Identification of a Human Epidermal Growth Factor Receptor-associated Protein Kinase as a New Member of the Mitogen-activated Protein Kinase/Extracellular Signal-regulated Protein Kinase Family*

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A putative mitogen-activated protein kinase (MAPK) has recently been identified, which potentially phosphorylates the human epidermal growth factor (EGF) receptor at a physiological site (Thr-669) and is distinguished from other MAPKs/EGF receptor/tyrosine kinase ERKs on the basis of chromatographic, immunological, and kinetic data. Here we report that this newly discovered MAPK is physiologically associated with the EGF receptor in A431 cells and with the related receptor/tyrosine kinase HER2 (encoded by c-neu) in enzyme preparations obtained from Wilm's tumors. This human EGF receptor-associated kinase is characterized as a 40-kDa Thr-669 kinase that exists in a high molecular mass complex with the respective growth factor receptor. EGF treatment of A431 cells stimulates the tyrosine phosphorylation of p40 and increases Thr-669 kinase activity in p40-containing fractions. The 40-kDa kinase is recognized by affinity-purified polyclonal antibodies directed against the sea star p44 erk1 and/or the rat p42 erk2 MAPKs, and by antibodies selective for the rat p44 erk1 and/or the p42 erk2 isoforms, thus identifying the EGF receptor-associated kinase as a novel MAPK that may regulate receptor function in vivo.

Mitogen-activated protein kinases (MAPKs), also known as extracellular signal-regulated protein kinases (ERKs), constitute a rapidly growing family of protein-serine/threonine kinases implicated in a diversity of cell signaling pathways (1–4). Often stimulated directly by phosphorylation on both tyrosyl and seryl/threonyl residues (5–10), MAPKs may function as convergence points in integrated protein kinase cascades that mediate cellular activation (11–13). Several serine/threonine kinases that represent distal targets in growth factor action, such as p74 erk1 (14, 15) and the ribosomal S6 kinases p70 sak (16, 17) and p70 sak (18), are directly phosphorylated in vitro by MAPKs at physiological sites. However, MAPK-related enzymes have also been implicated in the regulation of more proximal aspects of molecular signaling by governing the biological activity of growth factor receptors. The human EGF class of receptors in particular are known to be phosphorylated at Thr-669 in vivo (19, 20), a site that is phosphorylated by a number of MAPK isoforms in vitro (21–23). Recently, we identified a novel putative MAPK, which potentially phosphorylates the human EGF receptor Thr-669 site and is distinguished from other MAPKs/ERKs on the basis of chromatographic, immunological, and kinetic data (23). In the present study we report that this newly discovered MAPK is physically associated with the EGF receptor in human A431 cells and with the related receptor/tyrosine kinase HER2 (encoded by c-neu) in enzyme preparations obtained from Wilm's tumors. We describe the physiological activation, tyrosine phosphorylation, and partial purification of the enzyme. In addition, we characterize a profile of immunoactivity that identifies this human EGF receptor-associated kinase as a MAPK but distinguishes this enzyme from previously identified members of the mammalian MAPK/ERK family.

EXPERIMENTAL PROCEDURES

Materials

Cells and Tissues—Human A431 epidermoid carcinoma cells obtained from the American Type Culture Collection (ATCC CRL 1555) and maintained as monolayers in log phase growth (25) were incubated in the absence of 10% fetal calf serum for 3 h prior to stimulation with 5 ng/ml murine epidermal growth factor (EGF) and harvesting as described by Sanghera et al. (23). Human pediatric Wilms' tumors provided by Timothy Triche (Chief of Pathology) and Phyllis Pepe of Children's Hospital Los Angeles were flash-frozen in liquid nitrogen and stored frozen at −70 °C prior to homogenization and enzyme purification.

Specific antibodies—Rabbit polyclonal antibodies directed against subdomains I, the ATP-binding site (GLAYIGEGAYGMV) of the sea star p44 erk (19–21, 22) and the purified p44 erk protein (anti-p44 erk), the rat erk1 sequences PFEIQTYCQRTLREIQILLGFRHENVIGIRDIL-RAP (erk1-III Ab) and PFTDKMELDLPLKERKELIQFQETARQFGAPA-FPEAP (erk1-CT Ab), and the carboxy-terminal domain (including sub-
domain XI) of mouse p34cdc2 (Upstate Biotechnologies Inc., Cdc2-CT Ab), have been previously characterized (23, 24). Extreme COOH-terminal (DNQIKKK) anti-Cdc2 antibodies, as well as anti-cyclin A antibodies, are described elsewhere (25, 32). Rabbit polyclonal antibodies were also raised against the sequence QLQYIGEGAYGMVSSA, representing subdomain I of the rat p44 MAPK (anti-Erk 1). Additionally, Pan-ERK antibodies were raised against the highly conserved sequence GFLTEYVATRWWYR contained within subdomain VIII of both human and murine p42MAPK/ERK2 and p44 MAPK. Polyclonal antibodies specific for the human EGF receptor were obtained from Cambridge Research Biochemicals (oncoprotein antibody OA-11–852). A monoclonal antibody specific for the HER-2 receptor was purchased from Oncogene Science (Ab-2). Monoclonal anti-phosphotyrosine antibodies (clone PY-20) were specific for the HER-2 receptor was purchased from Oncogene Science. Extreme COOH-terminal antibodies were raised against the highly conserved sequence XI) of mouse (Upstate Biotechnologies Inc., Cdc2-CT). The Thr-669hIBP phosphotransferase activity 1 mg/ml MBP (Sigma) and 1 mM CaCl2, followed by step elution with 700 mM NaCl, and application to a second Fast Q column (25 ml), which was washed and developed with a linear 350–800 mM NaCl gradient. The respective peak of Wilm's tumor Thr-669 peptide kinase activity eluted from this column at approximately 500 mM NaCl. Concentrated aliquots were stored at –70 °C.

