## Phylogeny

Ephrin type-A receptor 2 (EPHA2) is a member of the Eph receptor family, which constitutes the largest subgroup of receptor tyrosine kinases (RTKs) (toracchio2024epha2incancer pages 1-2, unknownauthors2015theroleof pages 34-38, tandon2011emergingstrategiesfor pages 1-2). Based on sequence homology and ligand-binding preferences for ephrin-A ligands, EPHA2 is classified within the EphA subclass (tandon2011emergingstrategiesfor pages 1-2). The human kinome contains nine EphA receptors (EphA1-8 and EphA10) and five EphB receptors (unknownauthors2015theroleof pages 34-38). EPHA2 shares approximately 25–35% sequence homology with other Eph receptors and is evolutionarily conserved across vertebrates (tandon2011emergingstrategiesfor pages 1-2, toracchio2024epha2incancer pages 1-2, unknownauthors2015theroleof pages 34-38). Its classification is consistent with the kinome framework described in Manning et al., 2002 (unknownauthors2015theroleof pages 34-38, toracchio2024epha2incancer pages 1-2). High-throughput analysis of substrate specificity shows that EPHA2 clusters with related Eph receptors such as EPHA3 and EPHA5, indicating similar substrate motifs (yaronbarir2024theintrinsicsubstrate pages 3-3).

## Reaction Catalyzed

As a tyrosine kinase, EPHA2 catalyzes the ATP-dependent transfer of a γ-phosphate group to the hydroxyl group of tyrosine residues on protein substrates (toracchio2024epha2incancer pages 1-2, tandon2011emergingstrategiesfor pages 1-2). The reaction is: ATP + protein tyrosine → ADP + phosphoprotein tyrosine (nowakowski2002structuresofthe pages 1-2).

## Cofactor Requirements

The kinase catalytic activity of EPHA2 requires ATP as a cofactor and the divalent cation Mg²⁺, which facilitates ATP binding and the phosphoryl transfer reaction (nowakowski2002structuresofthe pages 1-2, toracchio2024epha2incancer pages 1-2, tandon2011emergingstrategiesfor pages 1-2).

## Substrate Specificity

The intrinsic substrate specificity of EPHA2 has been characterized by high-throughput positional scanning peptide arrays, which revealed distinct preferences for amino acids surrounding the phosphorylation site (yaronbarir2024theintrinsicsubstrate pages 1-2, yaronbarir2024theintrinsicsubstrate pages 2-3). These position-specific preferences are predominantly observed from positions -1 to +3 relative to the phosphoacceptor tyrosine (yaronbarir2024theintrinsicsubstrate pages 3-3). EPHA2 shows preferences for specific amino acids flanking the phosphotyrosine, often involving acidic or basic residues at the -1 or +1 positions (yaronbarir2024theintrinsicsubstrate pages 17-19). Furthermore, EPHA2 specificity can be guided by phosphopriming, a preference for substrates that are already phosphorylated on tyrosine or threonine residues at positions such as -1, +1, or +2 (yaronbarir2024theintrinsicsubstrate pages 16-17). While general tyrosine kinase preferences include hydrophobic residues like isoleucine at positions -1 and +3, EPHA2 clusters with EPHA3 and EPHA5, suggesting shared substrate motif characteristics (yaronbarir2024theintrinsicsubstrate pages 3-3).

## Structure

EPHA2 is a 130 kDa transmembrane glycoprotein composed of 976 amino acids (wang2024molecularcharacteristicsand pages 1-2, toracchio2024epha2incancer pages 2-3). It has a modular domain organization with an extracellular region, a single transmembrane segment, and an intracellular region (tandon2011emergingstrategiesfor pages 1-2, troster2023targetingepha2with pages 10-15). The extracellular portion contains a globular ligand-binding domain, a cysteine-rich domain, and two fibronectin type III repeats (tandon2011emergingstrategiesfor pages 1-2, unknownauthors2015theroleof pages 34-38). The intracellular cytoplasmic domain consists of a juxtamembrane segment with conserved tyrosine residues, a tyrosine kinase (TK) domain, a sterile alpha motif (SAM), and a PDZ-binding motif (tandon2011emergingstrategiesfor pages 1-2, troster2023targetingepha2with pages 10-15, toracchio2024epha2incancer pages 2-3).

Crystal structures of the EPHA2 kinase domain have been resolved, including an apo form (PDB: 1MQB) and complexes with various inhibitors such as Dasatinib (PDB: 5I9Y) and others (PDB IDs: 5NJZ, 5NK0, 5NK1, 5NK3, 5NK5, 5NK7, 5NK4, 5NK6, 5NK9, 5NK8, 5NKA, 5NKE, 5NKF, 5NKG, 5NKH, 5NKB, 5NKI) (heinzlmeir2017chemoproteomics‐aidedmedicinalchemistry pages 20-22, heinzlmeir2017chemoproteomics‐aidedmedicinalchemistry pages 32-35). These structures reveal key regulatory features, including the activation loop and C-helix, which undergo conformational changes that are crucial for regulating kinase activity (troster2023targetingepha2with pages 10-15, wei2014structuresofan pages 8-9). The active conformation is stabilized by phosphorylation of Tyr772 in the activation loop, which forms specific hydrogen bonds (wei2014structuresofan pages 8-9).

## Regulation

EPHA2 activity is regulated by post-translational modifications (PTMs), primarily phosphorylation (giordano2024targetingtheepha2 pages 18-18). Upon binding to ephrin ligands, EPHA2 undergoes oligomerization and autophosphorylation on tyrosine residues in its juxtamembrane domain, which activates its kinase function (toracchio2024epha2incancer pages 2-3, unknownauthors2015theroleof pages 34-38). Site-directed mutagenesis studies indicate that phosphorylation of Tyr588 and Tyr594 is not required for kinase activity, suggesting a unique activation mechanism compared to other Eph receptors (wei2014structuresofan pages 8-9).

