RYK, a Catalytically Inactive Receptor Tyrosine Kinase, Associates with EphB2 and EphB3 but Does Not Interact with AF-6*

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RYK is an atypical orphan receptor tyrosine kinase that lacks detectable kinase activity. Nevertheless, using a chimeric receptor approach, we previously found that RYK can signal via the mitogen-activated protein kinase pathway. Recently, it has been shown that murine Ryk can bind to and be phosphorylated by the ephrin receptors EphB2 and EphB3. In this study, we show that human RYK associates with EphB2 and EphB3 but is not phosphorylated by them. This association requires both the extracellular and cytoplasmic domains of RYK and is not dependent on activation of the Eph receptors. It was also previously shown that AF-6 (afadin), a PDZ domain-containing protein, associates with murine Ryk. We show here that AF-6 does not bind to human RYK in vitro or in vivo. This suggests that there are significant functional differences between human and murine RYK. Further studies are required to determine whether RYK modulates the signaling of EphB2 and EphB3.

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† The abbreviations used are: RTK, receptor tyrosine kinase; NGF, nerve growth factor; EGFR, epidermal growth factor receptor; PDGF, platelet-derived growth factor; GFP, green fluorescent protein; HA, hemagglutinin.

to the lack of catalytic function. However, a tyrosine-phosphorylated 75-kDa protein was co-immunoprecipitated with the receptor, independently of stimulation, and activation of the mitogen-activated protein kinase pathway was observed following stimulation of the receptor. Interestingly, when the invariant lysine in subdomain II of the catalytic domain was mutated to alanine (K334A), abolishing any existing kinase activity, the receptor failed to activate the mitogen-activated protein kinase pathway and to bind and/or phosphorylate the 75-kDa protein. Further, RYK is overexpressed in ovarian tumors, and this correlates adversely with survival (12).

The exact function of RYK is unknown, and its ligand has not been identified. A mouse model, where Ryk was homozygously deleted by homologous recombination, revealed an unusual, completely penetrant phenotype (13). The mice were small, compared with wild type, and died within a week because of cleft palate. Such a phenotype has been found in other knock-out mice, including those lacking members of the Ephrin receptor family (14). It was recently shown that although Ryk cannot autophosphorylate, it can associate and be phosphorylated by two members of the Eph family of receptors: EphB2 and EphB3 (13). The Ephrin receptor family, which consists of 14 members, has been shown to mediate contact-dependent cell interactions that regulate the repulsion and adhesion mechanisms involved in the cell guidance and assembly of multicellular structures (15, 16). They are important in the development of a range of vertebrate species, in the formation of blood vessels, axonal guidance, and metastasis of transformed cells (15, 17). Yeast two-hybrid analysis, using the cytoplasmic domain of murine Ryk as a probe, has identified an interaction with AF-6, a PDZ domain-containing protein (13). This interaction involved the PDZ domain of AF-6 and the C-terminal region of Ryk, especially the critical C-terminal valine residue. This valine residue is completely conserved among RYK homologues from Drosophila to human. AF-6 is a scaffold protein found at sites of cell-cell contact and a target of the Ras family of proteins (18, 19). Interestingly, a subset of activated Eph receptors, including EphB2, EphB3, and EphA7, also bind to AF-6 (20, 21). The purpose of this study was to investigate further the interactions between RYK and the Eph receptors and between RYK and AF-6.

EXPERIMENTAL PROCEDURES

Plasmids—The following plasmids were received as gifts: Myc-AF-6 from Kozo Kaibuchi (Nara Institute of Science and Technology, KOMA, Japan) (19), HA.EphB3 and HA.EphB3K665R from Steven Stacker (Ludwig Institute for Cancer Research, Victoria, Australia) (21), and EphB2 from Tony Pawson (Samuel Lunenfeld Research Institute, Toronto, Canada) (22, 23). The TrkA-RYK chimeric construct has been described previously (2). The RYK-V5 plasmid was constructed by cloning RYK (GenBank™ accession number NM002958) in pTracer (Invitrogen) in frame with the V5 tag. A Not1 site was created at the 3′ end of the cDNA, and the TGA stop codon was mutated to TGG by PCR using the primer TGAGCGGCCGCGAGAGGAGCCAGACGTAGG.
The RYKEC construct was made by cloning an EcoRI-HindIII fragment (nucleotides 1–1201; GenBank™ accession number NM002958) in frame with GFP in pEGFP vector (CLONTECH).

