

Therapeutic targeting of EPH receptors and their ligands

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Abstract | Critical roles for EPH receptor (EPH)–ephrin signalling in a range of chronic and regenerative diseases are increasingly being recognized. In particular, the complex roles of EPHs and ephrins in tumour growth and progression, and in nerve injury and regeneration have been studied extensively. This has led to considerable progress in developing strategies for their therapeutic targeting, with some anticancer agents already in clinical trials. Promising leads for non-malignant diseases are also emerging, with compelling preclinical data encouraging clinical development. We discuss this rapidly developing area of drug discovery, highlighting the associated challenges and limitations.

Ephrins

EPH receptor interacting proteins.

Tissue patterning

A self-organizing principle during development that allows the generation of defined groups of cells, which are spatially segregated from (groups of) phenotypically distinct cells; such segregation gives tissues their characteristic morphology. Patterning relies on interplay between cell-intrinsic activity of the actin–myosin network and the modulation of cell–cell adhesion mechanisms.

EPH receptors (EPHs) are the largest family of receptor tyrosine kinases. In cooperation with cell-bound ephrins, EPHs orchestrate cell positioning, tissue patterning and organ patterning, also controlling cell survival during normal and neoplastic development^{1–3}. Structural features and ephrin-binding preferences in humans distinguish nine EPHA and five EPHB family members, which interact promiscuously with five glycosyl phosphatidylinositol (GPI)-linked cell surface-bound type-A ephrins and three transmembrane type-B ephrins, respectively. Their interactions initiate the clustering, tyrosine phosphorylation and signalling of EPHs (referred to as ‘forward signalling’)^{1,2} (BOX 1; FIG. 1), while concurrent clustering of ligated ephrins on the opposite cell surface also elicits signalling in ephrin-bearing cells^{4,5} (referred to as ‘reverse signalling’)⁵ (BOX 2; FIGS 1, 2). In this way, mutually dependent outcomes from EPH–ephrin interactions are usually relayed into both cell populations. Frequent co-expression of EPHs and ephrins on the same cell, co-clustering and co-signalling of divergent EPH family members⁶ and crosstalk with other cell signalling pathways^{1,7} (BOX 3; FIG. 2) leads to considerable diversity in the output from these EPH–ephrin signalling clusters. This diversity is further enhanced by feedback signalling mechanisms that dynamically control cluster size and lifespan⁸. Cellular responses to EPH–ephrin signalling include changes in the actin cytoskeleton, in cell–cell or cell–substrate adhesion and in cell motility, as well as modulation of cell survival and proliferation. These diverse signalling outcomes are thus highly dependent on cell type and tissue context, and seemingly contradictory responses have been described in different cells even from the same EPH–ephrin interaction^{1,9,10}.

Research on EPHs and ephrins initially focused on embryonic tissue patterning and revealed crucial roles of several family members during all phases of development^{2,11,12}. Indeed, targeted deletions of EPHs and ephrins have been shown to cause severe embryonic defects in the cardiovascular^{13–15}, skeletal^{16,17} or neural^{18–20} systems. However, EPHs and ephrins also have continued crucial roles in adult tissue and organ maintenance, regeneration and pathogenesis, including synaptic plasticity and response to nerve injury^{21,22}, bone and vascular remodeling^{23,24}, stem cell positioning and proliferation^{12,25}, and cancer^{1,3}. Not surprisingly, the activities of EPHs and ephrins in adult life reflect their dichotomous functions and signalling activities during development. This is especially prevalent in oncogenesis, in which, depending on the tumour type, disease stage and kinase function, EPH overexpression can promote or inhibit tumour progression, and can even perform both functions within the same tumour type^{25,26}. Overall, it seems that increased EPH forward signalling promotes cell segregation and is mostly tumour suppressive, whereas reverse signalling through ephrins is often tumour promoting, driving neoangiogenesis and invasion^{9,27}.

The central role of EPHs and ephrins in many pathological conditions has triggered mounting interest in exploring their potential as therapeutic targets for a broad range of applications, ranging from anticancer therapeutics to modulators of synaptic plasticity, and for the control of regenerative and pathological neoangiogenesis, bone homeostasis and remodelling, immune modulation, glucose homeostasis and stem cell biology. A host of therapeutic approaches are being developed to modulate EPH–ephrin function, including

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Box 1 | **EPH structure, function and forward signalling concepts**

Extensive genetic evidence suggests that spatial information from EPH receptor (EPH) and ephrin expression gradients accurately defines cell position, tissue boundaries and cell survival^{10,12,72,261}. Many of the functions of EPHs in embryonic and oncogenic patterning are conveyed by responses to bidirectional signalling into the EPH- and ephrin-expressing cells^{3,28} and involve known regulators of cytoskeletal dynamics and morphology, including focal adhesion kinases (FAKs), paxillin, p130CAS (also known as BCAR1), CR2 kinase (CRK2; also known as CDK6) and RHO family GTPases and their guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs)^{9,262}. A default cellular response to EPH activation is local actin stress fibre depolymerization, which results in rapid cytoskeletal collapse and loss of focal adhesions, in turn leading to cell detachment and cell–cell segregation^{34,67}. Global phospho-proteome analysis of interacting EPH- and ephrin-expressing tissue-culture cells has confirmed that activated EPHs and ephrins function upstream of RAS/RHO GTPases, phospholipase C γ 2 (PLC γ 2), phosphoinositide 3-kinase (PI3K), ABL, SRC and mitogen-activated protein kinases (MAPKs)^{8,28,263}. EPHs also function independently of kinase activation: for example, by initiating integrin-mediated cell adhesion and migration^{53,54}. In turn, persistent EPH–ephrin clusters that form between interacting cells owing to a lack of endocytosis of cytoplasmic-truncated or kinase-dead EPHs were shown to cause cell–cell adhesion^{264–266}. Depending on the cell surface density⁶⁸, EPH kinase signalling capacity^{59,267} and regulation by protein tyrosine phosphatases^{268–270}, EPH–ephrin interactions result in diametrically opposing responses¹¹, such as cell adhesion and contraction/substrate detachment^{33,34}, proliferation and apoptosis^{36,37}, and tumour promotion and suppression^{27,38,40,41}. In general, EPH kinase-independent signalling often promotes cell–cell adhesion, migration and cell intermingling⁹, whereas signalling from activated EPHs and ephrins triggers cell rounding and cell–cell segregation. EPH kinase activation can also promote cell proliferation or apoptosis, with the outcome apparently depending on cell type and the duration of the EPH signal^{41,42,44}.

A prerequisite for cell–cell repulsion to occur is disruption of the molecular tether between the interacting cells that is formed by the EPH–ephrin complex. *Trans*-endocytosis describes an incompletely defined internalization concept that involves the uptake of full-length transmembrane EPHB–ephrin B signalling complexes into either cell²⁶⁴, although it remains unclear how this mechanism can trigger the bidirectional signalling in both interacting cells that is required for synchronized cell–cell segregation. An alternative mechanism reveals that ephrin-induced EPH clustering and phosphorylation triggers the EPH-associated metalloproteinase ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) to catalyse shedding of cell surface ephrins²⁷¹ and cadherin⁵⁶, a feedback-controlled mechanism that facilitates cell–cell segregation and endocytosis of ephrin-ligated EPH signalling complexes into the EPH-expressing cell^{218,265,266}. Increased Ca²⁺ flux may also trigger ADAM-mediated EPHB2 extracellular domain shedding and ensuing γ -secretase cleavage to release a cytosolic EPHB2 fragment that regulates *N*-methyl-D-aspartate (NMDA) signalling²¹⁸; a similar mechanism generates a short intracellular domain with an as-yet-unknown function²⁷².

Although it has been shown that a range of EPH and ephrin functions are dependent on endocytosis⁸, the intracellular fate of internalized EPHs is largely unknown. Degradation might be an end point of some EPHA and EPHB receptors^{44,111,273}, but there is also evidence for EPH and ephrin signalling from endosomes in a similar manner to other receptor tyrosine kinases^{44,218,268}.

small-molecule kinase inhibitors, ephrin-mimetic peptides, peptide vaccines, short hairpin RNAs, function-modulating fusion proteins and monoclonal antibodies (mAbs), which are at various stages of preclinical or early clinical development.

This Review discusses EPH–ephrin signalling mechanisms and function in normal and pathophysiological settings as a basis for a comprehensive discussion of therapeutic strategies that are currently being pursued in different disease contexts. Although the dichotomy of EPH–ephrin functions continues to challenge the explicit prediction of drug responses and of obvious paths to clinical translation, promising outcomes from the initial phases of clinical testing confirm the notion that suitable targeting of EPH–ephrin function holds considerable promise for the treatment of a range of diseases.

EPH–ephrin signalling

EPH forward signalling. Many basic and highly specialized EPH and ephrin functions in tissue and organ genesis, homeostasis and pathogenesis^{2,28} are accomplished through signalling mechanisms that rely on the dynamic assembly of oligomeric EPH and ephrin clusters between

interacting cells (BOX 1; FIG. 1). Owing to the large number of possible EPH–ephrin interactions, combined signalling by co-expressed A- and B-type EPHs^{6,29} and cross-talk with other cell signalling pathways⁷ (BOX 3; FIG. 2), there is a considerable diversity in the composition and signalling activities of these clusters. In addition, interactions between EPHs and ephrins that are co-expressed on the same cell³⁰ (*cis* interactions) have been reported to control EPH forward signalling³¹, although the physiological significance of these *cis* interactions has been disputed³².

This complexity of EPH–ephrin signalling mechanisms has hampered attempts to unambiguously define the signalling pathways that underlie many of the EPH functions. For example, to what extent the involvement of cytoskeletal signalling proteins is a cause or consequence of EPH-induced cytoskeletal changes remains to be demonstrated. It is, however, apparent that cellular responses are highly dependent on specific cell, tissue and organ contexts, and that signalling from the same EPH–ephrin pair can result in diametrically opposing responses, such as cell adhesion and contraction^{33,34}, proliferation and apoptosis^{35–37}, and tumour promotion

Glycosyl

phosphatidylinositol (GPI). A glycosylated lipid anchor that links various proteins at their free carboxyl end to a terminal mannose unit of the GPI moiety. GPI-anchored proteins account for approximately 20% of membrane proteins, including the A-type ephrins, which partition to specific sphingolipid- and cholesterol-enriched membrane domains.

Synaptic plasticity

The ability of synapses (the cellular junctions between pre- and postsynaptic neurons) to change in strength and thereby to modulate signal flow. Whereas intrinsic plasticity is due to changes in the signal transmitting capacity of synaptic receptors, extrinsic plasticity is due to synaptic interaction with a regulatory cell. The regulatory cell modulates the ability of the presynaptic cell to release neurotransmitter (signal) into the postsynaptic cell.

Fusion proteins

(Also known as chimeric proteins). Recombinant proteins that are composed of two unrelated, functionally intact components.

Cis interactions

Cell surface receptors (such as EPH receptors) that bind to cell-bound ligands (such as ephrins) on neighbouring cells communicate in *trans*. If these receptors can also interact with ligands that are expressed on the same cell surface, this is defined as a *cis* interaction. The biological consequences of the *cis* and *trans* interactions are often different if not contrary to each other.