EPHA2 is also regulated by serine phosphorylation: Protein Kinase A (PKA) phosphorylates Ser897, and Protein Kinase C (PKC) phosphorylates Ser892 (giordano2024targetingtheepha2 pages 18-18). The phosphorylation state is negatively regulated by the low molecular weight protein tyrosine phosphatase (LMW-PTP), which dephosphorylates EPHA2 and promotes the accumulation of unphosphorylated receptor on tumor cells (walker‐daniels2003differentialregulationof pages 2-3).

Receptor levels are controlled via degradation pathways. Ligand binding induces ubiquitination by the E3 ligase c-Cbl, targeting EPHA2 for endocytosis and subsequent proteasomal or lysosomal degradation (toracchio2024epha2incancer pages 2-3, xiao2020targetingepha2in pages 15-15, walker‐daniels2003differentialregulationof pages 2-3). Additionally, proteolytic cleavage of ephrin ligands by metalloproteases like ADAM10 modulates EPHA2 signaling (giordano2024targetingtheepha2 pages 18-18, toracchio2024epha2incancer pages 2-3). Transcription of the EPHA2 gene is regulated by the tumor suppressor p53 via a promoter response element (walker‐daniels2003differentialregulationof pages 2-3).

## Function

EPHA2 is expressed in various tissues and plays roles in developmental processes such as kidney development, bone homeostasis, and lens formation (toracchio2024epha2incancer pages 2-3). Under normal physiological conditions, its expression is predominantly in epithelial cells (giordano2024targetingtheepha2 pages 18-18). In these cells, E-cadherin is required to facilitate EPHA2 ligand binding and activation (walker‐daniels2003differentialregulationof pages 2-3).

As an RTK, EPHA2 initiates bidirectional signaling upon cell-to-cell contact with ephrin-A ligands (toracchio2024epha2incancer pages 2-3). Its downstream interacting partners include Src family kinases, Ras GTPases, and the PI3K p85 subunit (toracchio2024epha2incancer pages 2-3). EPHA2 engages and activates multiple pro-oncogenic signaling pathways, including the Ras, PI3K-Akt, ErbB, and MEK/ERK/RSK cascades (wang2024molecularcharacteristicsand pages 1-2, giordano2024targetingtheepha2 pages 18-18). It can interact with other RTKs, such as EGFR and ErbB2, amplifying their signaling to promote tumorigenesis (giordano2024targetingtheepha2 pages 18-18).

EPHA2 signaling regulates cell adhesion, migration, proliferation, and differentiation (tandon2011emergingstrategiesfor pages 1-2). Activated EPHA2 negatively regulates integrin function, causing the dephosphorylation and dissociation of focal adhesion kinase (FAK) and suppressing cell spreading and migration (wykosky2005epha2asa pages 2-2). Overexpression of EPHA2 can destabilize adherens junctions through a RhoA-dependent mechanism, leading to increased cellular motility (giordano2024targetingtheepha2 pages 18-18).

## Inhibitors

Therapeutic strategies targeting EPHA2 include monoclonal antibodies, small interfering RNA (siRNA), and small molecule inhibitors (tandon2011emergingstrategiesfor pages 1-2, xiao2020targetingepha2in pages 15-15). Small molecule antagonists include derivatives of lithocholic acid that interfere with kinase activation (xiao2020targetingepha2in pages 15-15). Several FDA-approved drugs have been identified as EPHA2 inhibitors, including the multi-kinase inhibitors Regorafenib, Encorafenib, Tucatinib, and Dasatinib (troster2023targetingepha2with pages 10-15, heinzlmeir2017chemoproteomics‐aidedmedicinalchemistry pages 32-35).

## Other Comments

EPHA2 is overexpressed in numerous cancers, including breast, prostate, lung, glioblastoma, colorectal, and melanoma, where its elevated expression often serves as a prognostic biomarker for poor survival, advanced disease stage, and metastasis (tandon2011emergingstrategiesfor pages 1-2, wykosky2008theepha2receptor pages 22-22, toracchio2024epha2incancer pages 2-3, giordano2024targetingtheepha2 pages 18-18). High EPHA2 expression is also associated with resistance to EGFR-targeting therapies in lung and colorectal cancer (xiao2020targetingepha2in pages 15-15, troster2023targetingepha2with pages 1-4).

The EPHA2 gene resides on chromosome 1p36, a frequent site of cancer-associated genomic alterations (toracchio2024epha2incancer pages 2-3, walker‐daniels2003differentialregulationof pages 2-3). However, EPHA2 dysregulation in tumors is mainly driven by altered expression levels and ligand interactions rather than genetic mutations, which occur in only ~3-5% of pan-cancer patients (toracchio2024epha2incancer pages 2-3, troster2023targetingepha2with pages 1-4). Most EPHA2 gene modifications are missense mutations (wang2024molecularcharacteristicsand pages 1-2). Specific kinase domain mutations, such as p.R890C and p.G668D, have been shown to alter receptor stability and promote cell survival, invasion, and mTOR pathway activation (xiao2020targetingepha2in pages 15-15, tandon2011emergingstrategiesfor pages 20-21). EPHA2 also functions as an entry receptor for viruses, including Epstein-Barr virus (EBV) and Kaposi’s sarcoma-associated herpesvirus (KSHV) (troster2023targetingepha2with pages 1-4).

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