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Eph/ephrin signaling in epithelial development and homeostasis

Hui Miao and Bingcheng Wang*

Rammelkamp Center for Research, MetroHealth Campus, Case Western Reserve University
School of Medicine, 2500 MetroHealth Drive, Cleveland, Ohio 44109

Abstract

Eph receptors and ephrin ligands are widely expressed during embryonic development with well-defined functions in directing neuronal and vascular network formation. Over the last decade, evidence has mounted that Ephs and ephrins are also actively involved in prenatal and postnatal development of epithelial tissues. Their functions beyond developmental settings are starting to be recognized as well. The diverse functions of Eph/ephrin are largely related to the complementary expression pattern of the Eph receptors and corresponding ephrin ligands that are expressed in adjacent compartments, although overlapping expression pattern also exists in epithelial tissue. The interconnection between Ephs or ephrins and classical cell junctional molecules suggests they may function coordinately in maintaining epithelial structural integrity and homeostasis. This review will highlight cellular and molecular evidence in current literature that support a role of Eph/ephrin systems in regulating epithelial cell development and physiology.

Keywords

Eph; Ephrin; epithelium

Introduction

The Eph family of receptor tyrosine kinases encompasses 14 members, constituting 25% of all the known human RTKs. They are divided into EphA and EphB kinases according to sequence homology and binding specificity of their membrane-bound ligands called ephrins. EphA receptors preferentially bind to GPI-anchored ephrin-A ligands; EphB receptors target transmembrane ephrin-B ligands. Two known exceptions are EphA4 and EphB2 that cross-react with ephrin-B2 and ephrin-A5 respectively (Takemoto et al., 2002; Himanen et al., 2004). Ephs and ephrins are expressed in almost all embryonic tissues and have been implicated in neuronal and vascular development (Adams and Klein, 2000; Flanagan and Vanderhaeghen, 1998; Pasquale, 2005). As both are membrane bound, interactions of the Eph receptors and ephrin ligands normally occur at the interface of adjacent cells and mediate cell contact-dependent signaling (Kullander and Klein, 2002; Arvanitis and Davy, 2008; Himanen et al., 2007). Eph/ephrin signaling takes place in a bidirectional manner. Not only is there signaling by the Eph kinases (forward signaling), ephrins on the opposing cells are also capable of receptor-like signaling (reverse signaling) (Davy et al., 1999; Holland et

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*Correspondence to B.W.: bxw14@case.edu, Rammelkamp Center for Research, MetroHealth Medical Center, Case Western Reserve University, 2500 MetroHealth Drive, Cleveland, OH 44109, Phone (216) 778-4256, FAX: (216) 778-4321.

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al., 1996) (Fig. 1). The complementary expression pattern is related to the diverse functions of Eph/ephrin in tissue assembly and in maintaining tissue homeostasis (Poliakov et al., 2004). There is growing evidence that they are also actively involved in embryonic and postnatal development of epithelial tissues, as well as maintaining epithelial tissue homeostasis in adult. Here we review the current knowledge in this specific aspect. Readers are referred to extensive previous reviews on general topics of signaling networks and cellular processes controlled by Ephs and ephrins (Adams and Klein, 2000; Flanagan and Vanderhaeghen, 1998; Pasquale, 2005; Arvanitis and Davy, 2008; Himanen et al., 2007; Kullander and Klein, 2002; Lackmann and Boyd, 2008).