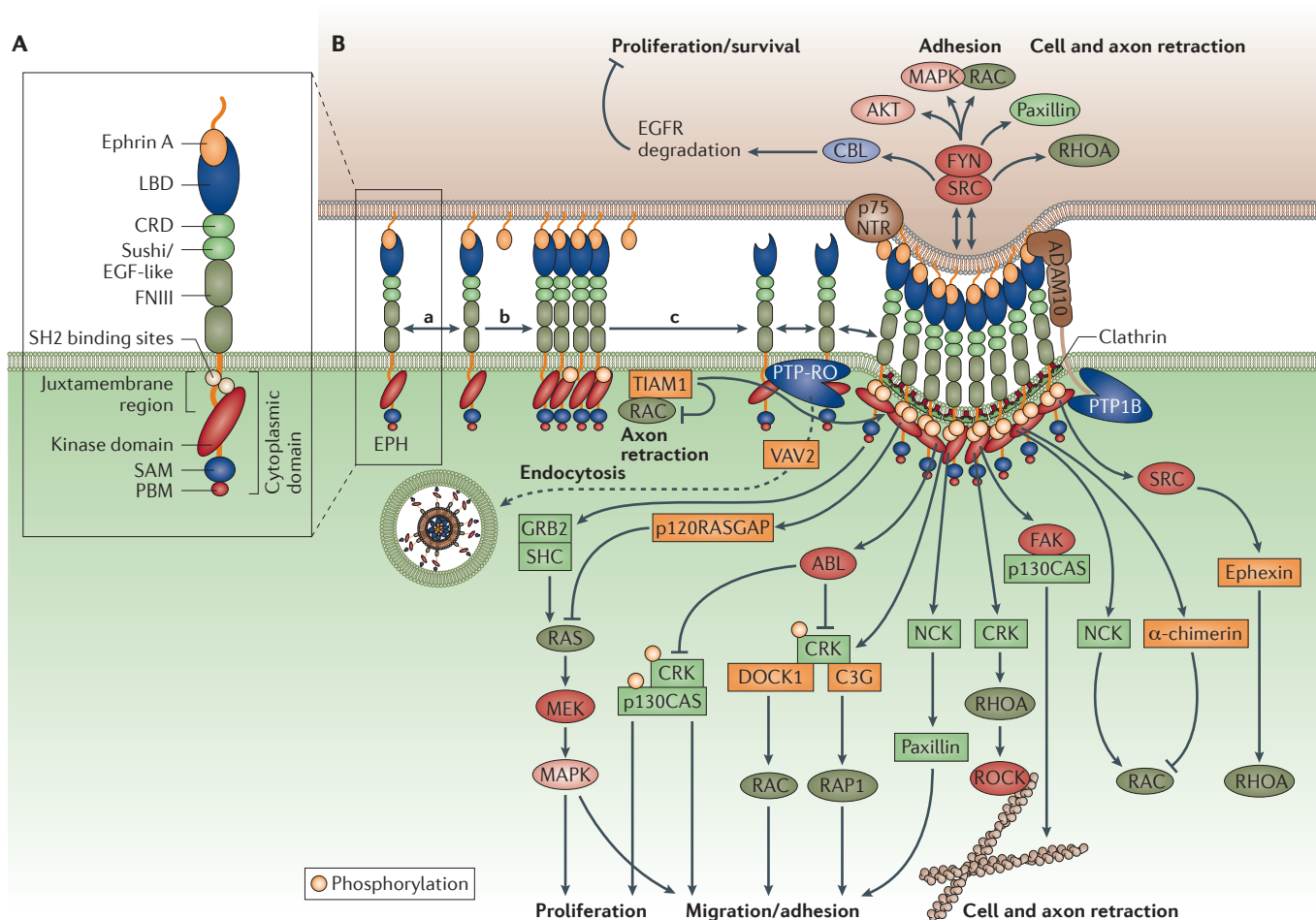


Figure 1 | EPH structure and signalling concepts. **A** | EPH receptors (EPHs) consist of an amino-terminal ligand-binding domain (LBD), a cysteine-rich domain (CRD) with Sushi (also known as short consensus repeat; SCR) and EGF-like motifs and two fibronectin III (FNIII) repeats, a cytoplasmic domain containing SRC homology 2 (SH2) binding sites, a juxtamembrane region that regulates kinase activity, a typical tyrosine kinase domain, a sterile alpha motif (SAM) protein–protein interaction domain and a PDZ binding motif (PBM)²⁸. **Ba** | Generally, only membrane-bound or artificially clustered soluble ephrins can activate EPHs, and tetrameric EPH clusters are effective in initiating signalling^{150,298}. Conversely, soluble monomeric ephrins and dimeric ephrin–Fc proteins mostly act as agonists by interfering with endogenous EPH–ephrin interactions^{41,42,67,81,299}. **Bb** | Signal activation involves oligomeric EPH–ephrin signalling clusters between interacting cells; EPH–ephrin heterodimers assemble into complexes of interacting ephrins and EPHs³⁰⁰ and — via EPH–EPH interactions — into clusters comprising four EPHs and four ephrins as a functional signalling unit¹⁵⁰. EPH activation involves the release of the EPH juxtamembrane domain from inhibiting the EPH kinase and extension of the cytoplasmic domain²⁶⁶. **Bc** | Depending on EPH and ephrin cell surface densities, additional EPH–EPH interactions within the LBD and CRD expand this oligomer into multimeric signalling clusters^{267,301,302}. Activated EPHs recruit the metalloproteinase ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10), which cleaves ephrins from the interacting cell surface to allow endocytosis to proceed, probably via a clathrin-mediated mechanism. EPH phosphorylation is controlled by protein tyrosine phosphatases (PTPs), including PTP-RO, PTP1B and LMW-PTP (not shown). Ephrin A signalling of glycosyl tyrosine phosphatidylinositol (GPI)-anchored ephrin As remains poorly defined⁵: clustering causes recruitment and activation of SRC kinases and facilitates RAC-, RHOA- and paxillin-facilitated cytoskeletal changes. Ephrin association with the p75 neurotrophin receptor (p75NTR) and inhibition of epidermal growth factor receptor (EGFR) degradation might be implicated in survival. For simplicity, signalling mediators of A- and B-type EPHs are illustrated together, including tyrosine kinases (red), serine/threonine kinases (pink), SH2 adaptors (light green), RHO GTPases (green) and their GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) (orange). Principle EPH signalling pathways¹ include: RHOA-, RAC- and repressor/activator protein 1 (RAP1; also known as TERF2IP)-mediated modulation of the cell cytoskeleton, which results in cell contraction or spreading and adhesion and involves a range of kinases and signalling mediators such as SRC kinases and focal adhesion kinases (FAKs); and SH2 adaptors such as p130CAS, CT10 regulator of kinase (CRK), CRK2, non-catalytic region of tyrosine kinase adaptor protein 1 (NCK1) and GAPs and GEFs (including ephexin, α -chimerin and C3G). EPH activation via ABL phosphorylation of CRK disrupts CRK–p130CAS and CRK–dedicator of cytokinesis protein 1 (DOCK1) complexes, thereby inhibiting cell migration, adhesion and proliferation²⁷, whereas FAK phosphorylation of p130CAS promotes its binding to CRK³³. EPH activation via p120RASGAP inhibition of RAS–MAPK/ERK kinase (MEK)–mitogen-activated protein kinase (MAPK) signalling inhibits cell proliferation¹. GRB2, growth factor receptor-bound protein 2; ROCK, RHO-associated protein kinase; TIAM1, T lymphoma invasion and metastasis-inducing protein 1.

Box 2 | Ephrin reverse signalling

As anticipated from closely interdependent responses of interacting cells, ephrin reverse signalling is tightly linked to EPH receptor (EPH) forward signalling^{4,5}; indeed, global phosphotyrosine network analysis confirmed that signalling patterns in ephrin-expressing cells are dependent on the presence or absence of EPH cytoplasmic domains in the interacting EPH-expressing cell, and vice versa²⁶³.

Signalling of clustered ephrins^{4,5} is initiated by recruitment of SRC family kinases, phosphorylating cytoplasmic ephrin B tyrosines and substrates implicated in downstream signalling of A- and B-type ephrins^{77,274}. In addition, the ephrin B PDZ-binding domain facilitates distinct ephrin signalling functions²⁷⁵ that are essential for embryonic midline patterning and (lymph)angiogenic sprouting^{62,63,276}, and mutations in ephrin B1 cause skeletal and neural patterning defects (such as cleft palate, craniofrontonasal dysplasia and craniosynostosis)^{60,277}.

In addition to SRC, ephrin B *trans*-phosphorylation via activation of platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), epidermal growth factor receptor (EGFR) and TIE2 (also known as angiopoietin 1 receptor) indicates important crosstalk with these signalling pathways⁴. In agreement, ephrin A5 reportedly inhibits tumour growth by causing increased ubiquitin-mediated EGFR degradation^{278,279}. Ephrin B2 co-clustering with vascular endothelial growth factor receptor 2 (VEGFR2) in endothelial stalk cells during sprouting angiogenesis is crucial for VEGFR endocytosis, signalling and biological effects^{62,63}.

In the case of activated B-type ephrins, SRC homology 2 (SH2)-dependent docking of the adaptor growth factor receptor-bound protein 4 (GRB4; also known as NCK2) results in focal adhesion kinase (FAK) activation and cell rounding²⁸⁰, whereas additional SH3-dependent binding of dedicator of cytokinesis protein 1 (DOCK1; also known as DOCK180) and p21-activated kinase (PAK) triggers RAC activation and axon retraction²⁸¹. Furthermore, association of signal transducer and activator of transcription 3 (STAT3) with activated ephrin B1 leads to its Janus kinase 2 (JAK2)-dependent dimerization and activation of transcriptional activity^{263,282}, a signalling path that is potentially involved in ephrin B1-mediated vascular assembly⁷⁷. Also, PDZ domain-mediated interactions are implicated in ephrin B signalling, including regulator of G-protein signalling 3 (RGS3) association with activated ephrin B1, which blocks stromal cell-derived factor 1 (SDF1)-induced signalling of the G protein-coupled CXC-chemokine receptor 4 (CXCR4)²⁸³. SH2- and PDZ-independent interactions between activated B-type ephrins and dishevelled (DSH), partitioning defective 6 (PAR6) and atypical protein kinase C (aPKC) are reportedly implicated in adhesion, migration and the formation of gap junctions⁴.

Little is known about the signalling mechanisms of glycosyl phosphatidylinositol (GPI)-anchored A-type ephrins, and so far only the p75 neurotrophin receptor (p75NTR) has been identified as a co-receptor that is implicated in EPHA7-induced axon retraction²⁸⁴. However, the involvement of A-type ephrins in modulating cell adhesion and morphology has been well established, and SRC family members — in particular, FYN and LCK, as well as mitogen-associated protein kinase kinase 3 (MAPKK3) and MAPKK1 (also known as ERK1 and ERK2, respectively), RAC, AKT, integrins and paxillin — have been found to be associated with clustered A-type ephrins^{4,5}.

Kinase-independent signalling

Canonical signalling by receptor tyrosine kinases occurs with tyrosine phosphorylation of their cytoplasmic residues as an initiating event that triggers downstream signalling cascades. However, receptor tyrosine kinases, such as EPH receptors, contain additional protein interaction domains, such as PDZ binding motifs, which initiate distinct signalling pathways in the absence of kinase activity.

Axon pathfinding

A cell-intrinsic mechanism of nerve cells that allows them to navigate by extending their growth cones over long distances to find the correct destination within the body. This 'axon guidance' is facilitated by molecular cues provided via cell surface proteins such as EPH receptors and ephrins, which guide the direction of extending growth cones by exerting cell contact-dependent adhesive or repulsive forces.

and suppression^{38–41} (FIGS 1, 2). In general, activated EPHs and ephrins trigger cell rounding and cell–cell segregation, whereas EPH kinase-independent signalling (see below) causes cell–cell adhesion and promotes intermingling²⁹. The responses in interacting EPH- and ephrin-expressing cells are tightly synchronized (BOX 1). In addition to the tissue patterning functions, EPH signalling promotes cyclin D1- or mitogen-activated protein kinase (MAPK)-dependent cell proliferation^{42–44} (BOX 3) and controls the survival of (tumour) stem and progenitor cells^{37,45–48}.

Kinase-independent EPH signalling. EPHs and ephrins have the unique property of being able to signal in the absence of phosphotyrosine-mediated interactions. Indeed, two EPH family members, EPHA10 and EPHB6, have various functions in normal and oncogenic development but lack the amino acid residues that are essential for kinase activity⁴⁹, and expression of kinase-dead mutant EPHA4 rescues several functions of the wild-type receptor in neural development³⁰. Moreover, EPHB receptors lacking the cytoplasmic domain compensate for the defective retinal axon pathfinding in EPHB2- and EPHB3-null mice⁵¹ and for defects in synaptic plasticity of EPHB2-null mice⁵², whereas kinase-independent EPH regulation of cell–cell adhesion was shown to involve signalling crosstalk with phosphoinositide 3-kinase (PI3K), integrins and cell–cell adhesion proteins^{53–56} (FIG. 2).

Interestingly, by activating separate, kinase-independent (PI3K-facilitated) and kinase-dependent (cyclin D1) pathways, the same EPH, EPHB2, can independently regulate intestinal stem cell migration and positioning and stem/progenitor cell proliferation, respectively⁴¹.

Although most of the cell autonomous and non-autonomous signalling mechanisms controlling the dynamic switch between kinase-dependent and independent functions remain to be elucidated, it is likely that the avidity of contributing EPH–ephrin interactions and the composition of the resulting signalling clusters are principle factors that together determine the size and persistence of the signalling complexes underlying the various EPH activities^{9,28}.

Ephrin reverse signalling. Reverse signalling by ephrins remains less explored than EPH forward signalling^{4,5}. In particular, there is a scarcity of knowledge on GPI-linked A-type ephrins, which lack a cytoplasmic signalling domain and hence are reliant on the association of co-receptors, such as the p75 neurotrophin receptor (p75NTR; also known as TNFRSF16), among others (FIG. 1; BOX 2). As for EPHs, the clustering of ephrin within discrete membrane microdomains seems to be necessary for their signalling via the recruitment of SRC kinases^{57,58}. Signalling outcomes are often matched to those of corresponding EPH signals; for example, EPH–ephrin-mediated cell–cell segregation at tissue boundaries relies

