's effects under these conditions.

8. Eph/ephrin signaling in skin cancer, wound healing and disease

Given the broad control that EphA/ephrin-A signaling complexes possess on the normal behavior of keratinocytes, one would anticipate a role for these receptors and ligands in skin disease. This has been most extensively studied in the context of skin cancer for EphA2 and depends on the particular tumor type being examined. The skin is constantly exposed to challenges from the environment, including exposure to chemical carcinogens and UV radiation. The most deadly of skin cancers are pigmented lesions arising from melanocytes, which primarily reside within the basal layer of the epidermis and matrix of hair follicles [111]. EphA2 has a prominent role in melanoma, where it functions as an oncogene [21,112–118]. In particular, EphA2 silencing in melanoma cell lines promotes tumor cell proliferation and invasion; this RTK is further required for the survival of these tumor cells. Surprisingly, EphA2 induction in normal melanocytes leads to their death, suggesting this RTK initially provides an early crisis signal in these neural crest derived cells but eventually melanomas rely on its signaling for their survival.

For keratinocytes, Eph/ephrin bidirectional signaling serves important tumor suppressive functions. Specifically, the homozygous deletion of EphA2 leads to dramatically increased tumor susceptibility in classical DMBA-TPA, two-stage chemical carcinogenesis models [15]. EphA2 null mutant mice develop skin tumors with an average of four-fold increased frequency and three weeks shorter latency. Moreover, tumors in the homozygous knockout mice grow faster and are more likely to show invasive, malignant progression. Haploinsufficiency of EphA2 causes an intermediate phenotype in tumor development. Activation of the Ras/Raf/Erk1/2-MAPK signaling pathway is known to stimulate EphA2 expression [58] and virtually all DMBA exposed skin tumors harbor a specific activating mutation in H-Ras (A61T) [119]. Coincidently, EphA2 is overexpressed in tumors relative to normal epidermis from wild-type mice at different stages of development. Because deletion of EphA2 increased tumor multiplicity and growth rate, the overexpressed EphA2 is likely to play an important role in suppressing the development and progression of the initiated keratinocytes with mutant Ras.

Additional EphA receptor subtypes may serve tumor suppressor roles in the epidermis, particularly EphA1, which is down-regulated in both basal and squamous cell carcinomas [43]. Moreover, EphA1 is redistributed into the cytoplasm of keratinocytes in skin ulcers, implicating a role for this RTK in wound healing [43]. Interestingly, EphA1 has been shown to inhibit cell migration by interacting with integrin-linked kinase (ILK) and activating RhoA-mediated actin reorganization events [120]. Ligand stimulation of keratinocytes also limits their ability to seal scratch wounds in vitro which may be related to changes in extracellular-cell matrix (ECM) adhesion. In particular, ephrin-A1 reduces integrin gene expression with a concomitant increase in collagen synthesis [17]. Keratinocytes migrating at the leading edge of wounds may therefore receive a stop signal for the integrin-based motility machinery when ultimately confronted by ephrins on adjacent cells.

There may be a role for Eph/ephrin signaling in some of the most common inflammatory skin diseases, such as psoriasis and atopic dermatitis. Although these polygenic, dermatological disorders are characterized by a strong immunoregulatory component along with changes in vasculature, marked alterations in epidermal morphology, homeostasis and barrier function are also evident [121]. In psoriatic plaques, keratinocyte proliferation is dramatically increased and extends inappropriately into the suprabasal layers. Abnormal keratinocyte differentiation and accelerated transit through the viable cell layers leads to a marked expansion of the spinous layer, a poorly developed granular layer and a thickened stratum corneum where the nuclei are frequently retained instead of being destroyed. Microarray gene expression studies indicate that EphA2 is up-regulated in psoriatic lesions [122,123], which is perhaps not surprising given its regulation by the EGFR–Erk1/2 pathway and cytokines (e.g., IL-1, TNF -α, Oncostatin) known to be increased in this disease [17,124]. Whether the increase in EphA2 contributes to the pathogenesis of psoriasis remains unknown. Since ephrin-A1 is capable of targeting EphA2 to restrain proliferation and bolster differentiation, therapeutic approaches that harness this pathway may prove useful for restoring tissue architecture and homeostasis in patients. Ligand-mediated activation of keratinocytes may also limit their ability to secrete cytokines that act on the underlying dermal compartment and exacerbate the disease [17].

Atopic dermatitis is a complex disease that involves changes in epidermal architecture and function within the rashes that characterize this chronic disorder [121]. In particular, the epidermal barrier is leaky in these individuals owing to defects in the tight junction and lipid barrier [104]. If Eph/ephrin signaling complexes are required for late stage differentiation events or enhance tight junction function, alterations in this cell–cell communication pathway may contribute to these epidermal defects. Since the spongiotic epidermis of active lesions exhibits widened intercellular spaces [125], it would seem likely that the efficiency of Eph/ephrin juxtacrine signaling is impaired in atopic dermatitis. In a related manner, abnormal cell–cell communication via the Notch signaling pathway has been described in these rashes [126]. Studies on the Eph/ephrin system in the etiology or progression of this disease would therefore seem warranted.