Eph/ephrin signaling is involved in prenatal epithelial development

Epithelium is the first tissue to form in the embryo. Expression of Eph/ephrin proteins or messengers has been documented at several important developmental stages including gastrulation, segmentation or somitogenesis, and organogenesis. All these developmental events involve dispersal and rearrangement of the epithelia, which are accompanied by epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET). EphA1, the founding member of Eph family, is found to be expressed in varying combinations with ephrin-A1 and ephrin-A3 at different times and different regions during primitive streak formation. For instance, *Epha1* and *Efna3* are first found in pre-gastrulation embryos when the epiblasts undergo epithelialization. Following the onset of gastrulation, the expression level of *Epha1* is significantly increased and colocalizes with *Efna1* whereas *Efna3* is expressed in a different region in the primitive streak, where germ layer emerge from (Duffy et al., 2006). Eph/ephrin signaling is further involved in the segmentation stage of embryonic development by controlling somite border morphogenesis. Embryonic somites form in a head-to-tail manner from the paraxial mesenchymal presomitic mesoderm (PSM). After the establishment of the segmental pattern, the cells in the rostral presomitic mesenchymal mesoderm undergo MET. This process delineates the segments by forming an epithelial somite boundary. Somitogenesis is a key part of segmentation in embryonic development and is precisely timed. Significant evidence of Eph/ephrin functions in somitogenesis has come from zebrafish. Early studies by Durbin and colleagues show that EphA4 receptor and ephrin-A1 and ephrin-B1 ligands are expressed in a segmental pattern in the rostral PSM. This pattern establishes a receptor/ligand interface at each site of somite furrow formation. Interruption of Eph/ephrin signaling results in abnormal somite development with absent or aberrant furrow formation between somites (Durbin et al., 1998). Later, Barrios and colleagues used the mutant *fused somites (fss)* as an in vivo system to demonstrate that restoration of Eph/ephrin signaling in the paraxial mesoderm of *fss* mutants rescued most aspects of somite morphogenesis (Barrios et al., 2003). The *fss* gene encodes Tbx24, a T box transcription factor involved in maturation of the PSM. The mesenchymal cells of the PSM in *fss* mutant embryos fail to undergo MET and form somites. Barrios and colleagues found that EphA4 and ephrin-B2b were absent and the segmental expression pattern of ephrin-B2a and ephrin-A1 were disrupted in *fss* mutant embryos, which resulted in the loss of Eph/ephrin interface in the rostral PSM. They then introduced wild type or *fss* mutant cells that expressed exogenous EphA4 to the perspective *fss* PSM that expressed endogenous ephrins. Restoration of the Eph/ephrin interface induced a repulsive response and detachment between donor cells and host *fss* cells and formed a boundary at the interface. Furthermore, activation of exogenous EphA4 in wild type cells led to MET of both EphA4-expressing donor cells and ephrin-expressing host *fss* cells, which was characterized by the acquisition of a columnar morphology with an apical-basal polarity and polarized relocalization of the centrosome and nucleus. There was also an apical distribution of β -catenin in the EphA4-expressing donor cells. However, activation of EphA4 in *fss* cells could only induce MET of the receptor-expressing donor cells but not ephrin-expressing host *fss* cells on the opposite side of the forming boundary. These results

suggest that an Fss-dependent component downstream from EphA4 activation may be required to signal inside-out in EphA4-expressing cells and trigger a parallel pathway promoting MET in the ephrin-expressing cells, and this pathway is functionally compromised in *fss* mutant cells. Thus, EphA4 activation results in the autonomous MET of the receptor-expressing cells and nonautonomous MET in ephrin-expressing cells at the boundary (Barrios et al., 2003). Interestingly, expression of EphA4 and ephrin-B2 can be induced by forkhead transcription factor Foxc1, which is essential for formation of epithelial somites during somitogenesis. Interruption of Foxc1 translation results in loss of ephrin-B2 expression and causes a defect in somite border formation (Topczewska et al., 2001).

A recent computer stimulation study by Glazier et al. has shown that EphA2 pairs with ephrin-B2 to function as a repulsive cue at the forming somite boundary. On the other hand, N-cadherin and N-CAM function as adhesive molecules during somitogenesis. The coordinate actions of the adhesive and repulsive molecules give rise to the dynamic morphological changes during somitogenesis (Glazier et al., 2008).

Embryonic kidney development is another process that involves extensive MET. Renal epithelia arise from two distinct sources. The collecting ducts develop by growth and repeated branching of an existing ureteric bud, which is an outgrowth of the Wolffian duct. The nephrons develop by MET of metanephric mesenchyme. Like the epithelia of glandular organs, collecting duct development relies on signals emanating from mesenchyme surrounding it (Saxen, 1987; Davies et al., 1999) (Fig. 2). In mouse, EphA2 is expressed in the ureteric buds of embryonic kidneys. In an in vitro 3-D culture system, we find that EphA2 signaling negatively regulates hepatocyte growth factor (HGF)-induced branching morphogenesis of MDCK cells. Activation of EphA2 by providing exogenous ephrin-A1 can not only prevent branching morphogenesis, but also cause a collapse of existing branched structures (Miao et al., 2003) (Fig. 2). Moreover, EphA2 kinase activation inhibits HGF-induced chemotactic migration and scattering of MDCK cells. Collectively, HGF induces EMT that is required for the rearrangement and remodeling of MDCK cells during branching morphogenesis, whereas EphA2 signaling can reverse this process and ensuring the branching morphogenesis takes place at the right place. Biochemical analysis of the tissue extracts from embryonic kidneys showed the expression of several ephrin ligands (Wang unpublished data). It is yet to be determined which of them could function as a partner of EphA2 in regulating kidney branching morphogenesis in vivo. Previous studies by Takahashi and colleagues revealed that ephrin-B2 is transiently expressed in glomerular epithelial cells at the comma stage of glomerulogenesis. The expression is restricted to a subpopulation of epithelial cells representing podocyte progenitor cells (Takahashi et al., 2001). Since disruption of EphB signaling or ablation of ephrin-B2 is known to cause abnormal projection of intersomitic vessels (Adams et al., 2001; Helbling et al., 2000), the authors speculate that glomerular epithelial ephrin-B2 may guide endothelial progenitors expressing EphB receptors to glomerular sites.

