# Eph receptors and ephrins in cancer: bidirectional signalling and beyond

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Abstract | The Eph receptor tyrosine kinases and their ephrin ligands have intriguing expression patterns in cancer cells and tumour blood vessels, which suggest important roles for their bidirectional signals in many aspects of cancer development and progression. Eph gene mutations probably also contribute to cancer pathogenesis. Eph receptors and ephrins have been shown to affect the growth, migration and invasion of cancer cells in culture as well as tumour growth, invasiveness, angiogenesis and metastasis *in vivo*. However, Eph signalling activities in cancer seem to be complex, and are characterized by puzzling dichotomies. Nevertheless, the Eph receptors are promising new therapeutic targets in cancer.

Eph receptors and their Eph receptor-interacting (ephrin) ligands together form an important cell communication system with widespread roles in normal physiology and disease pathogenesis[1]. Links between Eph receptors and cancer date back to the first identified Eph family member[2]. EPHA1 was cloned from a carcinoma cell line in a screen for new oncogenic tyrosine kinases. This novel receptor was found to be upregulated in tumour tissues compared with normal tissues and its overexpression caused the oncogenic transformation of NIH3T3 fibroblasts[2,3]. The first ephrin ligand, ephrin-A1, was also identified from cancer cells a few years later[4]. The evidence implicating Eph receptors and ephrins in cancer is now extensive and continues to grow.

The activities of the Eph system in cancer are complex, and intriguing in their paradoxical effects. For example, multiple Eph receptors and/or ephrins are present in most cancer cells. However, both increased and decreased Eph expression has been linked to cancer progression. Consistent with this dichotomy, there is good evidence that Eph receptors and ephrins can both promote and inhibit tumorigenicity. The factors responsible for these divergent activities are only now beginning to be uncovered.

Following a brief overview of the Eph and ephrin families and their bidirectional signalling mechanisms, the factors that regulate their expression and the remarkable multiplicity of their roles in cancer are discussed, and the strategies under evaluation to target the Eph system for cancer therapy outlined. Other reviews provide more in-depth information on Eph signalling mechanisms in development and adult physiology[1,5–8].

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## Eph and ephrin families

In the human genome there are nine EphA receptors, which promiscuously bind five glycosylphosphatidylinositol (GPI)-linked ephrin-A ligands, and five EphB receptors, which promiscuously bind three transmembrane ephrin-B ligands[5]. Exceptions are the EPHA4 and EPHB2 receptors, which can also bind ephrin-Bs and ephrin-A5, respectively, and EPHB4, which preferentially binds ephrin-B2 only. Eph receptors typically interact with the cell surface-associated ephrins at sites of cell–cell contact (FIG. 1). In addition, soluble ephrin-As released from the cell surface retain the ability to activate EPHA2 (REFS 4,9,10).

Eph–ephrin complexes emanate bidirectional signals: forward signals that depend on Eph kinase activity propagate in the receptor-expressing cell, and reverse signals that depend on Src family kinases propagate in the ephrin-expressing cell. Ephrin-dependent but kinase-independent Eph signals can also occur[11–13]. Eph signalling controls cell morphology, adhesion, migration and invasion by modifying the organization of the actin cytoskeleton and influencing the activities of integrins and intercellular adhesion molecules[1,5]. Recent work has also uncovered Eph effects on cell proliferation and survival as well as specialized cellular functions such as synaptic plasticity, insulin secretion, bone remodelling and immune function[1].

Bidirectional signals can lead to the removal of the adhesive Eph–ephrin complexes from cell contact sites through an unusual endocytic mechanism that involves their internalization, together with patches of the surrounding plasma membranes, into the receptor- or ephrin-expressing cell[5]. This enables the separation of

**At a glance**

- The Eph receptors are the largest family of receptor tyrosine kinases. They bind glycosylphosphatidylinositol (GPI)-linked and transmembrane ephrin ligands, generating bidirectional signals at sites of cell–cell contact.
- Eph receptors and/or ephrins are widely expressed in cancer cells and tumour stroma, but they can be downregulated at advanced cancer stages. Often Eph receptor and ephrin levels are discordantly regulated. In addition to changes in expression levels, Eph receptor mutations are also likely to have a role in cancer pathogenesis.
- In many cellular contexts, Eph bidirectional signalling promotes an epithelial phenotype and suppresses cancer cell–substrate adhesion, migration, invasion and growth. Consistent with this, Eph receptor signalling seems to be low in many cancer cells owing to an imbalance of Eph and ephrin expression or the inability of receptor and ligand to interact effectively.
- Eph receptors and ephrins can also promote cancer progression through poorly understood mechanisms that do not involve reciprocal association but rather depend on crosstalk with oncogenic signalling pathways. In addition, Eph bidirectional signals promote tumour angiogenesis.
- Eph receptors and ephrins are promising new therapeutic targets in cancer, and many Eph-based approaches show promise for prognosis and therapy.

the engaged cell surfaces to produce the characteristic Eph-repulsive responses. Another mechanism allowing cell separation involves protease-mediated cleavage of the Eph or ephrin extracellular domains[14–18]. Internalization and cleavage result in degradation, which can profoundly downregulate Eph levels. However, in certain cellular contexts Eph–ephrin complexes persist at intercellular junctions and emanate prolonged bidirectional signals that favour adhesiveness. For example, the cell adhesion molecule E-cadherin promotes EPHA2–ephrin-A1 localization at epithelial cell junctions and the metalloprotease ADAM19 stabilizes EPHA4–ephrin-A5 at neuromuscular junctions independently of its proteolytic activity[19–21]. A combination of Eph-dependent repulsive and adhesive forces can drive the segregation of cell populations expressing different combinations of Eph receptors and ephrins, which may include transformed and normal cells or divergent subpopulations of tumour cells[5,22,23].

There is also increasing evidence that other signalling modalities beyond 'conventional' bidirectional signalling contribute to the multiple activities of the Eph system in cancer. For example, an initial extracellular Eph or ephrin cleavage by metalloproteases followed by γ-secretase-mediated cleavage in the transmembrane segment releases intracellular domains that can generate distinctive signals[14,16,24,25]. Eph receptors and ephrins can also signal independently of each other, through crosstalk with other signalling systems, which produces yet more distinctive outcomes. In addition, they participate in feedback loops that may switch between different outputs depending on the state of other cellular signalling networks (FIG. 2).

## Eph and ephrin dysregulation in cancer

The Eph and ephrin families have grown in complexity during evolution, keeping pace with the increasingly sophisticated tissue organization of higher organisms. Finely coordinated spatial and temporal regulation of Eph receptor and ephrin expression controls many processes that are crucial for development and tissue homeostasis, including the formation of tissue boundaries, assembly of intricate neuronal circuits, remodelling of blood vessels and organ size[1,5]. Many Eph receptors and/or ephrins are also expressed in both cancer cells and the tumour microenvironment, where they influence tumour properties by enabling aberrant cell–cell communication in and between tumour compartments[26–32]. Mutations dysregulating Eph function are also likely to have a role in cancer progression.

***Expression in cancer cells.*** Many studies have correlated Eph and ephrin expression levels with cancer progression, metastatic spread and patient survival (TABLE 1). EPHA2, for example, is upregulated in many cancers and its expression has been linked to increased malignancy and a poor clinical prognosis[27,28,31,33]. Furthermore, EPHA2 seems to be preferentially expressed in malignant breast and prostate cancers with a basal phenotype[34,35]. EPHB4 is also widely expressed in cancer cells and its increased abundance has been correlated with cancer progression[29,36,37]. However, decreased Eph or ephrin levels in malignant cancer cell lines and tumour specimens have also been reported. For example, EPHA1 is downregulated in advanced human skin and colorectal cancers[38,39], EphB receptors in advanced colorectal cancer[23,40–42] and ephrin-A5 in glioblastomas[43]. Furthermore, EPHB6 expression is lower in metastatic than non-metastatic lung cancers[44]. Reconciling these discrepancies, recent studies show that an initial Eph receptor upregulation (that is due to activated oncogenic signalling pathways and other factors) can be followed by epigenetic silencing in more advanced stages owing to promoter hypermethylation, as shown for several EphB receptors and EPHA1 in colorectal cancer[23,39–41]. Transcriptional repression, such as repression of *EPHB2* by REL (a member of the nuclear factor-κB family) in colorectal cancer, may also play a part in Eph silencing[45]. Intriguingly, differential transcriptional regulation has been reported for *EPHB2* and *EPHB4* during colorectal cancer progression[37]. This was attributed to a switch in the association of β-catenin from the p300 co-activator (which induces *EPHB2* transcription) to the CREB binding protein (CBP) co-activator (which induces *EPHB4* transcription). An inverse expression pattern has also been observed for EPHA2 compared with ephrin-A expression in breast cancer cell lines, owing at least in part to feedback loops (FIG. 2a), and for several EphB receptors compared with ephrin-Bs in early colorectal tumours and breast cancer cell lines[23,46,47].