Eph/ephrin-mediated signaling may also be relevant to genetic disorders that perturb epidermal homeostasis, autoimmune skin blistering diseases and hair follicle defects. The latter has been examined to a certain extent, with a reduction in ephrin-A3 being found in the dermal papilla of individuals with androgenic alopecia [50]. Since ligand targeting of EphA2 can bolster desmosomal adhesion [16], this approach may prove useful for preventing blistering in pemphigus foliaceus and vulgaris where auto-antibodies are produced against the desmosomal cadherins, Dsg1 and Dsg3, respectively [16,127,128]. Finally, gene expression changes elicited by ephrin ligands in human keratinocytes resemble a transcriptional program evident in ichthyosis patients, suggesting relevance of the pathway to this group of genetic diseases that leads to barrier defects and dry, flaky skin [17,129].

9. Conclusions and perspectives

The importance of establishing a good line of communication is critical in all relationships and the same might be said for keratinocytes. Although Eph receptors and ephrin ligands are renowned for their roles in neuronal patterning events and oncogenic progression, it is now quite clear that the Eph/ephrin signaling pathway also mediates epidermal cell–cell communication. This review has focused primarily on the role of EphA/ephrin-A sub-types in epidermal and follicular keratinocytes under normal and pathological conditions since more is known about these family members but there are just as likely to be key contributions from EphB/ephrin-B signaling complexes to epidermal homeostasis and disease. A better understanding of when and where individual receptor and ligand subtypes

are expressed in the skin will help guide future studies. In particular, delineating specific pathways downstream of individual Eph receptor subtypes will be required to understand how they regulate aspects of keratinocyte proliferation, differentiation and survival.

Eph/ephrin complexes also probably play a role in heterotypic interactions between keratinocytes and other resident epidermal cells, including Langerhans cells, melanocytes, and lymphocytes. A longstanding question that remains in the field of skin biology is how Langerhans cells and melanocytes extend processes through the tightly packed keratinocytes in the epidermis; the ability of Eph/ephrins to dynamically regulate intercellular junctions and actin cytoskeleton may contribute to this process. Finally, knowledge gained from the epidermal Eph/ephrin signaling axis may bear relevance to other stratified epithelial tissues that undergo constant remodeling such as the corneal epithelium, which is known to express many of these family members [41].

In conclusion, Eph/ephrin signaling complexes play a key role in epidermal cell–cell communication. These bidirectional signaling complexes regulate normal keratinocyte behavior but the wiring between receptor and ligand appears to be faulty in various dermatological disorders. This opens up the possibility that Eph receptors can be therapeutically targeted using agonist or antagonist-based approaches to improve epidermal homeostasis and architecture in patients with skin disease.

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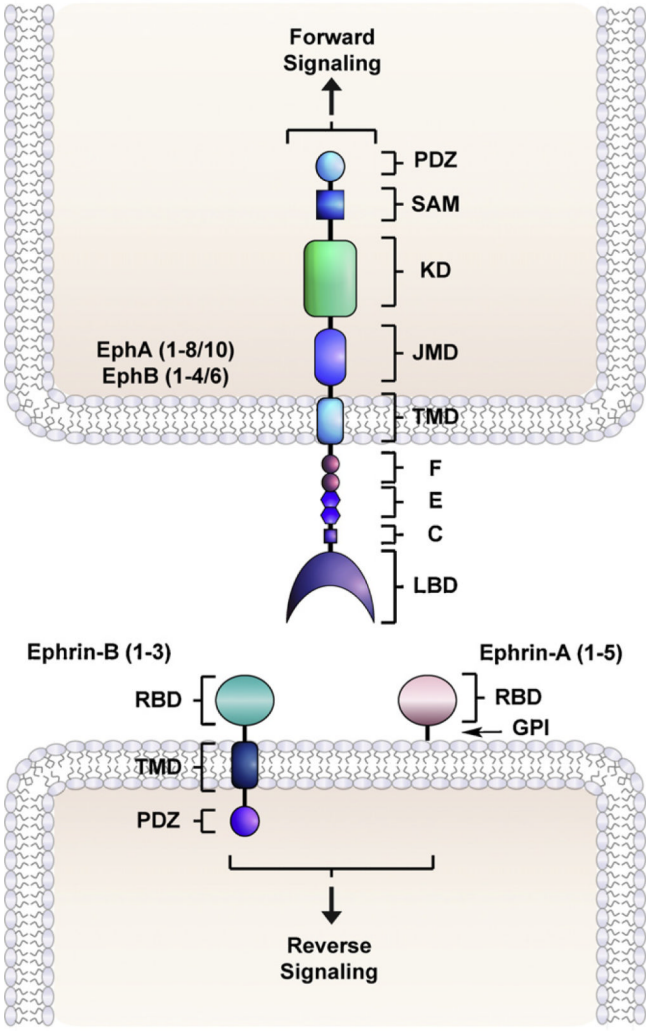

Fig. 1.

