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Review

Concepts and consequences of Eph receptor clustering

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ABSTRACT

Polymeric receptor–ligand complexes between interacting Eph and ephrin-expressing cells are regarded as dynamic intercellular signalling scaffolds that control cell-to-cell contact: the resulting Eph–ephrin signalling clusters function as positional cues that facilitate cell navigation and tissue patterning during normal and oncogenic development. The considerable complexity of this task, coordinating a multitude of cell movements and cellular interactions, is achieved by accurate translation of spatial information from Eph and ephrin expression gradients into fine-tuned changes in cell–cell adhesion and position. Here we review emerging evidence suggesting that the required combinatorial diversity is not only achieved by the large number of possible Eph–ephrin interactions and selective use of Eph forward and ephrin reverse signals, but in particular through the composition and signal capacity of Eph–ephrin clusters, which is adjusted dynamically to reflect overall Eph and ephrin surface densities on interacting cells. Fine-tuning is provided through multi-layered cluster assembly, where homo- and heterotypic Eph and ephrin interactions define the composition – whilst intracellular signalling feedbacks determine the size and lifetime – of signalling clusters.

Contents

1. Introduction

1.1. A global cell positioning system

Eph receptors (Ephs) are the most numerous family of the receptor tyrosine kinases (RTKs), type I trans-membrane allosteric enzymes, containing a unique ligand binding domain on the extracellular side and a structurally conserved tyrosine kinase domain on the intracellular side [1–3]. Whilst many RTKs bind soluble ligands, such as growth factors, a distinguishing feature of Ephs is their binding to cell surface-bound ligands, called ephrins. Their interaction thus relies on direct contact between neighbouring cells, where by Ephs and ephrins transduce interdependent signals into each interacting cell [4–6].

Unlike other RTKs, the principle function of Ephs is not in mitogenesis but in navigating and connecting cells during developmental tissuegenesis [6–8]. Whilst generally down regulated in adult tissues, Ephs and their activities often re-emerge in cancers,

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where over-expressed and mutated receptors are implicated in invasion and metastasis [9,10]. At a cellular level the diverse patterning functions of Ephs are a consequence of their action on the cytoskeleton that modulates cell morphology and cell–cell adhesion.

Eph–ephrin interactions trigger either cell contraction and segregation between Eph- and ephrin-expressing cells, or initiate the opposite – cell–cell adhesion, spreading and intermingling of the two cell populations [11–13]. The major determinant of this important switch in cellular interaction is the degree of Eph- and ephrin clustering, activation and phosphorylation [8]. Unligated RTKs generally associate only randomly, their transient phosphorylation is tightly controlled by phosphatases; ligand-induced oligomerisation is required for productive tyrosine phosphorylation, generating docking motifs for SH2 or PTB domain-containing downstream effectors [14]. However, the concepts regulating interactions between Ephs and cell-bound ephrins are more complex: here, a nucleating hetero-tetrameric complex between Ephs and ephrins on opposing cells initially triggers lateral extension of Eph/ephrin clusters via distinct ephrin/Eph and Eph/Eph interactions; only then association of signalling/adaptor proteins modulating focal adhesion-, Ras-MAP kinase- and PI3-kinase signalling circuits follows, whereby the overall size and composition of these clusters determines the signal outcome [8,15]. Under conditions where Eph tyrosine kinase signalling is muted, Eph and ephrin clustering will promote Eph kinase-independent cell–cell adhesion rather than kinase-dependent segregation. It is increasingly apparent that conventional depiction of seemingly 'linear signalling pathways' from individual Eph/ephrin pairs has lead to confusing and even contradictory findings. Meaningful insights into Eph/ephrin biology will require consideration of all molecular components that are recruited into their signalling clusters and thus contribute to the type, magnitude and duration of responses emanating synchronously into both interacting cells. Here we review recent findings describing some of the principle players and molecular concepts implicated in the assembly of Eph signalling clusters.

1.2. Eph and ephrin structures and classification

Ephs are classified on the basis of sequence homology and binding preferences: nine mammalian EphAs (EphA1–A8, EphA10) preferentially bind GPI-linked A-type ephrins-A1–A6, whilst EphBs (EphB1–EphB4, EphB6) preferentially bind transmembrane ephrins-B1–B3 [16,17]. Ephs and ephrins interact promiscuously within each subclass, but cross-class interactions are known for EphA4, interacting with B-type ephrins [18,19], and ephrin-A5, activating EphB2 as well as EphAs [20].