**Growth Factor and Antibodies**—The recombinant human NGF was purchased from R & D Systems. The N-terminal TrkA-specific monoclonal antibody 5C3 was a gift from U. Saragovi, the N-terminal anti-Ryk antibody RYK3 was a gift from Steven Stacker (Ludwig Institute for Cancer Research) (24), and the anti-human Ryk polyclonal antibody 15.2 was raised against the C-terminal region of RYK. The antibodies and protein A- or G-agarose beads were used according to the manufacturer’s instructions. For double transfections, RYK.V5 and HA-EphB3 or EphB2 were used in the ratio 4:1. The cells were treated with 1 mM sodium butyrate, a nonspecific transcriptional inducer, 5 h after transfection. 24–48 h after transfection, the cells were lysed on ice for 15 min in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA) containing 1 mM sodium butyrate, a nonspecific transcriptional inducer, 5 h after transfection. 24–48 h after transfection, the cells were lysed on ice for 15 min in lysis buffer (1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA) containing 0.1% SDS, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA) was used to lyse cells. Insoluble material was removed by centrifugation for 20 min at 13,000 rpm at 4 °C. The cell lysates were incubated with antibodies and protein A- or G-agarose beads at 4 °C. All of the immunoprecipitations were washed twice in lysis buffer containing 1 mM NaCl and once in unsupplemented lysis buffer. For immunoblotting, the immunoprecipitates were resolved by SDS-PAGE according to standard protocols and then transferred onto nylon membranes. The antibodies were used according to the manufacturer’s conditions. The 15.2 and RYK3 antibodies were used at 1:500 in 1% milk and 0.2% Tween 20 for IB, and once in unsupplemented lysis buffer. For immunoblotting, the immunoprecipitates were resolved by SDS-PAGE according to standard protocols and then transferred onto nylon membranes. The antibodies were used according to the manufacturer’s conditions. The 15.2 and RYK3 antibodies were used at 1:500 in 1% milk and 0.2% Tween 20 for blotting.

For the induction studies with NGF, cells were washed once with phosphate buffered saline and then incubated for 16 h in serum-free medium 30 h after transfection. The quiescent cells were stimulated with recombinant human NGF (100 ng/ml) for different time intervals (0, 5, 15, 30, or 60 min) before being lysed.

**purification of the His-tagged catalytic domain of Ryk**—The catalytic domain of Ryk (nucleotides 1019–2138; GenBank™ accession number NM002958) was generated by PCR using the following primers: 5′-TAACTCGAGGAAGGTGACTCTTGAGAGGAG-3′ and M13 reverse primer from pBluescript. The cDNA was cloned as a Pst/EcoRI frag-
ment in a baculovirus vector (pBlueBacHis2; Invitrogen) in frame with a His tag on the N-terminal end and expressed in SF9 cells. The cells were infected with a multiplicity of infection of 10, grown at 27 °C, and l lysed after 4 days in 20 mM Tris, pH 8, 0.5 M NaCl, 1% Triton, 1% Tween 20, 0.5% Nonidet P-40, and protease inhibitors (pepstatin, leupeptin, aprotinin, trypsin inhibitor, and phenylmethylsulfonyl fluoride). After centrifugation at 14,000 rpm for 30 min, the protein was bound on a cobalt-Sepharose column (Talon, CLONTECH) for 1 h at 4 °C in lysis buffer containing 10 mM imidazole. The resin was washed thoroughly with lysis buffer containing 10 mM imidazole, and the protein was eluted in 20 mM Tris, pH 7.5, 0.5 M NaCl, 50 mM imidazole. The protein was concentrated using a Centriplus YM-10 (Amicon), dialyzed against a 20 mM Tris, pH 8, solution, and stored at -70 °C in 10% glycerol.