The epidermis of the *C. elegans* has been a simple model for analyzing epithelial morphogenesis. Null mutations of the single Eph receptor (VAB-1) or its ephrin ligands cause defects in the migration of epidermal cells during ventral closure of the epidermis, resulting in failure of ventral closure or embryo arrest (George et al., 1998; Wang et al., 1999; Chin-Sang et al., 1999). Since VAB-1 is expressed in neuronal cells, but not in epidermal cells, it is likely that the VAB-1-expressing cells might provide guidance cues for epidermal cells. However, all ephrin ligands identified so far in *C. elegans* are also expressed in neuronal cells. The molecular mechanisms underlying the guidance function of VAB-1 is yet to be elucidated. It has been suggested that VAB-1 may function together with other guidance molecules such as PTP-3, a LAR-like receptor tyrosine phosphatase, and SAX-3, the *C. elegans* homologue of Robo, in regulating epidermal cell migration (Harrington et al.,

2002; Ghenea et al., 2005). In mammalian embryos, Eph forward signaling and ephrin-B reverse signaling have been reported to have an important role in caudal midline development. EphB2 and ephrin-B2 are coexpressed in the same epithelial cells that pattern the terminal hindgut. These cells move toward the midline and seal the midline through both receptor and ligand signaling. Mutation of either receptor or ligand causes incomplete septation of the urethra leading to a hypospadias phenotype similar to a common human birth defect (Dravis et al., 2004).

Eph/ephrin signaling in postnatal development of epithelial tissues

Unlike other branched organs that develop in embryos, the mammary gland develops mainly postnatally and is subjected to continuous cyclic remodeling according to functional demands. The key structure that drives branching morphogenesis is the terminal epithelial buds (TEB). The TEBs are highly invasive and motile. They intrude into surrounding mesenchyme to elongate the duct and initiate branch points. At the same time, they generate a hollow lumen for ductal transport (Sternlicht et al., 2006). Many of the key mechanisms underlying these processes are believed to be conserved among all branched organs.

The most extensively studied Eph receptors in mammary gland is EphB4, which was originally isolated from the mouse mammary gland (Andres et al., 1994). EphB4 is expressed primarily in myoepithelial cells in a hormone-dependent manner, whereas its ligand ephrin-B2 was expressed in luminal epithelial cells (Fig. 3) (Nikolova et al., 1998). The expression is induced at puberty during mammary gland development and is regulated during different phases of estrous cycle. It is down-regulated during pregnancy and transcriptionally silent during lactation, and reinduced thereafter (Munarini et al., 2002). Together these observations suggest EphB4 expression is turned on in response to cell proliferation. The involvement of EphB4 in regulating patterning of mammary epithelial structure is evidenced by the study of transgenic mice expressing EphB4 under control of the MMTV-LTR promoter. In these mice EphB4 gene was turned on during pregnancy and lactation when the endogenous gene was down-regulated. This unscheduled expression of EphB4 led to a delayed and abnormal mammary epithelium (Munarini et al., 2002).

Recent gene profiling studies by Kouros-Mehr and Werb have suggested the involvement of other Eph receptors and ephrin ligands in the development of mammary gland. They utilized a β -actin-green fluorescent protein (GFP) reporter mouse model, which displayed high GFP expression throughout mammary epithelium. RNA analysis from micro-dissected epithelial and stromal compartments revealed that EphA2 is the only Eph receptor enriched in TEB. One would expect that some of the ephrin-A members may be expressed in the TEB microenvironment. However, ephrin-B1 is found to be the only ephrin ligand that is enriched in TEB stroma (Fig. 3). Expression of both EphA2 and ephrin-B1 is confirmed by in situ hybridization (Kouros-Mehr and Werb, 2006). Although there is binding specificity between Eph receptors and ephrin ligands, for example, EphA receptors generally bind to ephrin-A ligands while EphB receptors preferentially target transmembrane ephrin-B ligands, there are indeed exceptions. One is that EphA4 interacts with ephrin-B and plays a critical role in somite boundary formation as we discussed in previous section (Barrios et al., 2003), and nervous system development (Takemoto et al., 2002). Another exception is that ephrin-A5 binds to and activates EphB2 leading to growth cone collapse and neurite retraction (Himanen et al., 2004). The compartmentalized expression pattern of EphA2 and ephrin-B1 in TEB raises the possibility that more cross family interactions between Eph receptors and ephrin ligands may exist. Further biochemical and structural studies are needed to verify the possible interaction between EphA2 and ephrin-B1. Alternatively, these data also suggest that EphA2 may have a ligand-independent function in mammary

branching morphogenesis. It should be noted however, that mammary glands in EphA2 knockout mice develop normally, with no signs of lactating problems (our unpublished observations), possibly as a consequence of compensatory upregulation of other EphA kinases.

