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Review

The role of EPH receptors in cancer-related epithelial-mesenchymal transition

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Abstract

Erythropoietin-producing hepatoma (EPH) receptors are considered the largest family of receptor tyrosine kinases and play key roles in physiological and pathologic processes in development and disease. EPH receptors are often overexpressed in human malignancies and are associated with poor prognosis. However, the functions of EPH receptors in epithelial-mesenchymal transition (EMT) remain largely unknown. This review depicts the relationship between EPH receptors and the EMT marker E-cadherin as well as the crosstalk between EPH receptors and the signaling pathways involved EMT. Further discussion is focused on the clinical significance of EPH receptors as candidates for targeting in cancer therapeutics. Finally, we summarize how targeted inhibition of both EPH receptors and EMT-related signaling pathways represents a novel strategy for cancer treatment.

Key words EPH receptor, E-cadherin, EMT, cancer, targeted therapy

Erythropoietin-producing hepatoma (EPH) receptors have been recognized for their roles in tumor suppression and promotion. Their roles in tumors is essential but complex because of their ligand-dependent and ligand-independent activity. However, the function of EPH receptors in human malignancies remains unclear. Recently, a number of studies that implicate the role of EPH receptors in the epithelial-mesenchymal transition (EMT) process have emerged. In this review we focus on the relationship between EPH receptors and EMT-related signaling pathways, and discuss the inhibition of both EPH receptors and EMT-related signaling pathways as anticancer therapy.

EMT

EMT has recently garnered attention from pathologists and cancer researchers due to its crucial role in both embryogenesis and tumor progression[1,2]. Tumor cells undergoing EMT display characteristics that promote distal metastasis and chemotherapy resistance, including resistance to cell death and senescence, evasion of immune surveillance, and the acquisition of stem cell properties[3]. During EMT, tumor cells initially exhibiting epithelial phenotypes lose their polarity, change their morphologies and cytoskeletal organization, and dissolve their cell-cell contacts. Consequently, the tumor cells acquire a mesenchymal phenotype. A number of EMT-related markers exhibit concomitantly altered expression. Among these are N-cadherin, vimentin, fibronectin, and matrix metalloproteinases (MMPs) such as MMP-2, MMP-3, and MMP-9, all of which are up-regulated; and E-cadherin, desmoplakin, cytokeratin, and occludin, which are down-regulated. Other concomitant changes include the activation of β-catenin, Smad-2/3, nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), and the transcription factors Snail, Slug, and Twist[4]. Therefore, impairing the up-regulation of EMT markers would prevent EMT, and as such, these markers might represent therapeutic targets for human malignancies[5-7].

EMT, as well as its related markers, is associated with cancer development and chemotherapy resistance[8,9]. A prominent hallmark of EMT is the loss of E-cadherin, the main component of the tight junctions that connect adjacent cells[10]. The loss of E-cadherin alone can facilitate metastasis via the induction of EMT, invasiveness, and resistance to anoikis[11]. Moreover, the loss of E-cadherin expression represents a rate-limiting step in the progression from well-differentiated adenomas to invasive carcinomas in a transgenic mouse model[12]. The loss of E-cadherin promotes the nuclear relocation of β-catenin through the Wnt signaling pathway, and β-catenin in the nucleus ultimately induces EMT via targeting tumorigenic genes[13]. On the other hand, tumor cells undergoing EMT acquire resistance to primary therapeutic modalities. For example, ovarian cancer cells undergoing EMT develop resistance to platinum-based chemotherapies, which facilitates relapse[14]. Other examples

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of EMT associated with therapy resistance can be found in bladder cancer[15], lung cancer[16], prostate cancer[17], and hepatocellular carcinoma (HCC)[18].

Multiple oncogenic signaling pathways, such as Wnt, Ras/mitogen-activated protein kinase (MAPK), Akt-mammalian target of rapamycin (mTOR), and Notch, have emerged as potent participants of EMT during malignant tumor progression. Diverse extracellular signaling molecules, including epidermal growth factor (EGF), transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), activate their corresponding receptors and downstream signaling pathways, such as Wnt/β-catenin, TGF-β/Smad, Ras/MAPK, and Notch, which eventually down-regulate or even completely abrogate the expression of E-cadherin by increasing the expression of transcriptional repressors of E-cadherin, including Snail, Slug, zinc finger E-box–binding homeobox 1/2 protein (ZEB1/2), and Twist. These processes have been extensively reviewed[4,10,19-22].

Canonical Wnt/β-catenin has been identified as an important signaling pathway in EMT. E-cadherin/β-catenin protein complexes are essential for maintaining epithelial integrity. Ectopic activation of Wnt/β-catenin singaling results in translocation of β-catenin from cytoplasm to nucleus, triggering the release of β-catenin from E-cadherin[13]. As a result, E-cadherin levels decrease in cell-cell junction adhesions, a phenotype that is a hallmark of EMT. On the other hand, excess β-catenin enters the nucleus and up-regulates the transcription of Slug and Snail, which promotes EMT by suppressing E-cadherin expression[10].