Eph receptors and ephrin ligands mediate cell–cell communication. Eph receptor tyrosine kinases interact with ephrin ligands residing on adjacent cell surfaces. Humans possess nine EphA (1–8/10) and five EphB (1–4/6) receptor sub-types, five ephrin-A (1–5) and three ephrin-B (1–3) ligands. Eph receptors are type I transmembrane spanning proteins that consist of a ligand binding domain (LBD), a cysteine-rich region (C), an epidermal growth factor (EGF)-like motif (E), fibronectin type-III repeats (F), a transmembrane domain (TMD), a juxtamembrane domain (JMD), a kinase domain (KD), a sterile-α-motif (SAM)-domain, and a PSD95/Dlg/ZO1 (PDZ)- protein binding site. Both subclasses of ephrin ligands consist of an N-terminal receptor binding domain (RBD). However, ephrin-A ligands are glycosylphosphatidylinositol (GPI)-linked whereas ephrin-B ligands are trans-membrane proteins and contain a cytoplasmic PDZ-tail. Upon engaging ephrins, bidirectional signaling is initiated where forward and reverse signaling take place in the receptor and ligand expressing cell, respectively.

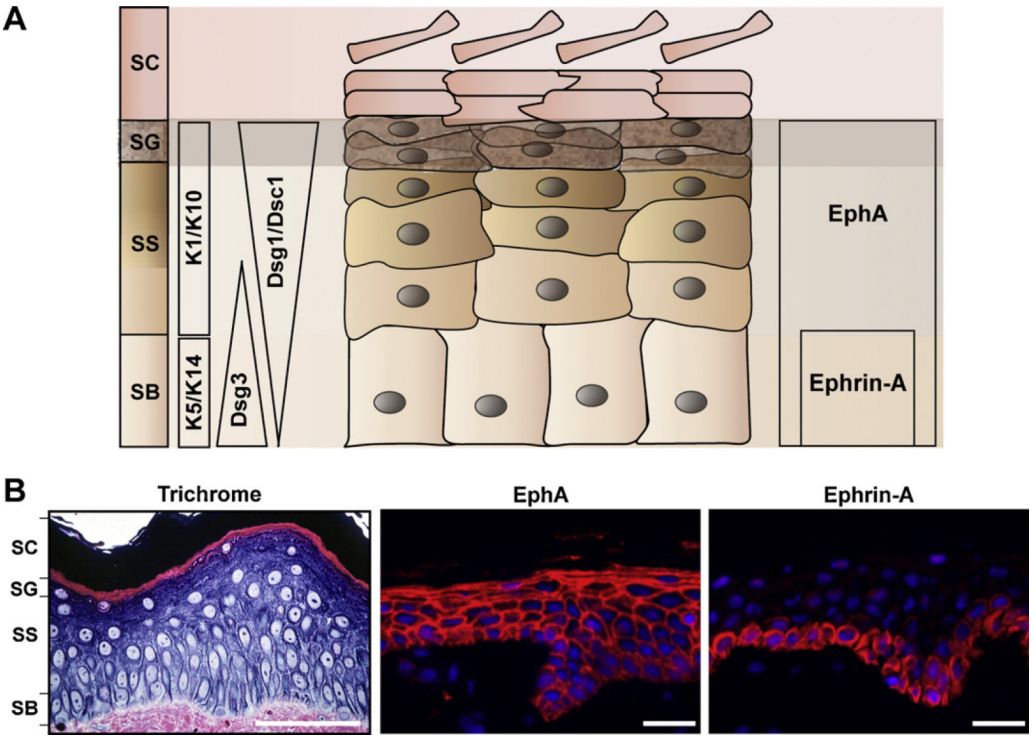

Fig. 2.

Eph receptors and ephrin ligands are compartmentalized in the epidermis. (A) The epidermis consists of four histologically distinct layers which are termed stratum: basale (SB), spinosum (SS), granulosum (SG), and corneum (SC). The epidermal layers are characterized by the expression of several markers of differentiation. Keratin intermediate filaments 5/14 (K5/14) and desmoglein 3 (Dsg3) are present in the basal layer while K1/10, desmoglein 1 (Dsg1), and desmocollin 1 (Dsc1) are concentrated in the more differentiated suprabasal layers of the epidermis. A summary of the distribution of EphA receptors and ephrin-A ligands is presented to the right of the schematic. (B) A 1 μm plastic section of human skin was stained with a trichrome solution (left panel). Black hyphens delineate all four layers of the human epidermis. Human skin samples were immunostained for A class receptors (EphA4) and ligands (ephrin-A1) (right two panels). (Scale bar: 50 μM) Ephrin-A ligands are restricted to the basal layer whereas EphA receptors are expressed throughout all layers of the epidermis.