Chromosomal alterations and changes in mRNA stability also regulate Eph and ephrin expression in cancer cells (TABLE 1). Several Eph receptor and ephrin genes are located in chromosomal regions that are frequently lost in cancer cells. For example, *EPHA2*, *EPHA8* and *EPHB2* are clustered at chromosomal region 1p36, which undergoes loss of heterozygosity in many cancers[48,49]. Some Eph genes, however, are in amplified regions[50]. Nonsense-mediated mRNA decay and interaction with mRNA-binding proteins can also regulate Eph mRNA

Basal phenotype
Highly aggressive breast and prostate cancers with gene expression profiles similar to basal cells. Basal-type breast cancers are typically negative for oestrogen, progesterone and ERBB2 receptors. Basal-type prostate cancers have high expression of cytokeratin 5 and low expression of androgen receptor and prostate-specific antigen.

Nonsense-mediated mRNA decay
The process by which mRNA molecules carrying premature stop codons are degraded by a regulated pathway, thereby limiting the synthesis of abnormal proteins.

Cyclic stretch
Periodic stretch (or strain) to which vascular endothelial cells are subjected as a result of the rhythmic changes in vessel diameter caused by pulsatile blood flow.

Shear stress
The physical force exerted on endothelial cells as a result of blood flow.

stability in cancer cells[49,51]. These complex mechanisms of regulation parallel the multiplicity of Eph activities in cancer cells.

***Expression in the tumour microenvironment.*** Several Eph receptors and ephrins are upregulated in vascular cells by tumour-derived factors and hypoxia. For example, tumour necrosis factor-α (TNFα), vascular endothelial growth factor A (VEGFA) and hypoxia-inducible factor 2α (HIF2α) have been shown to upregulate ephrin-A1 in cultured endothelial cells[52–54]. Endothelial ephrin-B2 is upregulated by VEGF through the Notch pathway and by cyclic stretch, hypoxic stress and contact with smooth muscle cells, whereas shear stress seems to decrease ephrin-B2 expression in endothelial cells but increase it in endothelial precursors by inducing their differentiation[55–59].

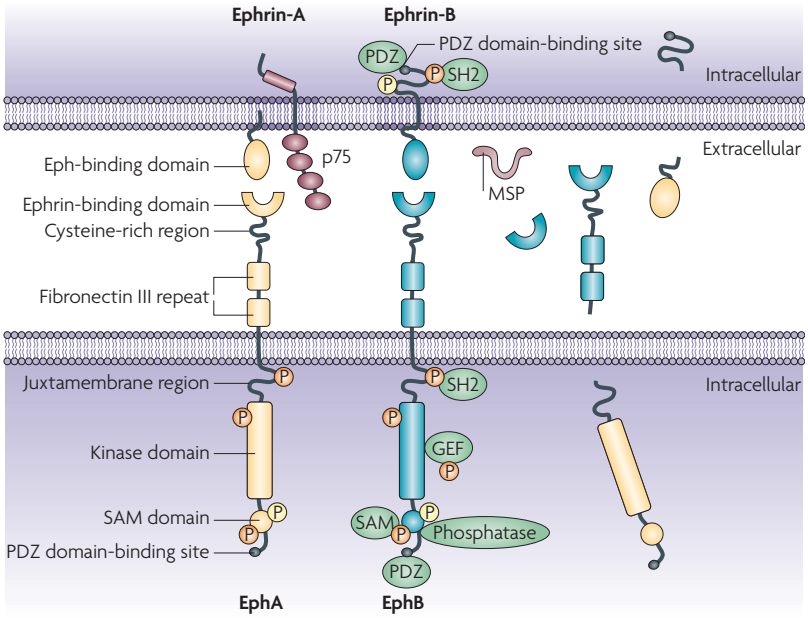



Figure 1 | **Eph receptor and ephrin domain structure and signalling interactions.** Domain structures of Eph receptors and ephrins. Alternative splicing or proteolysis can generate extracellular and intracellular domain fragments of Eph receptors and ephrins of both the A and B classes. The major sperm protein (MSP) domain from vesicle-associated membrane protein (VAMP)-associated protein (VAP) proteins is another Eph ligand that can compete with ephrins for binding[84]. Eph receptor forward signalling involves ephrin-induced clustering, autophosphorylation and association with signalling effectors containing protein interaction domains such as Src homology 2 (SH2), PSD95, DLG, ZO1 (PDZ) and sterile alpha motif (SAM)[1,5]. Some signalling proteins, such as certain guanine nucleotide exchange factors (GEFs) for Rho family GTPases, can constitutively associate with Eph receptors[175]. The activities of some effectors are modified by activated Eph receptors, for example through tyrosine phosphorylation (orange P). Phosphotyrosine phosphatases dephosphorylate Eph receptors and ephrins to dampen or terminate their activity. Eph receptors are also phosphorylated on serine/threonine residues (yellow P)[176], which can have dramatic functional consequences[72]. The transmembrane ephrin-Bs mediate reverse signals, which involve Src-dependent tyrosine phosphorylation of their cytoplasmic segment and association with SH2 and PDZ domain-containing proteins[5,6]. EphB binding can also affect ephrin-B function by inducing serine phosphorylation, as shown in neurons[7]. The glycosylphosphatidylinositol (GPI)-linked ephrin-As also mediate reverse signals, through poorly understood signalling interactions that may occur in lipid rafts (dark purple). In neurons, ephrin-As can use the p75 nerve growth factor receptor as a signalling partner to activate the Src family kinase FYN[177]. Most domain names are shown on EphA and ephrin-A, and signalling interactions are shown on EphB and ephrin-B, but each applies to the other.

Moreover, ephrin-B2 is expressed in pericytes and vascular smooth muscle cells[57,60]. Expression of EPHA2–ephrin-A1 and EPHB4–ephrin-B2 in tumour blood vessels has been most extensively characterized, but other Eph receptors and ephrins are also present in the tumour vasculature[54–57,61,62]. By contrast, little is known about Eph and ephrin expression in other tumour compartments, such as activated fibroblasts and infiltrating immune and inflammatory cells. Nevertheless, Eph-dependent communication between these cells and tumour cells probably has an important role in tumour homeostasis.

***Eph mutations with cancer relevance.*** Screens of tumour specimens and cell lines have recently identified mutations in the genes encoding all the Eph receptors, whereas cancer-related ephrin mutations have not yet been reported, perhaps in part because many of the screens have focused on the kinome[63–67] (see Further information; Catalogue of somatic mutations in cancer). Mutations of at least some Eph receptors are predicted to have a role in cancer pathogenesis. For example, *EPHB2* mutations have been identified in human prostate, gastric and colorectal tumours, and melanoma[40,49,67–69]. Some of these mutations can impair kinase function, and some are accompanied by loss of heterozygosity, suggesting a tumour suppressor role for EPHB2 forward signalling. Furthermore, several Eph receptors — particularly *EPHA3* and *EPHA5* — are frequently mutated in lung cancer[63,70]. The mutations are typically scattered throughout the Eph domains, including the ephrin-binding domain and other extracellular regions[67,70]. Elucidating the effects of the mutations will provide important insight into the functional roles of the Eph system in cancer.

## Tumour suppression

In many cancer cell lines, Eph receptors seem to be highly expressed but poorly activated by ephrins, as judged by their low level of tyrosine phosphorylation[1,29,37,47,71,72]. This was one of the first clues that ephrin-dependent Eph forward signalling might be detrimental to tumour progression. Furthermore, recent expression profiling of $Apc^{Min/+}$ intestinal tumours from wild-type and $Ephb4^{+/-}$ mice has revealed an extensive transcriptional reprogramming that suggests anti-proliferative and anti-invasive activities of EPHB4 in colorectal cancer[73].

***Eph forward signalling inhibits cell transformation.*** Forcing Eph receptor activation with soluble Fc fusion proteins of ephrin ligands can inhibit proliferation, survival, and migration and invasion of many types of cancer cells in culture as well as tumour growth in several mouse models[5,29,42,47,74]. Conversely, a dominant-negative form of EPHB4 has been shown to promote colorectal cancer proliferation and invasion[73]. These studies demonstrate that Eph forward signalling pathways can lead to tumour suppression (FIG. 3). Indeed, Eph receptors that are activated by ephrins acquire the remarkable ability to inhibit oncogenic signalling pathways, such as the HRAS–Erk, PI3K–Akt and Abl–Crk pathways. Interestingly, this may reflect a physiological function of

Pericytes
Mesenchymal cell precursors to vascular smooth muscle that associate with endothelial cells during angiogenesis and provide support to small capillaries.

*Apc*$^{Min/+}$
Mouse that carries the multiple intestinal neoplasia (Min) point mutation in one *Apc* allele and spontaneously develops intestinal adenomas. Commonly used model for human familial adenomatous polyposis and human sporadic colorectal cancer.

Mesenchymal-to-epithelial transition
The conversion of non-polarized and motile mesenchymal cells into polarized epithelial cells. Typically associated with increased E-cadherin levels and low cancer cell invasion and metastasis. It is the reverse of the better known epithelial-to-mesenchymal transition.

the Eph system in epithelial homeostasis by promoting contact-dependent growth inhibition and decreasing motility and invasiveness. These changes are reminiscent of mesenchymal-to-epithelial transition (BOX 1).

