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Ephs and Ephrins in Cancer: Ephrin-A1 Signaling

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Abstract

Ephrin-A1 and its primary receptor, EphA2, are involved in numerous physiological processes and have been intensely studied for their roles in malignancy. Ephrin-Eph signalling is complex on its own and is also cell-type dependent, making elucidation of the exact role of ephrin-A1 in neoplasia challenging. Multiple oncogenic signalling pathways, such as MAP/ERK and PI3K are affected by ephrin-A1, and in some cases evidence suggests the promotion of a specific pathway in one cell or cancer type and inhibition of the same pathway in another type of cell or cancer. EphrinA1 also plays an integral role in angiogenesis and tumor neovascularization. Until recently, studies investigating ephrins focused on the ligands as GPI-anchored proteins that required membrane anchoring or artificial clustering for Eph receptor activation. However, recent studies have demonstrated a functional role for soluble, monomeric ephrin-A1. This review will focus on various forms of ephrin-A1-specific signalling in human malignancy.

Keywords

Ephrin; Eph; Receptor; Ligand; Signalling; Angiogenesis

1. Introduction

Since their discovery, ephrins and Ephs have been extensively studied for their role in normal physiology and development. Initial indications of ephrin-A1 upregulation during melanoma progression [1] and *eph* overexpression in multiple human malignancies pointed toward the Eph/ephrin family as important players in tumorigenesis [2]. Ephrin-A1, the first ephrin protein, was discovered in 1990 as a novel TNF-inducible protein in human umbilical vein endothelial cells (HUVECs) [3], but it was not until 1994 that it was identified as a ligand for the EphA2 receptor, which was at that time considered an orphan receptor tyrosine kinase (RTK) since its discovery in 1987 [4, 5]. Several reviews have been published specifically focusing on EphA2 and ephrin-A1 in carcinogenesis as well as outlining ways in which the ephrin-A1/EphA2 system can be utilized for cancer therapies [6–9]. In this review, we will describe more in detail the role of ephrin-A1 in signalling

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events potentially leading to the initiation and progression, or inhibition of human malignancy.

2. Ephrin-A1 structure-function relationship

The ephrin family consists of eight members, divided into A and B subclasses based on their mode of cell membrane attachment. Ephrin-A1-A5 are linked to the membrane via a Glycosylphosphatidylinositol (GPI) moiety, while ephrin-B1-B3 are anchored by a transmembrane domain and contain a cytoplasmic tail [10]. Due to their membrane localization, ephrins are able to engage in both forward and reverse signalling [11]. While more is known about reverse signalling through the ephrin-B cytoplasmic domain, recent studies are beginning to shed light on the mechanism by which members of the ephrin-A family are able to induce reverse signalling within cells of the central nervous system [11, 12].

Eph receptors comprise the largest family of receptor tyrosine kinases and, like their ligands, are divided into two groups, A and B. Unlike the ephrins, however, their subclass division is based on sequence homology of their extracellular domains. In general, EphA receptors bind to ephrin-A ligands, and EphB receptors to ephrin-B ligands. Some exceptions to this rule include a functional ephrin-A5-EphB2 interaction [13] and EphA4 binding to both ephrin-A and ephrin-B family members [14, 15]. Ephrin-A1 exerts its function largely through interactions with EphA2. Based on the recently solved crystal structure of the ephrin-A1 and EphA2 complex, the G-H loop of ephrin-A1, a highly conserved region of 15 amino acids that connects the G and H β -strands, is inserted into a channel on the surface of EphA2 to form a heterodimeric, 1:1 ligand/receptor complex [16]. Ligand binding of ephrin-A1 induces EphA2 autophosphorylation and interaction with c-Cbl followed by internalization and degradation of the receptor [17].

3. Ephrin-A1 expression in malignancy

In addition to playing an important role in normal cellular processes, ephrin ligands and Eph receptors have come under intense scrutiny for their roles in human malignancy. Paradoxically, ephrin-A1 and EphA2 have been shown to influence both tumor initiation and progression [8, 9, 18]. Ephrin-A1 and EphA2 are upregulated during melanoma progression [1], and high expression of the receptor and ligand have been correlated with poor patient survival in ovarian cancer [19]. Similar increased expression has been reported in bladder [20], gastric [21], and cervical cancer [22]. Interestingly, ephrin-A1 expression is evident at varying levels in esophageal carcinoma, while EphA2 is overexpressed and correlates with lymph node metastases and poor patient survival [23].

