1. Phylogeny  
   Ephrin type‐B receptor 6 (EPHB6) is a member of the Eph receptor family within the receptor tyrosine kinase (RTK) superfamily. It clusters with the EphB subgroup and is evolutionarily conserved in mammals, sharing a common domain architecture with other Eph receptors that originated early in metazoan evolution. Comparative analyses indicate that the Eph receptor family expanded from an ancestral RTK by gene duplication events, and although many family members retain catalytic activity, EPHB6 is distinguished by its kinase‐defective status. Orthologs of EPHB6 are found across vertebrate species, and its evolutionary relationships are framed by the same duplication and divergence events that gave rise to both catalytically active and pseudokinase Eph receptors (liang2021theintracellulardomains pages 1-2, krishnan2019thefirstidentification pages 12-13, krishnan2019thefirstidentification pages 4-7).
2. Reaction Catalyzed  
   Unlike catalytically active kinases, EPHB6 does not catalyze a phosphorylation reaction. The canonical reaction for active receptor tyrosine kinases—ATP + [protein] → ADP + [protein]-phosphate—is not performed by EPHB6 because its pseudokinase domain lacks key catalytic residues required for phosphotransfer (liang2021theintracellulardomains pages 1-2, liang2021theintracellulardomains pages 14-17).
3. Cofactor Requirements  
   Conventional receptor tyrosine kinases typically require divalent metal ions such as Mg²⁺ to coordinate ATP binding and catalysis; however, because EPHB6 is catalytically inactive, no cofactor is necessary for enzymatic activity. Notably, biophysical studies have shown that the presence of Mg²⁺ can alter the conformational stability of the EphB6 pseudokinase domain, even though this protein does not perform catalytic phosphoryl transfer (liang2021theintracellulardomains pages 5-6).
4. Substrate Specificity  
   EPHB6 does not exhibit intrinsic substrate specificity for catalyzing phosphorylation because it is kinase defective. Instead, its function is largely based on its role as a phosphorylation substrate for catalytically active Eph receptors such as EphB4. The intracellular juxtamembrane (JM) region of EPHB6 contains conserved tyrosine residues—including Y644, Y645 (JX1), Y651 (JX2), and Y669—that, once phosphorylated, create docking sites for SH2 domain-containing adaptor proteins like Abl, Src, Vav3, Nck, Grb7, and Grb10. In this manner, the phosphorylation-dependent motifs in the JM region mediate the assembly of downstream signaling complexes rather than serving to define a catalytic substrate motif (liang2021theintracellulardomains pages 13-14, andretta2015roleofepha3 pages 48-52).
5. Structure  
   EPHB6 displays a domain organization that closely resembles other members of the Eph receptor family. Its extracellular region comprises an ephrin ligand-binding domain (Eph-LBD), a cysteine-rich domain, and several fibronectin type III repeats that facilitate ephrin recognition and ligand-induced receptor clustering; these regions have been characterized by crystallographic studies of Eph receptor ectodomains (liang2021theintracellulardomains pages 17-19). The protein is anchored in the plasma membrane via a single transmembrane segment. The intracellular portion is organized in a modular fashion, beginning with a juxtamembrane (JM) region that contains several conserved tyrosine residues (notably Y644, Y645, Y651, and Y669) critical for phosphorylation by associated kinases. Immediately following the JM region is the pseudokinase domain, which retains the overall fold typical of active kinases—including the N- and C-lobes, an activation loop, and a C-helix—but lacks essential catalytic residues (i.e., within the DFG, HRD motifs) and is consequently catalytically inactive. Despite its lack of enzymatic activity, the pseudokinase domain of EPHB6 has been shown to bind ATP with an estimated dissociation constant of approximately 94 μM and can interact with ATP-competitive inhibitors, a property that may allow for conformational switching (liang2021theintracellulardomains pages 1-2, liang2021theintracellulardomains pages 5-6, liang2021theintracellulardomains pages 6-8). Connected to the pseudokinase domain by a flexible ~40 amino acid linker is a sterile α-motif (SAM) domain, which is implicated in protein–protein interactions and receptor oligomerization. Finally, a C-terminal PDZ-binding motif is present, allowing for further intracellular scaffold formation (liang2021theintracellulardomains pages 8-10, liang2021theintracellulardomains pages 17-19, andretta2015roleofepha3 pages 48-52, liang2024coclusteringofephb6 pages 1-2).