Thymus is another organ that continues to develop after birth in response to T cell development demand. Almost all Eph receptors and ephrin ligands have been found to be expressed in thymus in a compartmentalized pattern (Vergara-Silva et al., 2002; Munoz et al., 2002). Thus far, only EphA4 seems to have a definitive function in thymus development. Munoz and colleagues recently show that EphA4 is critical for establishing an epithelial network in thymus. Abnormal organization of thymic epithelial cells is observed in EphA4 knockout mice, which starts in fetal thymus and persists in postnatal life. As a result, T cell development was severely affected (Munoz et al., 2006).

Role of Eph/ephrin in maintaining epithelial integrity

A characteristic of epithelial cells is that they make intercellular connections through specialized membrane structures including tight junctions, adherens junctions, desmosomes, and gap junctions (Fig. 4) (Perez-Moreno et al., 2003). Evidence is accumulating suggesting that there is an intimate relationship between Ephs or ephrins and epithelial cell junctional molecules. Eph/ephrin systems and junctional molecules appear to work coordinately to accomplish cell sorting processes and regulate epithelial integrity (Table 1, Fig. 4).

Tight junctions are the most apical intercellular junctional structure in polarized epithelial cells, and consist of three distinct types of transmembrane proteins: occludins, claudins, and junctional adhesion molecules. Occludins form complexes with zonula occludens (ZO)-1 protein. Claudins have a PDZ-binding domain and directly interact with ZO-1, ZO-2, and ZO-3. Junctional adhesion molecules localize to both tight junction and adherens junctions, and mediate Ca^{2+} -independent adhesion. Recently, claudin 4 has been reported to interact with EphA2 and ephrin-B1 in normal epithelial cells and epithelial carcinoma cells (Tanaka et al., 2005b; Tanaka et al., 2005a). The interaction between Claudin 4 and EphA2 leads to the phosphorylation of Claudin 4, whereas the interaction between Claudins (Claudin 4 or Claudin 1) and ephrin-B1 causes phosphorylation of ephrin-B1. Phosphorylation of claudin 4 by EphA2 leads to reduced association between claudin 4 and ZO-1 and delayed assembly of claudin 4 to tight junctions (Tanaka et al., 2005a). Ephrin-B1 interacts with claudins 1 and 4 on the same cell in *cis* via its extracellular domains. The subsequent phosphorylation of ephrin-B1 is dependent on Src kinase upon formation of cell-cell contact, but independent of Eph receptors. The interaction of claudins with ephrin-B1 seems to also affect tight junction integration (Tanaka et al., 2005b).

Adherens junctions are composed of at least two distinct adhesion structures: E-cadherin-based adherens junctions and Nectin-based cell-cell adhesions. The cadherin-based adherens junctions are Ca^{2+} -dependent, whereas the nectin-based cell-cell adhesions are Ca^{2+} -independent. Nectins are linked to the actin cytoskeleton through actin-binding protein afadin/AF-6. Afadin can also interact with catenins, the components of E-cadherin complex in epithelial cells where nectin-afadin system and cadherin-catenin system assemble adherens junctions cooperatively (Miyoshi and Takai, 2005). Two early studies show that AF-6 interacts with a subset of Eph receptors including EphA7, EphB2, EphB3, EphB5 and EphB6. The interaction is mediated by the PDZ domain of AF-6 and is dependent on tyrosine phosphorylation of Eph kinases. By interacting with Eph receptors, AF-6 is recruited to cell-cell contacts in MDCK and 293T cells (Buchert et al., 1999; Hock et al., 1998). The nectin-afadin complex is important for the formation of not only adherens junctions, but also tight junctions in epithelial cells. Studies using afadin-knockout mice

have revealed that afadin is indispensable for embryonic development by organizing the formation of cell-cell junctions (Ikeda et al., 1999; Komura et al., 2008; Miyoshi and Takai, 2005). The physical interaction between afadin and multiple Eph receptors and subsequent clustering of afadin to cell-cell adhesion sites may provide additional mechanisms for these molecules to regulate epithelial architecture.