In Vitro Kinase Assay—HA.EphB3 was transiently expressed in HEK 293T cells and immunoprecipitated using an anti-HA antibody. A, the anti-phosphotyrosine antibody blot shows phosphorylation of EphB3 and AF-6 when they are present in the kinase assay. B, presence of RYK detected by the anti-V5 antibody. C, presence of AF-6 detected by the anti-AF-6 antibody. D, presence of EphB3 detected by the anti-HA antibody. IB, immunoblot.

For the kinase assay using full-length RYK as a substrate, HA.EphB3, Myc.AF-6, and RYK.V5 were transiently expressed in HEK 293T cells and immunoprecipitated using an anti-HA tag, and anti-
Interaction of RYK with EphB2, EphB3, and AF-6

RESULTS

Expression of RYK—Transfection of HEK 293T cells with a C-terminal V5-tagged RYK, followed by immunoprecipitation with an anti-V5 antibody and immunoblotting with the same antibody, identified two major signals: a set of bands at around 80 kDa (comprising three bands of 70, 75, and 80 kDa) and another at 45 kDa. The 45-kDa band was detected by the anti-V5 antibody and immunoblotting with the same antibody, identified two major signals: a set of bands at around 80 kDa (comprising three bands of 70, 75, and 80 kDa) and another at 45 kDa. The 45-kDa band was detected by the anti-V5 antibody and immunoblotting with the same antibody.

AF-6 and anti-V5 antibodies, respectively. The immunoprecipitates were washed twice in lysis buffer containing 1 M NaCl and twice in kinase buffer. HA.EphB3 was resuspended with Myc-AF-6 or RYK-V5 in 100 μl of kinase buffer containing 200 μM ATP and incubated at 30 °C for 45 min. The immunoprecipitates were washed in kinase buffer and resuspended in sample buffer. The proteins were resolved by SDS-PAGE and blotted and phosphorylation was detected using the antiphosphotyrosine antibody 4G10.

Binding Assay—A Myc-tagged AF-6 construct was transfected in HEK 293T cells and immunoprecipitated using an anti-Myc antibody. The cell lysate from untransfected HEK 293T cells was also immunoprecipitated with the anti-Myc antibody. The protein G-agarose beads were resuspended in phosphate buffered saline, and 1 μg of the purified RYK catalytic domain, RYKcat, was added. After 1 h of incubation at 4 °C, the beads were washed in lysis buffer and resuspended in sample buffer before being resolved by SDS-PAGE and blotted.

RYK Associates with EphB2 and EphB3 but Is Not Phosphorylated—We transfected HEK 293T cells with RYK.V5 together with HA.EphB3 or a kinase-inactive mutant of EphB3 (EphB3K665R) (21). Upon immunoprecipitation of RYK by the anti-V5 antibody, both wild type EphB3 and EphB3K665R were detected (Fig. 3, A–C). This shows that EphB3 and RYK interact and that this association is not dependent on activation and phosphorylation of the Eph receptor. However, although the binding with RYK was demonstrated under stringent conditions using radioimmune precipitation buffer (data not shown), there was no phosphorylation of RYK (Fig. 3D) when it is co-expressed with EphB3. To further evaluate the ability of EphB3 to phosphorylate RYK, we performed an in vitro kinase assay on immunoprecipitated proteins. HEK 293T cells were transfected with HA.EphB3, RYK.V5, or Myc-AF-6, and these proteins were immunoprecipitated using the appropriate antibodies. An in vitro kinase assay was performed using immunoprecipitated EphB3 and RYK or AF-6, and their phosphorylation was detected with 4G10 antibody. EphB3 failed to phosphorylate full-length RYK (Fig. 4, A, B, and D), whereas the positive control AF-6 was phosphorylated (Fig. 4, A, C, and D). Furthermore, EphB3 was unable to phosphorylate the catalytic domain of RYK in an in vitro kinase assay (data not shown).

A converse experiment where we co-expressed EphB3 and RYK in HEK 293T cells and immunoprecipitated EphB3 (Fig. 5) showed the same result. Interestingly, only the 80-kDa form of RYK associated with EphB3, suggesting an essential role of the extracellular domain of RYK for this interaction.