Another oncogenic signaling pathway involved in EMT is the Ras/MAPK pathway, a downstream target of TGF-β signaling. TGF-β was the first known inducer of EMT, and its relationship to the Smad family is already well characterized. Upon TGF-β ligation, its receptor phosphorylates Smad2/3 and activates c-Jun N-terminal kinase (JNK) and p38MAPK. Activated JNK and p38MAPK can act in a Smad-dependent or Smad-independent manner to regulate EMT by controlling downstream transcription factors[23]. TGF-β signaling can also activate extracellular signal-regulated kinase 1/2 (ERK1/2) through Ras, Raf, and MAPK/ERK kinase 1/2 (MEK1/2). The activation of ERK1/2 can also control EMT by down-regulating E-cadherin via Slug, Snail, and Smad[23].

In addition, the AKT-mTOR and Notch signaling pathways have come under intense scrutiny for their roles in EMT. Signals transduced by receptor tyrosine kinases (RTKs) lead to EMT via the phosphatidylinositol 3-kinase (PI3K)/AKT pathway[10]. New evidence shows that stable knockdown mTOR complex 1 (mTORC1) components in SW480 cells increases E-cadherin levels and decreases vimentin levels, whereas transcription factors that suppress E-cadherin—Snail and Twist—are decreased[24]. These observations demonstrate that the AKT-mTOR signaling pathway is involved in EMT processes. Notch activation in endothelial cells results in morphologic, phenotypic, and functional changes consistent with mesenchymal transformation. These changes include down-regulation of endothelial markers (e.g., VE-cadherin) and up-regulation of mesenchymal markers (e.g., fibronectin)[22]. Activation of membrane receptors like TGF-β receptor (TGFβR), FGF receptor (FGFR), PDGF receptor (PDGFR), and Notch up-regulates the expressions of Snail, Slug, Twist, and ZEB1/2 through their common intracellular Notch signals[22]. Up-regulation of these transcription factors promotes EMT by repressing the expression of E-cadherin. Moreover, a fundamental yet complex crosstalk exists among these signal transduction pathways[25]. For instance, TGF-β activates other EMT-related signaling pathways, such as Notch, Wnt/β-catenin, and integrin[10]. Recently, a number of studies including ours have suggested that EPH receptors play a role in the EMT process[26-28]. However, the mechanism by which EPH receptors regulate EMT is still largely unknown. Therefore, we focus this review article on the role of EPH receptors in cancer-related EMT.

EPH Receptors

Since the first EPH family member was cloned in an EPH cell line in 1987[29], abundant studies regarding the function of EPH receptors in cancer have been published. The EPH family is considered the largest family of RTKs, which play pivotal roles in cell growth, differentiation, and motility. To date, several members of the EPH family have been discovered. These members are divided into the classes EPHA and EPHB based, in part, on their sequence homologies and ligands (EPHRIN-As and EPHRIN-Bs)[28]. The receptors contain extracellular domains, including a ligand-binding domain, a cysteine-rich domain, two fibronectin type III repeats, and an intracellular cytoplasmic domain that consists of a juxtamembrane region, a tyrosine kinase domain, a sterile alpha motif (SAM), and a C-terminal PSD95/Discs large/Zona Occludens 1 protein (PDZ)–binding motif[30]. Although EPHAs and EPHBs are generally known to bind to EPHRIN-As and EPHRIN-Bs, respectively, cross-class interactions have nonetheless also been identified[31].

Unlike the classical concept of "receptor" and "ligand," both EPHs and EPHRINs are membrane-bound and act as receptors as well as ligands to induce EPH-initiated forward signaling and EPHRIN-initiated reverse signaling. This function enables EPH receptors and their ligands to play complex roles in tumor cells. In many cancer cell lines, EPH receptors act as tumor promoters in the absence of ligand, whereas these receptors assume a tumor suppressor role when ligand is present[32,33]. However, new evidence shows that tumor cells use EPH receptors to migrate to ligand-expressing stromal cells, thus facilitating tumor invasion and metastasis[34]. Notably, the same EPH receptor can play both tumor promotion and tumor suppression roles in different cancer types. For example, overexpression of EPHA2 can promote ovarian cancer cell growth, whereas *Eph2A*-null mutant mice exhibit accelerated skin tumor growth[35,36]. EPHB3 also functions as both a tumor promoter and tumor suppressor in non–small cell lung cancer (NSCLC) cells and colon cancer cells, respectively[37]. Furthermore, several EPH ligands are up-regulated during human malignancy. Reverse signals initiated by EPH ligands also play dual roles in tumor inhibition and promotion. For instance, EPHRIN-A1 affects the biological behavior of tumor cells through multiple oncogenic signaling pathways, such as MAP/ERK and PI3K; in some cases, a specific pathway is promoted in one cell or cancer

type but inhibited in another type of cell or cancer[38]. These findings indicate that the function of EPH receptors/EPH ligands in cancer cells depends not only on the EPH receptor profile of the cancer cell and the reciprocal EPHRIN ligands expressed by neighbor cells[39] but also on the specific cancer type.