Studies on embryonic stem (ES) cells by Orsulic and Kemler reveal that expression of several Eph receptors and ephrin ligands is regulated by E-cadherin. For instance, loss of E-cadherin in ES cells results in the upregulation of ephrin-A1, ephrin-A2, ephrin-B1, ephrin-B2, ephrin-B3, and EphB4, and downregulation of EphA2. Rescue of E-cadherin-null ES cells with E-cadherin but not N-cadherin restore the wild-type expression pattern of EphA2 and ephrin-B2, suggesting the specific connection between E-cadherin and Eph receptors and ephrin ligands. A similar observation was made in NIH3T3 fibroblastic cells where E-cadherin expression induced EphA2 protein synthesis (Orsulic and Kemler, 2000). However, the physical interaction between E-cadherin and EphA2 has not been established. One possibility is that the two molecules may not directly interact with each other or the interaction is too weak to be preserved during lysis. Another possibility is that EphA2 is passively brought to the cell-cell contact while E-cadherin-mediated adhesion is established. In normal breast epithelial MCF-10A cells, disruption of E-cadherin-mediated cell-cell adhesion with either calcium-chelating agent or functional blocking antibody of E-cadherin results in reduced phosphorylation and cell-cell contact localization of EphA2. On the other hand, expression of E-cadherin in metastatic breast cancer MAD-MB-231 cells enhances the phosphorylation of EphA2 and redistribution of EphA2 from membrane rafts to cell-cell contacts. Moreover, the activation of EphA2 leads to reduced cell-matrix adhesion (Zantek et al., 1999). This study suggests that while the function of EphA2 is dependent on the expression of E-cadherin, EphA2 activation may facilitate epithelial phenotype conversion.

Cortina and colleagues recently demonstrate that E-cadherin is required for EphB receptor-mediated compartmentalization in colorectal epithelial cancer cells (Cortina et al., 2007). These investigators found that EphB/ephrin-B interaction prevents receptor-positive tumor cells from invading and colonizing ligand-positive normal tissue. Knocking down E-cadherin causes intermingling of tumor cells and normal cells, although activation of EphB remains. This study provides strong evidence that in epithelial cancer cells, EphB signaling restricts the capacity of malignant cells to expand into ephrin-B-positive surrounding tissue by enforcing E-cadherin-mediated cell-cell adhesion (Cortina et al., 2007). Similar compartmentalization behavior is also reported in the coculture of E-cadherin-null and wild-type ES cells by Orsulic and Kemler. As mentioned above, loss of E-cadherin results in significant downregulation of EphA2 and upregulation of multiple ephrins. When E-cadherin-null ES cells are mixed with wild type cells in culture, the two populations segregated. The E-cadherin-expressing wild type ES cells aggregate into distinct clusters surrounded by the E-cadherin-null ES cells (Orsulic and Kemler, 2000). The molecular mechanism, however, is not provided in their study. It is possible that this sorting and compartmentalization behavior is mediated by the interaction between EphA2 on the wild-type ES cells and one of the ephrins that is induced in the E-cadherin-null ES cells.

Functional compartmentalization of tissues is characterized by coupling of cytoplasm between cells in the same compartment to permit direct cell-cell communication through gap junctions, whereas such flow of information does not occur between adjacent compartments. Mellitzer et al. were the first to report that Ephs and ephrins had dual roles in facilitating cell sorting into opposing compartments and in shutting down inter-compartmental communications through gap junctions (Mellitzer et al., 1999). Exogenous EphA4 or EphB2 was expressed in Zebrafish animal cap cells and placed next to the same cells expressing exogenous ephrin-B2 to observe cell intermingling and communication between the two

fluorescently tagged cell populations. Interestingly, while bidirectional signaling was required for preventing intermingling of cells expressing EphA4/B2 kinases and ephrin-B2, unidirectional signaling was sufficient to restrict communications across adjacent compartments by inhibiting gap junction complex.

Physiologically, a recent study by Konstantinova et al. demonstrates that stimulation of endogenous ephrin-As on pancreatic β -cells with EphA5-Fc promoted insulin secretion; the effects required connexin 36, a component of gap junction complex (Konstantinova et al., 2007). It is tempting to speculate if secretory functions of other exocrine epithelial cells are also dependent on ephrin-A-connexin 36 interactions. In contrast, activation of endogenous EphA kinases, including EphA5, by ephrin-A5-Fc inhibited insulin secretion independent of connexin 36. Thus, EphA and ephrin-A have opposite effects on insulin secretion on β -cells, and crosstalk with connexin 36 seem to contribute to stimulatory effects of ephrin-As (Konstantinova et al., 2007). Since EphAs and ephrin-As are both expressed on β -cells, their diametrically opposite effects of insulin secretion are particularly noteworthy, and point to the intricacy and adaptability of the Eph/ephrin system in mammalian epithelial physiology.

Glomerular epithelial cells (podocytes) are highly specialized terminally differentiated cells, which are characterized by interdigitating foot processes and slit diaphragms connecting adjacent foot processes. Since neighboring foot processes are derived from different podocytes, the slit diaphragm is a highly developed variant of a cell-cell junction. A recent study by Hashimoto and colleagues shows that ephrin-B1 is expressed in slit diaphragm, where it colocalizes with nephrin and CD2-associated proteins (CD2AP). CD2AP is an adaptor protein that facilitates interaction between nephrin and actin cytoskeleton. Hashimoto and colleagues demonstrate that knocking down ephrin-B1 in podocytes results in altered subcellular localization of CD2AP. They suggest that ephrin-B1 may play a role in maintaining the slit diaphragm structure through the proper arrangement of CD2AP (Hashimoto et al., 2007).