In breast cancer and in glioblastoma multiforme (GBM), a brain tumor of dismal prognosis, ephrin-A1 and EphA2 are differentially expressed. Ephrin-A1 is downregulated in cell lines and patient specimens and EphA2 is highly overexpressed [24], a pattern which correlates with more invasive and tumorigenic breast cancer cells [25]. Additionally EphA2 is associated with higher astrocytoma grade [26] and decreased patient survival [27]. Additionally, there is a correlation between increased EphA2 mRNA levels in Her2-positive breast cancer patients and a decrease in overall and disease-free survival [28]. Similarly, EphA2 is upregulated in pancreatic cancer [29] and renal carcinoma [30], as well as in lung cancer, in which increased expression correlates with shorter patient survival and is a predictor of brain metastasis [31]. Moreover, EphA2 knockdown inhibits migration and proliferation in non-small cell lung cancer (NSCLC) cells [32]. EphA2 has also been studied and proposed as a therapeutic target in colorectal cancer [33], adding to the plethora of malignancies in which ephrin-A1 and its preferred receptor play a pivotal role.

4. Evidence for functional, soluble ephrin-A1

Previous studies investigating the function of ephrin-A1 and EphA2 have focused on the ligand as a membrane-bound, GPI-anchored protein capable of mediating juxtacrine signalling and requiring membrane attachment or clustering/oligomerization [34]. This requirement was thought to be due to the necessity of Eph receptors themselves to undergo clustering in order to be activated [35]. This review underlines the importance of a functional form of ephrin-A1 that is released into the extracellular environment. Until recently, there has been no documented evidence of a functional, soluble, monomeric form of any member of the ephrin-A family. In fact, soluble, unclustered ephrin-A5 was shown to stimulate weak autophosphorylation of EphA5 and proposed to be an antagonist of axon bundling [36]. Ephrin-A1 was found to be released into the cell medium after interaction with exogenous EphA2-Fc, but functionality was not tested [37]. Therefore, most experimental studies utilize a homodimeric recombinant chimeric ephrin fused to the Fc of IgG. Our lab has recently presented evidence for the existence of a functional, soluble, unclustered monomeric ephrin-A1 [38]. This monomer is believed to be cleaved from the plasma membrane of ephrin-A1-expressing cells and acts in a similar fashion to the recombinant homodimeric ephrin-A1-Fc. Monomeric ephrin-A1 induces phosphorylation and internalization of EphA2, elicits morphological changes in tumor cells, and causes a decrease in the oncogenic potential of GBM cells [38]. A subsequent study from another group identified soluble ephrin-A1 released from HeLa and SK-BR3 cells and demonstrated its importance for cell growth and transformation [39].

Importantly, this study shed some light on the seemingly contradictory role of ephrin-A1 in both tumor promotion and inhibition. Ephrin-A1 was found to promote or inhibit growth in a manner dependent on whether ephrin-A1 was presented to the receptor as soluble or membrane bound, respectively [39]. In support of these findings, ephrin-A1 has also been found in the serum of patients with hepatocellular carcinoma, confirming its existence as a soluble form and suggesting a possible role of ephrin-A1 as a biomarker for human malignancy [40]. However, as these are recent discoveries, most experiments described in subsequent sections of this review utilized the artificially dimerized form of ephrin-A1, ephrin-A1-Fc. One would expect practically only monomeric ephrin-A1 to exist long-term, even after addition of homodimeric ephrin-A1-Fc to cell culture due to ephrin-A1 proteolytic cleavage. Therefore, at least part of the long lasting effect of ephrin-A1-Fc is most likely due to the activity of monomeric ephrin-A1.