Crosstalk between EPH receptors and other oncogenic RTKs represents a new possible mechanism for the function of EPH receptors during tumor development[40]. For example, EPHA2 forms a complex with ErbB2 to promote tumor progression in mice by enhancing both Ras/MAPK signaling and the activation of RhoA GTPase[41]. Moreover, EPHA2 overexpression contributes to breast cancer resistance to trastuzumab, a humanized monoclonal anti-HER2 antibody[42], which indicates that EPHA2 contribute to therapy resistance. In addition, the expression of EPHA2 in cancer cells is restricted to those growing adherently and is dependent upon the activation of epidermal growth factor receptor (EGFR)[43]. Furthermore, crosstalk between EPH receptors and the VEGF signaling pathway promotes tumor-associated angiogenesis in a ligand-dependent manner[44]. Therefore, inhibiting the activation of EPHA2 not only blocks tumor progression but also increases tumor cell sensitivity to chemotherapy. Thus, EPHA2 represents a promising therapeutic target for cancer treatment[45,46].

Other mechanisms can help us to more comprehensively understand the function of EPH receptors in cancer progression. For instance, EPH receptors and their ligands can regulate stem/progenitor cell proliferation in adult tissues as well as tumor progression; this is a relevant function of EPH receptors in the context of oncology[47]. Moreover, EPH receptors can promote the dissemination of tumor cells by guiding amoeboid movement toward EPH ligands that are expressed on endothelial cells at sites of future metastatic tumor formation[48,49]. Furthermore, natural killer cells and monocytes triggered a larger reduction in xenograft tumor volume when EPHA2 antibodies were present, suggesting that the immune system can also contribute to the function of EPH receptors in cancer[50]. Moreover, microRNAs involved in the metastatic process can target the 3'-UTR of EPH transcripts, thereby regulating EPH gene expression[51-53]. Although these results are encouraging, the function of EPH receptors in human malignancies remains unclear. Therefore, we have attempted to summarize the functional role and clinical significance of EPH receptors in EMT in the following sections.

The Role of EPH Receptors in EMT

Recently, EPH receptors have been found to play an important role in many aspects of EMT[26-28], including induction of a mesenchymal-like phenotype (e.g., an E-cadherin–negative and vimentin-positive phenotype) and inhibition of epithelial characteristics (e.g., an E-caherin–positive and vimentin-negative phenotype)[46]. Moreover, EPH receptors crosstalk with EMT-related signal transduction pathways such as those induced by NF-κB and PI3K[54,55]. Furthermore, ligand-stimulated activation of the EPHA2 receptor enhances cell-cell adhesion and inhibits growth factor-mediated scattering, thereby promoting mesenchymal-epithelial transition (MET)—the reverse process of EMT—or inhibiting EMT, respectively. As a result, EPH receptors are believed to be important during the induction of EMT. Therefore, the discussion in the following subsections is focused on the role of EPH receptors in EMT.

EPH receptors and E-cadherin

During the acquisition of the EMT phenotype, the loss of E-cadherin expression appears to be a crucial step, resulting in reduced cell-cell adhesion and ultimately the destabilization of the epithelial architecture. The relationship between EPH receptors and E-cadherin has been investigated in both development and cancer progression. The EPHB receptor interacts with E-cadherin and with the metallo-proteinase a disintegrin and metallopeptidase 10 (ADAM10) at the sites of epithelial cell-cell adhesion. The activation of this complex induces shedding of E-cadherin by ADAM10. As a result, cell sorting is triggered by the asymmetric location of E-cadherin and the affinity between EPHB-positive and EPHRIN-B–positive cells[56]. This process suggests that EPH/EPHRIN signaling plays a crucial role in the compartmentalization of cells in epithelial tissues.

Both EPH receptors and E-cadherin are involved in the progression of human malignancies. For example, EPHA2 is overexpressed in gastric and colorectal cancers exhibiting liver metastasis and lymphatic vessel invasion, whereas the expression levels of E-cadherin are inversely associated with cancer stage and lymphatic vessel invasion[26,27,57]. Nevertheless, simultaneous expression of E-cadherin and EPHB3 was significantly inversely associated with the stage of esophageal adenocarcinoma[58], indicating that *EPHB3* is a tumor suppressor gene. The tumor suppressor function of EPHB3 can be explained in two ways. On one hand, EPHB3 might affect the redistribution of cellular E-cadherin to the plasma membrane, thus enhancing E-cadherin–mediated cell-cell adhesion through the Rac1 pathway[37]. On the other hand, EPHB-mediated compartmentalization restricts the spreading of EPHB-expressing tumor cells into EPHRIN-B1–positive regions[59]. The different roles of EPH receptors in cancer progression are consistent with a model in which one EPH gene becomes dominant, whereas other EPH members are lost through epigenetic or genetic mechanisms. As a result, elevated levels of EPH signaling in primary lesions are gradually attenuated by reduced EPH expression[60]. Therefore, the expression profile of EPH receptors in different tumor types and stages should be established.

