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Review

Ephs and ephrins in cancer: Ephrin-A1 signalling

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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. Ephrin-A1 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.

Contents

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 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 events potentially

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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 the E3 ubiquitin ligase 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 has 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-A1 fused to the Fc of human 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*