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Current Opinion in Cell Biology

Ephrins and Eph receptors in stem cells and cancer

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Eph tyrosine kinase receptors and their ephrin ligands are expressed in most adult stem cell niches and in many types of tumors. They maintain tissue homeostasis by controlling the proliferation of stem and progenitor cells, although in divergent ways in different tissues. Eph receptors can also act as both tumor promoters and suppressors in different contexts. The recent characterization of the signaling pathways employed by Eph receptors has resulted in new suggestions for therapeutic strategies.

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Current Opinion in Cell Biology 2010, **22**:611–616

This review comes from a themed issue on
Cell-to-cell contact and extracellular matrix
Edited by Peter Friedl and Jennifer Zallen

0955-0674/$ – see front matter

DOI 10.1016/j.ceb.2010.08.005

Introduction

Eph receptors constitute the largest family of tyrosine kinase receptors with 14 members in mammals. Their ephrin ligands are membrane bound, restricting the interaction to sites of direct cell-to-cell contact. Ephs and ephrins are widely expressed during embryogenesis and regulate developmental processes such as axon guidance, angiogenesis, and boundary formation [1]. Many of the effects are mediated by signaling cascades modulating the actin cytoskeleton, affecting cell adhesion or cell movement, although they also may control cell survival, proliferation, and differentiation [2]. More recently, there has been an increasing interest in their role in regulating the physiology of adult organs and they have been implicated in the pathogenesis of several diseases. In particular, an increasing number of studies point to roles for Ephs and ephrins in the regulation of adult stem cell function as well as in tumorigenicity.

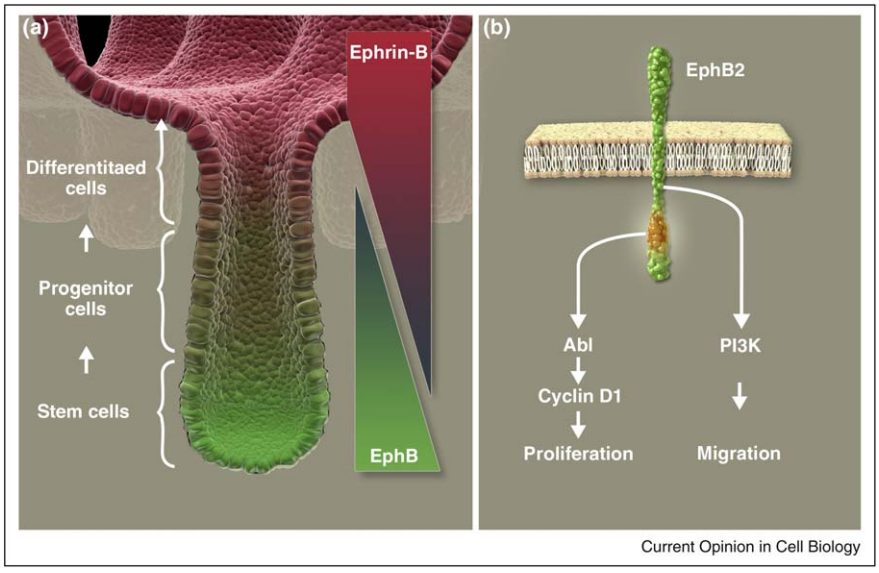
Ephrins and Eph receptors are each divided into two classes based on sequence homology and binding specificity. EphA receptors bind glycosylphosphatidylinositol-anchored ephrin-A ligands and EphB receptors bind transmembrane ephrin-B ligands, although interclass binding has been demonstrated [3–5]. Eph receptors have not only a high affinity ephrin-binding domain allowing for direct interaction between receptors and ligands upon cell contact, but also two sites of lower ephrin affinity, which are believed to facilitate higher order clustering of Eph–ephrin complexes [2]. The interaction of Eph receptors with ephrins can result in bidirectional signaling, where signals are conveyed not only into the receptor expressing cell (forward signaling) but also into the ligand expressing cell (reverse signaling) [6,7]. Forward as well as ephrin-B mediated reverse signaling can be mediated through tyrosine phosphorylation of intracellular residues, whereas the mechanism of ephrin-A reverse signaling is less clear, but probably requires a transmembrane co-receptor [8••]. The outcome of the ephrin–Eph interaction can be modulated at several levels, for example by varying degrees of multimerization of ligand–receptor complexes, which together with both kinase-dependent and independent signaling and forward and reverse signaling adds complexity and may explain why the physiological effect varies with cellular context.