Accumulating evidence demonstrates a mutual regulation between EPH receptors and E-cadherin–based cell-cell adhesion. EPHA2 and E-cadherin co-localize along the lateral membranes of normal epithelial cells, specifically within sites of cell-cell contract. The loss of E-cadherin can result in the loss of EPHA2 at intercellular contacts and within membrane ruffles, whereas the expression of E-cadherin is required for EPHA2 phosphorylation and localization[61]. Moreover, vascular endothelial cadherin (VE-cadherin) regulates the expression of EPHA2 at the plasma membrane by mediating its ability to become phosphorylated through its interaction with its membrane-bound ligand, EPHRIN-A1[62]. This evidence shows that E-cadherin can regulate the expression and subcellular localization

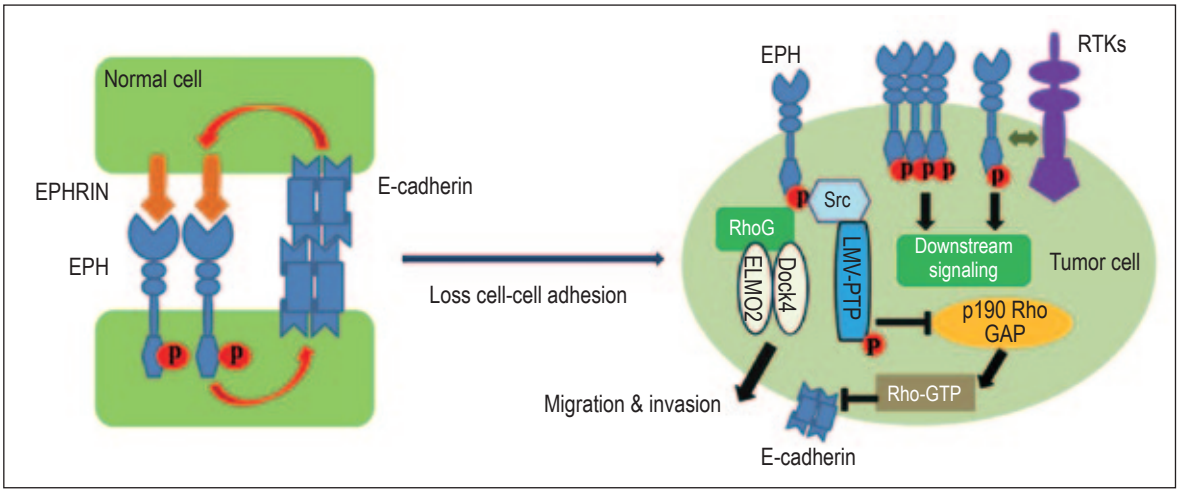

Figure 1. **The relationship between the erythropoietin-producing hepatoma (EPH) receptors and E-cadherin in normal and tumor cells. The mutual regulation between ligand-dependent EPH function and E-cadherin is important for morphologic maintenance of epithelial cells (left). However, epithelial tumor cells undergoing the epithelial-mesenchymal transition (EMT) exhibit loss of cell-cell adhesion and altered shape due to cytoskeletal rearrangement. EPH receptors are overexpressed, promote migration and invasion, and inhibit E-cadherin through ligand-independent mechanisms including EPH receptor overexpression, clustering, and autophosphorylation/phosphorylation by other RTKs (right). LMW-PTP, low molecular weight phosphotyrosine phosphatase; Red P, phosphorylation; RTKs, receptor tyrosine kinases.**

of EPHA2[63], as well as facilitate the interactions between EPHA2 and its ligands by stabilizing cell-cell contacts. Conversely, the ligand-mediated activation of EPHA2 enhances E-cadherin–based cell-cell contact, as well as the apical-basal polarization of epithelial cells[64]. These results indicated that a positive feedback loop exists between E-cadherin and the EPHA2 receptor (**Figure 1**).

However, EPHA2 does not co-immunoprecipitate with E-cadherin, suggesting that EPHA2 and E-cadherin interact indirectly[63]. Moreover, although EPHA2 is overexpressed in metastatic breast cancer cells, its phosphorylation and E-cadherin expression level decreased. These data demonstrate that EPHA2 activation depends on autophosphorylation and receptor aggregation–induced phosphorylation rather than E-cadherin–induced phosphorylation in metastatic cells[61]. Therefore, other factors are likely to also modulate EPHA2 phosphorylation in cancer cells. A model in which EPHA2 overexpression promotes the destabilization of adheren junctions has been proposed (**Figure 1**). In this model, EPHA2 recruits the low molecular weight phosphotyrosine phosphatase (LMW-PTP) and Src kinase. Increased LMW-PTP phosphatase activity inhibits p190 RhoGAP activity by dephosphorylating p190 RhoGAP. The decreased p190 RhoGAP activity destabilizes the adheren junctions by up-regulating Rho-GTP activation[65]. Furthermore, EPHA2 interacts with Ephexin4, one of guanine nucleotide exchange factors (GEFs) for RhoG, and activates RhoG in a ligand-independent manner. The activation of RhoG recruits its effectors ELMO2 and Dock4 to form a complex with EPHA2 at the tips of cortactin-rich protrusions to promote breast cancer cell migration and invasion[66]. In addition, other growth factors stimulate the phosphorylation of EPH receptors. For example, growth factors including EGF, bFGF, PDGF, and HGF are all capable of inducing the phosphorylation of EPHA2 on serine 897 in U373 glioma cells[33]. These results indicate that E-cadherin and ligand-independent mechanisms represent a predominant approach to activate EPH receptors in human malignancies, consistent with the elevated expression levels of EPH receptors. However, evidence has shown that interplay between EPH receptors and other oncogenic signaling pathways that trigger EMT is a crucial part of EPH receptor function in EMT.