5. Ephrin-A1-independent functions of EphA2

Multiple studies have documented low levels of EphA2 phosphorylation in malignant cells compared to normal cells despite its overexpression [7]. In addition to a deficiency in cell-cell contact, which is common in cancer cells, a lack of sufficient amounts of ephrin-A1 on tumor cells could result in the decrease in EphA2 phosphorylation [7, 24]. Evidence suggests that in cases with sufficient ligand and receptor expression, EphA2 is activated by ephrin-A1 and phosphorylated, but is quickly acted upon by a phosphatase such as low molecular weight protein tyrosine phosphatase (LMW-PTP), contributing to the lack of detectable EphA2 phosphorylation in tumor cells [41]. EphA2 appears to play a role in tumorigenesis in its non-phosphorylated state and possesses ligand-independent kinase activity *in vitro* [42–44]. EphA2 mediates ligand-dependent inhibition and ligand-independent promotion of prostate cell migration and invasion [44]. The ligand-independent effects are a result of EphA2 phosphorylation on Serine897 by activated AKT (Fig. 1A). Ephrin-A1 stimulation causes S897 dephosphorylation and simultaneous inactivation of Akt, inhibiting lamellipodium protrusion and cell migration (Fig. 1B) [44]. This model explains how an overexpressed, albeit largely unphosphorylated RTK can have tumorigenic effects in

the absence of ligand stimulation and provides an explanation for the paradox of EphA2 as an oncogene and a tumor suppressor. In endothelial cells, however, phosphorylation of conserved juxtamembrane tyrosines 587 and 593 of EphA2 are required for optimal kinase activity [45]. Therefore, the necessity of ligand-mediated phosphorylation of EphA2 for kinase activity is likely cell type dependent and/or depends on the expression and activity of Akt or other yet unidentified EphA2-phosphorylating proteins.

6. Ephrin-A1 signalling in inhibition and/or promotion of malignancy

6.1. Ephrin-A1 and cytoskeletal organization and cell migration

Ephrin-A1 and EphA2 play an important role in cell migration by influencing cell-cell and cell-extracellular matrix (ECM) interactions. EphA2 activation by ephrin-A1 decreases cell attachment to ECM and counteracts integrin signalling in multiple cell types leading to Rac-mediated upregulation of Rho activity [46, 47]. It has also been proposed that reactive oxygen species (ROS) play a role in this process whereby ephrin-A1 interaction with EphA2 leads to the downregulation of Rac1-dependent ROS production. A lack of ROS prevents the oxidation and inactivation of LMW-PTP, which dephosphorylates p190RhoGAP leading to the sustained activation of Rho and inhibition of cell adhesion and an increase in contractility and cell rounding [46].

Ephrin-A1-mediated activation of EphA2 has been shown to both inhibit [48, 49] and stimulate [50, 51] the MAP/ERK kinase signalling cascade. One proposed mechanism of stimulation is via interaction of ligand-activated EphA2 with the adaptor protein SHC, which binds GRB2 and elicits signalling involving activation and nuclear translocation of ERK [50]. ERK activation is transient and is concomitant with the destabilization of cell-ECM attachments in malignant mesothelioma cells [51] and breast cancer cells [50]. However, in prostate cancer and endothelial cells, ephrin-A1 stimulation of EphA2 leads to the inhibition of both basal and EGF-induced MAPK activity (Fig. 1B) [48, 52]. This disparity again highlights the importance of cell type and microenvironment in Ephrin/Eph signalling. Also of note, while Pratt *et al.* tested the effect of both ephrin-A1-Fc as well as an EphA2-specific monoclonal antibody in order to ensure the positive effect on the MAP/ERK pathway was ephrin-A1/EphA2 specific [50], the studies documenting negative regulation of the same pathway utilized only ephrin-A1-Fc [48, 49, 52]. Therefore, another possible explanation for such disparate results is the stimulation of other EphA receptors with ephrin-A1-Fc ligand.

Focal adhesion kinase (FAK), a nonreceptor tyrosine kinase involved in cell adhesion [53], contributes to crosstalk between Eph kinases and integrins, which likely plays an important role in the pro- and anti-migratory effects of the Ephrin/Eph system. In prostate cancer cells, unstimulated EphA2 constitutively associates with FAK. Upon ephrin-A1 stimulation the PTPase SHP2, or one of the myriad of PTPases interacting with focal adhesions, is recruited, resulting in dephosphorylation of FAK and its downstream effectors and disruption of the EphA2/FAK complex [54]. FAK inactivation induced by EphA2 stimulation contributes to the inhibitory effects of ephrin-A1 on cancer cell migration [54]. EphA2 promotes FAK-dependent invasion of pancreatic adenocarcinoma cells believed to be due to an increase in the expression of matrix metalloproteinase-2 (MMP-2), which is consistent with the known role of protease-mediated ECM degradation in the process of invasion. [55]. Treatment of EphA2-overexpressing cells with ephrin-A1, in addition to inhibiting invasion [54, 55], downregulates MMP-2 [55]. Additionally, transfection of high EphA2/ low ephrin-A1 expressing cells with full-length human ephrin-A1 results in the downregulation of malignant properties of cancer cells through the phosphorylation and downregulation of EphA2 [38, 56] and dephosphorylation and downregulation of FAK [56]. Thus, ephrin-A1-mediated inhibition of the oncogenic input to cancer cells likely involves multiple

mechanisms, including not only receptor downregulation and subsequent prevention of EphA2 involvement with FAK and other proteins, but also by direct signalling through EphA2.