We also investigated the interaction between RYK and EphB2 (Fig. 6). When both receptors were expressed transiently in HEK 293T cells and EphB2 was immunoprecipitated, only the 80-kDa form of RYK was detected (Fig. 6, A–C), although it is possible that the 45-kDa band was not detected because of unspecific bands. A phosphotyrosine blot (Fig. 6D) showed that EphB2 was activated but did not phosphorylate RYK. This suggests that, similar to EphB3, the association approach (2). We undertook to study the ability of RYK to autophosphorylate using a biochemical approach. We cloned the catalytic domain in a baculoviral vector, expressed it in SF9 insect cells, and purified it under nondenaturing conditions. An in vitro kinase assay using the purified protein showed that the catalytic domain of RYK is unable to autophosphorylate (Fig. 2). As a positive control, EphB3 was phosphorylated in the presence of ATP.

RYK Is Unable to Autophosphorylate—RYK has not been shown to have catalytic activity, in vitro on a peptide substrate, using the whole protein or the catalytic domain on its own produced in bacteria (1, 4) or in vivo using a chimeric receptor

Fig. 7. Absence of interaction between EphB3 and TrkA-RYK. HEK 293T cells were transfected with the indicated constructs, and EphB3 was immunoprecipitated using an anti-HA antibody. A, the anti-Trk antibody detects TrkA and TrkA-RYK in the cell lysate (shown by arrows) but not in the immunoprecipitates. B, the anti-HA antibody shows that EphB3 is expressed and immunoprecipitated. IP, immunoprecipitation; IB, immunoblot.

Fig. 8. Absence of interaction between EphB3 and RYK. HEK 293T cells were transfected with the indicated constructs, and EphB3 was immunoprecipitated using an anti-HA antibody. A, the anti-GFP antibody shows that RYK.EC was expressed. B, the anti-HA antibody shows that EphB3 was expressed and immunoprecipitated. IP, immunoprecipitation; IB, immunoblot.
between RYK and EphB2 is dependent on the extracellular domain of RYK and does not lead to the phosphorylation of RYK.

To investigate further the association between RYK and EphB3 and assess whether the extracellular domain of RYK is required for the interaction to take place, we co-expressed EphB3 and a TrkA-RYK chimeric receptor or EphB3 and TrkA as a negative control. The TrkA-RYK chimeric receptor contains the extracellular domain of TrkA, the NGF receptor, and the transmembrane and intracellular domains of RYK (2). When expressed, TrkA is a 120-kDa protein, and TrkA-RYK is 140 or 120 kDa, depending on its state of glycosylation. The chimeric receptor, lacking the extracellular domain of RYK, did not associate with EphB3 (Fig. 7).

We then wanted to assess whether the extracellular domain of RYK on its own was able to bind EphB3. We constructed a GFP-tagged cDNA (RYKEC) containing the extracellular, transmembrane, and juxtamembrane domains of RYK and co-expressed it with EphB3 in HEK 293T cells (Fig. 8). The predicted size of RYKEC protein, together with GFP (30 kDa), is 70 kDa. On transfection, the protein migrated at 85 kDa on SDS-PAGE, probably because of glycosylation. When EphB3 was immunoprecipitated, RYKEC did not associate with it, suggesting that the whole receptor is required for the interaction between RYK and EphB3.

**RYK Does Not Associate with AF-6**—The C-terminal end of RYK possesses a consensus sequence for the binding of a PDZ domain (Fig. 9). The C-terminal tyrosine and valine residues are completely conserved. Because RYK is devoid of catalytic activity and PDZ domains do not bind to phosphorylated amino acids (26–28), it is possible that RYK may signal via a PDZ domain-containing protein. Of several PDZ domain-containing proteins, only AF-6 has been shown to bind RTKs such as EphB2 and EphB3. In the light of previous results (13), we wished to evaluate whether AF-6 bound to RYK.