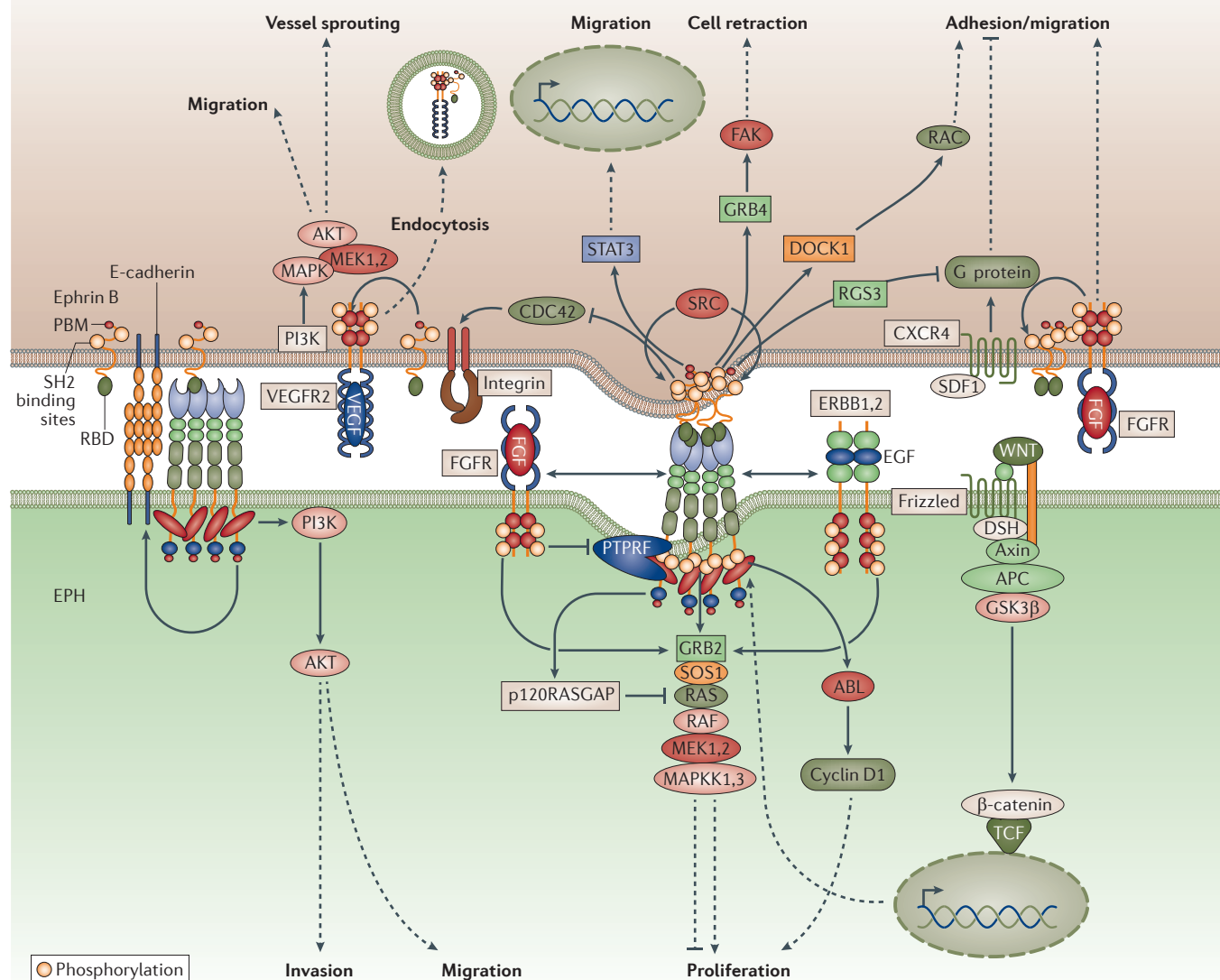


Figure 2 | EPH and ephrin B crosstalk with cell signalling pathways. Cell adhesion and growth factor receptor signalling pathways cooperating^{1,7} with signalling by EPH receptors (EPHs)^{1–3} and B-type ephrins^{4,5}. B-type ephrin signalling domains include the amino-terminal receptor binding domain (RBD), an intracellular signalling domain with SRC homology 2 (SH2) binding sites and a carboxy-terminal PDZ binding motif (PBM). Cell autonomous ephrin B2 signalling is considered to be responsible for endothelial cell migration²⁴⁸. Furthermore, ephrin B2 signalling is thought to be responsible for endothelial tip cell extension and vessel sprouting by facilitating vascular endothelial growth factor (VEGF)–VEGF receptor 2 (VEGFR2) endocytosis signalling and downstream activation of AKT and mitogen-activated protein kinase (MAPK) signalling⁶³. EPH signalling modulates E-cadherin-mediated adhesion^{54,260}. It either stimulates or inhibits (via the 120kDa RAS GTPase-activating protein p120RASGAP) fibroblast growth factor receptor (FGFR)–ERBB1 or ERBB2-induced growth factor receptor-bound protein 2 (GRB2)–RAS–MAPK signalling, whereas EPH activation of p120RASGAP results in RAS inhibition. Alternatively, EPH signalling can activate RAS–MAPK signalling directly. EPHB signalling via ABL and cyclin D1 drives cell proliferation⁴¹. Signal transducer and activator of transcription 3 (STAT3) recruitment to ephrin B1 and Janus kinase 2 (JAK2)-dependent dimerization and activation of transcriptional activity^{263,282} might be involved in regulating migration during ephrin B1-mediated vascular assembly⁷⁷. Ephrin B signalling can be cross-activated by FGFR. Intestinal EPHB2 expression is regulated by WNT signalling⁸². Association of regulator of G-protein signalling 3 (RGS3) with activated ephrin B1 blocks stromal cell-derived factor 1 (SDF1)-induced migration induced by the G protein-coupled CXC-chemokine receptor 4 (CXCR4)²⁸³. APC, adenomatous polyposis coli protein; CDC42, cell division control protein 42; DOCK1, dedicator of cytokinesis protein 1; DSH, dishevelled; EGF, epidermal growth factor; GSK3β, glycogen synthase kinase 3β; MAPKK, MAPK kinase; MEK1,2, MAPK/ERK kinases 1 and 2 (also known as MAPKK1 and MAPKK2, respectively); PI3K, phosphoinositide 3-kinase; PTPRF, receptor-type tyrosine protein phosphatase F (also known as LAR); SOS1, son of sevenless homologue 1; TCF1, transcription factor 1 (also known as HNF1α).

Box 3 | EPH signalling crosstalk with MAPK, PI3K and WNT signalling pathways

Activated EPH receptors (EPHs) engage in extensive crosstalk with the major cytosolic signalling pathways that control cell survival, migration and differentiation, of which the involvement of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) signalling has been studied most extensively. Thus, EPHB2-induced neurite retraction depends on inhibition of 120 kDa RAS GTPase-activating protein (p120RASGAP)–MAPK signalling²⁸⁵, whereas activation of MAPK signalling is required for EPHB2-driven cell–cell repulsion²⁶⁹ and for P19 mouse carcinoma cell migration. The signalling complex containing EPHA2 and oncogenic ERBB2 enhances RAS–MAPK and RHO GTPase signalling in breast cancer²⁶. By contrast, ephrin A1-activated EPHA2 inhibits MAPK phosphorylation and reduces tumour cell viability^{35,286}, and whereas EPH-mediated MAPK inhibition triggers apoptosis during *Caenorhabditis elegans* oocyte maturation²⁸⁷, ephrin B1–EPHB3 signalling in the palatal shelves promotes MAPK-driven cell proliferation⁴⁴. Thus, in contrast to other receptor tyrosine kinases, which generally stimulate MAPK signalling, the dichotomous roles of EPHs in inhibiting or activating RAS–MAPK signalling confirm a more general concept that signalling outcomes are highly context dependent².

PI3K–AKT signalling, which was initially shown to be responsible for EPH kinase-independent activities *in vitro*⁵³, has now also been implicated in (endothelial) cell migration *in vitro*⁹³ and *in vivo*⁴¹. By contrast, in phosphatase and tensin homologue (PTEN)-deficient tumour cells with constitutively active AKT and mammalian target of rapamycin (mTOR), activated EPHA2 is thought to suppress AKT phosphorylation and cell migration¹²⁰.

Crosstalk between EPHB and WNT, adenomatous polyposis coli protein (APC) and β -catenin controls cell positioning in intestinal crypts, where an increasing WNT expression gradient towards the crypt base induces EPHB2 and EPHB3 expression and represses ephrin B1 and ephrin B2 expression. The resulting cell–cell repulsion prevents EPHB2- and EPHB3-positive (EPHB2/3⁺) intestinal stem cells at the crypt base from entering the ephrin B⁺ territory of more differentiated cells towards the top of the crypt⁸²; this compartmentalization controls expansion of EPHB2/3⁺ cancer cells during early stages of tumour progression, so that mutational loss of EPHB expression or function during tumour progression is required to allow tumour expansion and invasion^{39,69}. In addition to this transcriptional control of EPHs, emerging evidence suggests that initiation of epithelial–mesenchymal transition in gastric cancer by EPHA2 involves its cross-activation with WNT and β -catenin signalling²⁸⁸.

on bidirectional signalling and cytoskeletal contraction in both interacting cells¹⁰. There are well-established examples in which developmental patterning relies on ephrin functions that are activated by EPHs functioning as agonistic ligands; for example, neural tube closure relies on ephrin A5-mediated cell–cell adhesion⁵⁹, whereas the essential patterning functions of ephrin B1 during secondary palate fusion are revealed by the severe skeletal defects (craniofacial syndrome) in heterozygous ephrin B1 loss-of-function mutations^{44,60,61}. In this case, it was recently demonstrated that ephrin B1 controls proliferation in the palate shelf by regulating MAPK-mediated cell proliferation (BOX 3), so that altered proliferation rates correlate with severe skeletal dysmorphogenesis⁴³. In addition, there is a growing list of ephrin functions that are independent of EPH signalling or presence⁴, and a crucial role of ephrin B2 in controlling vascular endothelial growth factor receptor (VEGFR) internalization was discovered by the demonstration that altered ephrin B2 expression or deficiency in PDZ signalling caused similar defects in vascular endothelial growth factor (VEGF)-dependent neoangiogenesis in mice^{62,63} (FIG. 2; BOX 4).

EPH-controlled cytoskeletal remodelling. EPH activation and phosphotyrosine-dependent signalling commonly leads to rapid depolymerization and reorganization of actin fibres^{64,65} and the loss of focal adhesions and cell–cell contacts, resulting in loss of substratum contact and cell segregation^{66,67}. The opposite response, cell–cell adhesion³³, is likely to reflect lower effective EPH–ephrin signalling cluster density and thus reduced signal strength^{59,68}, whereby accurate cell positioning relies on well-defined transitions between the two opposing responses⁶⁸. This concept also applies to oncogenic patterning, in which tumour cells expressing active EPHs

are prevented from spreading until mutations reduce or ablate EPHB kinase function and thus allow tumour cell invasion and spreading^{39,69}.

The underlying bidirectional EPH–ephrin signalling pathways involve EPH interactions with well-known regulators of cytoskeletal dynamics (FIGS 1, 2). EPH-mediated cell morphology changes occur via the modulation of integrin expression and function⁷⁰ and the modulation of focal adhesion signalling^{34,70}, whereas the activation of ephrin reverse signalling via cell division control protein 42 (CDC42) inactivation promotes cell polarization and mesenchymal–epithelial transition (MET) during tissue patterning⁷¹. SRC homology 2 (SH2)-adaptor proteins are thought to link EPHs to cytoskeletal and focal adhesion proteins and to facilitate actin cytoskeletal changes by modulating the activity of RHO family proteins^{9,28}.

Functions of EPH–ephrin signalling

Extensive research has established a leading role of EPHs and ephrins in developmental tissue patterning^{72–74}. Their functions are also crucial in maintaining the plasticity and regenerative capacity of adult tissues and organs. Among other functions, EPH–ephrin signalling is implicated in: synaptic remodelling during learning, memory and in response to brain and central nervous system injuries^{21,22}; physiological and pathological vascular and bone remodelling^{23,24}; and the control of stem cell positioning and proliferation^{12,25} (TABLE 1). Interestingly, emerging findings suggest that it is the unique capability of the EPH–ephrin system for interdependent forward and reverse signalling that dynamically regulates the contact between interacting cell types and thus mediates the plasticity that is characteristic of these tissues and their physiological functions.

PDZ

A structural protein domain that binds to the carboxy-terminal consensus sequence Gly-Leu-Gly-Phe, which is present in a range of transmembrane receptors and facilitates the formation of protein signalling complexes. The name stems from the first three proteins identified to contain a PDZ domain: post-synaptic density protein 95 (PSD95), disc large tumour suppressor 1 (DLG1) and zonula occludens 1 (ZO1).

Box 4 | EPH and ephrin function in pathological neoangiogenesis

The critical functions of EPHs and ephrins, particularly EPHB4 and ephrin B2, in embryonic cardiovascular patterning and angiogenesis, are re-emerging during postnatal vascular remodelling at sites of physiological and pathological neovascularization^{63,178,248,289}, where ephrin B2 in particular is essential for endothelial–stromal cell interactions^{77,289}. Ephrin B2 expression, which *in vitro* is induced by vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF; also known as FGF2) and hepatocyte growth factor (HGF)²⁹⁰, is upregulated at sites of adult neovascularization, such as ischaemia, wounds and tumours^{24,63,248,289}. Cell autonomous ephrin B2 signalling is considered to be responsible for endothelial cell motility²⁴⁸ and, via VEGF receptor 2 (VEGFR2) signalling, for endothelial tip cell extension and vessel sprouting⁶³, whereas endothelial EPHB4 activation by stromal or exogenous ephrin B2 enhances their angiogenic sprouting activity^{291,292}. Ephrin B2 is also required for VEGFR3 endocytosis in lymphatic endothelial cells⁶³ and sprouting angiogenesis of lymphatic vessels²⁷⁶, so that downregulation of its Notch-controlled expression leads to defects in lymphatic vessel regrowth²⁹³. Thus, localized ephrin B2 silencing inhibits alveolar and vascular growth in the rat lung, whereas ephrin B2 treatment of hyperoxic rats preserved alveolar and vascular growth²⁶⁰. EPHB4 signalling during retinal angiogenesis seems to switch the angiogenic programme from branching angiogenesis to maturation into vessels with a larger circumference and reduced vascular permeability, probably via angiopoietin 1–TIE2 pathway activation⁷⁶. Deregulated EPHB4–ephrin B2 function is implicated in neoangiogenesis, and inhibition of ephrin B2 signalling in a Kaposi sarcoma model using an EPHB4–human serum albumin fusion protein, alone or in combination with VEGF inhibition, led to reduced blood vessel density and reduced pericyte recruitment¹⁵³.

Other EPH and ephrin family members also participate in cardiovascular assembly, maturation and maintenance²³⁶, including EPHA2, ephrin A1 and EPHA3 (REFS 294,295). For example, EPHA2 is not expressed in or required for the development of the embryonic vasculature⁹³, but together with ephrin A1 it guides endothelial cell migration during adult angiogenesis and neoangiogenesis^{93,96,242}. EPHA2 or ephrin A1 deficiency reduces tumour angiogenesis¹³⁵, and systemic infusion of soluble EPHA2–Fc or EPHA3–Fc to neutralize ephrin A1–EPHA2 interactions inhibits tumour growth and endothelial cell migration *in vivo*¹⁵⁵. EPHA2 activation on vascular smooth muscle cells might also induce the retraction of perivascular supporting cells via the inhibition of RAC and p21-activated kinase (PAK), thus allowing endothelial cells to respond to angiogenic cues²⁹⁶. EPHA2-null mice with reduced density of tracheal capillaries and reduced pericyte coverage suffer from enhanced inflammatory responses to airway inflammation²⁹⁷.

In view of the pivotal functions of EPH–ephrin signalling in physiological and pathological tissue homeostasis, the increasing interest over the past decade in the potential of several family members as therapeutic targets in a broad range of applications is of little surprise. Areas of particular attention include cancer, inflammation, stem cell biology, nerve injury and degeneration, neoangiogenesis and tissue remodelling.

EPH–ephrin-facilitated adult tissue remodelling. Within the adult hippocampus and cerebral cortex, EPH forward signalling and, importantly, ephrin reverse signalling have crucial roles in synapse formation and synaptic plasticity, which are fundamental mechanisms that underlie aspects of learning and memory. Thus, ephrin B–EPHB forward signalling, in cooperation with *N*-methyl-D-aspartate (NMDA) receptors and the RAC1 guanine exchange factor T lymphoma invasion and metastasis-inducing protein 1 (TIAM1), regulates the actin dynamics that underlie dendritic spine morphogenesis²². Conversely, forward signalling of EPHA4 on dendritic spines, which is triggered by ephrin A3 located on neighbouring astrocyte processes, controls spine length and morphology²². Importantly, ephrin A3 reverse signalling from the same EPH–ephrin complex is crucial for synaptic plasticity and hippocampal long-term potentiation (LTP)⁷⁵, processes that are essential for learning and memory and that are dysfunctional in neurodegenerative diseases.