Stem cells

Ephs and ephrins are commonly expressed in adult stem cell niches, although the large number of members of each family does not allow for the pinpointing of a specific receptor or ligand as being more commonly expressed than others. Most studies have to date focused on the nervous system and the intestine, although EphB4 and ephrin-B2 are expressed in a complementary pattern in the mammary gland [9], where they are implicated in the development of the mammary epithelium [10]. Moreover, hair follicle bulge stem cells express high levels of EphA4, EphB4, and ephrin-B1 [11], and EphA2 and ephrin-A1 are expressed in a complementary pattern in the epidermis allowing for receptor–ligand interaction only at the proliferative basal layer of the epidermis [12]. Both the A and B classes negatively regulate proliferation of hair follicle and epidermal progenitor cells in the adult mouse [13]. It is not yet known whether this is mediated by forward or reverse signaling.

Neural stem/progenitor cells are influenced by Eph–ephrin signaling both during development and in adulthood. Genetic ablation of ephrin-B1 in neuroepithelial progenitor cells results in cell cycle exit and consequent loss of neural progenitor cells during cortical neurogenesis [14••]. The two neurogenic areas of the adult brain, the subventricular zone (located subjacent to the ependymal layer in the lateral ventricle wall) and the dentate gyrus of the hippocampus, display complementary expression

Figure 1



EphB signaling regulates both proliferation and migration in the adult intestinal stem cell niche. **(a)** Stem cells reside at the bottom of the crypt, where they divide and give rise to progenitor cells, which migrate up the crypt as they differentiate. Stem cells express high levels of EphB receptors (green), whereas the more differentiated cells express ligand (red). **(b)** EphB2 regulates proliferation in a kinase-dependent manner through Abl and cyclin D1, whereas migration is mediated in a kinase-independent signaling cascade through PI3-kinase.

patterns of receptors and ligands. Progenitor cells and neuroblasts in the subventricular zone express ephrin-A2, whereas quiescent ependymal cells, as well as some GFAP-positive putative stem cells, express EphA7. EphA7 induces ephrin-A2 reverse signaling, negatively regulating adult neural progenitor cell proliferation and the addition of new neurons in the olfactory bulb [16]. Furthermore, cells in the subventricular zone express all three ephrin-B ligands, as well as EphB1, EphB2, and EphB3. Blocking the interaction between B class ephrins and Eph receptors leads to an increase in the number of dividing cells in the subventricular zone [15,16], and genetic removal of ephrin-B3, results in increased progenitor cell proliferation in the adult lateral ventricle wall [17]. p53 negatively regulates the self-renewal of neural stem/progenitor cells in the subventricular zone [18], and EphB3 may repress proliferation by increasing p53 expression [19]. Thus both the A and B classes negatively regulate neural progenitor proliferation in the adult subventricular zone. Hippocampal neural stem and progenitor cells present in the subgranular layer of the dentate gyrus express EphB1 receptors and EphB forward signaling positively regulates neurogenesis and migration of progenitor cells when stimulated by the ephrin-B3 expressing mature granule cells in the dentate gyrus inner molecular layer [20••]. Increased neurogenesis in the dentate gyrus of ephrin-A5 null mice indicates a similar role also for A class Eph receptors [21]. In addition to influencing the neural stem/progenitor cells in the adult brain, EphB signaling also regulates the phenotype of niche cells and plays a critical role in the maintenance and self-renewal of the stem cell niche [22••].