When the TrkA-RYK chimeric receptor, which contains the entire cytoplasmic domain of RYK, and AF-6 were co-expressed in HEK 293T cells, they did not associate, whether AF-6 (Fig. 10, A and B) or the chimeric receptor (Fig. 10, C and D) was immunoprecipitated. EphB3, which was used as a positive control for the interaction, was immunoprecipitated with AF-6 (Fig. 10, E and F). The possibility that AF-6 may bind to RYK when the latter is stimulated was excluded when no association was observed after stimulation of TrkA-RYK with NGF (data not shown). Transfection with full-length untagged RYK could not be used in these experiments because the 15.2 antibody has a low affinity for immunoprecipitation.

To further verify whether there is direct interaction between the proteins, we used the purified His-tagged catalytic domain of RYK. AF-6 was immunoprecipitated from transfected HEK 293T cells and mixed with RYK.cat under physiological conditions. Subsequently, after washing the agarose beads, the proteins were resolved by SDS-PAGE and blotted. As shown (Fig. 11), AF-6 does not bind in the presence of excess amounts of the catalytic domain of RYK.

**DISCUSSION**

RYK is a receptor tyrosine kinase that belongs to a small family of individually distinct receptors that are devoid of catalytic activity (29). The mechanism of signaling by these receptors is best understood for ErbB3 (30).

We have observed several forms of the RYK protein in transfected cells with different molecular masses: 45, 70, 75, and 80 kDa. The three high molecular mass bands are full-length receptors, because they are recognized by the V5 antibody and the RYK3 antibody specific to the extracellular domain. The three different sizes could be due to differences in the glycosylation status of the protein, although other post-translational

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**FIG. 9.** Comparison of the C-terminal end of RYK across species. The C-terminal residues of Drosophila, human, and mouse RYK (GenBank accession numbers NP477341, S58885, P34925, and Q01887, respectively) were aligned using Clustal-X and displayed with MacBoxShade. DNT, doughnut. DRL, derailed.

**FIG. 10.** Absence of interaction between AF-6 and TrkA-RYK. HEK 293T cells were transfected with the indicated constructs; EphB3 was used as a positive control. A and B, AF-6 was immunoprecipitated with an anti-AF-6 antibody, but TrkA-RYK is not detected in the immunoprecipitates, although it is present in the cell lysate. In the untransfected lane, the anti-AF-6 antibody detects endogenous AF-6. C and D, TrkA-RYK was immunoprecipitated, but AF-6 is not detected in the immunoprecipitates, although it is present in the cell lysate. E and F, AF-6 was immunoprecipitated using an anti-Myc antibody, and EphB3 is detected in the immunoprecipitate and the cell lysate. IP, immunoprecipitation; IB, immunoblot.

**FIG. 11.** Absence of interaction between AF-6 and the catalytic domain of RYK. A binding assay was carried out between AF-6 and the purified catalytic domain of RYK (RYK.cat) as described under “Experimental Procedures.” In the presence of immunoprecipitated AF-6 (Myc-AF-6) from transfected HEK 293T cells and purified RYK catalytic domain (RYK.cat) in phosphate buffered saline, no binding was detected after blotting with the anti-His antibody (A). B and C show that AF-6 and RYK.cat, respectively, were present in the reaction. IP, immunoprecipitation; IB, immunoblot.
modifications could occur because the 75- and 80-kDa bands are not detected by the 15.2 antibody. The epitope against which this antibody is directed is a 17-mer peptide located at positions 548–564 of the RYK protein. It contains a cysteine that could be converted to formylglycine; this modification occurs in sulfatases at a late stage of co-translational protein translocation into the endoplasmic reticulum (31). There are also two glutamate residues in the peptide that could be converted to carboxylglutamate by a carboxylase (32). If such post-translational modifications occur in the RYK protein, the modified forms of RYK may not be recognized by the 15.2 antibody. The 45-kDa band is not detected by the RYK3 antibody directed against the extracellular domain and must be a truncated form of the receptor, presumably resulting from the cleavage of the extracellular domain. This cleavage is very likely to take place at the tetrabasic protease cleavage site (KRRK). Ectodomain cleavage occurs frequently among cell surface transmembrane proteins, including receptor tyrosine kinases such as ErbB4 (33), colony-stimulating-factor-1 receptor (34), e-Kit (35, 36), Met (37), or TrkA (38). It seems to be a general mechanism to modulate receptors by down-regulating ligand-induced signaling (39, 40). Because only the full-length RYK and not the 45-kDa form co-immunoprecipitates with EphB3 and EphB2, it seems likely that, like with other RTKs, the ectodomain cleavage of RYK regulates the receptor, either by preventing binding of the unknown ligand or the binding of other receptors.