In a similar fashion, ephrin reverse signalling is crucial for the remodelling of the adult vasculature, which allows for vessel regeneration after ischaemia and during pathological neoangiogenesis^{8,24} (BOX 4). In addition to the essential function of EPH–ephrin signalling

during developmental angiogenesis^{13–15}, adult sprouting angiogenesis and vessel remodelling rely on EPHB4-activated or cell-autonomous ephrin B2 reverse signalling; transgenic loss of function of ephrin B2 in adult mice results in impaired physiological and pathological neoangiogenesis^{62,63}. In line with this role, ephrin B2 expression is positively regulated by VEGF and Notch signalling and, in turn, the full activation and endocytic signalling of VEGFR2 and VEGFR3 relies on endothelial ephrin B2 driving their effective endocytosis (FIG. 2; BOX 4). In addition to these endothelial functions, cell contact- and EPHB4-dependent ephrin B2 signalling in mural cells facilitates smooth muscle and pericyte positioning and interactions with the emerging endothelium, which is of particular relevance during tumour angiogenesis^{76,77} (see below).

Bidirectional ephrin B2–EPHB4 signalling also provides direct communication between neighbouring osteoclast and osteoblast precursors, and by controlling their differentiation, it regulates bone homeostasis⁷⁸.

EPH–ephrin-controlled stem and progenitor cell maintenance. Although mostly overlooked in early studies, EPH–ephrin signalling also modulates cell proliferation and survival, especially in neural and intestinal adult stem cell niches^{12,25} (TABLE 1; BOX 3). As with other functions, signalling outcomes are dichotomous. For example, in epithelial and endothelial cells EPHA forward signalling inhibits MAPK signalling and proliferation *in vitro*³⁵, and in the developing brain it controls progenitor cell expansion by inducing apoptosis⁴⁴. Meanwhile, in the adult brain, EPHA7–ephrin A2 reverse signalling seems to control neural progenitor proliferation^{45,46}. In the adult

Dendritic spine morphogenesis

The process in which the anatomical structures of a dendritic spine are generated and organized. The process involves bidirectional signalling of surface receptors, such as EPH receptors, RHO GTPases, scaffold proteins and actin-binding proteins. Dendritic spines are small protrusions from the dendrites of most principal mammalian brain neurons, and they contain all of the essential molecules and organelles that are involved in postsynaptic signalling and plasticity.

Long-term potentiation (LTP)

The long lasting strengthening of signal transmission between presynaptic and postsynaptic neurons that results from their synchronous stimulation. LTP is one of several concepts underlying synaptic plasticity, which is considered to be the cellular basis for memory and learning.

Sprouting angiogenesis

One of the processes in which new blood vessels develop from pre-existing vessels. It involves specification of endothelial cells into morphologically, phenotypically and functionally distinct migratory cells at the tip of the new vessel sprout and into proliferating stalk cells that form the nascent vascular lumen of the emerging vessel. The phenotypic specialization of endothelial cells as tip or stalk cells is transient and reversible and depends on the balance between pro-angiogenic factors, including vascular endothelial growth factor, Notch and EPH receptors.

Stem cell niches

The tissue or organ microenvironments that support the establishment and maintenance of stem cell populations and regulate their involvement in tissue generation, maintenance and repair. A range of factors in the stem cell niche tightly controls a fine balance between stem cell proliferation and differentiation.

Table 1 | Functions in adult physiology and pathology regulated by EPH forward and ephrin reverse signalling

Biological function	EPH and/or ephrin involved	EPH/ephrin role	Refs
Craniofacial patterning	Ephrin B1–EPHB3 forward signalling	Differentiation, proliferation of osteogenic precursors	16,43,59
Osteoclast–osteoblast homeostasis	EPHB4 forward and ephrin B2 reverse signalling	Activation (forward) and inhibition (reverse) of osteoblast precursor differentiation	78,111
Neural progenitor proliferation	EPHB1, EPHB2 forward signalling	Regulation of hippocampal neural progenitor proliferation	42
Neural progenitor proliferation	Ephrin A2, ephrin A5 reverse signalling	Inhibition of progenitor proliferation	45
Neural progenitor apoptosis	Ephrin A5–EPA7 forward signalling	Induction of progenitor apoptosis	44
Spine morphogenesis	EPHB forward signalling (NMDA crosstalk)	Actin reorganization	22
Spine length, morphology	Ephrin A3–EPA4 forward signalling	Controls spine length, positioning and morphology	22
Hippocampal long-term potentiation	EPA4–ephrin A3 reverse signalling	Controls glutamate receptor expression levels	75
Plasticity of neural stem cell niche	EPHB2 forward signalling	Inhibits differentiation of ependymal cell into astrocytes	79
Retinal stem cell proliferation	EPA4 forward signalling	Inhibits proliferation of retinal stem cells	78
Intestinal stem cell proliferation and positioning	EPHB2, EPHB3 forward signalling	ABL–cyclin D1 mediated proliferation of multipotent intestinal stem cells	41,42,82
Control of intestinal tissue boundary	Ephrin B1, ephrin B2–EPHB2 forward signalling	Compartmentalization of EPHB ⁺ tumour cells; mutation or loss of expression leads to invasion	38,25
Tumour growth and metastasis	EPA2 forward signalling	Crosstalk with ERBB2, stimulation of MAPK signalling	26,94,96
Tumour neovascularization	Ephrin A1–EPA2 forward signalling	Promotes tumour neovascularization	93–95
Neoangiogenesis	Ephrin B2 reverse signalling (via PDZ)	Facilitates VEGFR endocytosis, promotes VEGF-induced endothelial tip cell extension, vascular growth	60,61
Breast cancer progression	EPHB4 forward signalling	RAS–MAPK-dependent MCF7 cancer cell proliferation	104
Breast cancer progression	EPHB4 forward signalling	Via ABL, dampens viability, proliferation and invasion of breast cancer cells	37
Glioma progression	EPA3 kinase-independent signalling	Maintenance of glioma-initiating cells	47
Leukaemia progression	EPA3 signalling	Maintenance of leukaemic stem cells	112
Follicular lymphoma progression	Soluble EPA7 exodomain	Blocks oncogenic EPH signalling	119
Vascular inflammation	Ephrin B2	Monocyte adhesion to endothelium, transmigration	245
Endothelial inflammatory response	EPA2	Leukocyte adhesion, destabilization of adherens junctions	236–238
Henipavirus infection	Ephrin B2, ephrin B3	Cell surface receptors for virus adhesion and entry	246,247

EPH, ephrin receptor; MAPK, mitogen-activated protein kinase; NMDA, N-methyl-D-aspartate; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

hippocampus, ephrin B3-triggered EPHB1 and EPHB2 forward signalling promotes neural progenitor proliferation⁴³, whereas EPA4 forward signalling, by suppressing WNT3A– β -catenin signalling, reportedly inhibits proliferation of retinal stem cells⁷⁹. In addition, EPHB2 signalling regulates the plasticity of the neural stem cell niche by controlling lineage conversion between ependymal cells and astrocytes, presumably serving to maintain the balance between these niche cell populations following injury, such as stroke⁸⁰.

EPH–ephrin reverse signalling also controls proliferation in skin and hair follicles⁸¹, whereas in the intestinal stem cell niche and during adenoma formation, EPHB2 forward signalling exerts pro-mitogenic effects^{42,82}

through regulation of ABL–cyclin D1 signalling^{41,42}. Importantly, selection for EPHB2 expression allows the isolation of undifferentiated, long-term, proliferating, multipotent intestinal stem cells from the human colon, which have considerable regenerative therapeutic potential⁸³.

EPHs and ephrins in cancer

Deregulated expression and function during tumour progression. With the discovery of most EPH family members in cancer cell lines², it is not surprising that a re-emergence of their embryonic functions in cancers is implicated in tumour progression, neoangiogenesis and metastasis of a large range of epithelial and mesenchymal

cancers^{1,3}. EPHA2, EPHB2 and EPHB4 have attracted by far the greatest interest^{25,27,84}, although it is apparent that the de-regulated expression of other family members, including EPHA3 (REFS 48,85), EPHA4 (REFS 86,87), EPHA7 (REF. 88) and EPHB6 (REFS 89,90), is also implicated in the progression of various cancers (TABLE 1).

EPHA2 is broadly expressed in mesenchymal and epithelial embryonic⁹¹ and adult tissues and is frequently overexpressed in melanoma, gliomas, breast, prostate, lung, cervical, colon, oesophageal, gastric, ovarian, bladder and renal cell carcinomas^{84,92}. In agreement with a role in adult angiogenesis that has been defined in EPHA2-knockout mice⁹³, EPHA2 forward signalling promotes tumour neovascularization^{94,95}. In breast cancer, increased EPHA2, EPHB2 and EPHB4 expression levels^{96–98} have been associated with poor prognosis^{26,90,98,99}. EPHA2 is one of the four most highly phosphorylated proteins in basal breast carcinoma cells¹⁰⁰, and in non-transformed MCF10A mammary epithelial cells its overexpression facilitates anchorage-independent growth and tumorigenicity *in vivo*⁹⁷. In transgenic MMTV-Neu mice, EPHA2 cooperates with oncogenic ERBB2 (which is overexpressed in mammary epithelium) to enhance RAS–MAPK signalling (FIG. 3), and genetic ablation of EPHA2 impairs tumour initiation and metastatic progression²⁶. Likewise, in melanoma cells with low endogenous levels of EPHA2, overexpression enhances proliferation, colony formation and invasive capacity, and silencing of EPHA2 significantly reduces tumorigenicity^{101,102}.

The roles of EPHB2 and, in particular, EPHB4 during tumour progression are controversial²⁷. Both are interaction partners of ephrin B2, which is a prominent marker of normal and tumour vasculature²⁴, and increased expression of EPHB2 and EPHB4 causes the tumorigenic activation of angiogenic reverse signalling¹⁰³. In addition, high levels of EPHB4 are considered to be prognostic for poor survival of patients with breast cancer⁹⁰. By contrast, ephrin B2-activated EPHB4 signalling decreases the viability, proliferation and invasion of MDA-MB-435, MDA-MB-231 and MCF7 breast cancer cells³⁷, although a more recent report suggests the opposite, indicating that EPHB4 forward signalling triggers RAS–MAPK-dependent proliferation of MCF7 cancer cells¹⁰⁴. Likewise, although progressive loss of EPHB2 (REF. 38) or EPHB4 (REF. 104) expression during carcinoma progression^{39,105} seems to correlate with reduced survival rates¹⁰⁶, a recent survey of mRNA transcripts of paired stage I–IV colon cancer samples identified EPHB2 and EPHB4 together with EPHA1 as the most significantly increased EPHs¹⁰⁷. A possible explanation for the contradictory responses to EPH signalling has been provided though detailed analysis of EPH-mediated prostate carcinoma cell migration, in which the obvious lack of contact inhibition of metastatic PC3 cells was found to be due to adhesive ephrin B2–EPHB3/EPHB4 signalling overriding repulsive ephrin A5–EPHA2/EPHA4 signalling in the same cells. This would seem to suggest that the overall EPH expression profile, and thus the sum of EPH signalling capacities in a tumour cell, determines its invasive and metastatic properties²⁹. In addition,

some of these apparent disparities might be due to differences in the disease stages of the analysed tumours and variability in methods, reagents and mRNA stability; this would explain conflicting reports on the role and expression of EPHA3 in colon and lung carcinoma, in which marked differences were found between reverse transcription PCR-based analysis and immunohistochemical analysis of patient tumour tissues and matched normal tissues^{107–110}. High levels of EPHA2 and EPHA3 protein and mRNA correlate with poor patient outcomes in an aggressive mesenchymal subtype of glioblastoma, in which both EPHs seem to have kinase-independent roles in maintaining the glioma-initiating cell population in an undifferentiated, proliferative state^{48,111}. A similar role of EPHA3 in the maintenance of leukaemic stem cells¹¹² could suggest a conserved function in mesodermal-derived stem cells. In support of this, EPHA3, which was originally isolated from pre-B acute lymphocytic leukaemia cells¹¹³, is significantly increased in a broad range of haematopoietic tumours but is undetectable in normal human lymphocytes and haematopoietic progenitors⁸⁵, making it an attractive therapeutic target for clinical development¹¹⁴.

EPHs as tumour suppressors. Although the correlation of EPH overexpression with poor disease outcomes in cancer patients argues for EPHs being tumour promoters, considerable evidence suggests that often the same family member can also act as a potent tumour suppressor^{1,3}. This dichotomy of oncogenic function is best described for EPHB2 and EPHB4 (REFS 10,27,115); overexpression of these EPHs in colon, breast and endometrial cancers^{90,107,116} correlates with tumour cell survival, disease progression and poor disease outcomes^{36,98}, but other studies suggest that activated EPHB4 triggers breast cancer cell apoptosis³⁷ and is tumour suppressive in colon cancer^{105,117}. This bimodal concept of pro- and anti-oncogenic EPH function has been confirmed for B-type EPHs during tumour progression in the adenomatous polyposis coli multiple intestinal neoplasia (*Apc*^{Min}) mouse (BOX 3): WNT-induced EPHB expression⁸² in tumour-initiating stem cells⁴⁰ of intestinal adenomas (FIG. 2) restricts tumour expansion by compartmentalization of EPHB⁺ tumour cells from surrounding ephrin B1⁺/B2⁺ stroma, and loss of EPHB allows invasive growth and carcinoma progression³⁹. Also, in contrast to its tumour-promoting roles, targeted deletion of EPHA2 increases susceptibility to carcinogen-induced skin tumours and promotes their invasive progression¹¹⁸. Furthermore, in follicular lymphoma, truncated soluble EPHA7 expressed by normal B lymphocytes seems to act as a tumour suppressor through its inhibitory interaction with EPHA2 and other EPHs¹¹⁹. Frequent loss of EPHA7 promotes EPHA2 activity and oncogenesis, and targeting lymphoma cells with an EPHA7 exodomain–rituximab fusion protein showed significant therapeutic efficacy in a xenograft model¹¹⁹. On the whole, however, there is accumulating evidence to suggest that EPH expression and function in the absence of kinase activity is tumour promoting, whereas EPH forward signalling is tumour suppressive^{36,37,97,120} and is accompanied with loss of receptor or cognate ligand expression¹²¹.