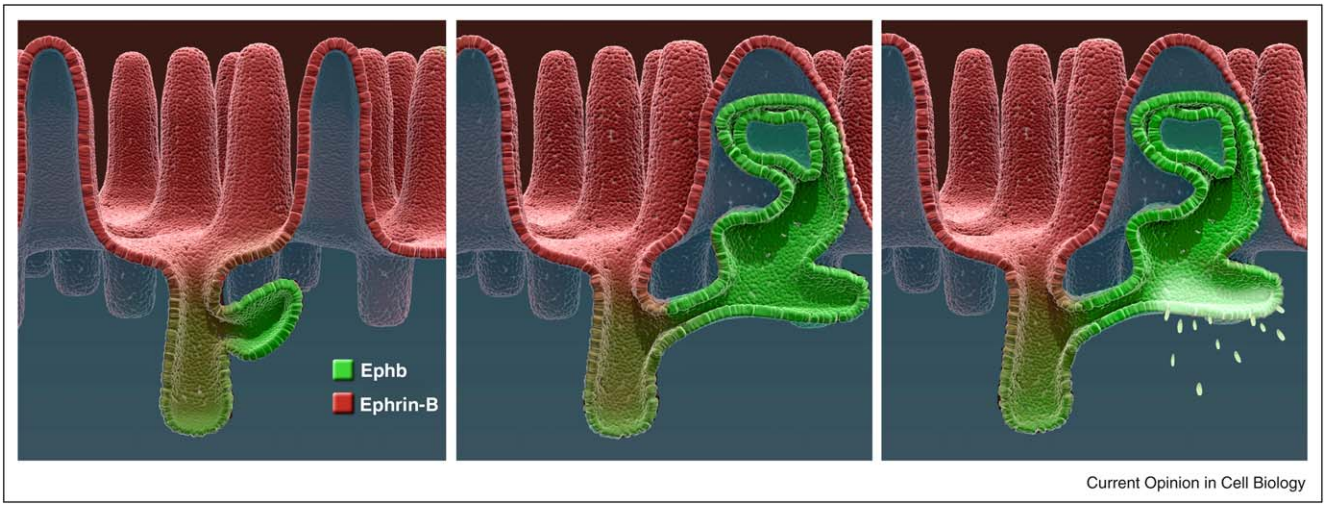
Stem cells in the intestine are situated at the bottom of the crypts in both the small intestine and colon, where they divide frequently and give rise to progenitor cells, which continue to go through the cell cycle as they migrate up the crypt [23••]. As cells exit the crypt, they become postmitotic and differentiate. Stem and progenitor cells in the adult intestinal crypts express high levels of EphB receptors whereas more differentiated cells express ephrin-B ligands resulting in an Eph–ephrin counter gradient (Figure 1a), orchestrated by restricted Wnt production at the bottom of the crypts [24,25]. EphB forward signaling regulates the directed migration of progenitor cells up the crypt and differentiated Paneth cells to the crypt bottom [24,26], through an EphB kinase-independent signaling cascade mediated via PI3-kinase [27••]. In parallel, EphB receptors positively regulate progenitor cell proliferation via Abl and cyclin D1, a pathway dependent on the kinase activity of the EphB receptor [27••] (Figure 1b).

Cancer

The expression of Ephs and ephrins is frequently altered in tumors when compared to the tissue of origin. There is evidence for Eph receptors both promoting tumorigenesis and acting as tumor suppressors in different contexts.

The tumor promoting roles of Ephs in breast cancer have been rather extensively studied. Both EphA2 and EphB4 levels are elevated in human breast cancer as compared to the normal mammary epithelium [29,28] and high EphA2 expression levels are associated with a poor prognosis

Figure 2



EphB receptors have dual roles acting both as tumor promoters and tumor suppressors. Adenomas arise as a consequence of mutations in stem and progenitor cells in the intestinal crypts. Tumor cells express high levels of EphB receptors (green) and are highly proliferative. Outpocketings are formed by the tumor cells from the normal crypt into the surrounding stroma (left panel), which eventually fold under the normal, untransformed ephrin-B (red) expressing villi (middle panel). As tumors progress, the EphB expression is lost, and the tumor cells gain the ability to invade the surrounding tissue (right panel).