6. Regulation  
   Regulatory control of EPHB6 is achieved primarily through post-translational phosphorylation events that are mediated by catalytically active Eph receptors, such as EphB4, rather than through auto-catalysis. The juxtamembrane region of EPHB6 harbors conserved tyrosine residues (Y645 [JX1], Y651 [JX2], along with adjacent sites such as Y644 and Y669) that, when phosphorylated, serve as docking platforms for SH2 domain-containing signaling molecules including Abl, Src, Vav3, Nck, Grb7, and Grb10. These phosphorylation events are critical for the modulation of downstream signaling cascades and facilitate the assembly of dynamic signaling complexes. In addition, although the pseudokinase domain is catalytically inactive, it is capable of binding ATP and various ATP-competitive inhibitors; such interactions induce conformational changes that may contribute to allosteric regulation of the receptor’s signaling scaffold. Ligand-induced receptor clustering upon binding to ephrin-B ligands further influences the regulatory state of EPHB6, promoting hetero-oligomerization with active Eph receptors and thereby integrating diverse signaling inputs (liang2021theintracellulardomains pages 13-14, liang2021theintracellulardomains pages 14-17, palese2021invivoand pages 10-14, andretta2015roleofepha3 pages 128-131).
7. Function  
   EPHB6 functions primarily as a kinase-defective receptor for ephrin-B ligands, specifically binding ephrin-B1 and ephrin-B2 through its extracellular domain to mediate cell–cell communication. Upon stimulation with ephrin-B2, EPHB6 modulates cell adhesion and migration through both positive and negative regulatory effects. This modulation is achieved via the phosphorylation of tyrosine residues in the juxtamembrane region by co-expressed, catalytically active Eph receptors (e.g., EphB4), which in turn creates docking sites for SH2 domain-containing adaptor proteins. In addition to its role in orchestrating cell adhesion and migratory responses, EPHB6 has been shown to inhibit intracellular signaling pathways; for example, it suppresses c-Jun N-terminal kinase (JNK) activation as well as T-cell receptor-induced interleukin-2 (IL-2) secretion and CD25 expression upon ephrin-B2 engagement. These functions position EPHB6 as a dynamic signaling hub that integrates extracellular ligand binding with intracellular adaptor recruitment to regulate processes relevant to cell migration, adhesion, and immune modulation. Its expression patterns in various tissues suggest roles in both developmental signaling and the maintenance of tissue homeostasis, while its downregulation has been associated with aggressive cancer phenotypes (liang2021theintracellulardomains pages 1-2, andretta2015roleofepha3 pages 58-61, liang2024coclusteringofephb6 pages 1-2, palese2021invivoand pages 121-123, palese2021invivoand pages 127-129).
8. Other Comments  
   Despite lacking intrinsic kinase activity, EPHB6 retains the ability to bind ATP as well as ATP-competitive inhibitors through its pseudokinase domain, indicating that its conformational state can be modulated by small molecules. This property raises the possibility of pharmacologically targeting the pseudokinase domain to alter the receptor’s non-catalytic signaling functions. EPHB6 is of particular interest in cancer biology; studies have shown that its expression is frequently downregulated in aggressive malignancies, and its role in modulating cell adhesion and migration suggests that it may function as a tumor suppressor. In addition, EPHB6 influences immune cell behavior by inhibiting JNK activation and blunting T-cell receptor-mediated responses, highlighting its potential relevance in immune regulation. No selective inhibitors specific for EPHB6 have been reported, and its function is instead modulated by hetero-oligomerization with catalytically active Eph receptors and by the phosphorylation status of its intracellular tyrosine residues (liang2021theintracellulardomains pages 2-4, liang2021theintracellulardomains pages 14-17, andretta2015roleofepha3 pages 64-68).
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