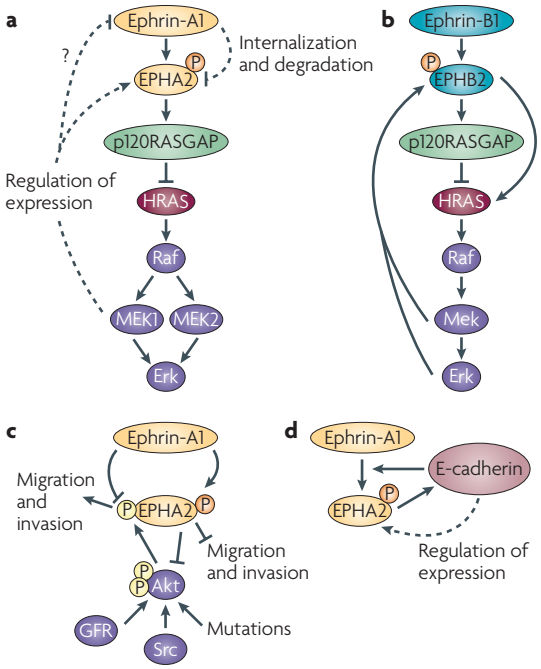



Figure 2 | **Eph feedback loops. a** | EPHA2–HRAS–Erk negative feedback loop. Activation of the HRAS–Erk pathway increases EPHA2 expression through MEK1 and decreases ephrin-A1 expression, although it is not known whether this also occurs through MEK1 (REFS 46,178,179). In turn, ephrin-dependent EPHA2 activation inhibits HRAS–Erk signalling and also downregulates EPHA2 levels by causing receptor internalization and degradation. **b** | Positive and negative EPHB2–MAPK feedback loops. In a positive feedback loop, ephrin-B-dependent EPHB2 activation stimulates the HRAS–Erk pathway, and the increase in Mek and/or Erk activity in turn enables enhanced responsiveness of EPHB2 to ephrin-B stimulation through unknown mechanisms[79]. However, in a different cellular context, EPHB2 can also inhibit the HRAS–Erk pathway[5,180], which may in turn reduce EPHB2 activation by ephrin. Ephrin-B1 stimulation can also downregulate EPHB2 levels by causing internalization and degradation (not shown). **c** | EPHA2–Akt negative feedback loop. Akt (activated by growth factor receptors (GFRs), Src family kinases, or mutations in upstream proteins or Akt itself) phosphorylates S897 in the carboxy-terminal tail of EPHA2 leading to increased EPHA2-dependent cell migration and invasion[72]. In turn, ephrin-A1-induced EPHA2 signalling inactivates Akt by causing its dephosphorylation at T308 and S473, thus decreasing EPHA2 phosphorylation at S897 and, consequently, cell migration and invasion. Other pathways downstream of EPHA2 can also inhibit migration and invasion. **d** | EPHA2–E-cadherin positive feedback loop. E-cadherin expression increases EPHA2 expression, surface localization, interaction with ephrin-A1 and consequently forward signalling[19,20] (BOX 1). In turn, EPHA2 signalling enhances E-cadherin-mediated adhesion. Dotted lines indicate the regulation of protein levels rather than activity. Orange P, tyrosine phosphorylation; yellow P, serine/threonine phosphorylation.

***Silencing of Eph forward signals in cancer cells.*** Cancer cells seem to use various mechanisms to minimize the tumour suppressor effects of Eph forward signalling. For example, the high EPHA2 or EphB expression and low ephrin expression observed in some cancers result in low bidirectional signalling[23,46,47]. Furthermore, co-expressed Eph receptors and ephrins often do not interact effectively in cancer cells[20]. This may be because they engage in lateral interactions that silence their signalling function, as has been shown in neurons and transfected cells[5]. Alternatively, loss of E-cadherin or VE-cadherin impairs endogenous EPHA2–ephrin-A1 interaction in malignant breast cancer and melanoma cells, respectively[20,75]. The two cadherins seem to promote EPHA2–ephrin-A1 interaction by stabilizing intercellular contacts and promoting the localization of EPHA2 at cell–cell junctions. Phosphotyrosine phosphatases also negatively regulate Eph receptor forward signalling in some cancer cells[76]. For example, the low-molecular-weight protein tyrosine phosphatase (LMW-PTP; also known as ACP1) has been implicated in cell transformation through its ability to dephosphorylate EPHA2, thus counteracting ephrin-dependent activation[77]. The receptor-type PTPs PTPRO and PTPRF, and PTEN in *Caenorhabditis elegans*, also dephosphorylate Eph receptors[78–80]. However, it is not known whether this plays a part in cancer. Eph mutations may also contribute to disrupting forward signalling by impairing ephrin binding or kinase activity. For example, the EPHA3 E53K mutation in the MeWo melanoma cell line abrogates ephrin binding[66,81], and the EPHB2 G787R mutation found in colorectal cancer impairs kinase activity[69]. It will also be interesting to investigate whether soluble Eph ectodomains that are generated by alternative splicing[82,83] or proteolysis[14–18,24] and proteins containing a major sperm protein (MSP) domain[84] (FIG. 1) can decrease Eph signalling in cancer cells by functioning as naturally occurring antagonists.

***Tumour confinement by surrounding ephrins.*** The tumour suppressor effects of Eph forward signalling can be active at the tumour periphery if the surrounding tissues express ephrins. In mouse tumour models, ephrins present in normal tissues have been proposed to inhibit expansion and invasiveness of incipient colorectal and skin tumours expressing Eph receptors[23,85,86]. In addition, recent experiments in the developing zebrafish hindbrain raise the possibility that increased Eph or ephrin levels may drive the segregation of tumour cells from surrounding normal tissues, thereby decreasing invasiveness, not only through repulsive mechanisms but also by promoting adhesiveness between tumour cells[22]. Eph receptors may further decrease tumour invasiveness by promoting the formation of tight junctions in neighbouring epithelial cells through the stimulation of ephrin-B reverse signalling[87] (discussed below). Indeed, recent systems-level studies have implicated complex, asymmetric signalling networks in the sorting of ephrin-B1-expressing HEK293 cells from EPHB2-expressing cells[88]. It is tempting to speculate that Eph receptors may contribute to tumour dormancy through these types of bidirectional signalling mechanisms that

Table 1 | **Examples of the regulation of Eph receptor and ephrin expression in cancer cells**

| Mechanism | Eph or ephrin | Change | Cell type and cancer type | Refs |
|---|---|---|---|---|
| ***Frequent chromosomal abnormalities that may lead to altered Eph or ephrin expression**** | | | | |
| 1p36 loss | EPHA2, EPHA8 and EPHB2 | ↓ | Various cancers | 48,49,69, 187–190[‡] |
| 1q21-q22 gain | Ephrin-A1, ephrin-A3 and ephrin-A4 | ↑ | Various cancers | See footnote[‡] |
| 2q36.1 loss | EPHA4 | ↓ | Cervical cancer | 191 |
| 3p11.2 loss | EPHA3 | ↓ | Lung and other cancers | 187[‡] |
| 3q21-qter gain | EPHB3 | ↑ | Early-stage squamous cell lung carcinoma | 50 |
| 5q21 loss | Ephrin-A5 | ↓ | Myeloid cancers and prostate cancer | 187,192[§] |
| 6q16.1 loss | EPHA7 | ↓ | Various cancers | 187,192[§] |
| 7q22 loss | EPHB4 | ↓ | Myeloid cancers and colon cancer | 187[§] |
| 7q22 gain | EPHB4 | ↑ | Various tumours and cancer cell lines | 187,193,194 |
| 7q33-35 loss | EPHB6 and EPHA1 | ↓ | Myeloid cancers | 187 |
| 7q33-35 gain | EPHB6 and EPHA1 | ↑ | Neuroblastoma and glioblastoma | 187 |
| 13q33 loss | Ephrin-B2 | ↓ | Multiple myeloma, chronic lymphocytic leukaemia, and head and neck cancer | 187[‡§] |
| 17p13.1-p11.2 loss | Ephrin-B3 | ↓ | Various cancers | See footnote[‡§] |
| 19p13.3 loss | Ephrin-A2 | ↓ | Various cancers | 195[§] |
| ***Epigenetics*** | | | | |
| Promoter hypermethylation | EPHA1 | ↓ | Advanced colorectal cancer | 39 |
| | EPHA3 | ↓ | Leukaemias and haematopoietic tumour cells | 196 |
| | EPHA7 | ↓ | Prostate, gastric and colorectal cancer | 197–199 |
| | Soluble EPHA7 ectodomain | ↓ | B cell lymphomas | 200 |
| | EPHB2 | ↓ | Colorectal cancer | 40,189,201 |
| | EPHB4 | ↓ | Colorectal cancer | 41 |
| | EPHB6 | ↓ | MDA-MB-231 breast cancer cells | 202 |
| ***mRNA stability*** | | | | |
| Nonsense-mediated mRNA decay | EPHB2 | ↓ | Prostate cancer | 49 |
| Binding sites for RNA binding protein ELAVL1 in 3′ untranstaled region | EPHA2, EPHA4 and ephrin-A2 | ↓ | HeLa cervical cancer and U373MG glioma cells | 51 |
| microRNA-210 | Ephrin-A3 | ↓ | Endothelial cells | 62,203 |
| ***Transcription*** | | | | |
| Ras–MAP kinase (MEK1) | EPHA2 | ↑ | Breast cancer cells and activated BRAF-transfected fibroblasts | 46,179 |
| p53 | EPHA2, EPHB4 and ephrin-A1 | ↑ | Various p53-transfected cell lines | 204–206 |
| TWIST1 | EPHA4 and ephrin-A4 | ↑ | Developing skull and possibly Sézary's lymphoma | 207,208 |
| REL | EPHB2 | ↓ | SW620 colon cancer cells | 45 |
| Wnt, β-catenin and TCF | EPHB2, EPHB3 and EPHB4 | ↑ | Early colorectal cancer | 23,209 |
| Wnt, β-catenin, p300 and TCF | EPHB2 | ↑ | Early colorectal cancer | 37 |
| Wnt, β-catenin, CBP and TCF | EPHB4 | ↑ | Advanced colorectal cancer | 37 |
| Oestrogen | EPHB4 and ephrin-B2 | ↑ | Mouse mammary epithelium | 210 |
| Ras and MAPK | Ephrin-A1 | ↓ | MCF-10A mammary epithelial cells | 46 |
| Wnt, β-catenin and TCF | Ephrin-B | ↓ | LS174T colon cancer cells | 209 |