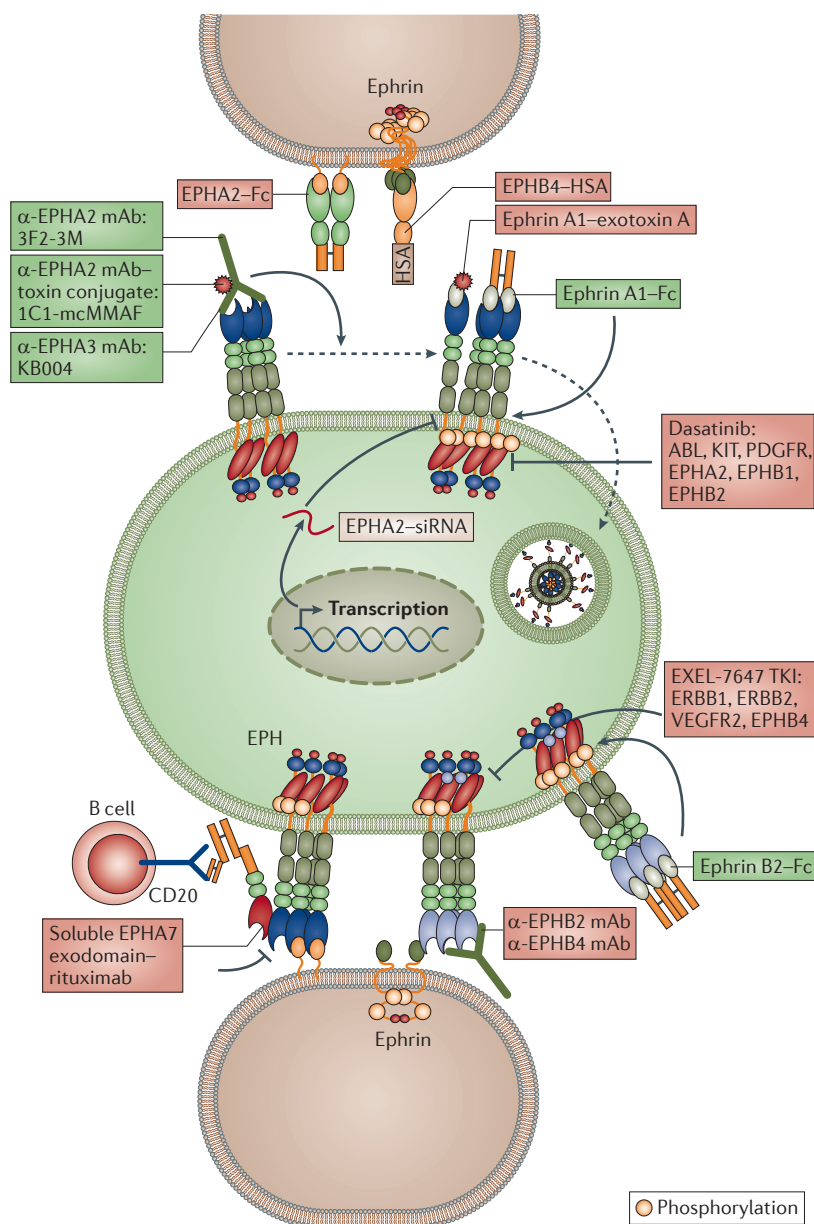


Figure 3 | Strategies for therapeutic targeting of EPH and ephrin functions. Signalling agonists and antagonists are indicated in green and red boxes, respectively. Agonists include the ephrin receptor A2 (EPHA2)-activating monoclonal antibody (mAb) 3F2-3M¹³⁸, which reduces neoangiogenesis and tumour growth by promoting EPHA2 internalization and degradation, whereas its auristatin-conjugated humanized version, 1C1-maleimidocaproyl-auristatin phenylalanine (mcMMAF)^{140,142,303}, acts via toxin-mediated tumour cell killing. The anti-EPHA3 mAb IIIA4 triggers sustained EPHA3 activation¹⁴⁹ and its humanized version KB004 causes antibody-dependent cell-mediated cytotoxicity (ADCC)-mediated and apoptotic leukaemic stem cell killing¹⁵⁰, whereas the ephrin B2-Fc fusion protein activates EPHB4-driven anti-oncogenic ABL-CRK2 (CT10 regulator of kinase 2) signalling³⁶ and blocks Nipah and Hendra virus attachment^{250,251}. Intramyocardial injection of ephrin A1-Fc activates EPHA signalling and expression and reduces infarct size and functional deficit²⁴⁵. Antagonists include mAbs, EPH and ephrin fusion proteins and small molecule inhibitors of EPH kinases. The anti-EPHB2 mAb 2H9 blocks ephrin binding and promotes internalization and degradation of non-activated EPHB2, and the anti-EPHB4 MAb47 inhibits tumour angiogenesis and tumour growth¹⁴⁸. Non-clustered EPHA2-Fc inhibits neovascularization, tumour cell proliferation, migration and invasiveness downstream of EPHA2 signalling^{155,156}, and reduces lung oedema²⁴⁶. The EPHB4-human serum albumin (HSA) fusion protein competitively blocks angiogenic EPHB4-ephrin B2 signalling^{146,147} and tumour growth^{145,146}. An ephrin A1-exotoxin A fusion protein administered by EPHA2-mediated uptake reduces glioblastoma growth¹⁵⁹, and a soluble EPHA7 exodomain-rituximab fusion protein acts as a dominant-negative inhibitor of oncogenic EPH signalling in follicular lymphoma¹¹⁹. The multi-tyrosine kinase inhibitor (TKI) EXEL-7647 inhibits tumour cell proliferation and neoangiogenesis by blocking epidermal growth factor receptor (EGFR), ERBB2, vascular epithelial growth factor receptor 2 (VEGFR2) and EPHB4 activation¹⁶⁴, whereas dasatinib inhibits oncogenic breakpoint cluster region protein (BCR)-ABL, platelet-derived growth factor receptor (PDGFR), KIT¹⁶⁸, EPHA2, EPHB1 and EPHB2 signalling. EPHA2 small interfering RNA (siRNA) is effective in models of ovarian cancer treatment¹⁸⁰.

Modulation of expression and activity by hypermethylation and mutation. Among the mechanisms that alter EPH expression and function during tumour growth and progression, epigenetic silencing and modulation of function through somatic mutations are well documented. Hypermethylation is responsible for silencing the expression of the soluble EPHA7 splice variant in mouse and human B cell lymphomas¹²², as well as for EPHA7 silencing in colon and gastric tumours^{123,124} and in 50% of analysed prostate carcinomas¹²⁵. In agreement with the role of EPHB receptors as tumour suppressors in advanced colon carcinoma, loss of EPHB2 and EPHB4 expression by hypermethylation and frame-shift mutations correlates with reduced patient survival¹²⁶. Conversely, hypermethylation of EPHA3 (REF. 127) together with many other EPHs and ephrins¹²⁸ is thought to contribute

to the pathogenesis of acute lymphoblastic leukaemia. Furthermore, genome-wide screens for diagnostic somatic mutations in lung and colon cancers have particularly emphasized EPHA3 among other family members as being one of the most frequently mutated genes in colon¹²⁹ and lung cancer¹³⁰, as well as in glioblastoma, melanoma and pancreatic carcinoma¹³¹. Accordingly, a survey of somatic mutations by genome-wide association studies for 'driver kinases' identified EPHB1, EPHA3 and EPHA7 as candidate cancer risk genes in breast cancer¹³². Overall, these findings seem to indicate a tumour suppressive role of kinase signalling-competent EPHs such as EPHA3, a notion that is supported by the finding that most EPHA3 mutations map to the ligand-binding, receptor-clustering or kinase domains and affect its signalling capacity¹³³.

Therapeutics targeting EPHs and ephrins in cancer

It is apparent that EPH and ephrin functions are implicated in tumour development and progression, although many of the underlying signalling mechanisms are only partially understood, in particular those controlling the direction of dichotomous responses during stem and progenitor cell proliferation, invasion and neoangiogenesis. However, despite the considerable complexity of EPH–ephrin signalling concepts and functions, their key roles in the progression of a large range of metastatic cancers have maintained EPHs and ephrins as candidate prognostic markers and potential therapeutic targets^{84,134}. In addition to the use of synthetic peptides that inhibit ephrin–EPH interactions and EPH–exodomain and ephrin–exodomain fusion proteins that modulate EPH and ephrin activity and function, small molecule kinase inhibitors and therapeutic mAbs targeting EPHs are at various stages of preclinical and clinical development (FIG. 3; TABLE 2).

Monoclonal antibodies. Owing to its prominent expression in a large range of tumours^{84,92} and role in adult angiogenesis^{94,135}, EPHA2 was the first EPH to be considered for therapeutic antibody development. From a panel of agonistic monoclonal antibodies (agonistic mAbs)¹³⁶, mAb EA5 was selected for its ability to reduce microvascular density and tumour growth and increase survival in ovarian cancer xenografts¹³⁷, and to inhibit breast cancer xenograft growth and metastasis¹³⁸. From the same mAb panel, the humanized version of mAb B233 with improved FcγRIII binding capacity (3F2-3M) suggested promising antibody-dependent cell-mediated cytotoxicity (ADCC)-mediated antitumour effects in ovarian, lung and breast cancer xenografts¹³⁹. Treatment with 3F2-3M mAbs restored drug sensitivity in trastuzumab-resistant tumour cell lines by targeting the previously reported oncogenic ERBB2–EPHA2 crosstalk²⁶, and in combination with trastuzumab, there was synergistic inhibition of trastuzumab-resistant tumour growth *in vivo*¹³⁹. Surprisingly, a fully human EPHA2 agonist mAb, 1C1 (MedImmune), which was generated by phage display library panning¹⁴⁰, did not affect tumour cell viability¹⁴⁰ despite maintaining the agonistic properties of the previous mAbs, including EPHA2 phosphorylation, internalization and degradation. However, a subsequently generated 1C1 antibody–drug conjugate with the microtubule inhibitor auristatin (1C1–maleimidocaproyl-auristatin phenylalanine (mcMMAF)) was reported to convincingly block tumour xenograft growth^{140,141}, reduce metastasis and improve survival rates in ovarian and endometrial carcinoma xenografts¹⁴² without any obvious adverse effects. This prompted an open-label first-in-human trial (ClinicalTrials.gov identifier: NCT00796055) investigating safety and maximum tolerated dose of 1C1–mcMMAF (MEDI-547) in relapsed or refractory solid tumours. Surprisingly, the trial had to be terminated owing to drug-related bleeding and coagulation events affecting all six patients in the lowest dose cohort. The study report¹⁴³ suggested that bleeding and coagulation events in these patients were consistent with those reported in the preclinical toxicology studies

in rats and primates, which had revealed increased activated partial thromboplastin time and disseminated intravascular coagulation as dose-limiting toxicities in primates¹⁴³.

The development of the anti-EPHB2 mAb 2H9 (Genentech), which antagonizes EPHB2–ephrin B1 interactions and causes internalization of non-phosphorylated EPHB2 (REF. 144), was motivated by the prominent overexpression of EPHB2 in colon cancer samples and the key role of EPHB2 in tumour progression, as suggested from a spontaneous mouse tumour model⁸². Although it exhibited no antitumour activity alone, a cytotoxic monomethylauristatin-E antibody–drug conjugate was found to effectively inhibit human colon carcinoma xenograft growth¹⁴⁴. However, clinical development has not yet been reported.

Given the essential roles of EPHB4 and ephrin B2 in embryonic vasculogenesis^{13,14} and tumour angiogenesis^{76,103,145–147}, they have been considered as therapeutic antibody targets by Vasgene (California, USA) and CNIO Biotechnology (Madrid, Spain), respectively. The Vasgene mAbs cause EPHB4 degradation, inhibit tumour angiogenesis and tumour growth and, in combination with bevacizumab, cause tumour regression in xenografted mice¹⁴⁸; their clinical development is yet to be reported. Likewise, the CNIO single-chain (scFv) anti-ephrin B2 antibodies reduce tumour growth¹⁴⁹ by inhibiting formation of tumour blood and lymphatic vessels, and they have been suggested as potential therapeutics. However, the prominent expression of both targets on normal vasculature might limit the therapeutic window of these antibodies. A recent study further challenged the potential of this therapeutic approach by suggesting that EPHB4 has strikingly dichotomous functions in cancer; in endothelial cells, activation of EPHB4 facilitated 120 kDa RAS GTPase-activating protein (p120RASGAP)–MAPK-mediated inhibition of proliferation while simultaneously stimulating MCF7 breast cancer cell proliferation¹⁰⁴.

The prominent overexpression of EPHA3 in a range of solid^{48,107–109} and haematological⁸⁵ cancers, including glioblastoma-initiating cells⁴⁸ and leukaemic stem cells¹¹², and its lack of expression on normal haematopoietic cells or their progenitors prompted development of the agonistic anti-EPHA3 mAb IIIA4 (REF. 150) as an anticancer therapeutic¹⁵¹. The IIIA4 mAb, which alone or in synergy with ephrin A5 triggers sustained EPHA3 activation, tumour cell contraction and apoptosis, effectively targets EPHA3⁺ tumour xenografts without binding to non-tumour mouse tissues¹⁵⁰. A recombinant, de-fucosylated human immunoglobulin G1κ (IgG1κ) therapeutic mAb version, KB004 (REF. 151) (KaloBios Pharmaceuticals, California, USA), exhibited antitumour activity in solid tumour models by disrupting EPHA3⁺ tumour stroma and vasculature, and it triggered apoptosis and ADCC on leukaemic blasts and tumour stem cells in a range of leukaemias, including acute myeloid leukaemia, chronic myeloid leukaemia, chronic lymphocytic leukaemia, myelodysplastic syndromes and myeloproliferative disorders¹⁵¹. In contrast to a recent report suggesting expression of EPHA3 in

Agonistic monoclonal antibodies

(Agonistic mAbs). Antibodies that enhance the function of an antigen of interest by recognizing and binding to a single specific epitope of the antigen (hence 'monoclonal'). In the case of a receptor tyrosine kinase, binding of an agonistic antibody triggers tyrosine kinase activation and signalling.