In sum, current literature provides strong evidence that Ephs or ephrins function coordinately with cell junctional molecules to accomplish cell sorting processes and regulate the epithelial integrity and physiology (Fig. 4). More studies, however, are needed for better understanding the precise role and molecular mechanisms of Eph/ephrin signaling in the dynamics of cell-cell junctional structures.

Eph/ephrin signaling in epithelial homeostasis

Epithelial cells are not only closely linked to each other but also closely associated with basement membrane through integrin-mediated adhesion to extracellular matrix. Epithelial cells can move within the epithelial layer, and this activity is often seen in epithelial homeostasis and wound healing (Schock and Perrimon, 2002). Damaged or dead epithelial cells require constant replacement throughout the life of an organism. Epithelial stem cells play a critical role in this process and maintain epithelial tissue homeostasis (Blanpain et al., 2007; Pinto and Clevers, 2005b). Recently, substantial insights have been gained into the molecular mechanisms controlling epithelial stem cell proliferation and differentiation. The small intestinal epithelium is composed of crypt-villus units, which function to absorb water and nutrients and also functions as a barrier protecting against ingested pathogens. Intestinal stem cells reside near the base of the crypt region. There are several different cell lineages in the villus region, which are continually renewed and shed into the intestinal lumen during tissue homeostasis (Pinto and Clevers, 2005a; Pinto and Clevers, 2005b; Sancho et al., 2003). Replacement comes from stem cell progeny that migrate out of their niche. As they migrate up the crypt to the tip of the villus they differentiate into cells of different lineages. β -catenin, the major component of the Wnt signaling pathway, is present in different pools

in cells. It predominantly resides at the adherens junctions where it functions as a bridging molecule between E-cadherin and α -catenin. The cytoplasmic pool of β -catenin forms a multi-protein complex with Axin, APC and GSK-3 β and β -catenin is phosphorylated by GSK-3 β . The phosphorylated β -catenin is then targeted for ubiquitin-mediated degradation. When Wnt proteins bind to their receptors, the multi-protein complex formation is inhibited, which leads to the accumulation of β -catenin in the cytoplasm and translocation into the nucleus where β -catenin binds to Tcf/Lef transcription factors (de Lau et al., 2007; Pinto and Clevers, 2005a; Pinto and Clevers, 2005b). In vivo studies and gene profiling experiments have shown that the genes encoding EphB2 and EphB3 are among the target genes of the β -catenin-Tcf/Lef, and are upregulated in proliferative cells in the crypt. In contrast, their ligand, ephrin-B1, is absent in the proliferative cells and is upregulated in the differentiated cells in which the receptors are downregulated. Interaction of EphB2 and EphB3 with ephrin-B1 prevents proliferating cells from migrating into the differentiating cell territory, thereby promoting compartmentalization of epithelial cells along the crypt-villus axis (Batlle et al., 2002; Batlle et al., 2005; Clevers and Batlle, 2006). Consistent with this finding, we find that ephrin-B1 potently inhibits colorectal epithelial cell motility in vitro (Miao et al., 2005).

A comprehensive genomic analysis of genes expressed in human colon by Kosinski and colleagues reveals a distinct expression gradient of several Eph receptors and ephrin ligands along the human colon crypt axis (Kosinski et al., 2007). In human colon, genes encoding EphB1, EphB2, EphB3, EphB4, and EphB6 are expressed in the crypt base, whereas the gene encoding ephrin-B2 is expressed at the crypt tips. This is consistent with the gene expression profile reported in the mouse intestinal crypt-villus axis by Clevers, Batlle and colleagues (Batlle et al., 2002; Batlle et al., 2005; Clevers and Batlle, 2006). In addition to the members of EphB family, the authors show that multiple EphA receptors and their ligands are also expressed in human colon. Unlike EphB receptors which are mostly localized to the crypt base, EphA receptors are differentially expressed in human colon. For instance, genes encoding EphA1, EphA4, and EphA7 are found to be highly expressed at the crypt base, the compartment that has proliferative activity, whereas EphA2, EphA5, and ephrin-A1 genes are highly expressed in crypt tips where maturation and elimination of intestinal epithelial cells occur (Kosinski et al., 2007). The abundant expression of EphA2 and ephrin-A1 was also reported by Hafner in human small intestine and colon mucosa (Hafner et al., 2005b). The differential expression pattern of EphA receptors suggests they may have diverse roles in colon epithelial homeostasis. In particular, the villus localization of EphA2 receptor and ephrin-A1 ligand may have implications in stress response in the human intestinal epithelia.