CBP, CREB-binding protein. *Chromosomal locations from the NCBI Human Genome Resources website . [‡]Cancer GeneticsWeb. [§]Atlas of Genetics and Cytogenetics in Oncology and Haematology.

restrict tumour expansion. Accordingly, high EPHA5 levels have been detected in various dormant but not fast-growing tumour xenograft models[89].

***Ephrin reverse signalling in tumour cells.*** Ephrin reverse signalling in cancer cells may in some cases also contribute to tumour suppression (FIG. 3). In the *Xenopus laevis* system and HT29 colon cancer cells, ephrin-B1 tyrosine phosphorylation (which can be induced by interaction with EphB receptors or by activated growth factor receptors and Src) disrupts binding of the ephrin to the scaffolding protein PAR6, promoting the formation of tight junctions between cells[87,90]. Similar to its role in neurons, ephrin-B reverse signalling may also inhibit the migratory and invasive effects of the CXCR4 G protein-coupled chemokine receptor in cancer cells[5,6]. Ephrin-A5 can downregulate epidermal growth factor receptor (EGFR) levels in glioblastoma cells[43].

## Tumour promotion

Conversely, forward and/or reverse Eph–ephrin signals can enhance malignant transformation in some cases. There is also increasing evidence that the Eph receptors are capable of unconventional signalling activities that do not depend on activation by ephrin ligands and that support cancer progression. Moreover, it is well established that the Eph–ephrin system promotes tumour angiogenesis.

***Eph forward signalling.*** In certain cellular contexts, Eph receptors that are activated by ephrins may have lost the ability to suppress tumorigenicity, and may have

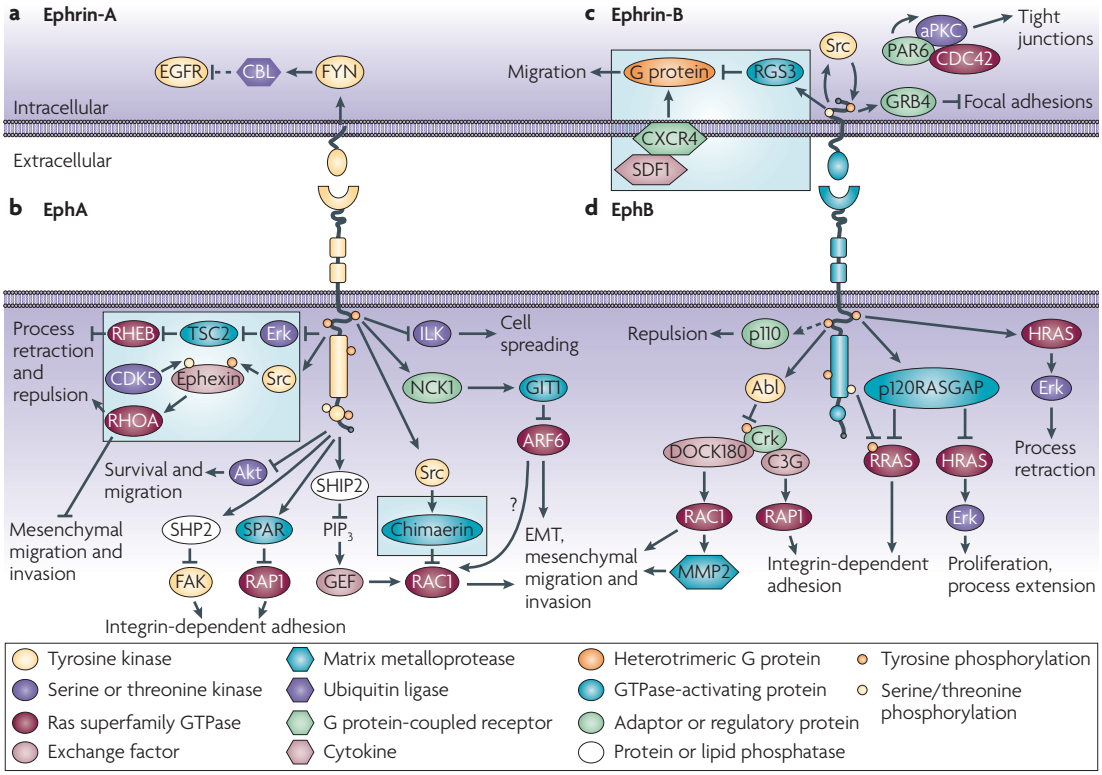



Figure 3 | **Tumour suppression through bidirectional signalling. a** | Ephrin-A5 reverse signalling downregulates epidermal growth factor receptor (EGFR) levels in glioma cells[43]. **b** | EphA receptors activate tuberous sclerosis complex 2 (TSC2) in neurons to inactivate RHEB[181]. EphA activation of RHOA involves Ephexin family exchange factors and other pathways (FIG. 4). EPHA2 inhibits Akt[72,179] and inactivates focal adhesion kinase (FAK) through the SHP2 phosphatase[5]. EPHA4 inhibits RAP1 through spine-associated RAPGAP (SPAR)[1,7]. Recruitment of the lipid phosphatase SHIP2 by EPHA2 inhibits RAC1 and EPHA2 internalization[182]. EPHA4 inhibits RAC1 through Chimaerins[1,183]. EPHA2-mediated inhibition of ADP-ribosylation factor 6 (ARF6) in epithelial cells inhibits epithelial-to-mesenchymal transition (EMT)[170]. EPHA1 inhibits integrin-linked kinase (ILK)[184]. **c** | Ephrin-B1 disrupts focal adhesions through GRB4 (REF. 5). Phosphorylation inhibits ephrin-B1 binding to PAR6, allowing PAR6 to bind GTP-bound CDC42 and activate atypical PKC (aPKC)[87]. Ephrin-Bs also inhibit signalling by the CXCR4 G protein-coupled chemokine receptor[5]. **d** | EphB signalling increases expression of the p110 subunit of PI3K[91]. EphB receptors (and EPHA2) activate Abl, which ultimately inhibits RAP1 and RAC1 (REFS 42,47,107). EPHB2 inactivates RRAS through phosphorylation[5]. EPHB2 (and EPHA2) activates p120RASGAP to inhibit HRAS and RRAS[5,180]. EPHB2 can also activate Erk[79]. Some pathways are assembled from different sources, so the complete pathways are hypothetical. Pathways identified in neurons, and predicted to have tumour suppressing activity, are in blue boxes. Most other pathways were identified in cultured cells and their importance in cancer also remains to be proved. Dotted lines indicate the regulation of expression levels. For more details see REFS 1,5–7,88,127. CDK5, cyclin-dependent kinase 5; GIT1, G protein-coupled receptor kinase-interacting ARFGAP 1; MMP2, matrix metalloproteinase 2; RAPGEF1, Rap guanine nucleotide exchange factor 1; RGS3, regulator of G protein signalling 3; SDF1, stromal cell-derived factor 1.

Ameboid-type migration
Motility frequently exhibited by cancer cells and leukocytes that is characterized by high speeds, lack of stable polarity and a relatively amorphous cell shape. Does not require stable integrin-dependent adhesion for traction but depends on RHOA to increase actomyosin contractility and allow invasion in the absence of extracellular proteolysis.

Mesenchymal-type migration
Movement of cells with elongated morphology and a front-back polarity, with traction generated through integrin-dependent adhesion. Requires extracellular proteolysis for cell invasion and is thought to depend on RAC1.

even acquired oncogenic ability. For example, activating mutations may render oncogenic signalling pathways resistant to inhibition by Eph forward signalling. Furthermore, EPHB2 can promote proliferation in mouse intestinal progenitor cells and *Apc*$^{Min/+}$ adenomas through an ABL1-mediated increase in cyclin D1 levels even though it inhibits invasiveness through other pathways[91] (FIG. 4). Activation of RHOA downstream of EPHA2 and EPHB4 promotes ameboid-type migration of cancer cells and destabilizes epithelial adherens junctions in various cancer cell lines (FIG. 4), even though RHOA inhibits mesenchymal-type migration[92–94] (FIG. 3). EPHA2 forward signalling in malignant melanoma and ovarian cancer cells can also promote vasculogenic mimicry[75,95].