EPH receptors crosstalk with EMT-related growth factors

Growth factors such as HGF, FGF, EGF, and PDGF are widely accepted to induce EMT by activating specific signaling pathways[19]. Crosstalk of EPH receptors with these molecules or their cognate receptors might effectively demonstrate that EPH receptors participate in EMT in human malignancies. EGF, FGF, PDGF, and HGF can phosphorylate EPHA2 and activate its downstream effector Akt, whereas treating cells with EPHRIN-A1-Fc significantly inhibited both growth factor-induced EPHA2 phosphorylation and Akt activation[33]. Similarly, stimulation of EPHA with EPHRIN-A1 attenuated the activation of MAPK by the VEGF, EGF, and PDGF receptors[67]. Moreover, adhesion-induced EPHA2 expression is dependent on the activation of EGFR, MEK, and Src. Conversely, EPHA2 expression can be effectively decreased by inhibiting EGFR, MEK, or Src[43]. The expression of such ligands can be lost during tumor progression. Thus, EPH receptors can be activated by other RTKs and not just their own ligands. As a result, the ligand-dependent tumor suppressive function of EPH receptors was reported to switch to ligand-independent tumor-promoting function by activating downstream endogenous signaling pathways in breast cancer cells[68]. Therefore, the combined inhibition of both EPH receptors and other RTKs represents an encouraging target therapeutic strategy.

Recently, Yokote *et al*.[69] found that an N-terminal portion of the tyrosine kinase domain of EPHA4 interacts directly with the juxtamembrane domain of FGFR, facilitating receptor trans-phosphorylation and potentiating the common downstream MAPK signaling pathway. In addition, the adhesion of cancer cells to the extracellular matrix (ECM) or the activation of EGFR can induce the phosphorylation and activation of MAPK. The latter induces the expression of EPHA2 by increasing *EPHA2* mRNA synthesis[43]. Furthermore, EPHA has the ability to activate the HGF signaling pathway. A study has shown that HGF induced EMT-like morphologic changes as well as up-regulation of Snail1 and N-cadherin in HCC[70]. Ligand-dependent activation of EPHA inhibits HGF-induced sprouting of cellular protrusions, an early step in the branching morphogenesis of Madin-Darby canine kidney (MDCK) cells[71]. Thus, these findings suggest that the EPHA receptor promotes EMT through the HGF signaling pathway. Moreover, these results demonstrate that EPH receptor crosstalk with other growth factors is dependent not only on the type of EPH receptor but on the oncogene context within which the EPH receptor functions.

EPH receptors and the Wnt signaling pathway

The Wnt/β-catenin pathway initiates a signaling cascade that is critical during both normal tissue development and cancer initiation and progression[72,73]. Studies to date have focused on the relationship between EPH receptors and Wnt signaling in physiological processes and tumor-related EMT. In the convergent extension of the zebrafish notochord, EphB is phosphorylated through non-canonical Wnt signaling and interacts with the planar cell polarity gene product of Dvl2 and disheveled-associated activator of morphogenesis (Damm1). This complex subsequently mediates cellular repulsion by removal of EphB molecules[74]. Moreover, the expression of EPHB receptors is essential for correct positioning of intestinal epithelial cells along the crypt/villus axis via the Wnt signaling pathway[75]. However, the activation of Wnt signaling induces EPHB expression in initial intestinal tumor cells. As a consequence, the tumor is compartmentalized within the epithelium when these EPHB-positive tumor cells encounter EPHRIN-B–positive epithelial cells[76]. Although Wnt signaling remains constitutively active, losing EPHB receptors accelerates the progression of colorectal cancer[77]. These studies indicate that EPHB and Wnt signaling play a crucial role in the maintenance of intestinal homeostasis and resistance to the development of initial tumor cells. Further studies are needed to investigate the mechanism by which EPHB expression is lost during the switch from tumor suppression to impaired tumor control.

Stimulation of the Wnt/β-catenin signaling pathway results in the translocation of β-catenin from the cytoplasm to the nucleus, where, in complex with T-cell factor (TCF), it regulates several EMT-inducing transcription factors[4]. Subsequently, TCF/β-catenin forms complexes with transcriptional co-activator cyclic AMP-responsive–related binding protein (CBP) or its closely related homologue p300. Studies in SW480 colon cancer cells have shown that *EPHB2* is a TCF/β-catenin–regulated gene that is dependent on the co-activator p300, whereas EPHB4 expression relies on the co-activator CBP[78]. Moreover, β-catenin preferentially binds to CBP rather than p300, an interaction that contributes to cancer progression and metastasis by increasing the expression of EPHB4 and decreasing the expression of EPHB2[78]. In addition, EPHB3 overexpression induces up-regulation of E-cadherin and an increase of cytoplasmic β-catenin, as well as a decrease in nuclear β-catenin. Moreover, TCF/β-catenin activity was found to be reduced in EPHB3-overexpressing colon cancer cells[37]. These results indicate that EPHB3 receptor acts as cancer suppressor through the inhibition of the Wnt signaling pathway (**Figure 2**). As a result, type-B EPH receptors exhibit a dual role as a tumor promoter and suppressor via their common downstream Wnt signaling pathway.