There is also a suggested role for ephrin-A1 in cytoskeletal remodeling which involves FAK phosphorylation [47, 57–59]. Whereas Miao *et al.* demonstrated the rapid induction of cell rounding and de-adhesion upon ligand stimulation [54], Carter and colleagues provide evidence that ephrin-A1 stimulates cell adhesion and spreading [58]. In NIH3T3 cells grown on ephrin-A1 coated dishes, FAK, and p130^{cas} were tyrosine phosphorylated, and both FAK^{-/-} and p130^{cas-/-} MEFs were unable to spread on ephrin-A1, indicating the importance of functional FAK and p130^{cas} in ephrin-A1-mediated responses in fibroblasts [58]. The discrepancy in results could be due to a difference in ephrin-A1-mediated dynamics in fibroblasts as opposed to tumor cells. However, Carter *et al.* did not observe any decrease in cell adhesion and FAK phosphorylation in PC3 prostate cells [58], highlighting the possible differences in various clones of even the same cell line. Another possibility is that plating cells on immobilized ephrin-A1-Fc induces changes within cellular architecture that are not present when cells are plated, allowed to adhere, and then treated with ephrin-A1-Fc. In addition, ephrin-A1 leads to the recruitment and activation of an EphA2 associated Src-FAK complex in which ephrin-A1-mediated FAK phosphorylation is Src-dependent [47]. The cytoskeletal contractility and migratory response elicited by ephrin-A1 is driven by the suppression of Rac1 signalling [60] and the Rho-mediated phosphorylation of myosin light chain II [47, 60]. While these studies were unable to replicate previous studies showing FAK dephosphorylation in response to ephrin-A1 stimulation [54], cell rounding and cell body retraction were similarly reported [38, 47, 54]. Also of note, ephrin-A1 was found to stimulate cell attachment and FAK phosphorylation in human endometrial carcinoma-derived Ishikawa cells [57]. However, these results could conflict with previous data due to differences in receptor signalling in that in this study, ephrin-A1 treatment caused phosphorylation of EphA4 in addition to EphA2 [57].

6.2. Ephrin-A1 in proliferation/cell survival

In some cases, ephrin-A1 influences cancer cell proliferation and survival through the alteration of oncogenic signalling pathways directly downstream of EphA2, and in others as a result of crosstalk of EphA2 with other RTKs [61, 62]. EphA2 is capable of direct interaction with EGFR and is a gene target of EGFR oncogenic signalling through Src and MAPK [62]. As previously mentioned, EphA2 expression and stimulation by ephrin-A1 has effects on ERK activation [61] [62]. Moreover, EphA2 is a direct transcriptional target of the Ras-Raf-MAPK pathway (Fig. 1A) [63]. Interestingly, while Ras stimulates the transcription of EphA2, ephrin-A1 attenuates Ras activation in a negative feedback loop (Fig. 1B) [63, 64]. In fact, the MAPK pathway has been implicated in the inhibition of ephrin-A1 expression, thereby contributing to the differential expression of ligand and receptor (Fig. 1A,B) [63]. Not long after its discovery, ephrin-A1 was demonstrated to stimulate PI3K activity via direct interaction of EphA2 with the p85 subunit of PI3K [65]. More recent studies attribute ligand-dependent activation of EphA2 to inhibition of the Akt-mTOR pathway in cancer cells [44], likely due to crosstalk of EphA2 with a serine/threonine phosphatase that dephosphorylates Akt [66].

Although ephrin-A1-mediated EphA2 activation has tumor-suppressing properties, ephrin-A1 treatment of hepatocellular carcinoma cells promotes cell proliferation, and suppression of ephrin-A1 evokes the opposite [67]. Furthermore, reduction of ephrin-A1 in HT29 colon carcinoma cells attenuates their growth in 3D spheroids [68], but not in 2D culture, again highlighting the importance of microenvironment to Ephrin/Eph function [68]. In the mouse model of intestinal cancer, (APC^{min/+}), ephrin-A1 promotes the growth and invasiveness of

tumors [69]. Additionally, transcriptional repression of *ephrin-A1* by Hic1 decreases mammary tumor growth [70].