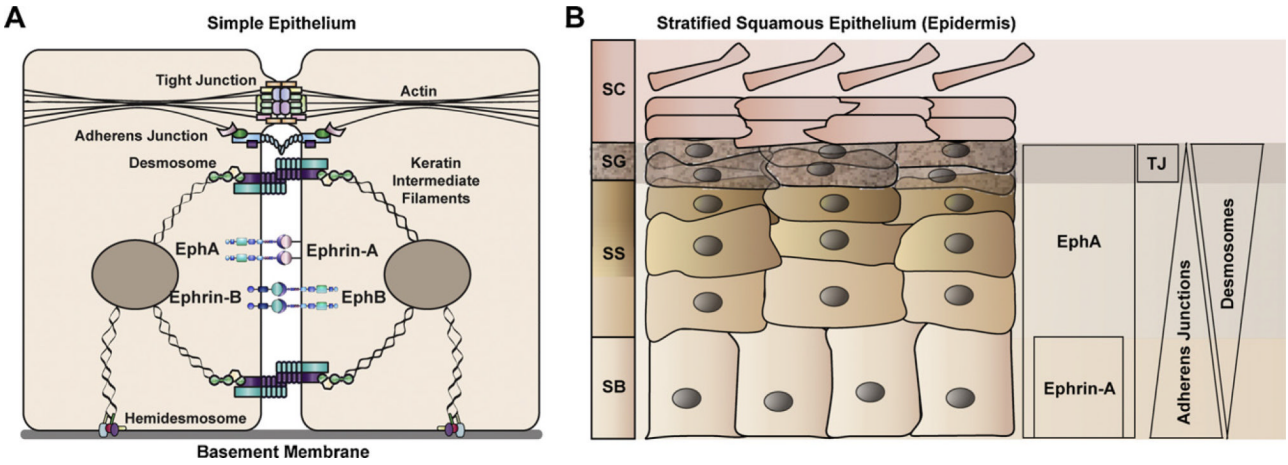

Fig. 3.

Eph receptors and ephrin ligands are functionally integrated with intercellular adhesion complexes. (A) A cartoon depiction of two polarized simple epithelial cells joined together by adherens junctions, desmosomes and tight junctions (TJ). Hemidesmosomes anchor these epithelial cells to the basement membrane. (B) Intercellular junctions undergo maturation in a differentiation-dependent manner that corresponds with the compartmentalized distribution of Eph receptors and ephrin ligands.

Table 1

EphA/ephrin-A distribution in the epidermis.

Eph/ephrin	Species	Epidermis layer(s)	Reference
Ephrin-Al	Human	Basal	Fig. 2
	Mouse	Basal	[15]
Ephrin-A3	Mouse	Present (no location specified)	[52]
Ephrin-A4	Rat	Present (strong basal expression P0-P7)	[19]
EphAl	Human	All	[43]
	Human	Basal	[17]
	Mouse	Basal	[51]
EphA2	Human	All	[43]
	Mouse	All (low basal to high suprabasal gradient)	[15]
EphA4	Human	All	Fig. 2
	Mouse	Basal	[18]
	Mouse	Present (no location specified)	[52]
EphA7	Human	Basal	[43]

Table 2

Eph/ephrin distribution in the hair follicle.

Eph/ephrin	Species	Hair follicle location	Reference
Ephrin-Al	Mouse	ORS, IRS, bulb	[15]
Ephrin-A3	Mouse	Dermal papilla	[50]
	Mouse	Hair pegs, bulge, hair matrix (anagen)	[52]
Ephrin-B1	Mouse	Bulge, hair matrix	[49]
Ephrin-B2	Mouse	Hair bud, follicular keratinocytes, dermal papilla, bulb, sebaceous gland (depends on stage ofdevelopment)	[18]
EphA1	Human	Present (no location specified)	[43]
EphA2	Human	Present (no location specified)	[43]
	Mouse	ORS, bulb, sebaceous gland	[15]
	Mouse	Mesenchymal cells surrounding hair follicle dermal papilla, bulge (depends on stage of hair cycle)	[52]
EphA4	Mouse	ORS, bulge (anagen)	[49]
	Mouse	Hair matrix (anagen)	[52]
EphB4	Mouse	ORS, bulge (anagen)	[49]
All	Mouse	ORS, hair matrix, dermal papilla, bulge, sebaceous gland (depends on stage of hair cycle and class subtype)	[14]