The heterodimerization of RTKs belonging to two different families is unusual, but it has been reported. The EGF and PDGF receptors have been shown to heterodimerize independently of receptor stimulation, and transactivation of EGFR was observed after stimulation with PDGF. EGFR transactivation by PDGF did not depend on PDGF receptor kinase activity, but phosphorylation of one of the receptors, or of a protein that links the receptors, by a Src kinase was required to maintain heterodimer formation (41). The interaction between RYK and the Eph receptors is unusual because RYK does not get phosphorylated and therefore may have a role different from that of analogous interactions involving typical RTKs. RYK could act as an inhibitor of EphB2 and EphB3 by preventing homodimerization of these receptors, but data provided by knock out mice for these receptors seem to suggest that RYK and these two Eph receptors may co-operate in vivo. RYK is required in normal development and morphogenesis of craniofacial structures and the limbs (13). Neonatal mice doubly deficient in EphB2 and EphB3 phenocopy RYK-deficient mice in terms of cleft palate development and morphogenesis of craniofacial structures for RYK and the Eph receptors. This suggests that regions outside the extracellular domain are required for heteromeric interactions, as is the case for RYK and the Eph receptors.

We have demonstrated in this study that the association between RYK and EphB2 or EphB3 does not result in the phosphorylation of RYK. However, a previous study (13) showed that, when the receptors are co-expressed, murine Ryk associates with and is phosphorylated by EphB2 and EphB3. In both studies, co-transfections were performed in HEK 293T cells with the same EphB3 cDNA construct, but we used a human RYK cDNA, whereas the authors used the murine homologue, Ryk. The ability of EphB3 to phosphorylate RYK could not be detected, either in vivo or in vitro. The identity between the human and murine cDNAs is very high (93%), but our results indicate that they do not signal in the same way. The species-dependent phosphorylation of a substrate by a kinase has been observed in the case of p53 (49). Murine p53 can be phosphorylated by mitogen-activated protein kinase, whereas human p53 is not, suggesting species-specific differences in the modification and therefore possibly the regulation of the protein.

Further, using a murine Ryk homologue, the authors of a previous study (13) were able to show that AF-6, a PDZ domain-containing protein, could interact with the receptor via its PDZ domain and that the C-terminal valve of Ryk was essential for this interaction. AF-6 is a Ras effector located at sites of cell-cell junction (18, 19) and has been shown to bind to EphB2 and EphB3 (20, 21). However, we were unable to show any interaction between the cytoplasmic domain of human RYK and AF-6. The co-transfection experiments, performed using the TrkA-RYK chimera to be able to immunoprecipitate RYK, did not correlate with the data obtained using murine Ryk, although only the extracellular domain of RYK was missing from the receptor. The observed differences in the data can only be explained as being due to species specificity. The C-terminal parts of the human and mouse receptors, which is the AF-6 binding peptide, are identical, but another motif, present only in the mouse homologue, could also be required for the interaction. AF-6 binds to EphB2 and EphB3 only if these receptors are activated and phosphorylated (20); the C-terminal motif of the receptors is therefore not the only feature needed for the interaction with AF-6.

In summary, our results suggest that the human homologue of RYK, although it binds to EphB2 and EphB3, is not phosphorylated by these receptors. Further studies exploring the mechanism by which RYK may modulate signaling by EphB2 and EphB3 are required.