7. Ephrin-A1 function in malignancy: role in angiogenesis and tumor neovasculature

Ephrin-A1 and EphA2 are not only expressed in multiple tumor types, but are also expressed and play an important role in normal angiogenesis and tumor neovascularization. Ephrin-A1 is expressed in the developing vasculature during embryogenesis [71] and in some normal microvascular endothelial cells [72].

7.1. Expression of ephrin-A1 in endothelial cells

Ephrin-A1 is expressed in the developing vasculature during embryogenesis [71] and in some normal microvascular endothelial cells [72]. Experimental evidence suggests that TNF- α -induced expression of ephrin-A1 in HUVECs is mediated through p38 MAPK and SAPK/JNK, but not NF- κ B [73]. This is in contrast to the TNF-induced expression of other angiogenic factors in which NF- κ B is a key player of expression [74]. On the other hand, *ephrin-A1* was identified as a cytokine-induced NF- κ B target gene in ovarian OVCAR-3, colon adenocarcinoma HCT 116, breast MCF7 A/Z cells, human microvascular endothelial cells (HMEC-1) [75]. However, ephrin-A1 protein levels were not investigated in this study.

7.2. Ephrin-A1-mediated activation of EphA2 in tumor angiogenesis

Ephrin-A1 treatment of EphA2-expressing HUVECs induces a specific angiogenic response in rat cornea, which is likely due to the ability of ephrin-A1 to stimulate migration of endothelial cells. In fact, the angiogenic effects of TNF- α are largely due to ephrin-A1-mediated activation of the EphA2 receptor [76]. The first indication of Eph function in tumor angiogenesis was the finding that ephrin-A1 and EphA2 co-localized with the vascular marker anti-CD34. In tumor xenografts, ephrin-A1 and EphA2 were found to be expressed in both endothelial and tumor cells. Compared to EphA2, however, ephrin-A1 was lower in tumor cells and higher in endothelial cells [77]. Consistent with previous reports that ephrin-A1 is a chemoattractant for endothelial cells [76] and is therefore a stimulant for vascular remodeling, EphA2 inhibition prevents morphological changes in endothelial cells required for the formation of blood vessels [77]. Additionally, blockage of the ephrin-A1-EphA2 interaction in several *in vivo* models results in a significant reduction in tumor vascular density [78].

7.3. Function of Rho family GTPases and ephrin-A1 in angiogenesis

Interestingly, there is a cell-type specific role of ephrin-A1/EphA2 even within the context of the different cell types which contribute to angiogenesis, as this process requires both endothelial cell migration and proliferation and smooth muscle cell destabilization [79, 80]. The stimulation of rat vascular smooth muscle cells (VSMCs) with ephrin-A1 inhibits cell spreading by preventing the activation of the Rho GTPase Rac1 and its downstream effector PAK [81]. However, treatment of endothelial cells with ephrin-A1 increases migration via PI3K-mediated activation of Rac1 [80]. Consistent with a Rac1-suppressive role for ephrin-A1, the ability of Slit2 to induce angiogenesis via activation of mTORC2/Rac and Akt is impaired in the presence of ephrin-A1 [82]. The interplay between ephrin-A1 and Slit2 regulates a balance between pro- and anti-angiogenic responses elicited by Slit2. Interestingly Slit2 promotes angiogenesis as a single agent, but inhibits angiogenesis the presence of ephrin-A1 [82].

7.4. Mechanism of action of ephrin-A1 in angiogenesis: requirement of EphA2

Multiple studies have addressed the question of how ephrin-A1 is able to act as a pro-angiogenic factor and whether or not EphA2 is required for this response. There is sufficient experimental evidence to suggest that ephrin-A1 exerts its angiogenic effects, at least in part, by activating EphA2 on host blood vessel endothelial cells *in vitro* and *in vivo* [77, 78, 80, 83–85]. Mutations in the juxtamembrane region, kinase domain, or SAM domain of EphA2 inhibit vascular assembly induced by ephrin-A1, demonstrating the importance of EphA2 activation in ephrin-A1 mediated angiogenesis [45]. Further, mice lacking EphA2 are deficient at vessel remodeling in response to exogenous ephrin-A1 or to ephrin-A1-expressing 4T1 mammary tumors, and endothelial cells isolated from these mice are unable to properly assemble and migrate in response to ephrin-A1 *in vitro* [80, 86]. Moreover, disruption of Ephrin-A1/EphA2 signalling results in the inhibition of angiogenesis in a rat aortic ring assay and *in vivo* in a porcine aortic endothelial cell Matrigel plug assay [85]. Taken together, these data suggest that functional EphA2 is required for the angiogenic effects mediated by the ephrin-A1 ligand.