Antibody-dependent cell-mediated cytotoxicity (ADCC). A mechanism of cell-mediated innate immunity in which Fc receptor-mediated binding of immune effector cells (such as macrophages and natural killer cells) to antibody-opsonized target cells (such as foreign cells or cancer cells) leads to effector cell activation, cytokine release and target cell killing.

Table 2 | Summary of clinical trials that involve EPHs or ephrins as therapeutic targets or as biomarkers

Therapeutic (company)	Target or patient population	Targeting strategy or observation	Trial design and stage	ClinicalTrials.gov identifier, refs
Cancer treatment: mAbs targeting various EPH⁺ tumours				
MEDI-547, human α -EPHA2 antibody–drug conjugate 1C1–mcMMAF ^{140,142,303} (MedImmune)	Patients with relapsed or refractory solid EPHA2 ⁺ tumours	EPHA2 activation and degradation; delivery of a cytotoxic drug	Phase I dose escalation study: terminated owing to dose-limiting toxicities	NCT00796055, REF. 143
KB004, humanized α -EPHA3 mAb ^{150,151} (KaloBios)	Patients with haematological malignancies (AML, ALL, CML, MDS, MPN)	EPH activation on leukaemic blasts and stem cells, tumour vasculature and tumour stroma; direct apoptosis and ADCC-mediated tumour cell killing	Phase I dose escalation study: recruiting	NCT01211691, REF. 114
Cancer treatment: soluble EPH fusion proteins targeting solid tumours				
Soluble EPHB4–HSA ^{153,154} (University of Southern California, Norris Comprehensive Cancer Center)	Patients with advanced (metastatic or recurrent) solid tumours	Competitive inhibition of EPHB4 signalling blocking tumour cell growth and tumour angiogenesis	Phase I dose escalation safety study: recruiting	NCT01642342
Cancer treatment: small molecule EPH kinase inhibitors				
XL647 (Kadmon Corporation)	Histologically confirmed metastatic or unresectable tumours	Inhibition of multiple receptor tyrosine kinases: EGFR, VEGFR2, ERBB2 and EPHB4	Phase I dose escalation safety study: completed, no results published	NCT00336765
XL647 (Kadmon Corporation)	Previously untreated patients with NSCLC	Inhibition of multiple receptor tyrosine kinases: EGFR, VEGFR2, ERBB2 and EPHB4	Phase II interventional study: completed, no results published	NCT00364780
XL647 (Kadmon Corporation)	XL647 versus erlotinib, stage IIIB–IV NSCLC with progression after first- or second-line chemotherapy	Inhibition of multiple receptor tyrosine kinases: EGFR, VEGFR2, ERBB2 and EPHB4	Phase III treatment study: recruiting	NCT01487174
J1-101 (Jubilant Innovation)	Advanced solid tumours	Inhibition of VEGFR2, PDGFR and EPHB4	Phase I treatment study: completed, no results published	NCT00842335
Dasatinib, carboplatin, paclitaxel (MD Anderson Cancer Center; NCI)	Advanced stage and recurrent endometrial cancer	Effect of combined inhibition of proliferation (carboplatin), cell division (paclitaxel) and oncogenic kinases (dasatinib): BCR–ABL, SRC, KIT and EPHA2	Exploratory treatment study: recruiting	NCT01440998
Dasatinib (NCI)	Patients with unresectable or metastatic squamous carcinoma or stage 0–I CLL	Inhibition of BCR–ABL, SRC, KIT and EPHA2 kinases	Phase II treatment study: active, not recruiting	NCT00563290
Dasatinib, radiotherapy and temozolamide (MD Anderson Cancer Center; Bristol-Myers Squibb)	Patients with newly diagnosed glioblastoma	Exploratory objective: tumour expression of dasatinib targets: SRC, EPHA2, KIT and PDGFR	Phase I/II treatment study: active, not recruiting	NCT00895960
Dasatinib and type I-polarized autologous DC vaccines targeting tumour blood vessel antigens ³⁰⁴ (University of Pittsburgh Cancer Institute; NCI)	Patients with unresectable stage IIIB/C or stage IV metastatic melanoma	Exploratory objective: level of EPHA2 expression in tumour biopsies	Phase II safety and efficacy study: active, not yet recruiting	NCT01876212
Cancer treatment: dendritic-cell based vaccines				
EPHA2 ^{833–891} as part of a type-1-polarized DC vaccine loaded with tumour-related peptide antigens (University of Pittsburgh Cancer Institute; NCI)	Patients with stage III/IV melanoma	Natural killer, CD4 ⁺ and CD8 ⁺ T cell response to either α -type-1 DC-based or cDC (non-polarized DC)-based intra-lymphatic vaccines	Phase I treatment study: completed, no results published	NCT00390338

Table 2 (cont.) | Summary of clinical trials that involve EPHs or ephrins as therapeutic targets or as biomarkers

Therapeutic (company)	Target or patient population	Targeting strategy or observation	Trial design and stage	ClinicalTrials.gov identifier, refs
Cancer treatment: siRNA				
siRNA–EPHA2–DOPC (MD Anderson Cancer Center; Ovarian Cancer Research Fund)	Patients with advanced solid tumours	Targeting of <i>EPHA2</i> gene expression using liposomal siRNA delivery	Open label Phase I treatment study to evaluate maximum tolerated dose of 2× weekly 0.45 mg/m ² i.v. injection of siRNA–EPHA2–DOPC: not yet recruiting	NCT01591356
Observational: effects of ephrin B1 expression and mutation				
Effect of ephrin B1 mutation on craniofacial (CFNS) development ^{16,17} (National Human Genome Research Institute)	Patients with CFNS	Determine whether all patients with CFNS harbour mutations in the ephrin B1 (<i>EFNB1</i>) gene in Xq12	Observational study: completed, no results published	NCT00339846
Ephrin B1 expression in heart disease (ephrin B1 regulation in human right appendage) ³⁰⁵ (University Hospital, Toulouse)	Dilated cardiomyopathy	Determine whether ephrin B1 expression is regulated in human heart and correlates with heart rate variability	Observational study: completed, no results published	NCT01080781

ADCC, antibody-dependent cell-mediated cytotoxicity; ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; BCR, breakpoint cluster region protein; CFNS, craniofrontonasal syndrome; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; DC, dendritic cell; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine; EGFR, epidermal growth factor receptor; EPH, EPH receptor; i.v., intravenous; mAb, monoclonal antibody; mMMAF, maleimidocaproyl-auristatin phenylalanine; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasms; NCI, US National Cancer Institute; NSCLC, non-small cell lung cancer; PDGFR, platelet-derived growth factor receptor; siRNA, small interfering RNA; VEGFR, vascular endothelial growth factor receptor.

non-malignant lung tissues¹¹⁰, preclinical safety studies demonstrated a lack of any reactivity of IIIA4 or KB004 in human or primate non-tumour tissues and an absence of off-target effects in primates. An on-going Phase I trial (ClinicalTrials.gov identifier: NCT01211691) at several US and Australian sites is investigating the maximum tolerated dose, clinical safety and clinical activity of KB004 in various haematological malignancies. Promising initial clinical responses in the absence of any dose-limiting toxicities or drug-related side effects have been reported¹¹⁴.

Soluble EPH and ephrin fusion proteins. The prominent tumour suppressor function of EPHB2 and EPHB4 in colon, breast and prostate cancers has encouraged the therapeutic evaluation of EPHB4 agonists and competitive EPHB4–ephrin B2 interaction inhibitors. Systemic use of ephrin B2–Fc as an agonist slows breast cancer xenografts by activating anti-oncogenic ABL–CRK (CT10 regulator of kinase) signalling and interfering with kinase-independent EPHB4 functions in vessel maturation^{37,76,103}, whereas soluble, monomeric EPHB4 attenuates tumour growth^{146,147} and melanoma metastasis¹⁵² by competitively blocking angiogenic EPHB4–ephrin B2 signalling^{146,147}. On the basis of these and other preclinical studies that have also demonstrated therapeutic effects of an EPHB4–human serum albumin (HSA) fusion protein in colon, lung, breast, glioma, melanoma and prostate tumours^{153,154}, soluble EPHB4–HSA has entered Phase I clinical trials in patients with refractory or metastatic solid tumours (ClinicalTrials.gov identifier: NCT01642342) (TABLE 2).

Oncogenic EPHA2–ephrin A1 signalling has been targeted with soluble EPHA2–Fc and ephrin A1–Fc proteins to inhibit proliferation, neovascularization, migration and invasiveness^{155,156}, and infusion of ephrin A1–Fc into the brain of glioblastoma xenograft-bearing mice suppressed tumour growth *in vivo*¹¹⁰ in a similar way to the silencing of EPHA2 by small interfering RNA (siRNA). Likewise, adenoviral delivery of ephrin A1–Fc into tumour cells reduced breast cancer growth *in vitro* and *in vivo*¹⁵⁷. Cytotoxic ephrin A1–drug conjugates in preclinical development include ephrin A1–exotoxin A, which effectively killed EPHA2⁺ glioblastoma, breast and prostate carcinoma cell lines¹⁵⁸. In addition, transfection of human mesenchymal stem cells with ephrin A1–PE38, which is a soluble ephrin A1–*Pseudomonas* exotoxin fusion, induced glioblastoma killing and reduction in tumour volume after intratumoural injection of the engineered mesenchymal stem cells¹⁵⁹.

Most recently, a soluble EPHA7 exodomain splice variant was identified as a tumour suppressor and an EPHA2 decoy receptor that controls proliferative signalling and is lost in follicular lymphoma¹¹⁹. A soluble chimera between the anti-CD20 antibody therapeutic rituximab and soluble EPHA7 has exhibited promising therapeutic activity against xenografted human lymphomas¹¹⁹, and clinical development of this fusion protein is anticipated.

EPH signalling inhibitors. A range of small molecule inhibitors of ephrin binding¹⁶⁰ has been developed, which might provide the basis for therapeutic development, although their *in vivo* efficacy remains to be demonstrated. 2,5-dimethylpyrrolyl benzoate¹⁶¹, salicylate¹⁶⁰ and

Ephrin B2–Fc

A chimeric protein composed of ephrin B2 extracellular domains fused to the Fc portion of human immunoglobulin. Fusing EPH receptors (EPHs) or ephrins with Fc produces an antibody-like fusion protein in which both of the antibody Fab fragments are replaced by the EPH or ephrin extracellular domains.

lithocholic acid¹⁶² derivatives compete for ephrin A binding to various EPHA and EPHB receptors and inhibit activation and cell rounding. Azurin, a *Pseudomonas aeruginosa* electron transfer protein, blocks ephrin binding to EPHB2 and has shown cytotoxic activity in EPHB2⁺ prostate carcinoma cells¹⁶³.

Several multi-targeted, tyrosine kinase inhibitors (TKIs) are currently in clinical trials for a range of cancers and have been evaluated for their potential effects on oncogenic EPH function. Among these, EXEL-7647 (XL647; Exelixis, California, USA), which is a novel 'second-generation' ATP-competitive small-molecule inhibitor of epidermal growth factor receptor (EGFR), ERBB2, VEGFR2 and EPHB4, inhibits both tumour cell proliferation and neoangiogenesis. EXEL-7647 robustly inhibits EGFR *in vivo*, including erlotinib-resistant, mutant EGFR[L858R/T790M] tumour cell lines¹⁶⁴, and in Phase I trials this molecule has shown antitumour activity in non-small cell lung cancer (NSCLC)¹⁶⁵. Although the overall outcome of a Phase II trial of EXEL-7647 in erlotinib- or gefitinib-resistant cancers did not confirm its antitumour activity as being significant¹⁶⁶, stratification suggested a 20% response rate in patients who were of Asian origin, were women or had minimal or no smoking history¹⁶⁷. The drug is now undergoing Phase III trials in this patient cohort (ClinicalTrials.gov identifier: NCT01487174). Dasatinib (BMS-354825), which was originally developed as an SRC inhibitor and effectively blocks breakpoint cluster region protein (BCR), ABL, platelet-derived growth factor receptor and KIT¹⁶⁸, also inhibits EPHA2, EPHB1, EPHB2 and EPHB4 kinases^{169,170} and reduces EPHA2 expression^{171,172}; its crystal structure in complex with the EPHA4 kinase domain has been elucidated¹⁷³. A Phase II trial of dasatinib in stage III and IV melanoma (ClinicalTrials.gov identifier: NCT00436605) revealed minimal clinical response and poor tolerability¹⁷⁴. The effect of combined carboplatin, paclitaxel and dasatinib treatment on tumour cell proliferation, cell division/motility, oncogenic (EPHA2, BCR, ABL, SRC and KIT) signalling and EPHA2 expression is currently being assessed in a pilot study (ClinicalTrials.gov identifier: NCT01440998) in stage III, IV or recurrent endometrial carcinoma. Similarly, two Phase I/II clinical trials will assess the correlation between EPHA2 expression and response to dasatinib treatment of squamous cell carcinoma (ClinicalTrials.gov identifier: NCT00563290), and dasatinib/radiotherapy/temozolamide combination treatment of glioblastoma (ClinicalTrials.gov identifier: NCT00895960). Other TKIs with *in vitro* EPH-inhibitory properties include the second-generation BCR and ABL inhibitor nilotinib, which inhibits EPHB1, EPHB2 and EPHB4 (REF. 170), the third-generation ABL and SRC inhibitor bosutinib, which preferentially inhibits EPHB4 over EPHB1 (REF. 169), and the ABL and LYN inhibitor bafetinib, which is active against EPHA2, EPHA5 and EPHA8 (REF. 175). Last, a range of new inhibitors has been sourced from kinase inhibitor libraries with specificity for EPHB4 (REF. 176) or broader specificities for EPHA2, EPHA4, EPHA8 and EPHB2 (REF. 177). Novartis have developed NVP-BHG712,

an EPHB4-specific inhibitor, which potently inhibits EPHB4 autophosphorylation and VEGF-driven *in vitro* vessel formation¹⁷⁸ but has not been evaluated clinically.