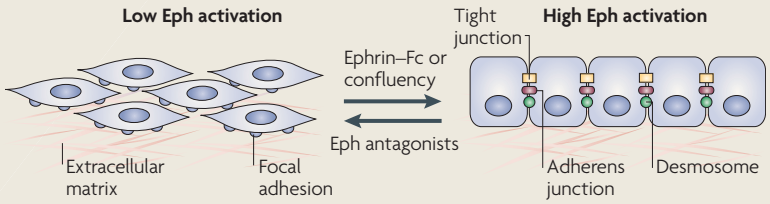
RRAS phosphorylation downstream of EPHB2 (FIG. 4) can enhance glioma cell invasiveness, possibly by decreasing cell–substrate adhesion[96], even though in other cell types Eph forward signals decrease cell adhesion and migration[5,47,97]. Instead of inhibiting the HRAS–Erk MAPK pathway, depending on the circumstances, EPHB2 can sometimes activate it[5,79]. In turn, activation of the Erk pathway enhances ephrin-dependent activation of overexpressed EPHB2 in cultured cells[79]. This may result in different EPHB2–MAPK feedback loops (FIG. 2b) that can either enhance or diminish cancer cell malignancy. Indeed, activation of an engineered membrane-anchored cytoplasmic domain of fibroblast growth factor receptor 1 (FGFR1) inhibits ephrin-dependent repulsive signalling by overexpressed EPHB2 through a mechanism involving downregulation of the HRAS–Erk pathway, suggesting that FGFR1 activation could neutralize the anti-invasive effects of EPHB2 in cancer cells[79]. By contrast, overexpressed EPHA4 and FGFR1 associate and potentiate each other's oncogenic activities in cultured glioma and other cell types[98,99]. It will also be interesting to determine whether Eph receptors can downregulate PTEN levels and perhaps activity in cancer cells, as suggested by a recent study in *C. elegans*[80].

***Unconventional Eph receptor activities.*** Downregulation of EPHA2 or EPHB4 by small interfering RNAs (siRNAs) or antisense oligonucleotides decreases cancer cell malignancy in culture and inhibits tumour growth in several mouse cancer models[36,37,100–102]. Furthermore, EPHA2 overexpression causes oncogenic transformation of mammary epithelial cells in culture as well as *in vivo*[71,103]. These experiments demonstrate positive effects of Eph receptors on cancer progression. Given the low levels of Eph forward signalling observed in many cancer cells, these tumour promoting activities are likely to be independent of ephrin stimulation and possibly also of kinase activity. Indeed, recent evidence shows that oncogenic signalling pathways can use Eph receptors to increase cancer cell malignancy.

Notable examples of how the altered signalling networks of cancer cells can subvert Eph function involve EPHA2. This receptor has been found to mediate some of the oncogenic activities of EGFR family members, including cancer cell migration in culture and tumour growth and metastasis in a transgenic mouse breast cancer model[104,105] (FIG. 4). EPHA2 also seems to be required for Src-dependent invasiveness of colorectal cancer cells in culture[90]. These effects may be ligand-independent and at least partly explained by the recently discovered crosstalk between EPHA2 and Akt, a serine/threonine kinase frequently activated in cancer cells[72] (FIGS 2c,4). Phosphorylation by Akt of a single serine in EPHA2 seems to promote cancer cell migration and invasion, an effect that interestingly does not require EPHA2 kinase activity and is reversed by ephrin-A1 stimulation[72]. It will be important to investigate the details of the Akt–EPHA2 crosstalk and whether other Eph receptors may contribute to cancer progression through analogous mechanisms. EPHA2 has also been recently shown to promote epithelial proliferation and branching morphogenesis in the developing mouse mammary gland by mediating hepatocyte growth factor (HGF)-dependent inhibition of RHOA activity[106], which is in contrast to the RHOA activation induced by EPHA2 overexpression, ephrin stimulation or crosstalk with the ERBB2 receptor[103,105,107]. It is not yet known whether an ephrin-independent EPHA2–HGF receptor crosstalk may have a role in cancer. Ephrin-independent activities of Eph receptors may also include the modulation of the

## Box 1 | **The Eph system can promote an epithelial phenotype**

Forward signalling by EPHA2 and several EphB receptors in epithelial and cancer cells can induce morphological changes reminiscent of mesenchymal-to-epithelial transition. For example, stimulation of EPHA2 forward signalling with an ephrin-A1–Fc fusion protein in sparse Madin-Darby canine kidney epithelial cells enhances the maturation of cell–cell junctions and cell compaction[170,171] (see the figure). In a positive feedback loop, E-cadherin can promote EPHA2 expression and surface localization in epithelial and cancer cells that have reached high density, thereby prolonging EPHA2 interaction with co-expressed ephrin-A1 and forward signalling[19,20,170] (FIG. 2d). Stimulation of EPHB2 forward signalling with ephrin-B1–Fc can also couple increased intercellular adhesion with cell contraction and apico-basal polarization in colorectal cancer cells by promoting the membrane localization of E-cadherin[86]. Interestingly, the consequences are dramatically different in colorectal cancer cells expressing EPHB2 but lacking E-cadherin, in which ephrin-B1–Fc stimulation causes cell contraction and separation instead of promoting cell–cell adhesion[86]. Stable transfection of EPHB3 in HT29 colon cancer cells, which endogenously express ephrin-Bs and E-cadherin, also causes changes consistent with mesenchymal-to-epithelial transition[42]. Furthermore, moderate ephrin-B expression and phosphorylation can promote the integrity of adherens and tight junctions in *Xenopus laevis* and HT29 cells[87]. Conversely, EPHB4 antagonists have been shown to disturb intercellular junctions in MCF-10A mammary epithelial cells[47]. Therefore, interplay with E-cadherin can convert Eph repulsive signals into signals that promote cell–cell adhesion. It is not known whether a similar interplay may occur with N-cadherin, which often replaces E-cadherin in malignant cancer cells that have undergone epithelial-to-mesenchymal transition. Studies in normal tissues suggest that Eph receptors can promote N-cadherin-dependent adhesion. For example, EPHA4 forward signalling is crucial for the N-cadherin-dependent mesenchymal-to-epithelial transition that occurs at the borders of developing zebrafish somites[172]. Interestingly, *EPHA2* mutations in humans and EPHA2 or ephrin-A5 loss in mice disrupt the N-cadherin-dependent intercellular junctions in the lens epithelium, causing cataracts[173,174].



Vasculogenic mimicry
The formation by the tumour cells of blood vessel-like channels that contribute to tumour blood perfusion.

Apico-basal polarization
Epithelial cells are polarized, with an apical membrane that faces the external environment or a lumen and is opposite the basolateral membrane, which functions in cell–cell interactions and contacts the basement membrane.

'Dependence' receptors
Structurally unrelated receptors that can induce cell death by apoptosis when unoccupied by ligand, thus creating cellular dependence on their ligands. In the presence of ligand, these receptors mediate survival, differentiation or migration.