Additional mechanisms contribute to intestinal epithelial repair, particularly in the case of minor breaks in epithelial continuity caused by mechanical strain associated with intestine motility and physiologic digestive trauma. It has been recognized that surface epithelium can be rapidly repaired in the absence of cell proliferation. This process is called “epithelial restitution”, which occurs in all regions of the intestinal tract. Epithelial restitution involves migration of the cells at the wound edge (Mammen and Matthews, 2003). Hafner and colleagues report that stimulation of ephrin-B with EphB1-Fc in intestinal epithelial cells significantly enhances wound healing process (Hafner et al., 2005b). Later, the same group demonstrate that ephrin-B reverse signaling can induce expression of wound healing associated genes, including c-Fos, Egr-1, Egr-2, and COX-2 (Hafner et al., 2005a). These genes are known to be involved in the injury response and are essential for rapid wound closure in diseased conditions. Therefore, while the forward signaling through EphB2 and EphB3 mediates the repulsive response between receptor-expressing cells and ephrin-B1 ligand expressing cells, thereby preventing intermingling between proliferating and

differentiating intestinal compartments, the reverse signaling through ephrin-B seems to have opposite effects, albeit in a different context.

Increasing evidence has shown the upregulation of certain Eph receptors and ephrin ligands in response to diverse stress conditions. EphA2 is among the most extensively studied (Ivanov et al., 2005; Nahm et al., 2002; Xu et al., 2004). EphA2 was first characterized as epithelial cell kinase (Eck) because it was primarily expressed in epithelial cells (Lindberg and Hunter, 1990). The role of EphA2/ephrin-A1 interaction in epithelial restitution was first reported by Rosenberg a decade ago (Rosenberg et al., 1997). It is shown that both EphA2 and ephrin-A1 are expressed on the same intestinal epithelial Caco-2 cells. Activation of EphA2 by adding exogenous ephrin-A1 enhanced the integrity of intestinal epithelial monolayer, promoted epithelial barrier function, and accelerated the repair process of epithelial monolayer after injury (Rosenberg et al., 1997).

Eph receptors also play an important role in epithelial tissue repair in the context of renal ischemia-reperfusion injury (Baldwin et al., 2006). EphA2 is highly upregulated in renal medulla and cultured IMCD-3 cells in response to hypertonicity and urea stress (Nahm et al., 2002; Nahm et al., 2002; Xu et al., 2004). It is suggested that embryonic developmental programs may be reactivated in the adult injured kidney. For example, reexpression of Pax-2 and increased expression of Wnt-4 are observed in renal injury. Bone Morphogenic Protein (BMP)-7 that induces formation of epithelial aggregates from metanephric mesenchyme during kidney development is also found to be capable of inducing MET in fibrotic kidney injury (Zeisberg et al., 2005). It would be interesting to determine how Eph/ephrin signaling may be involved in this type of renal epithelial regeneration.

Skin epithelium, by its location, is facing constant challenge from the environment. Eph/ephrin signaling has also been implicated in maintaining skin epithelial homeostasis. We recently report that EphA2 and ephrin-A1 are expressed in a complementary pattern in mouse skin. EphA2 is expressed in a basal to suprabasal gradient with lower expression in the basal layer and higher expression in spinous and granular layers. In contrast, ephrin-A1 expression is primarily restricted to the basal layer abutting the basement membrane. This expression pattern suggests that EphA2/ephrin-A1 interactions are localized to the basal layer, where most cell proliferation takes place. EphA2 activation by ephrin-A1 stimulation in primary keratinocytes results in the inhibition of Erk/MAP kinase activation. Deletion of EphA2 eliminates EphA2/ephrin-A1 interactions in the interface of the receptor-expressing and the ligand-expressing cells, which could contribute to the increased tumor susceptibility (Guo et al., 2006). This finding indicated that EphA2 signaling is important for epithelial homeostasis in the skin. At the same time, Hafner and colleagues have screened the mRNA and protein expression of a whole panel of Eph receptors and ephrin ligands in adult human skin. They have found that all tested members are expressed in human skin at varying levels. Among EphA receptors, EphA1, EphA2, and EphA4 display the highest expression levels. Among EphB receptors, EphB3 is most abundant. Ephrin-A3 has the highest expression levels among all ephrin ligands. They further reported that EphA1 was specifically expressed in keratinocytes of the epidermis and hair follicles. Interestingly, EphA1 was significantly downregulated in both basal cell carcinoma and squamous cell carcinoma (Hafner et al., 2006). It seems that EphA1 and EphA2 have similar expression pattern in the skin epithelia and both potentially function as tumor suppressor. However, distinct molecular mechanisms may be utilized by the two Eph receptors.