The correlation of EPHA2 overexpression in ovarian cancer with poor clinical outcome¹⁷⁹ and of EPHA2 downregulation with a reduction in tumorigenicity in breast and pancreatic cancer models^{26,155} provided the rationale for preclinical evaluation of siRNA-based EPHA2 silencing for ovarian cancer treatment¹⁸⁰. Considering the severe toxicity associated with the killing of EPHA2-expressing cells using an antibody–cytotoxin conjugate (ClinicalTrials.gov identifier: NCT00796055)¹⁴³, a scheduled Phase I trial assessing the maximum tolerated dose of twice-weekly siRNA infusion in patients with advanced cancers (ClinicalTrials.gov identifier: NCT01591356) will be of considerable interest.

Vaccine-based immunotherapy. Owing to the notable overexpression of EPHs in tumours, EPH-derived peptides have been considered for vaccine-based therapy, including EPHB6 peptides that induce cytotoxic T cells in peripheral samples from human leukocyte antigen A2-positive (HLA-A2⁺) glioma patients¹⁸¹ and EPHA2 peptide-pulsed dendritic cell vaccines that induce natural killer, CD4⁺ and CD8⁺ T cells and inhibit growth and metastasis in mouse EPHA2⁺ tumour models¹⁸². A Phase I/II trial in recurrent glioma, which evaluated the safety of and clinical response to pulsed dendritic cell vaccination with glioma-associated antigens, including the EPHA2 peptide T₈₃₃-LADFDPRV₈₉₁, demonstrated measurable CD8⁺ T cell and clinical responses in 58% of patients, although the specific contribution of particular peptides was not defined¹⁸³. Extending this concept, a currently scheduled randomized Phase II trial will assess the notion that dasatinib, in addition to its effect on the immune-suppressive tumour microenvironment, will trigger tumour cell EPHA2 degradation, thereby improving CD8⁺ T cell recognition of cell surface EPHA2 and enhancing the killing of remaining EPHA2⁺ tumour cells (ClinicalTrials.gov identifier: NCT01876212).

EPH-based biomedical imaging. Alongside anticancer strategies, the selective overexpression of some EPHs on cancers provides opportunities for use in imaging strategies. Conjugates of anti-EPHA2 (1C1) and anti-EPHA3 (IIIA4) antibodies or of the polyethylene glycol (PEG) conjugated EPHB4-binding peptide TNYL-RAW with radioisotopes such as ¹²⁵I, ¹¹¹In or ⁶⁴Cu have been used to identify EPHA2-, EPHA3- and EPHB4-overexpressing cells in small animal biodistribution studies using positron emission tomography, computed tomography or magnetic resonance imaging^{150,184,185}. Furthermore, TNYL-RAW peptide-conjugated polymeric micellar nanoparticles labelled with the near-infrared dye indocyanine 7 (Cy7) and with ¹¹¹In allow multimodal whole-body imaging of tumour-bearing mice by single-photon emission computed tomography and near-infrared microscopy¹⁸⁶, and this technique holds great promise for the development of non-invasive strategies for visualizing tumour lesions.

EPHs and ephrins in non-malignant disorders

The growing appreciation of the direct involvement of the EPH–ephrin system in normal tissue homeostasis and regeneration, and of its role in neuropathology (see below) has engendered a growing interest in its therapeutic targeting. However, in sharp contradiction to therapeutic goals in cancer, which focus on ablating tumour cells or the tumour microenvironment to control or stop tumour growth, the therapeutic goal in tissue injury, regeneration and neurodegenerative diseases is possible recovery of tissue structure and function to ameliorate the disease. Thus, antibody therapeutics that are valuable strategies for targeted anticancer therapies are generally not suited to non-malignant disease conditions. Similarly, although EPH TKIs (TABLE 2) are attractive anticancer modalities¹⁸⁷, their action is limited to kinase-dependent functions and thus they affect only some targetable EPH and ephrin activities. Moreover, in the case of neurological pathologies, significant off-target effects due to the acknowledged lack of selectivity of EPH TKIs complicate their clinical development, and most preclinical research has focused on selective inhibitors of specific members of the EPH and ephrin families. Among these, soluble EPH and ephrin proteins are powerful tools for *in vitro* and *in vivo* studies and provide a logical basis for the development of therapeutics. However, their large size limits their use, and efforts are being made to develop specific small molecular weight inhibitors.

EPHs in neurological disorders

Considering the crucial roles of EPHs and ephrins in the patterning of the developing central nervous system^{72,188} and their continuing role in the adult brain, particularly in synapse formation and neural progenitor cell maintenance and positioning^{188,189}, it is not surprising that they have a role in neurological disease. EPH expression is prominent, especially in regions of continued remodelling such as the hippocampus, where the number and size of synapses is important for learning and memory¹⁹⁰. EPHA4 and ephrin A3 control synaptic plasticity by modulating the neuron–glial cell communication that affects dendritic spine morphology¹⁸⁹, whereas EPHB2-null mice show memory deficits due to downregulation of NMDA-dependent LTP⁵². It is thought that the neuropsin-mediated EPHB2 cleavage, which abrogates the EPHB2–NMDA receptor interaction in the amygdala after stress, can lead to increased anxiety¹⁹¹. Interestingly, EPHB2 (and EPHA4) expression is downregulated in Alzheimer's disease¹⁹², and increased EPHB2 expression in human amyloid precursor protein transgenic mice reversed deficits in NMDA receptor-dependent LTP and memory impairments, suggesting potential therapeutic applications in Alzheimer's disease¹⁹³.

EPHs in central nervous system injury. A survey of EPH gene expression in rat tissues following brain injury demonstrated often inconsistent changes in EPH genes¹⁹⁴. Whereas one study showed significant EPHB3 downregulation accompanied by an increase in neural precursor

proliferation as a direct consequence of the inhibition normally exerted by EPHB3 (REF. 195), others reported unchanged EPHB3 expression¹⁹⁴. Most attention, however, has been placed on EPHA4 because its expression has been shown to be significantly upregulated after traumatic brain injury (TBI) in rodents and primates^{188,196,197}, suggesting that EPHA4 blockade might represent a therapeutic strategy in TBI. However, comparative analysis of TBI in EMX-Cre conditional *Epha4*^{-/-} versus undeleted control mice has revealed no significant differences in functional recovery or histological changes, including gliotic response¹⁹⁸, a caveat being that EPHA4 expression is already reduced to low levels in control mice⁷³.

EPHs in spinal cord injury. In comparison to TBI, the role of EPHs and ephrins in spinal cord injury (SCI) has been studied in detail. SCI causes the upregulation of several EPHA^{199,200} and EPHB²⁰¹ genes, of which EPHA4 emerged as a key therapeutic target owing to its crucial role in the development of the corticospinal tract¹⁸ and of central pattern generators¹⁹. Because EPHA4 activation inhibits axonal growth in these developmental processes, it was argued that EPHA4 might be re-expressed after SCI and could block neuronal regrowth and functional recovery^{199,202}. The ephrin B3–EPHA4 interaction was targeted in a hemisection injury model in wild-type and EPHA4-null mice, and the functional recovery and histological responses were compared¹⁹⁹; nerve tracing studies revealed extensive axonal regrowth through the injury site in EPHA4-null mice but not in wild-type mice, and this regrowth correlated with markedly enhanced functional recovery. EPHA4-null mice also showed a significantly reduced gliotic response, the significance of which has been questioned subsequently as a strain-specific defect^{203,204}. Interestingly, the EPHA4 ligand, ephrin B3, has also been proposed to inhibit nerve regeneration after injury²⁰⁵ by contributing to the myelin-associated inhibition of axonal regrowth. Accordingly, ephrin B3-null mice showed improved recovery after a dorsal SCI²⁰⁵. EPHA4 interaction with ephrin B2 expressed on astrocytes might also contribute to this effect because a recent study showed enhanced recovery in mice with conditional deletion of ephrin B2 in astrocytes²⁰⁶. In addition, ephrin B2 interaction with EPHB2 might contribute by altering scar formation, hence enabling axonal recovery²⁰⁷.

Clearly, EPHA4, probably through interaction with ephrin B2 and ephrin B3, seems to have a functional role in inhibiting nerve regeneration post-SCI, suggesting that pharmacological inhibition of these interactions might promote functional recovery. Indeed, treatment of SCI by blocking EPHA4–ephrin interactions with either ephrin A5–Fc, which binds EPHA4 with high affinity, or with EPHA4–Fc, which acts as a 'decoy receptor', resulted in significant functional recovery when compared to control Fc protein-treated animals²⁰⁸ (TABLE 3). Notably, direct application to the injury site and systemic administration gave equivalent results, suggesting that blood–brain barrier (BBB) disruption at the wound site allows entry of the Fc fusion proteins. More recently, EPHA4–Fc treatment resulted in significant functional recovery in a rat

Traumatic brain injury (TBI). Single or repeated impacts to the head that result in immediate post-traumatic sensory, motor and neurocognitive syndromes due to brain contusions, intracerebral haemorrhage and axonal shearing, as well as progressive neurological dysfunction after repetitive insult.

Spinal cord injury (SCI). A partial or complete injury to the spinal cord that is often caused by fracture or dislocation of the vertebrae, leading to tearing or complete severing of the nerve fibres of the spinal cord.

contusion model, an injury that mimics human SCI²⁰⁹. In support of these findings, local administration of EPHA4-blocking peptides also induced significant functional recovery after SCI in mice²¹⁰. In a clinical setting, the nature of the injuries and the need for immediate treatment make parenteral administration preferable, but peptides generally have unfavourable pharmacokinetic profiles when administered via this route. This can be overcome by conjugation to carriers such as PEG polymers, which have previously been used for an EPHB4-specific peptide²¹¹. However, Fc fusion proteins have the potential advantage of blocking the interactions of other relevant EPH and ephrin proteins that might be upregulated after SCI. Indeed, levels of EPHA7 have been found to be increased after SCI, and silencing of EPHA7 with antisense oligonucleotides was reported to enhance recovery after SCI²¹² (TABLE 3). By contrast, a similar approach in which ephrin A1 expression was knocked down after SCI was reported to give a worse outcome²¹³. Indeed, the involvement of ephrin A1 in the vascular response to injury would suggest that blocking its function might be deleterious. Currently, based on its preclinical safety profile and its nanomolar affinity to ephrin ligands, the recombinant EPHA4–Fc protein is arguably the lead therapeutic candidate.

EPHs in Alzheimer's disease. Genome-wide association studies in patients with Alzheimer's disease have identified *EPHA1* as a candidate gene in this disease^{214,215}. This was surprising because it had been reported that *EPHA1* is not expressed in the adult brain²¹⁶. Further studies are required to determine how *EPHA1* contributes to the risk of Alzheimer's disease and to determine whether it is a potential target for therapy. However, other EPHs, particularly EPHB2, which has a role in neural plasticity²¹⁷, have been more directly implicated in Alzheimer's disease. Following activation, EPHB2 is cleared from the cell surface by a two-step process: an initial cleavage that releases its ectodomain and a second cleavage that releases its intracellular domain²¹⁸. The second cleavage is catalysed by a presenilin-dependent γ -secretase and inhibited by Alzheimer's disease-associated mutations in presenilin. Also, in EPHA4-mediated neural plasticity, the γ -secretase-facilitated release of the intracellular domain²¹⁹ is implicated in RAC1-dependent dendritic spine formation. In this case, loss of protease function decreases EPHA4-dependent RAC1 activation and contributes to neurofibrillary tangle formation²²⁰. Together, these findings clearly identify the EPH–ephrin system as a potential therapeutic target in Alzheimer's disease. In a proof-of-concept mouse model of Alzheimer's disease, lentiviral EPHB2 expression in the dentate gyrus reversed defects in NMDA-dependent LTP and thus resulted in improved memory function¹⁹³. Although such studies have validated EPHB2 as a target, devising therapeutic approaches for Alzheimer's disease that bypass the BBB will be challenging.

EPHs in other neuropathologies. Increased expression of EPH and ephrin genes has been documented in active multiple sclerosis lesions²²¹. In particular, ephrin A1 and

EPHA3, EPHA4 and EPHA7 are reportedly increased in axons of multiple sclerosis lesions, and although earlier studies linked EPHA4 with the pathology of multiple sclerosis, subsequent genetic studies have not supported this finding²²². In a recent mouse model of demyelinating disease, EPHA4 inhibition showed at best a modest effect on disease progression²²³, although it was not examined whether this reflects poor penetration of the EPHA4–Fc protein into the demyelinating lesions rather than a lack of efficacy.

In Parkinson's disease, among other axon guidance genes, *EPHB1* has been linked with disease development and progression²²⁴. Intriguingly, in a rat model of Parkinson's disease, the injection of clustered ephrin A1–Fc into the lateral ventricles, thus bypassing the BBB, resulted in cell proliferation and the subsequent migration of dopaminergic neurons into the substantia nigra²²⁵. Its low affinity for EPHB receptors suggests that in this Parkinson's disease model, ephrin A1 is likely to be activating EPHA proteins. Defining which EPHA proteins are being targeted by ephrin A1 might lead to a more specific and effective therapy.

A role for EPHs in motor neuron disease (MND) was initially identified through the analysis of a dominant inherited mutation of the endoplasmic reticulum protein vesicle-associated membrane protein-associated protein B (VAPB; also known as VAMPB), which is the causative molecular lesion in a familial form of amyotrophic lateral sclerosis (ALS)²²⁶. In the mutant form of VAPB, its cleavage, which normally results in the release of a major sperm protein (MSP) domain fragment that binds to EPHs, is defective. A further VAPB mutation was discovered in a second ALS kindred that resulted in less severe disruption to protein structure but markedly reduced the affinity of the MSP fragment for EPHA4 (REF. 227). Also, analysis of patients with MND²²⁸ identified an inverse correlation between EPHA4 expression and survival, suggesting a direct role for EPHA4 as a disease modifier in ALS. Moreover, the generation of superoxide dismutase 1 (*Sod1*) transgenic²²⁹ and *Epha4*^{−/−} mice¹⁸ resulted in a significant delay in disease progression, identifying EPHA4 as a key therapeutic target in MND.