The conserved multi-domain Eph structure comprises an N-terminal globular ligand binding domain (LBD), a cysteine-rich domain (CRD), and two fibronectin type III (FNIII) repeats within the extracellular domain (ECD) [21] (Fig. 1). The CRD comprises a Sushi (or Complement Control Protein) domain, tightly packed to an EGF like module with homology to the TNFα receptor CRD [22,23]. The intracellular region contains a regulatory juxtamembrane region, a kinase domain, a SAM (sterile-α motif) domain and a PDZ-binding motif (PBM). All ephrins contain a conserved N-terminal receptor binding domain, and whereas ephrin-Bs contain also transmembrane and cytoplasmic sequences, including a PBM, A-type ephrins do not, but are inserted into the plasma membrane via a GPI-linkage.

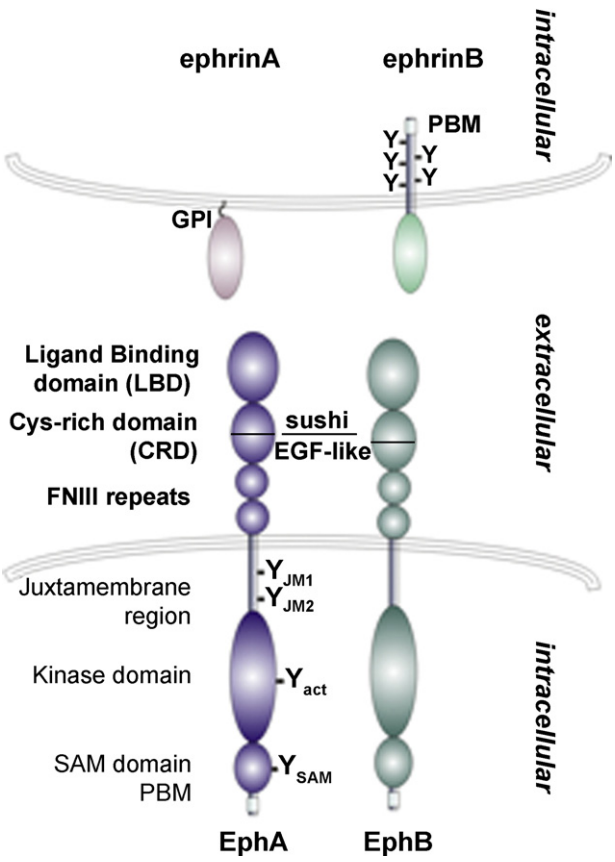

Fig. 1. Eph and ephrin domain structures. The extracellular region of Eph receptors comprises a globular ligand binding (LBD), a cysteine-rich (CRD) and two fibronectin III (FNIII) domains. The CRD incorporates tightly packed sushi and EGF-like modules. The intracellular region comprises a tyrosine kinase domain, as well as juxtamembrane and SAM domains with conserved tyrosine phosphorylation sites, and a PTB-domain binding motif, all of which mediate interaction with intracellular signalling effectors. A-type ephrins are GPI-linked, whilst B-type ephrins contain transmembrane and intracellular domains.

2. Ligand-induced receptor clustering

A defining characteristic of Ephs compared to other RTKs is that only membrane-bound or artificially clustered ligands can trigger receptor signalling [24] whilst non-clustered soluble ephrins are antagonists [25–28]. This reflects a propensity of membrane-anchored ephrins for clustering, supported by their localisation to membrane microdomains [29,30]. Thus, at cell–cell contacts, clustered ephrin will bind and cluster Ephs on the opposing cell, whereby the size of the resulting Eph clusters determines function: at least tetramerisation is pre-requisite for activation and biological responses [31–33]. This interdependent growth of signalling clusters reflects the function of Ephs, relaying spatial information encoded by Eph and ephrin expression gradients into fine-tuned changes in cell morphology and cell–cell adhesion, which accurately determine cell interactions and location within a tissue [34,35].

Prior to ephrin contact, Ephs are largely distributed across the plasma membrane and display minimal kinase activity [15,36], which however may become noticeable at elevated expression [37–39]. Signalling is initiated by 1:1 interactions between the Eph LBD and a conserved Eph-binding domain of ephrins [40], which rapidly assemble into Eph–ephrin hetero-tetramers and laterally expand into higher-order clusters [41], a mechanism confirmed in structures of ephrin-ligated Ephs (reviewed in [21]): initially defined for EphB2/ephrin-B2 complexes [32], Ephs bind ephrins through insertion of an extended ephrin loop into a deep channel of the Eph LBD. Lower affinity interfaces [42,43] join two Eph–ephrin dimers into a tetramer connecting each ephrin with two adjacent Ephs in a ring like formation. Recent elucidation of complete EphA2