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REFERENCES

1. Hovens, C. M., Stacker, S. A., Andres, A. C., Harpur, A. G., Ziemiecki, A., and Wilks, A. F. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11818–11822
2. Katso, R. M., Russell, B. R., and Ganesan, T. S. (1999) Mol. Cell. Biol. 19, 6457–6440
3. Wang, X. C., Katso, R., Butler, R., Hanby, A. M., Poulsom, R., Jones, T., Sheer, D., and Ganesan, T. S. (1996) Mol. Med. 2, 189–203
4. Tamagnone, L., Partanen, J., Armstrong, E., Lasota, J., Ohgami, K., Tazunoki, T., LaForgia, S., Huehner, K., and Alitalo, K. (1993) Oncogene 8, 2009–2014
5. Williams, A., and Barclay, A. (1988) Annu. Rev. Immunol. 6, 381–405
6. Petersen, T., Thogersen, H., Skorstengaard, K., Vibe-Pedersen, K., Sahl, P., Sottrup-Jensen, L., and Magnusson, S. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 137–141
7. Ulrich, A., Coisson, L., Hayflick, J., Dull, T., Gray, A., Tam, A., Lee, J., Yarden, Y., Libermann, T., and Schlessinger, J. (1984) Nature 309, 418–425
8. Schneider, R., Schneider-Scherer, E., Thurnher, M., Auer, B., and Schweiger, M. (1988) EMBO J. 7, 4151–4156
9. Schneider, R., and Schweiger, M. (1991) Oncogene 6, 1897–1811
10. Rothberg, J., Jacobs, J., Goodman, C., and Artavanis-Tsakonas, S. (1990) Genes Dev. 4, 2169–2187
11. Patton, L. (2000) Trends Biochem. Sci. 25, 12–13
12. Katso, R. M., Manek, S., Gajevi, H., Biddolph, S., Charnock, M. F., Bradburn, M., Wells, M., and Ganesan, T. S. (2000) Clin. Cancer Res. 6, 3271–3281
13. Halford, M. M., Armes, J., Buchert, M., Meskenaite, V., Grail, D., Hibbs, M. L.,...
Interaction of RYK with EphB2, EphB3, and AF-6

23043

Wilks, A. F., Farlie, P. G., Newgreen, D. F., Hovens, C. M., and Stacke, S. A. (2000) Nat. Genet. 25, 414–418

14. Orioli, D., Henkemeyer, M., Lemke, G., Klein, R., and Pawson, T. (1996) EMBO J. 15, 6035–6049

15. Holder, N., and Klein, R. (1999) Development 126, 2033–2044

16. Feng, C., Laskowski, M., Feldheim, D. A., Wang, H. M., Lewis, R., Frisen, J., Flanagan, J. G., and Sanes, J. R. (2000) Neuron 25, 295–306

17. Tessier-Lavigne, M. (1995) Cell 82, 345–348

18. Linnemann, T., Geyer, M., Jaitner, B. K., Block, C., Kuhlthau, H. R., Wittinghofer, A., and Herrmann, C. (1999) J. Biol. Chem. 274, 13556–13562

19. Kuriyama, M., Harada, N., Kuroda, S., Yamamoto, T., Nakafuku, M., Iwamatsu, A., Yamamoto, D., Prasad, R., Croce, C., Canaani, E., and Kaibuchi, K. (1996) J. Biol. Chem. 271, 607–610

20. Hock, B., Bohme, B., Karn, T., Yamamoto, T., Kaibuchi, K., Holtrich, U., Holland, S., Pawson, T., Rubsamern-Waigmann, H., and Strebhardt, K. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9779–9784

21. Buchert, M., Schneider, S., Meskenaite, V., Adams, M. T., Canaani, E., and Baechi, T., Moelling, K., and Hovens, C. M. (1999) J. Cell Biol. 144, 361–371

22. Holland, S. J., Gale, N. W., Mhamad, G., Yancopoulos, G. D., Henkemeyer, M., and Pawson, T. (1996) Nature 383, 722–725

23. Henkemeyer, M., Marengere, L. E., McGlade, J., Olivier, J. P., Conlon, R. A., Holmyard, D. P., Lettin, K., and Pawson, T. (1994) Oncogene 8, 1001–1014