Notably, we showed that overexpression of EPHA2 resulted in up-regulation of the EMT molecular markers N-cadherin and Snail and down-regulation of E-cadherin through canonical Wnt signaling in gastric cancer cells[79]. To our surprise, inhibition or activation of the Wnt/β-catenin pathway down-regulated or up-regulated EPHA2 expression, respectively. This observation indicated that EPHA2 may be a downstream target of the Wnt/β-catenin pathway.

EPH receptors and the Ras/MAPK signaling pathway

The Ras/MAPK pathway allows cells to interpret and respond to external signals appropriately, particularly during EMT. Moreover, MAPK family members such as JNK, p38MAPK, and ERK are all involved in TGF-β–induced EMT[23]. EPHA2 is a direct transcriptional target of the Ras-Raf-MAPK pathway, and a negative feedback loop exists between EPHA2 and the Ras/MAPK signaling pathway in normal cells[80]. However, in the absence of ligands, the crosstalk between EPH receptors and other RTKs, such as ErbB2 and EGFR, increases the activity of the Ras/MAPK pathway and enhances breast cancer malignancy[68]. On the other hand, a study has reported that in LNCaP prostate cancer cells and MDA-MB-231 breast cancer cells, the ligand-mediated activation of EPHA2 promotes the nuclear translocation and phosphorylation of ERK kinases, followed by the increased nuclear translocation of the Elk-1 transcription factor and the consequent destabilization of cell-ECM attachment[81] (**Figure 2**). These data suggest that EPHA2 is involved in EMT through the MAPK signaling pathway and promotes tumor progression in both ligand-independent and ligand-dependent manners.

EPH receptors and the Akt-mTOR signaling pathway

The mTOR signaling pathway serves as a crucial regulator for cell growth, metabolism, proliferation, and survival. mTOR functions as part of the mTOR complex 1 (mTORC1) and complex 2 (mTORC2) downstream and upstream of Akt, respectively[82]. Generally, mTORC2 phosphorylates and activates Akt, which promotes the activation of mTORC1 through the Ras family protein Rheb. The Akt-mTORC1 pathway is often activated in cancer cells due to loss of the tumor suppressor PTEN[83]. On the other hand, activated RTKs recruit and activate PI3K, leading to Akt binding to phosphatidylinositol-3,4,5-