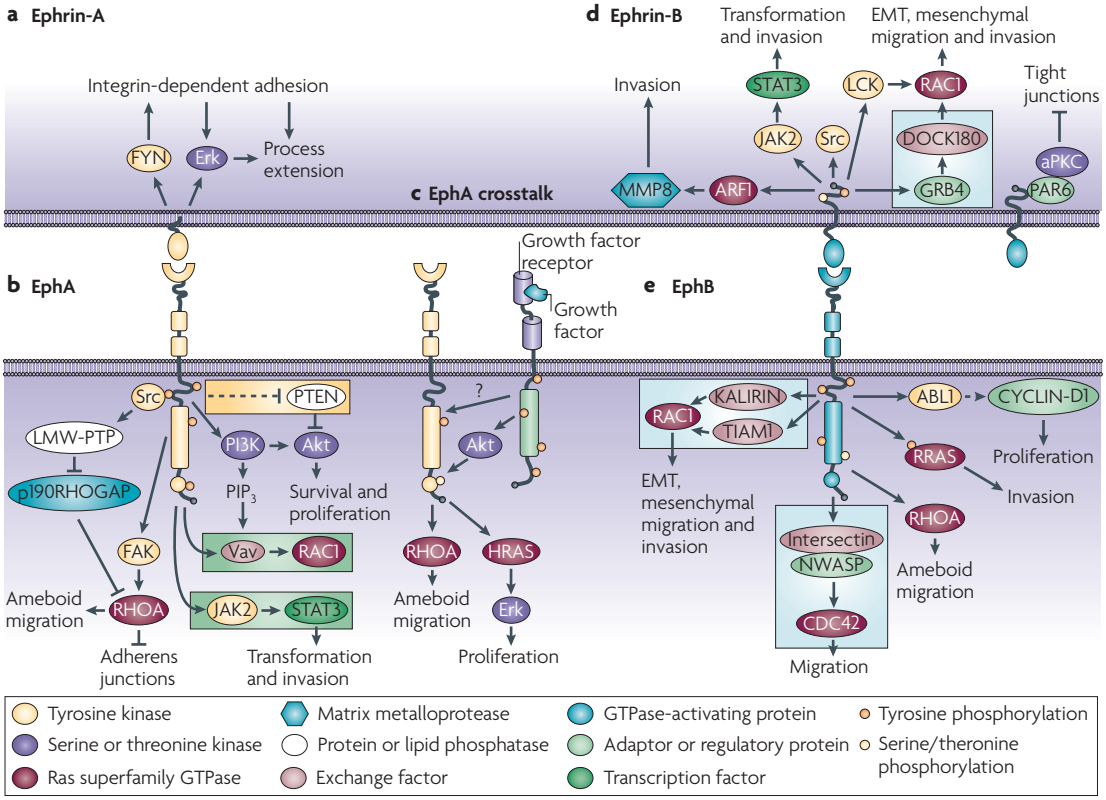



Figure 4 | **Eph tumour promoting pathways. a** | Ephrin-A5 reverse signalling promotes activation of FYN, β1-integrins and Erk in fibroblasts[5]. **b** | Low-molecular-weight protein tyrosine phosphatase (LMW-PTP) is activated by Src and dephosphorylates and inactivates p190RHOGAP. This increases RHOA activity to destabilize adherens junctions in EPHA2-overexpressing epithelial cells[103]. EPHA2 (and EPHB2) activate RHOA through focal adhesion kinase (FAK)[7,94]. EPHA4 activates signal transducer and activator of transcription 3 (STAT3)[185]. A pathway involving EPHA2, PI3K and Vav family exchange factors for RAC1 operates in endothelial cells[27,186]. Activation of EPHA2 activates Akt in pancreatic cancer cells[138]. The *Caenorhabditis elegans* Eph receptor inhibits PTEN expression[80]. **c** | EPHA2–ERBB2 crosstalk activates the HRAS–Erk pathway and RHOA in a mouse mammary tumour model, enhancing tumour growth and *in vitro* cell proliferation and migration[33,105]. Akt, activated by ERBB2 or other pathways, phosphorylates EPHA2. **d** | Ephrin-B reverse signalling affects pathways that promote invasiveness, including matrix metalloproteinase 8 (MMP8) secretion[17] and activation of STAT3,[118] Src and RAC1 (REFS 113, 114). By contrast, non-phosphorylated ephrin-B1 can bind PAR6 to inhibit atypical protein kinase C (aPKC)[87]. **e** | EphB forward signalling activates RAC1 and CDC42 exchange factors[5,7,127], which could promote cancer cell migration and invasion. EPHB4 activates RHOA[93]. EPHB2-mediated RRAS tyrosine phosphorylation increases glioma cell invasiveness[96]. EPHB2-mediated ABL1 activation increases cyclin-D1 levels[91]. Pathways identified in neurons, endothelial and muscle cells, or *C. elegans* that are predicted to have tumour promoting activity are in blue, green or yellow boxes, respectively. Most other pathways were identified in cultured cells and their importance in cancer remains to be proved. For more details see REFS 1,5–7,127. ARF1, ADP-ribosylation factor 1; EMT, epithelial-to-mesenchymal transition; JAK2, Janus kinase 2.

subcellular localization of signalling partners that are constitutively associated with an Eph receptor (FIG. 1) or become associated as a result of Eph phosphorylation by other kinases.

With regard to other receptors, the recently discovered ephrin-independent downregulation of β1-integrin levels and cell substrate adhesion by endogenous EPHB4 can inhibit migration in some cancer cell types, although EphB-dependent decreased adhesion can promote invasiveness in others[96,97]. Additionally, distinctive signalling activities of Eph intracellular domain fragments, which are generated by metalloprotease and γ-secretase cleavage, might promote cancer cell malignancy. For example, the EPHA4 cytoplasmic domain released by γ-secretase can enhance RAC1 activity in cultured cells independently of ephrin stimulation and kinase activity[24]. Furthermore, ephrin-B3 stimulation can block apoptosis that is caused by caspase-dependent cleavage of overexpressed EPHA4 in cultured cells, which interestingly suggests a role for EPHA4 as a 'dependence' receptor[108].

***Tumour promotion by ephrin signalling in cancer cells.*** Little is known about the effects of ephrin-A reverse signalling in epithelial cells. One study has shown that ephrin-A1 is highly upregulated in hepatocellular carcinoma and promotes the proliferation and expression of genes associated with proliferation and invasion in human liver cancer cells[109]. In fibroblasts, EphA-dependent stimulation of ephrin-A5 activates the Src family kinase FYN, integrin-mediated adhesion and Erk MAP kinases[5,6] (FIG. 4). Accordingly, ephrin-A5

overexpression can increase fibroblast growth in soft agar, as well as invasion and morphological transformation[110]. Ephrin-B reverse signalling also involves Src family kinases, which phosphorylate the ephrin-B cytoplasmic domain and so regulate its interaction with signalling molecules[5,6]. Src activation has been proposed to require the release of the ephrin-B intracellular domain by metalloprotease and γ-secretase cleavage following EphB binding, which decreases Src association with its inhibitory kinase CSK[14]. Furthermore, homophilic engagement of claudins, which are tight junction proteins, causes Src-mediated ephrin-B1 phosphorylation that slows down the formation of epithelial cell junctions and therefore might enhance invasiveness[111]. This is in contrast to the promotion of tight junction formation owing to ephrin-B1 phosphorylation (discussed above). Whether phosphorylation of different tyrosines, different levels of phosphorylation, or the cellular context might lead to positive or negative effects of ephrin-Bs on intercellular adhesion remains to be determined.

Other recurring themes in ephrin-B reverse signalling are a localization in lipid rafts and RAC1 activation, which can occur through multiple mechanisms and increase cancer cell migration and invasion[112–114] (FIG. 4). For example, ephrin-B3 is upregulated in invading cells of glioma biopsy samples and promotes RAC1-dependent invasion of glioma cell lines[112], and ephrin-B2 is upregulated in the invading cells of glioma and melanoma biopsy samples and its forced overexpression in the cultured cancer cells enhances integrin-mediated attachment, migration and invasion[115,116]. Furthermore, ephrin-B1 reverse signalling has been reported to induce secretion of matrix metalloproteinase 8 (MMP8) and promote invasion of glioma, pancreatic, gastric and leukaemic cancer cells *in vitro* and in mouse tumour models[17,113,117].

Ephrin-B reverse signalling may also modulate gene transcription in cancer cells. Ephrin-B1 binds and activates signal transducer and activator of transcription 3 (STAT3), a transcription factor involved in cancer progression[118] (FIG. 4). Furthermore, in neural progenitors ephrin-B1 intracellular domain fragments can localize to the nucleus and bind the ZHX2 transcriptional repressor, potentiating its activity, although it is not known whether this regulation also has a role in cancer[25].

***Tumour angiogenesis.*** Blood vessels are crucial for tumour growth and are an important venue for metastatic dissemination. Several Eph receptors and ephrins promote angiogenesis by mediating communication of vascular cells with other vascular cells as well as tumour cells. The interactions with tumour cells may occur during blood vessel growth and in tumour vessels with discontinuous endothelial lining. Furthermore, they may affect not only the endothelial cells but also, reciprocally, tumour cell behaviour[119].

Analysis of tumours grown in *Epha2*-deficient mice or mice treated with inhibitory EphA–Fc fusion proteins suggests that EPHA2 forward signalling promotes tumour angiogenesis[27,31,56]. By contrast, EPHA2 does not seem to have a major role in developmental angiogenesis, and only recently have abnormalities in capillary development that may be due to defective pericyte coverage been revealed in *Epha2*-deficient mice[120]. *In vitro* and *in vivo* data also show that EPHA2 forward signalling can increase blood vessel permeability, perhaps in part through phosphorylation of claudins[8,121]. A major ligand for endothelial EPHA2 is ephrin-A1; the upregulation of ephrin-A1 in endothelial cells and consequent activation of EPHA2 have been reported to have an important role in the angiogenic effects of VEGFA and TNFα[52,53]. In tumours, ephrin-A1 can be expressed by both endothelial and tumour cells[52,122,123]. Interestingly, the upregulation of EPHA2 and ephrin-A1 that is observed in pancreatic tumours of mice treated with VEGF inhibitors suggests that EPHA2-dependent angiogenesis might contribute to the development of resistance to anti-VEGF therapies, possibly by promoting endothelial coverage by pericytes and smooth muscle cells[120,124]. Curiously, ephrin-A3, another ephrin ligand for EPHA2, is downregulated in hypoxic endothelial cells in culture by the microRNA miR-210 and seems to inhibit angiogenic responses in hypoxic human umbilical vein endothelial cells[62]. It will be important in future studies to evaluate the combined activities of all relevant EphA receptors and ephrin-A ligands in the regulation of capillary sprouting, vessel permeability and pericyte coverage, as well as their possible redundancies and opposing functions in tumour blood vessels.