The proangiogenic factor VEGF induces the expression of ephrin-A1 in endothelial cells, leading to the phosphorylation of EphA2 [83, 84]. VEGF-induced angiogenesis is inhibited by blocking ephrin-A1-mediated activation of EphA2 as well as by antisense oligonucleotide inhibition of the receptor [83, 87]. Additionally, ephrin-A1 expressed on tumor cells, through its interaction with EphA receptors, is able to induce expression of VEGF and subsequently activate distant host endothelial cells, leading to angiogenesis and metastasis [88]. *In vitro* and *in vivo* studies revealed the additive effects of combined inhibition of VEGF and EphA2, leading to the conclusion that EphA and VEGFR signalling are both critical and nonredundant for the process of angiogenesis [85].

7.5. Inadequate angiogenesis: ephrin-A1 and the hypoxic environment

In addition to playing a critical role in tumor angiogenesis, ephrin-A1 is also involved in responses to hypoxia, which occur in tumors with inadequate blood supply [89]. HIF-1 α , a hypoxia-inducible transcription factor, can lead to the upregulation of ephrin ligands and Eph receptors in mouse skin [90]. Additionally, *ephrin-A1* expression was diminished in mice displaying a reduction in HIF-2 α expression [91]. Reduced ephrin-A1 expression was also observed in endothelial cells cultured from these mice, as well as defects in vascular remodeling that were reversed upon overexpression of ephrin-A1 [91]. Of note, VEGF is also upregulated by HIF transcription factors in response to hypoxic environments [89]. Therefore, not only is ephrin-A1 expression controlled directly by HIF-2 α , but its expression is also induced indirectly via HIF-induced VEGF upregulation. This can create a feedback loop whereby increased ephrin-A1 expression, through the interaction with its receptor, can promote the expression and activity of VEGF leading to an increase in angiogenesis within the hypoxic tumor core.

8. Conclusions

Overall, even though much research has been focused on ephrins and their receptors over the past couple of decades, their exact complex roles in malignancy have not been fully elucidated. What is apparent, however, is that their expression and the signalling pathways activated by that expression is cell-type and microenvironment dependent. In all tumor types ephrin-A1 and its primary receptor affect multiple oncogenic signalling pathways such as MAP/ERK, and PI3K. In addition, multiple proteins, such as E-cadherin, and pathways, such as integrins/FAK/paxillin involved in cellular architecture are affected by ephrin-A1. Further studies using more *in vivo* approaches and using ligands such as monomeric ephrin-A1, released into the extracellular space, in addition to clustered ephrin-A1 will also be

important for elucidating the role ephrin-A1 plays in neoplasia. Additionally, since ephrin-A1 has been determined to function as a soluble monomer, it is worth investigating other ephrins to explore whether they exist in a similar form and may act in similar or different ways to their membrane-bound counterparts. This adds an additional layer of complexity to an already challenging biological system that has yet to be fully understood. Finally, it is critical to tease out the functions of these receptors and ligands with respect to tumor type in order to therapeutically target them in an efficient manner in a clinical setting.

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Figure 1. The signalling effects of ephrin-A1-mediated activation of EphA2 on the PI3K/Akt and MAPK pathways

(A) Ligand-independent EphA2 signalling. Activated AKT can phosphorylate serine residue 897 on EphA2, leading to an increase in EphA2 kinase function and the promotion of malignancy. MAPK activation causes the suppression of ephrinA1 expression and the upregulation of EphA2, leading to an increased substrate for Akt phosphorylation. (B) Ephrin-A1-induced EphA2 signalling. Upon ephrin-A1 ligation of EphA2, EphA2 and Akt are dephosphorylated and tyrosine residues of EphA2 are phosphorylated. EphA2 activation by ephrin-A1 inhibits the Ras/MAPK pathway and decreases the oncogenic signalling within tumor cells.