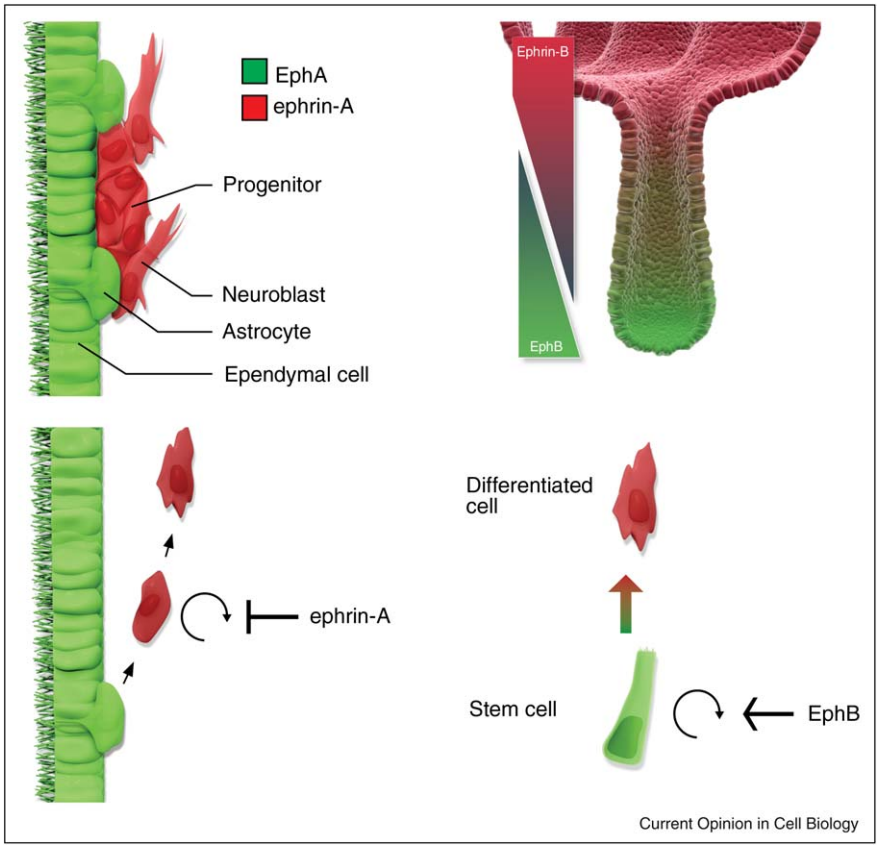
[31,30]. Loss of EphA2 or overexpression of EphB4 in a MMTV-Neu breast cancer transgenic mouse model impairs or accelerates tumor initiation and lung metastasis, respectively [10,32•], corroborating a role for Eph receptors in mammary tumor promotion. Surprisingly, administration of recombinant ligand for EphB4 inhibits tumor growth *in vivo* as well as growth and migration of breast cancer cells [33], suggesting that EphB4 functions as a tumor suppressor when activated by its ligand and that the tumor promoting functions of Eph receptors might be ligand-independent in this context. Similarly to the situation in breast cancer, EphA2 expression is increased in epidermal tumors. Despite this, EphA2 null mice exhibit increased epidermal tumor susceptibility when exposed to carcinogens and an accelerated rate of growth and progression to malignancy [12].