EPHB4 and ephrin-B2 also have a role in tumour angiogenesis. During development, they are characteristically expressed in the endothelial cells of veins and arteries, respectively, and enable arterial–venous vessel segregation and vascular remodelling[55–57,125]. The currently available information highlights the importance of ephrin-B2 reverse signalling in tumour angiogenesis, although little is known about the role of EPHB4 forward signalling[56,126–128]. Reverse signalling by ephrin-B2, and possibly other ephrin-Bs, in tumour endothelial cells, pericytes and smooth muscle cells probably depends on interaction with several EphB receptors that are expressed by vascular and/or tumour cells and has been shown to be important for blood vessel assembly, enlargement and decreased permeability both in cell culture and *in vivo*[57,126,127]. Ephrin-B2 signalling also promotes the interaction between endothelial cells and pericytes or vascular smooth muscle cells[60,128], suggesting that upregulation of this ephrin might stabilize the vessels of tumours recurring after anti-VEGF therapy[129]. Ephrin-B2 may also have additional roles in the tumour endothelium. For example, it might enhance the recruitment of bone marrow-derived endothelial progenitor cells that could participate in tumour vascularization, through a mechanism involving the EPHB4-dependent upregulation of selectin ligands[130]. It will be interesting to determine whether ephrin-B2 also promotes extravasation of EphB-positive metastatic tumour cells through the vascular endothelium, similar to its *in vitro* effect on monocytes[58,131].

## Eph proteins as therapeutic targets

Eph receptors and ephrins are promising new therapeutic targets in cancer. Various strategies are under

evaluation to interfere with their tumour-promoting effects or enhance their tumour-suppressing effects, although our limited mechanistic understanding of the dichotomous Eph activities is a challenge in the design of therapeutic agents. Other approaches that do not rely on interfering with Eph function involve using Eph receptor-targeting molecules for the selective delivery of drugs, toxins or imaging agents to tumours, as well as the use of Eph-derived antigenic peptides to stimulate anti-tumour immune responses.

***Interfering with Eph and ephrin function.*** Inhibiting the Eph–ephrin system may be particularly useful for anti-angiogenic therapies, and possibly to overcome resistance to anti-VEGF therapies[27,29,55,124,129]. Efforts to identify small molecules that target the Eph kinase domain have begun to yield some high affinity inhibitors[132–136] (TABLE 2). Furthermore, several inhibitors designed to target other kinases also inhibit Eph receptors. For example dasatinib, a multi-targeted kinase inhibitor already used in the treatment of chronic myelogenous leukaemia and under clinical evaluation

Table 2 | **Eph and ephrin targeting molecules**

| Molecules | Targets | Activity | Refs |
|---|---|---|---|
| ***Kinase inhibitors*** | | | |
| Anilinopyrimidine derivatives | EPHB4* | ATP competitors | 133,211 |
| Benzenesulfonamide derivative | EPHB4* | ATP competitor | 132 |
| XL647 (also known as EXEL-7647)‡ | EPHB4* | ATP competitor | 212 |
| Xanthine derivatives | Eph receptors | ATP competitors | 135,213 |
| LDN-211904 | Eph receptors | ATP competitor | 136 |
| Pyrido[2,3-d]pyrimidine PD173955 | Eph receptors | ATP competitor | 214 |
| Nilotinib and analogues‡ | Eph receptors | ATP competitors | 134,215 |
| Dasatinib | Eph receptors* | ATP competitor | 34,35, 137,138 |
| ***Inhibitors of Eph expression*** | | | |
| siRNA | EPHA2 | mRNA downregulation | 101,102,139 |
| Oligonucleotides | EPHA2 | Protein downregulation | 100 |
| siRNA | EPHB4 | mRNA downregulation | 36,37,194, 216,217 |
| Oligonucleotides | EPHB4 | Protein downregulation | 36,194, 216,217 |
| ***Inhibitors of Eph–ephrin interactions*** | | | |
| EPHA2–Fc and EPHA3–Fc | Ephrin-A | Eph competitor | 53,218–220 |
| sEPHB4 | Ephrin-B | Eph competitor | 142,221,222 |
| KYL and other peptides§ | EPHA4 | Ephrin competitor | 147,150,223 |
| SNEW and other peptides | EPHB2 | Ephrin competitor | 145,149 |
| TNYL-RAW peptide | EPHB4 | Ephrin competitor | 145,148,224 |
| Dimethyl-pyrrole derivatives | EPHA2 and EphA4 | Ephrin competitor | 150,151 |
| 2H9 antagonistic mAb | EPHB2 | Ephrin competitor | 143 |
| ***Activators of Eph forward signaling (also downregulate Eph expression)*** | | | |
| EA1.2 mAb | EPHA2 | Eph activation and degradation; possibly ADCC | 100 |
| EA2, B233 and 3F2-WT (humanized B233) mAbs | EPHA2 | Eph activation and degradation; possibly ADCC | 152,155 |
| EA5 mAb | EPHA2 | Eph activation anddegradation; reduced Src phosphorylation and VEGF levels; possibly ADCC | 153 |
| Ab20 and 1G9-H7 mAbs‖ | EPHA2 | Eph activation and degradation | 156 |
| mAB208 mAb | EPHA2 | Eph degradation and enhanced presentation of peptide antigens on tumour cell surface | 154 |
| YSA and SWL peptides | EPHA2 | Ephrin competitor; Eph activation and degradation | 146 |
| Dimerized IIIA4 mAbs | EPHA3 | Eph activation | 161 |
| Ephrin-A1–Fc | EphA receptors | Eph activation and degradation | 74 |
| Ephrin-B2–Fc | EPHB4 | Eph activation and degradation | 127 |

Neutral liposomes
Small vesicles made of neutral phospholipids (such as DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine), which can be filled with small interfering RNA for efficient *in vivo* intracellular delivery to tumour tissue.

to treat solid tumours, potently inhibits EPHA2 and other Eph receptors in addition to its primary targets Abl and Src family kinases[34,137,138] (EPHA2 clinical trials). Interestingly, EPHA2 has also been identified as a biomarker for dasatinib sensitivity of cancer cells[34,35]. Moreover XL647, an orally bioavailable EGF and VEGF receptor inhibitor being evaluated in clinical trials for lung cancer, also targets EPHB4 (EPHB4 clinical trials).

Downregulation of EPHA2 or EPHB4 expression with siRNAs or antisense oligonucleotides has been shown to inhibit malignant cell behaviour in culture and tumour growth *in vivo*[36,37,100–102] (TABLE 2). For example, the delivery of *EPHA2* siRNA to tumours using neutral liposomes inhibits tumour growth and metastasis in mouse models of ovarian cancer, particularly when combined with the delivery of siRNA silencing focal adhesion kinase (FAK) or with paclitaxel chemotherapy[102,139]. Eph receptor levels and function might also be reduced *in vivo*, as they are *in vitro*, by drugs that target the chaperone protein HSP90 (REFS 140,141), although other proteins will also be concomitantly downregulated.

Another strategy that shows promise for cancer anti-angiogenic therapy is to inhibit Eph–ephrin interactions. Various molecules can be used for this purpose (TABLE 2). The dimeric EPHA2 ectodomain fused to Fc (which inhibits EphA forward signalling but promotes reverse signalling) and the monomeric soluble EPHB4 ectodomain (which inhibits both forward and reverse signalling) can reduce tumour growth in mouse cancer models, at least in part by inhibiting tumour angiogenesis[27,31,57,142]. Antagonistic antibodies[143,144] and peptides that inhibit ephrin binding to individual Eph receptors or subsets of receptors[145–147] could be useful for inhibiting Eph–ephrin interactions and bidirectional signalling with a greater selectivity than the promiscuous Eph ectodomains. At least two of these peptides bind to the high-affinity ephrin-binding channel of their target receptor[148,149]. This Eph channel also seems to be suitable for targeting with chemical compounds, and two isomeric small molecules that preferentially inhibit ephrin binding to EPHA2 and EPHA4, albeit with low affinity, have been identified[150,151]. Structural characterization of additional small molecules and peptides in complex with Eph receptors could reveal general rules enabling the rational design of chemical compounds that are capable of selectively targeting Eph receptors with high affinity.

Intriguingly, ephrin ligands and agonistic antibodies have also been successfully used to inhibit tumour progression in mouse cancer models despite being activators rather than inhibitors of Eph–ephrin signalling (TABLE 2). These agonists have been proposed to function by stimulating Eph forward signalling pathways with tumour suppressor activity and/or receptor degradation in the cancer cells[47,152–154]. Antibody-dependent cell-mediated cytotoxicity may also contribute to the anticancer effects of some of the antibodies[155]; this perhaps explains the discrepancies in the effectiveness of different EPHA2 antibodies with similar agonistic properties[155,156]. Eph agonistic antibodies may also be useful in combination with chemotherapy[33,153].