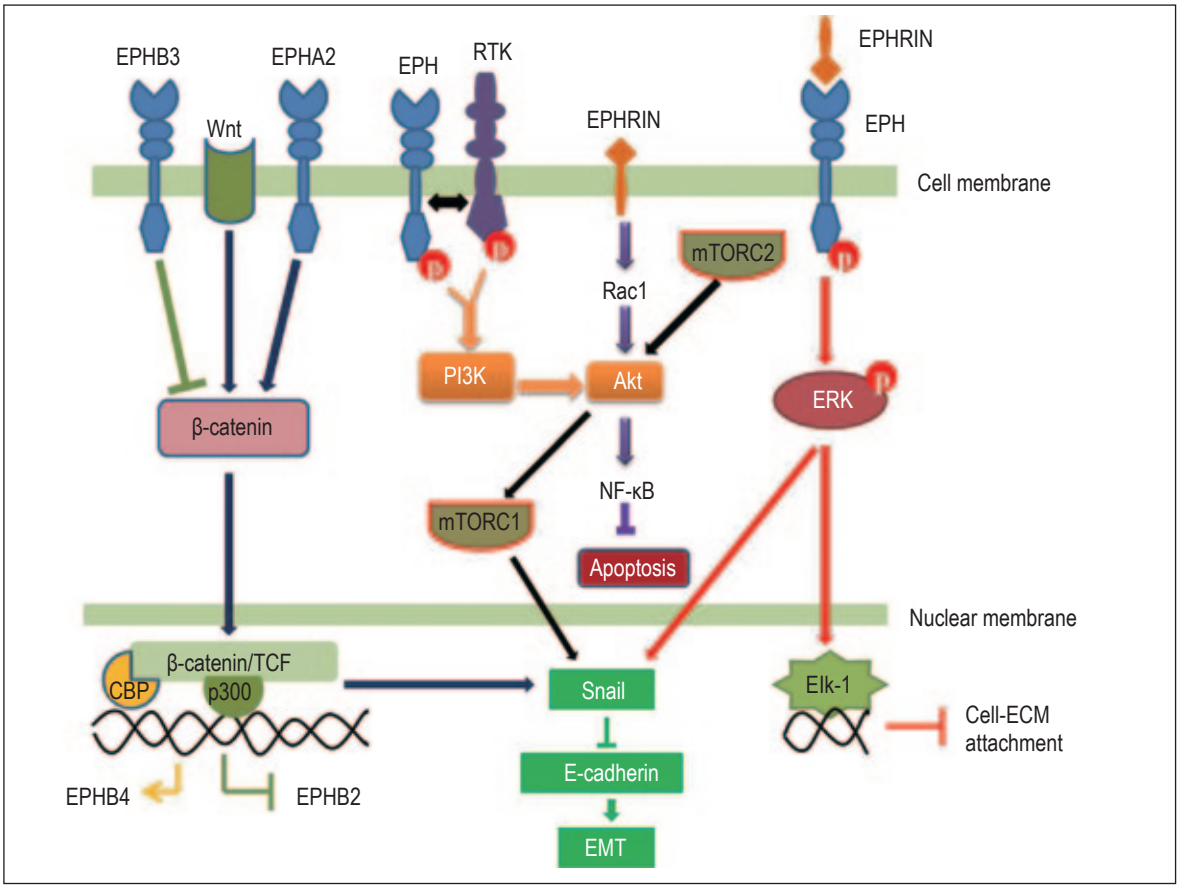

Figure 2. **EPH receptors crosstalk with EMT-related signaling pathways. EPH receptors initiate forward signaling to promote cancer progression via the MAPK signaling pathway in both ligand-dependent and ligand-independent manners. EPHRIN-mediated reverse signaling also contributes to tumorigenicity by inhibiting apoptosis. The Wnt/β-catenin signaling pathway promotes tumor progression by up-regulating EPHB4 and down-regulating EPHB2, whereas EPHB3 inhibits the Wnt signaling pathway. EPHA2 promotes EMT through the Wnt/β-catenin signaling pathway. CBP, cyclic AMP-responsive related binding protein; ECM, extracellular matrix; ERK, extracellular regulated protein kinase; NF-κβ, nuclear factor kappa-light-chain-enhancer of activated B cell; MAPK, mitogen-activated protein kinase; mTORC1, mammalian target of rapamycin (mTOR) complex 1; PI3K, phosphatidylinositol 3-kinase; Red P, phosphorylation; RTKs, receptor tyrosine kinases; TCF, T-cell factor.**

trisphosphate (PIP3), the levels of which are increased by activated PI3K[83]. The activation of mTORC1 is inhibited by tuberous sclerosis complexes 1 (TSC1) and 2 (TSC2), which can be regulated by Akt through PI3K-independent signaling[83]. It is conceivable that the PI3K signaling pathway crosstalks with the mTOR pathway at its crossroad with Akt.

A recent study has shown that stable knockdown of Raptor (a component of mTORC1) and Rictor (a component of mTORC2) increased E-cadherin levels and decreased the levels of the vimentin and E-cadherin transcription factors Snail and Twist, respectively, in SW480 colon cancer cells[24]. Aberrant activation of mTORC1 and mTORC2 has been reported to promote EMT in colorectal cancer cells. Several studies have shown that EPHA receptor crosstalks with the Akt/mTOR pathway and suppresses cancer cells in a ligand-dependent pattern[84] (**Figure 2**). Nie *et al*.[85] found that EPHA receptor activation by EPHRIN-A ligands in neurons inhibited ERK1/2 kinase activity, decreasing the ERK1/2-mediated inhibition of TSC2 and thus inactivating the mTOR pathway by enhancing TSC2 activity. Similarly, EPHRIN-A1–dependent activation of EPHA2 decreases the growth of PC3 prostate cancer cells and profoundly inhibits the Akt-mTORC1 pathway[86]. These data suggest that the EPHA receptor might affect EMT through the mTOR signaling pathway, although the underlying mechanism requires further detailed investigation.

In addition, EPH receptors are involved in the Rho GTPase and PI3K signaling pathways. For example, stimulating EPHA with EPHRIN-A1 induces endothelial cell migration and vascular assembly through PI3K-mediated activation of the Rac1 GTPase[55]. This observation suggests that EPHA receptor enhances tumor invasion and metastasis by promoting angiogenesis. Moreover, EPHA2-mediated activation of RhoG and PI3K suppresses anoikis, a suggested crucial barrier for cancer cell metastasis[87]. Other effector proteins also regulate the relationship between EPH receptors and downstream signals. EPHB3-binding protein, the receptor for activated C-kinase 1 (RACK1), forms a complex with

protein phosphatase 2A (PP2A) and Akt in response to EPHB3 activation, inhibiting the migration of NSCLC cells by reducing Akt phosphorylation[88]. Therefore, activating EPHB3 represents a potential therapeutic strategy for inhibiting tumor metastasis. Thus, it appears that EPH receptors might be linked to multiple signaling pathways in different tissues and cell types.

EPH receptors and the Notch signaling pathway

Notch signaling has been shown to take part in many physiological processes. In several human malignancies, the activation of Notch is abnormally regulated[89]. As summarized in a recent review[22], accumulating evidence suggests that Notch signaling regulates EMT during tumor development. Notch signaling induces down-regulation of epithelial markers such as E-cadherin, as well as up-regulation of mesenchyal markers such as fibronectin[22]. Moreover, Notch signaling regulates not only the EMT transcription factors Snail and Slug but also the crosstalk with EMT-related growth factors, such as TGF-β, FGF, and PDGF[22]. Recently, Feng *et al*.[54] found that a crucial EPH ligand, EPHRIN-A2, is up-regulated in HCC tissues and cell lines and regulates tumor growth and cell survival through the Rac/Akt/NF-κB pathway (**Figure 2**). Because NF-κB is a classical target of Notch signaling, it is possible that EPHRIN-A2–mediated reverse signaling is involved in the Notch signaling pathway in HCC. Moreover, EPHRIN-A2 is a cognate ligand to several EPH receptors including EPHA3, EPHA4, EPHA5, and EPHA7[90]. Thus, further investigation is needed to determine whether these EPHA receptors are expressed and regulate tumor cell biological function via Notch signaling in HCC. In addition, combined targeting of Notch receptor signaling induced by its ligand Delta-like-4 (Dll4) and EPHRIN-B2/EPHB4 signaling significantly reduced vascular density and tumor size compared with inhibition of either pathway alone[91]. This result demonstrates a new therapeutic anticancer strategy that inhibits both Notch- and Eph-mediated signaling pathways.