EphB receptor expression is commonly lost in colon carcinoma [34] and this correlates with poor survival in patients [35,36]. The intestine is, however, one example where EphB receptors have been clearly demonstrated to have dual roles, acting both as tumor promoters and suppressors in the same organ, although at different stages of tumor progression. Tumor initiation starts with gain-of-function mutations in the canonical Wnt signaling pathway, leading to a direct transcriptional upregulation of EphB receptors in tumor cells. As the mutated EphB positive adenoma cells expand, they bud out from the crypt, forming outpocketing pouches into the surrounding stroma [37] eventually resulting in a large continuous, but folded, layer of transformed cells under the untransformed villi cells (Figure 2). The transformed layer of EphB positive cells is repelled by the surrounding ephrin-B expressing villi cells, resulting in *in situ* adenoma growth [24,38•]. The same EphB–Abl–cyclin D1 proliferation signaling cascades present in normal untransformed epithelium still drives proliferation of adenoma cells [27••]. As tumor development progresses, EphB receptor expression is commonly lost and concomitantly cells gain the ability to invade the surrounding tissue (Figure 2). Reduced EphB activity accelerates the progression of colorectal cancer, since carcinomas develop in APCmin mice (a model for familial adenomatous polyposis) lacking EphB3, ephrin-B1 or expressing a dominant negative EphB2 receptor [34,38•]. EphB2 has also been implicated as a tumor suppressor in prostate cancer [39], consistent with a causal role for Eph receptors functioning as tumor suppressors in this context. Because of the accumulation of mutations in cancer cells, the EphB receptors are no longer necessary for the regulation of the tumor promoting EphB–Abl–cyclin D1 pathway, and carcinomas are able to maintain proliferation despite the downregulation of EphB receptors. Thus, EphB receptors in the intestine have dual roles, working both as tumor promoters, driving proliferation in adenomas, and as tumor suppressors, since downregulation of EphBs accelerates progression to carcinoma.

Conclusions

The result of the ephrin–Eph interaction is remarkably divergent in different contexts. The same molecules can promote proliferation of stem/progenitor cells in one

Figure 3



Ligand or receptor signaling regulates proliferation in adult stem cell niches. In the adult subventricular zone (SVZ) of the lateral ventricle wall, ependymal cells and astrocytes express EphA7 (green), whereas ephrin-A2 (red) is expressed by progenitors and neuroblasts (top left panel). Proliferation of progenitor cells is negatively regulated by EphA7-induced reverse ephrin-A2 signaling (bottom left panel). Intestinal crypts express EphB receptors and ephrin-B ligands in counter gradients, where stem and progenitor cells express high level of receptor, and more differentiated cells express ligands (top right panel). Forward EphB signaling promotes proliferation of stem and progenitor cells in the crypt (bottom right panel).

tissue and inhibit in another and can even act as a tumor promoter and suppressor within the same tissue.

When comparing the intestine and the two adult neurogenic niches in the adult brain, a common feature is that the initial cells in the lineage express Eph receptors and the more differentiated cells express ephrins (Figure 3). In spite of this similarity in expression pattern, the ephrin–Eph interaction promotes proliferation in the intestine and in the hippocampus, whereas it inhibits proliferation in the adult lateral ventricle wall. A difference between these systems that may explain this divergent outcome is at what position in the cellular lineage most of the cell proliferation occurs (Figure 3). In the intestine and hippocampus, almost all proliferation takes place in the Eph expressing initial cells in the lineage, whereas in the lateral ventricle wall neurogenic lineage most mitoses are in the ephrin expressing more differentiated transit amplifying cells. In the intestine and hippocampus, forward signaling promotes proliferation whereas reverse signaling in the lateral ventricle wall inhibits proliferation. The divergent outcomes of the ephrin–Eph interaction may thus be a result of where in the lineage the main cellular expansion takes place and whether reverse or forward signaling dominates (Figure 3).

The role of ephrins and Ephs in regulating both cell renewal from stem/progenitor cells in adult tissues and tumor progression will surely spur much further interest. Elucidation of the signaling pathways that ephrins and Ephs employ to regulate the generation of new cells from stem/progenitor cells in the adult and to influence tumorigenesis may contribute to the development of new therapeutic strategies in regenerative medicine and cancer.

Acknowledgements

Work in the authors' laboratory was supported by grants from the Swedish Cancer Society, Swedish Research Council, the Karolinska Institute, Tobias Stiftelsen and Knut och Alice Wallenbergs Stiftelse.

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