Eph-targeting agents probably function through a combination of multiple effects on cancer cells and the tumour microenvironment, which might explain the efficacy of agents with opposite mechanisms of action. For example, EPHA2 agonists would be expected

Table 2 (cont.) | **Eph and ephrin targeting molecules**

| Molecules | Targets | Activity | Refs |
|---|---|---|---|
| ***Cytotoxic molecules*** | | | |
| 1C1 mAb-mc-MMAF conjugate‡ | EPHA2 | Receptor-mediated internalization and disruption of microtubule dynamics | 157,158 |
| 3F2-3M mAb (mutated 3F2-WT with enhanced effector function) | EPHA2 | ADCC | 155 |
| bscEphA2xCD3 bispecific single-chain mAb | EPHA2 and CD3 | Redirection of unstimulated cytotoxic T cells to EPHA2-positive tumour cells | 162 |
| YSA-modified adenovirus§ | EPHA2 | Adenoviral transduction of EPHA2-expressing tumour cells | 225 |
| Ephrin-A1-PE38QQR *Pseudomonas* exotoxin A conjugate | EphA receptors | EphA-mediated internalization and exotoxin-dependent cell death | 226 |
| Ephrin-A1 gold-coated nanoshells | EphA receptors | Absorption of near infrared light for photo-thermal ablation of tumour cells | 159 |
| 2H9 mAb-vc-MMAE conjugate | EPHB2 | Receptor-mediated internalization and disruption of microtubule dynamics | 143 |
| ***Imaging agents*** | | | |
| $^{64}$Cu-DOTA-1C1 mAb | EPHA2 | Binding, which enables radioimmunoPET | 160 |
| YSA peptide-magnetic nanoparticles | EPHA2 | Binding, which enables cell capture | 227,228 |
| $^{111}$Indium-labelled IIIA4 mAb | EPHA3 | Binding, which enables tumour detection | 161 |

ADCC, antibody-dependent cell-mediated cytotoxicity; DOTA, 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid; mAb, monoclonal antibody; mc-MMAF, stable maleimidocaproyl linker-monomethylauristatin F; vc-MMAE, cathepsin B-cleavable valine-citrulline linker-monomethylauristatin E; PET, positron emission tomography; siRNA, small interfering RNA; VEGF, vascular endothelial growth factor. *Eph receptor selectivity has not been reported. ‡In clinical trials. §Tested *in vivo* in a model of spinal cord injury. ‖ Not effective *in vivo*.

to enhance tumour suppressor signalling pathways and receptor degradation in the cancer cells but promote tumour angiogenesis[31]. Conversely, some Eph kinase inhibitors with anti-angiogenic activity might also block possible Eph tumour suppressor activities. Such inhibitors could therefore be particularly effective for the treatment of tumours in which Eph forward signalling pathways with tumour suppressor activity are not activated. EPHB4 agonists that also antagonize ephrin binding may be particularly beneficial by both enhancing EPHB4-dependent tumour suppression in cancer cells and inhibiting ephrin-B2-dependent angiogenesis[47,127]. Ultimately, how a tumour will respond to a particular Eph-targeted strategy is likely to depend on the tumour type, stage and microenvironment. Selecting optimal strategies to interfere with Eph function for cancer therapy will therefore require a better understanding of Eph signalling mechanisms in the different cellular compartments of tumours. Eph-dependent oncogenic signalling networks may also be suitable therapeutic targets. Newly developed targeting molecules, in particular those with selectivity for individual Eph receptors or ephrins, in turn represent useful research tools to further our knowledge of Eph cancer biology.

***Targeted delivery of drugs, toxins or imaging agents.*** Because of their increased expression in many tumours compared with normal tissues, Eph receptors are attractive targets for the delivery of drugs, toxins or imaging agents to cancer tissue. Several chemotherapeutic drugs and toxins that are conjugated to Eph antibodies or an ephrin, which cause receptor-mediated drug internalization, seem to be promising in initial studies (TABLE 2). EPHA2- or EPHB2-targeting antibodies that are coupled to the derivatives of the peptide drug auristatin, which disrupts microtubule dynamics, inhibit the growth of several cancers in rodent models[143,157,158]. Another potential application is the targeted delivery of gold-coated nanoshells conjugated to ephrins for photothermal destruction of Eph-positive cancer cells[159]. Importantly, systemic toxic effects have not yet been apparent and the EPHA2 antibody coupled to an auristatin derivative is under clinical evaluation (NCT00796055). Notably, targeting Eph surfaces that are preferentially exposed on tumour cells, which may include the ephrin-binding channel, could further improve the therapeutic index[152].

Antibodies, ephrins and peptides can also be used to deliver imaging agents for diagnostic purposes. EPHA2 is a particularly attractive target for this application given its widespread expression in both cancer cells and tumour vasculature and low expression in most adult tissues[27,56,160]. Promising results have been obtained in animal models by using an EPHA2 antibody labelled with $^{64}$Cu through the chelating agent 1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid (DOTA) for radioimmunopositron emission tomography (PET) imaging and an EPHA3 antibody coupled to $^{111}$Indium for gamma camera imaging[160,161].

***Immunotherapy.*** In addition to the immune cell-mediated cytotoxicity that can be elicited by Eph-targeted antibodies, a bispecific single-chain antibody that simultaneously binds both EPHA2 and the T cell receptor–CD3 complex causes T cell-mediated destruction of EPHA2-positive tumour cells *in vitro* and decreases tumour growth *in vivo*[162] (TABLE 2). Eph receptors that are preferentially expressed in tumours compared with normal tissues are also attractive targets for cancer vaccines. EPHA2, EPHA3 and an EPHB6 isoform have been identified as sources of tumour-associated peptide antigens that are recognized by cancer-specific cytotoxic T cells[163–166]. Interestingly, agonists and drugs that stimulate Eph receptor degradation might inhibit tumour growth at least in part by enhancing the presentation of Eph-derived peptides that are recognized by effector T cells[141,154]. Vaccination with Eph-derived epitopes also shows promise as a strategy to elicit tumour rejection[167,168].

## Perspectives

Accumulating evidence implicates the deregulation of the Eph–ephrin cell communication system in cancer pathogenesis. The Eph receptors are emerging as master regulators capable of either potentiating the activities of oncogenic signalling networks or repressing them, depending on ephrin stimulation and other contextual factors. Remarkably, Eph receptors and ephrins can switch between contrasting activities by using bidirectional signalling as well as other signalling modalities to influence cancer cell behaviour. We still only know relatively little about how the Eph system regulates tumorigenesis at the molecular level, but clearly there is extensive cell context dependency for many Eph pathways. For example, many of the differences observed in Eph–ephrin signalling outcomes may relate to differences in spatial and temporal coordination of input signals and relays, and so vary between cell types as well as *in vitro* and *in vivo* environments[169]. An important step forwards will be to understand the Eph activities beyond bidirectional signalling and the crosstalk with oncogenic pathways in detail. Furthermore, systems-level studies will be instrumental for providing a comprehensive overview of the effects of Eph bidirectional and unconventional signalling mechanisms in cancer and stromal cells; comparing the signalling activities of different Eph and ephrin family members; examining the consequences of changes in Eph and ephrin expression, for example to compare the effects of Eph receptor downregulation by agonists and by transcriptional silencing; and elucidating the effects of cancer-relevant Eph and ephrin mutations. Indeed, a recent proteomic analysis combined with siRNA screening and data-driven network modelling has provided a wealth of tantalizing new information on the asymmetric bidirectional signalling networks that are initiated by ephrin-B1 and EphB2 at sites of cell–cell contact[88]. Another area of great interest is how the Eph system influences the metastatic process, including tissue invasion, dissemination through the vascular system, possible reversal of epithelial-to-mesenchymal transition at distant sites, and dormancy of Eph-expressing micrometastases seeded in ephrin-rich tissues.

Radioimmunopositron emission tomography (PET) imaging
PET imaging using a radioactively labelled antibody. It allows non-invasive *in vivo* visualization of a tissue of interest, such as tumour tissue, that expresses the antigen as well as quantification of antigen levels.

Gamma camera imaging
Imaging with a camera that detects radioisotopes emitting gamma radiation. It is also known as scintigraphy and allows non-invasive *in vivo* visualization of radioisotopes coupled, for example, to an antibody that targets tumour tissue.

Epithelial-to-mesenchymal transition
A complex process in which genetic and epigenetic events lead to epithelial cells acquiring a mesenchymal architecture concomitant with increased cell motility. Typically associated with the loss of E-cadherin expression, disruption of cell–cell junctions, and cancer cell invasion and metastasis.

To advance our understanding of Eph cancer biology, it will also be important to examine the effects of Eph or ephrin loss, increased expression and cancer relevant mutations in genetically engineered mouse models that mimic the progression of human cancers. Such *in vivo* models are key for studying the Eph system, given its penchant for regulating communication between different cell types, which is difficult to accurately recapitulate *in vitro*. The mouse models will also be useful for preclinical evaluation of new Eph-based therapies.

Eph and ephrin expression promises to be a powerful predictor of prognosis and perhaps drug sensitivity. For example, increased EPHA2 expression can confer sensitivity to dasatinib but resistance to the ERBB2-targeting antibody trastuzumab[33–35]. Therefore, there is a need for a comprehensive assessment of Eph and ephrin protein expression in large cohorts of human tumours in correlation with stages of malignancy and clinical outcome. Carefully validated antibodies and quantitative proteomics approaches are needed to ensure the reliability of such studies. Understanding the complexities of the Eph system will help to clarify the mechanisms of cancer development, progression and metastasis as well as aid in the development of new anticancer therapies.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**Competing interests statement**

The author declares no competing financial interests.

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