Combined Therapeutic Targeting of EPH Receptors and EMT

An ideal molecular marker for cancer-targeted therapy should be expressed in tumor cells but not in normal somatic cells. Using this principle guideline, cancer researchers have made significant progress in the search for molecular tumor markers. Recently, EPH receptor overexpression has been reported in many human cancer cell lines and tissues and has been associated with poor prognosis[60,92]. This strongly suggests that inhibiting EPH receptor activation in specific cancers will elicit a significant impact on tumor therapeutic outcome. Moreover, EPHA2-expressing cells have been shown to acquire the EMT phenotype by up-regulating mesenchymal markers and down-regulating epithelial markers[46]. Furthermore, in human colon cancer cells, overexpression of the tumor suppressor gene *EPHB3* induces the up-regulation of E-cadherin and the nuclear-to-cytoplasmic translocation of β-catenin, which demonstrates that the loss of EPHB3 expression promotes EMT in colon cancer cells[37]. These results indicate that inhibiting or activating MET by modulating specific EPH receptors in distinct cancer types might represent a novel strategy for individualized cancer-targeted therapeutic approaches.

Since the discovery of their crucial role in tumor-associated angiogenesis, EPH receptors and EPHRIN ligands have become an exciting new area of anti-angiogenesis drug development[28]. Simultaneously, many modalities have served as suitable candidates for cancer therapeutics, including monoclonal antibodies against the extracellular domain of EPHA2, adenoviral vectors as a gene delivery system[45], peptides and small molecules for competitive binding of the intracellular kinase domain of EPH receptors[93], immunoconjugates containing an anti-EPHA2 monoclonal antibody[94], doxorubicin-loaded hollow gold nanospheres that target EPHB4[95], and tyrosine kinase-targeted small interfering RNAs (siRNAs)[96]. Although targeting EPH receptors is an encouraging prospect, some limitations exist. Because of the variable EPH receptor expression pattern in different cancer types and because of the role of EPH receptors as both tumor promoters and suppressors, highly selective and specific agents are needed to target these receptors. More importantly, EPH receptors crosstalk with other RTKs and oncogenic signaling pathways, and blocking one pathway might inadvertently activate another through the acquired autonomous functions of tumor cells. This issue limits the targeting of a single EPH receptor for antitumor therapy. Therefore, combinatorial targeted therapy appears to be a more feasible approach.

Tumor cells undergoing EMT acquire stem cell characteristics and resistance to chemotherapy[8,15,16]. Therefore, inhibiting the EMT process also represents a promising approach for cancer therapy. Several EMT markers, such as N-cadherin[5] and vimentin[6,7], have been identified as therapeutic targets in cancer. Moreover, E-cadherin–mediated suppression of transcription factors is often deregulated in cancer, and thus these transcription factors might represent potential candidates for targeted therapeutics[9]. Furthermore, targeting the crucial orchestrators of several EMT signaling pathways, such as NF-κB, Akt-mTOR, Ras/MAPK, and Wnt/β-catenin, also represents a feasible strategy for controlling EMT and the progression of human epithelial cancers[97].

Combined inhibition of EPH-related and EMT-related signaling pathways for cancer treatment should be carefully considered. For example, inhibiting the Dll4/Notch as well as the EPHRIN-B2/EPHB4 signaling pathways was shown to result in a more efficient reduction of tumor size, vessel perfusion, and mural cell recruitment[91]. Moreover, the dual targeting of EPHA2 and HER2 restores trastuzumab sensitivity in HER2/EPHA2-positive breast cancers[42]. Considering the vast network between the EPH and EMT signaling pathways, large gray areas still remain in the field and require further investigation. Furthermore, EPH receptors interact with the immediate microenvironment to promote tumor invasion and metastasis by enhancing angiogenesis and the degradation of cell-cell adhesion. In contrast, the microenvironment plays an important role in promoting EMT in primary tumor cells and accelerating distant metastasis formation through MET[98]. Therefore, further study of the tumor microenvironment will enhance our understanding of the relationship between EPH receptors and EMT. Notably, several microRNAs are

known to regulate EPH receptors and EMT. Thus, investigating microRNAs that target specific EPH receptors might provide insight into the differential expression patterns of EPH receptors across cancer types. Moreover, the identification of the specific microRNAs that regulate both EPH receptors and EMT will be important for the design of future specific anti-cancer therapeutics.

Conclusions

In summary, EPH receptors affect several levels of EMT. Ectopic expression of EPH receptors led to E-cadherin relocation and ultimately loss of cell-cell adhesion. EPH receptors are activated by other RTKs and induce EMT through oncogenic singaling pathway. In addition, EPH receptors crosstalk with EMT-related signal transduction pathways such as those induced by Wnt, Ras/MAPK, Akt-mTOR, and Notch. These characters make it possible to combine therapeutic targeting of EPH receptors and EMT. Further studies are warranted to investigate the mechanism of EPH receptors in EMT.

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