EPHs and inflammation

EPH–ephrin interactions have multiple roles in cell adhesion-based processes during inflammation, immunity and atherosclerosis^{230,231}, such as the disruption of endothelial–epithelial barriers and adhesion of leukocytes to endothelial cells²³¹, in addition to their implication in the regulation of haematopoiesis²³², platelet aggregation²³³ and T cell physiology^{234,235}. Although a number of EPH and ephrin proteins have been implicated in these processes, the two interactions emerging from a review of the literature²³⁰ as prime candidates for therapeutic intervention are the interactions of EPHA2 with ephrin A1 and of EPHB4 with ephrin B2. This is not surprising given their important roles in the adult vascular system and the crucial role of the microvasculature in inflammation²³⁶. Ephrin A1 was originally described as a tumour necrosis factor response gene that is expressed in endothelial cells²³⁷ and was only later identified as the first known EPHA2

Demyelinating disease
A condition that results in damage to the protective myelin sheath surrounding nerve fibres in brain and spinal cord. This damage to the myelin sheath results in significant slowing or stopping of signal transmitting capacity, in turn causing deficiency in sensation, movement or cognition.

Table 3 | Summary of potential EPH- and ephrin-based therapeutic targets and emerging preclinical therapeutics

Candidate therapeutic	Disease targeted	Mechanism targeted	Potential for translation	Refs
EPH–ephrin fusion proteins				
EPHA4–Fc	Central nervous system and spinal cord injury	EPH–ephrin-mediated inhibition of nerve regrowth	Recombinant protein shown to be safe and effective in two animal models	206,207
EPHA4–Fc	Demyelinating disease (EAE)	Potential to promote nerve regeneration after demyelinating lesion	Marginal efficacy, potentially due to poor penetration into lesions	223
Ephrin A5–Fc	Spinal cord injury	EPH–ephrin-mediated inhibition of nerve regrowth	Effective but some stability issues might preclude development	208
Ephrin A1–Fc	Myocardial injury	Reduced cardiomyocyte apoptosis secondary to inflammatory response	Poor penetration into infarcted lesion might prevent effective treatment. Potential use post-reperfusion	245
Ephrin A1–Fc	Renal reperfusion injury	Vascular leak and leukocyte extravasation leading to tissue damage	Currently, only proof-of-concept data are available; recent findings support testing of EPH–ephrin-based inhibitors in reperfusion injury	244
Clustered ephrin A1–Fc	Parkinson's disease	Proliferation of dopaminergic neurons	Translational challenges owing to requirement for clustered agonist and localized, intraventricular injection	225
Ephrin B1–Fc	Arthritis	Multiple targets in the inflammatory process	Effects were consistent with agonist activity but indicate that targeting EPH–ephrin interactions can modulate inflammatory arthritis	253
Peptide inhibitors				
EPHA4 antagonist peptide	Spinal cord injury	EPHA4-mediated inhibition of nerve regeneration	Only suitable for direct infusion at injury site. Proof of principle only in present form	210
EPHA4 antagonist peptide	Motor neuron disease	Preservation of motor axons	Intracerebroventricular injection into rat brain delays motor neuron disease onset and prolongs survival	228
Antisense methods				
Antisense EPHA7 oligonucleotides	Spinal cord injury	EPH–ephrin-mediated inhibition of nerve regrowth	Not translatable in the form used but provides proof of principle for gene knockdown approaches	212

EAE, experimental autoimmune encephalomyelitis; EPH, ephrin receptor.

ligand²³⁸. EPHA2 induction by thrombin-mediated endothelial activation has a role in inducing intercellular adhesion molecule 1, which is a key ligand for integrin-mediated leukocyte attachment to the activated endothelium²³⁹. Moreover, increased EPHA2 expression on activated endothelium was shown to promote vascular cell adhesion molecule 1 expression and monocyte adhesion²⁴⁰. Induction of EPHA2 has also been shown to destabilize adherens junctions, thereby allowing leukocyte egress into the extracellular space and increasing the leakiness of the endothelial barrier^{241,242}. These findings provide a case for therapeutic targeting of the EPHA2–ephrin A1 interaction. In a model of ischaemic stroke-induced brain injury, EPHA2-null mice exhibited reduced disruption to the BBB and reduced severity of brain injury²⁴³. EPHA2 also seems to have a crucial role in renal ischaemia–reperfusion injury²⁴⁴. More directly relevant to therapeutic development, ephrin A1–Fc significantly reduced leukocyte infiltration and myocyte damage in a mouse model of myocardial infarction and significantly decreased infarct size and functional deficit²⁴⁵. In a rat model of lung injury, treatment with either EPHA2–Fc or an anti-EPHA2 antibody reduced lung oedema²⁴⁶.

There is also considerable interest in ephrin B2, which is a crucial player in vascular patterning during development²⁴⁷ and has on-going roles in adult physiological and pathophysiological endothelial functions; this suggests that it has potential as a therapeutic target in inflammatory diseases^{236,248}. Ephrin B2 activity is in part determined by interaction with the VEGF receptor⁶³, and one promising inhibitor is a human Fv single-chain antibody that effectively blocks VEGF-induced neoangiogenesis¹⁴⁹. Apart from mediating the vascular responses in inflammation, endothelial ephrin B2 was reported to facilitate transmigration of monocytes into inflammatory lesions²⁴⁹. Thus, therapies that inhibit the function of ephrin B2 would suppress a number of components of the inflammatory response. Haemorrhagic fever viruses enter endothelial cells by binding ephrin B2 and ephrin B3, which have been identified as cell surface receptors for the Henipaviruses^{250,251}. These include Nipah and Hendra viruses, both of which cause haemorrhagic fever in humans with a very high mortality. Agents that target the virus interaction with ephrin B2 and thus prevent endothelial cell binding and virus entry therefore have therapeutic prospects for these deadly infections.

This notion is supported by the finding that virus attachment and entry seem to be inhibited by soluble ephrin B2 *in vitro*^{250,251}.

Other EPH and ephrin proteins that are essential to the inflammatory process, including post-injury platelet adhesion and the release of pro-inflammatory molecules from platelet granules, are EPHA4, EPHB3 and ephrin B1, which have crucial roles in late-phase platelet aggregation and granule release^{233,252}. Given the plethora of anti-platelet agents currently available, a competitive role for EPH–ephrin inhibitors seems unlikely, although agents that block only the late events of platelet activation might have a better safety profile in terms of bleeding complications and provide the grounds for further investigation.

Interestingly, ephrin B1 activation of EPHB1 has been implicated in rheumatoid arthritis and the corresponding animal model, collagen-induced arthritis²⁵³. Ephrin B1–Fc was shown to enhance lymphocyte migration, promote tumour necrosis factor- α production from lymphocytes and stimulate the release of interleukin-6 from synoviocytes. Although increased EPHB1 phosphorylation suggested that the Fc protein seemed to act as an agonist rather than an antagonist, these studies raise the potential for ephrin B1 inhibitors in the treatment of inflammatory arthritis.

The optimal strategy for any EPH or ephrin-based therapeutic approach in inflammation is likely to be dictated by the panel of EPHs and ephrins expressed in normal tissues and in pathological circumstances^{254,255}. The EPH and ephrin family members discussed above are prime targets for therapy, although highly specific agents might be less effective than soluble forms of EPH or ephrin proteins acting as antagonists or decoy proteins. The redundancy of EPH–ephrin interactions — for example, soluble ephrin A1 can effectively block functions of all EPHA receptors — could mean that such a therapy is preferable to specific small molecule inhibitors or mAbs.

EPHs in tissue repair and remodelling

The emerging evidence of the crucial involvement of EPH and ephrin in the homeostasis, repair and remodelling of various adult tissues is of little surprise considering their extensive roles in embryonic tissues.

EPH–ephrin signalling has been implicated in various aspects of mammalian skin physiology: several family members are expressed in a hypoxia-regulated manner²⁵⁶ in the epidermis, hair follicles, sebaceous glands and underlying dermis²⁵⁷. In line with a role in controlling tissue homeostasis, lack of EPHA2 leads to increased proliferation of basal epidermal cells *in vivo* and of primary keratinocytes *in vitro*, and EPHA2-knockout mice are significantly more susceptible to chemical-induced skin carcinogenesis¹¹⁸. Expression of several EPHs is found together with the stem cell-associated marker CD34 in the bulge area in resting and growing hair follicles⁸¹. Interestingly, systemic injection of ephrin A2–Fc, monomeric ephrin A2, ephrin B2–Fc or EPHA7–Fc as a pan-specific antagonist of EPH–ephrin signalling results in increased proliferation within hair follicles and the basal layer of the epidermis, suggesting that

EPH–ephrin antagonists could be potential therapeutics for regenerative strategies in the skin⁸¹. Furthermore, increased EPHA2 expression in psoriatic lesions suggests that EPH–ephrin signalling is likely to be implicated in inflammatory skin diseases such as psoriasis and atopic dermatitis²⁵⁷.

Signalling between EPHB receptors and B-class ephrins is essential for skeletal patterning^{16,17}, and ephrin B1 loss-of-function mutations are the underlying cause of craniofrontonasal syndrome (CFNS), an X-linked developmental disorder that, in severe cases, results in frontonasal dysplasia and fusion of the coronal sutures in females⁶⁰. A recently completed clinical trial (Clinical Trials.gov identifier: NCT00339846) (TABLE 2) assessed the correlation between CFNS and ephrin B1 gene mutations in a total of 42 patients with spontaneous or inherited CFNS. Publication of the outcome of this trial is awaited.

In addition to its crucial role in skeletal patterning, EPH–ephrin signalling controls cellular interactions that are essential for bone growth and maintenance⁷⁴. Most EPH and ephrin members are expressed during osteoblast differentiation, whereas in osteoclasts, expression is limited to ephrin B1, ephrin B2, ephrin A2 and EPHA2 (REF. 74). Bidirectional ephrin B2–EPHB4 signalling provides the crucial coupling between osteoclastic and osteoblastic activities that maintains bone homeostasis — ephrin B2 reverse signalling into osteoclast precursors suppresses their differentiation, whereas EPHB4 forward signalling into osteoblasts enhances osteogenic differentiation⁷⁸. Interestingly, ephrin B2 expression in differentiating osteoblasts is regulated dose-dependently through the activation of parathyroid hormone receptor 1 (PTH1R) by PTH on the osteoblast cell surface²⁵⁸, suggesting that the anabolic PTH function might be mediated by EPHB4 signalling. Of note, EPHB4 is also expressed in osteoarthritic subchondral bone osteoblasts and osteocytes, where ephrin B2-activated EPHB4 forward signalling seems to inhibit abnormal bone resorption activity, suggesting therapeutic opportunities for an EPHB4 agonist in osteoarthritis²⁵⁹.

Ephrin B2–EPHB4 interactions also seem to have a crucial role in lung growth and repair. Superoxide-induced alveolar growth arrest in newborn rats and the resulting loss of lung capillaries and pulmonary hypertension is associated with decreased expression of ephrin B2 and EPHB4. In agreement, intranasal siRNA-mediated ephrin B2 silencing during postnatal alveolar development arrests alveolar and vascular growth, suggesting the application of EPHB4 agonists to prevent alveolar damage and pulmonary hypertension²⁶⁰.

Challenges of targeting EPHs and ephrins

Two decades after their initial discovery as orphan receptor tyrosine kinases, EPHs and ephrins are increasingly being recognized as therapeutic targets in a growing list of diseases, not surprisingly foremost in cancer. However, despite intense research and substantial progress in the understanding of some of the mechanisms underlying the biological effects of EPHs and ephrins,

we are still far from being able to reliably decipher their precise mechanisms of action and to confidently predict the outcomes of their modulation. A major reason for this uncertainty is the unusual complexity of EPH–ephrin signalling mechanisms, whereby both of the interacting cells receive interdependent signals from the same signalling complex, which in turn may contain several contributing EPH family members that often associate with other receptor tyrosine kinases. However, it is now undisputed that underlying the characteristic ability of EPHs to trigger diametrical opposing responses is an ability to initiate kinase-dependent and kinase-independent signalling pathways that can be differentially activated in a cell or tissue context-dependent manner. Thus, inhibiting kinase activity might be therapeutically beneficial in some settings, whereas in others the opposite might be useful. So how is it possible to effectively target such a complex signalling system for therapeutic benefit? There is now increasing evidence from a large number of preclinical studies, strongly supported by disease-specific genome profiling, that in many of the pathological conditions only a select number of EPHs or ephrins are driving the disease, even if other family members are present in the same cells. It seems that therapeutic targeting with EPH or ephrin exodomains, which until recently was thought to lack the required specificity for therapeutic targeting, seems to be a feasible approach in a number of applications. The remaining challenge — how to devise strategies that target pathological EPH functions without causing detrimental consequences in non-involved tissues or organs — will require rigorous analysis and consideration of corresponding expression patterns and activities in the

whole organism, rather than only in the target tissue. In this regard, EPHs are not different to any other therapeutic target with a defined therapeutic window, but it is now clear that their potent and essential biological functions during development often remain in adulthood, so that systemic ablation of their expression or function might lead to unwanted side effects.

Concluding remarks

Their crucial roles in tumour biology and in a range of non-malignant diseases have defined EPHs and ephrins as promising therapeutic targets, and their recently established functions in cell proliferation, survival and stem cell biology have further expanded the scope for clinical use of EPH-targeted drugs. Initial clinical experiences with EPH-targeted anticancer drugs, which are currently leading the drug development path, have taught us that — in line with their divergent roles — successful therapeutic strategies will vary for different EPH family members. Thus, promising clinical performances of an EPHA3-agonist antibody in Phase I trials and of a small-molecule multi-kinase inhibitor in Phase II trials foreshadow the scope for a range of EPH-targeted cancer therapeutics that are currently in late-stage preclinical development. Clearly, the clinical success of any of these drugs will rely on the comprehensive understanding of the signalling concepts underlying EPH and ephrin functions in a particular disease context. However, together with emerging clinical experiences with EPH-targeted therapeutics, this knowledge will pave the way for their clinical exploitation in non-malignant diseases, such as neurological disorders, tissue regeneration, inflammation and pathological neoangiogenesis.

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We respectfully dedicate this Review to the memory of Tony Pawson, a pioneering discoverer of tyrosine kinase function and a founder of the EPH kinase field.

Competing interests statement

The authors declare [competing interests](#): see Web version for details.

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