24. Halford, M. M., Oates, A. C., Hibbs, M. L., Wilks, A. F., and Stacker, S. A. (1999) J. Biol. Chem. 274, 7379–7390

25. Kato, K. M., Manek, S., Biddolph, S., Whittaker, R., Charnock, J. M., Wells, M., and Ganesan, T. S. (1999) Cancer Res. 59, 2265–2270

26. Songyang, Z., Fanning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chishiti, A. H., Crompton, A., Chan, A. C., Anderson, J. M., and Cantley, L. C. (1997) J. Biol. Chem. 272, 17577–17584

27. Kornau, H., Shenker, L., Kennedy, M., and Seeburg, P. (1995) Science 269, 1737–1740

28. Kim, E., Niethammer, M., Rothschild, A., Jan, Y. N., and Sheng, M. (1995) Nature 378, 85–88

29. Kreuter, M., Miller, M. A., and Steele, R. E. (2001) Bioessays 23, 69–76

30. Alroy, I., and Yarden, Y. (1997) FEBS Lett. 410, 83–86

31. Downing, J. R., Roussel, M. F., and Sherr, C. J. (1989) Mol. Cell. Biol. 9, 7390–7399

32. McCarthy, M. J., Burrows, R., Bell, S. C., Christie, G., Bell, P. R., and Brindle, K. (1994) J. Biol. Chem. 269, 3410–3418

33. Morris, D. P., Stevens, R. D., Wright, D. J., and Stafford, D. W. (1995) J. Biol. Chem. 270, 20589–20595

34. Downing, J. R., Roussel, M. F., and Sherr, C. J. (1989) Mol. Cell. Biol. 9, 2890–2896

35. Yee, N. S., Langen, H., and Besmer, P. (1993) J. Biol. Chem. 268, 14189–14201

36. Yee, N. S., Hsiau, C. W., Serve, H., Vosseller, K., and Besmer, P. (1994) J. Biol. Chem. 269, 31991–31998

37. Jeffers, M., Taylor, G. A., Weidner, K. M., Onuma, S., and Van de Woude, G. F. (1997) Mol. Cell. Biol. 17, 799–808

38. Cabrera, N., Diaz-Rodriguez, R., Becker, E., Martin-Zanca, D., and Pandiella, A. (1996) J. Cell Biol. 132, 427–436

39. McCarthy, M. J., Burrows, R., Bell, S. C., Christie, G., Bell, P. R., and Brindle, K. (1994) Nat. Genet. 7, 1740–1747

40. Jeffers, M., Taylor, G. A., Weidner, K. M., Omura, S., and Van de Woude, G. F. (1997) Mol. Cell. Biol. 17, 799–808

41. Saito, Y., Haendeler, J., Hojo, Y., Yamamoto, K., and Berk, B. (2001) Mol. Cell. Biol. 21, 6387–6394

42. Callahan, C. A., Bonkovsky, J. L., Scully, A. L., and Thomas, J. B. (1996) Development 122, 2761–2767

43. Callahan, C. A., Muralidhara, M. G., Lundgren, S. E., Scully, A. L., and Thomas, J. B. (1995) Nature 376, 171–174

44. Pinkas-Kramarski, R., Shelly, M., Guarino, B. C., Wang, L. M., Lyass, L., Alroy, I., Alimandi, M., Kuo, A., Moyer, J. D., Lavi, S., Eisenstein, M., Ratkini, E. J., Seger, R., Racus, S. S., Pierce, J. H., Andrews, G. C., Yarden, Y., and Alimandi, M. (1998) Mol. Cell. Biol. 18, 6090–6101

45. Pinkas-Kramarski, R., Soussan, L., Waterman, H., Levkowitz, G., Alroy, I., Klapper, L., Lavi, S., Seger, R., Ratzkin, B. J., Sela, M., and Yarden, Y. (1996) EMBO J. 15, 2452–2467

46. Ferguson, K. M., Darling, P. J., Mohan, M. J., Macatee, T. L., and Lemmon, M. A. (2000) EMBO J. 19, 4632–4643

47. Jardine, L. J., Milne, D. M., Dumaz, N., and Meek, D. W. (1999) Oncogene 18, 7602–7607
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