Conclusions and future directions

Much progress has been made in recent years in our understanding of the role of Eph receptors and their ephrin ligands outside of the nervous system. As outlined in this review,

these cell surface molecules play diverse roles that profoundly influence the epithelial tissue morphogenesis and integrity throughout the life. However, much remains to be learned to define the precise effects of these molecules in epithelia and to elucidate the molecular mechanisms. It seems that, in many cases, Eph receptors and their ligands trigger distinct or even opposite signaling events. Further studies will be required to distinguish the functions and signaling pathway of the receptors from those of the ligands, particularly in cases where they are coexpressed in the same cells. The functional significance of the coexpression of EphA receptors and ephrinA ligands within the same cells has been addressed in the nervous system (Flanagan, 2006). Two theories have been proposed. One is that EphA receptors and ephrinA ligands interact in *cis* on the same cell surface and this interaction downregulates or inhibits EphA forward signaling and function (Hornberger et al., 1999; Yin et al., 2004; Carvalho et al., 2006). Another is that coexpressed EphA receptors and ephrin-A ligands segregate into distinct membrane microdomains where they mediate forward and reverse signaling independently (Marquardt et al., 2005). Whether these theories also apply to epithelial cells needs to be addressed. Finally, both MET and EMT are also important for epithelial cell-derived tumor progression. While EMT is thought to be important for epithelial tumor cells to acquire an invasive and metastatic mesenchymal phenotype, MET is critical for the establishment of secondary tumors at the site of metastases (Thiery and Sleeman, 2006; Hugo et al., 2007). The abnormal expression of Eph/ephrin in various human cancers has been widely reported. How the compromised Eph/ephrin signaling may contribute to the EMT and MET during tumor progression should be one of the questions to be answered in the future studies.

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Figure 1. Domain structure of Eph kinases and ephrins, and some of the known interacting proteins. LBD., Ligand-binding domain; EGF: EGF-like motif; FN III, fibronectin type III repeat; TM, transmembrane domain; J.M. juxtamembrane domain; SAM, sterile α motif; PDZ-BS, PDZ domain binding site. Some of the reported Eph interacting proteins are indicated.

Figure 2.

A. Schematic diagram of early embryonic kidney development. The ureteric bud arises in Wolffian duct in response to signals from metanephric mesenchyme. It undergoes iterative branching, and eventually forms the collecting duct. The induced metanephric mesenchyme gives rise to nephron. B-C. EphA2 is expressed on ureteric buds of E12.5 embryonic kidneys. The ureteric buds are marked by cytokeratin (Green in C), where the β -gal reporter for *EphA2-LacZ* (blue in B) is present. The EphA2 expression is not detectable in metanephric mesenchyme that is marked by Pax-2 (Red in C). D-E. Activation of EphA2 by ephrin-A1 inhibits HGF-induced MDCK cell branching morphogenesis in 3-D collagen gel (Adapted from Miao et al., 2003).

Figure 3.

Schematic diagram of terminal end bud (TEB) in the developing mammary gland. The club-shaped TEB forms at the end of the pre-existing duct. TEB stroma surrounds the neck region of TEB, which mainly contains fibroblasts. EphA2 is detected in TEB epithelium, and Ephrin-B1 is detected in the TEB stromal cells during mammary branching morphogenesis (Kouros-Mehr and Werb, 2006). EphB4 is primarily expressed in myoepithelial cells. Ephrin-B2 is expressed in luminal epithelial cells (Nikolova et al., 1998).

Figure 4.

Interactions between Ephs and ephrins and epithelial cell adhesion and junction proteins. TJ, tight junction; AJ, adherens junction; GJ, gap junction; Des, desmosome; ECM, extracellular matrix; FA, focal adhesion; HD hemidesmosome. This review focuses on Eph/ephrin crosstalk with proteins in tight junctions, adherens junctions and gap junctions in epithelial cells. Readers are referred to other recent reviews for Eph/ephrin crosstalk with other adhesion and signaling molecules including integrins (Davy, 2008; Lackmann and Boyd, 2008; Pasquale, 2008).

Table 1

Effects of Eph or ephrin signaling on cell-cell junctional structures

Junctional components	Major binding partners	Eph/ephrin	Impact of Eph/ephrin
TJs:			
Claudins *	ZO-1, ZO-2, ZO-3	EphA2 Ephrin-B1	↓ TJs ↓ TJs
AJs:			
E-cadherin	α -, β -, γ -catenin, p120catenin	EphA2 EphB2, EphB3	↓ or ↑ cell-cell adhesion ↑ cell-cell adhesion
Nectin	Afadin/AF-6 *	EphA7, EphB3	Recruit Afadin to cell-cell adhesion
GJ			
Connexins	Connexins: Homo- or hetero-oligomers	EphA4, EphB2 Ephrin-B2, ephrin-A1	↓ GJs

* The direct interaction between these molecules and Eph or ephrin has been detected. TJ: tight junction. AJ: adherens junction.