RESULTS AND DISCUSSION

FPLC Mono Q fractionation of soluble extracts from human A431 epithelioid carcinoma cells previously afforded the resolution of four distinct peaks of EGF receptor Thr-669 phosphotransferase activity, three of which (peaks I, II, and III) were readily detected with either wheat germ agglutinin-Sepharose, chromatographic media, and HR 5/5 Mono Q columns were purchased from Pharmacia LKB Biotechnology Inc. Murine EGF was purchased from Collaborative Research.

Methods

FPLC Mono Q Chromatography—Human A431 cell extracts were prepared as described in Sanghera et al. (23). Approximately 4 mg of clarified, filtered detergent lysate was applied to an equilibrated HR 5/5 Mono Q column in Buffer A (5 mM Mops, pH 7.2, 5 mM EDTA, 1 mM sodium vanadate, and 1 mM dithiothreitol) at a flow rate of 0.8 ml/min on a Pharmacia FPLC system. The column was developed with a 10-ml linear 0–800 mM NaCl gradient in Buffer A mixed and delivered at the same flow rate; 0.25-ml fractions were collected. Selected column fractions were immediately analyzed by in vitro kinase assays or were preserved with SDS sample buffer for Western analysis.

Protein Kinase Assays and Western Analysis—Chromatographic fractions were assayed for their phosphotransferase activity toward 1 mg/ml myelin basic protein or 1 mg MGF receptor Thr-669 peptide substrate (ELVEPLTSGPQAPNALLKK), as described previously (23, 40). Mono Q fractions were subjected to SDS-PAGE, electrophoretic transfer to Immobilon P membranes (Millipore), and immunoblotting as described (23, 25, 32). Affinity-purified, alkaline phosphatase-conjugated secondary antibodies were generally used at a 1:1000 dilution in 1% bovine serum albumin. Enzymatic detection was routinely performed with 5-bromo-4-chloro-3-indolyl phosphate/ nitro blue tetrazolium or 3,3 ’-diaminobenzidine tetrahydrochloride respectively; however, an enhanced chemiluminescence (ECL) detection system (Amersham Corp.) was also utilized, as noted in legend to Fig. 4. Specific immunoprecipitations and affinity precipitations with wheat germ agglutinin-Sepharose were performed as described in the legend to Fig. 4.

Partial Purification of HER-2 Kinase from Wilm’s Tumors—One hundred tumor nodules of solid Wilm’s tumor were homogenized and sonicated in a 100-ml quaternary amine-Sepharose (Pharmacia Fast Q) column, which was washed extensively with 350 mM NaCl in equilibration buffer (as described in Ref. 32, plus vanadate) to elute the identified MAPKs (4, 6, 12, 23), followed by step elution with 700 mM NaCl, dilution to 350 mM NaCl, and application to a second Fast Q column (25 ml), which was washed and developed with a linear 350–800 mM NaCl gradient. The respective peak of Wilm’s tumor Thr-669 peptide kinase activity eluted from this column at approximately 500 mM NaCl. Concentrated aliquots were stored at –70 °C.

RESULTS AND DISCUSSION

FPLC Mono Q fractionation of soluble extracts from human A431 epithelioid carcinoma cells previously afforded the resolution of four distinct peaks of EGF receptor-Thr-669 phosphotransferase activity, three of which (peaks I, II, and III) were stimulated by treatment of A431 cells with EGF after a 3-h period of serum deprivation (23). These EGF-activated Thr-669 kinase peaks I, II, and IV were also readily detected with myelin basic protein (MBP) used as an alternative substrate (Fig. 1a and b). The Thr-669/MBP phosphotransferase activity in Mono Q peak II was previously attributed to p42MAPK (ERK2) and a 44-kDa MAPK that appears to be encoded by the erk1 gene (23). The Mono Q peak III kinase did not cross-react with any of five distinct MAPK antibodies at our disposal; however, Western analysis of peak III with monospecific antibodies detected a subset of p85γcyninA and p34cdc2, the regulatory and catalytic subunits, respectively, of the proline-directed protein kinase (PDPK) (25). The EGF stimulation of Mono Q peak III is remarkable in that p34cdc2/p58cytinA PDPK was initially identified in mammalian cells as a growth factor-sensitive enzyme system (26, 27), a finding that has been confirmed in a number of different cell lines (28, as well as platelets (29). Mono Q peak IV contains a novel member of the MAP kinase family that, in addition to chromatographic behavior, can be distinguished from other putative EGF receptor Thr-669 kinases on the basis of immunoreactivity and enzyme kinetics (23). This Mono Q peak IV kinase was tentatively identified as a 40-kDa protein that cross-reacts with a polyclonal antibody directed against the purified sea star MAP kinase (anti-p44 MAPK Ab) (see Fig. 1d) but not by any of a series of antibodies directed against defined epitopes within other identified MAPK/ERKs (23, 24). Subsequent studies were undertaken to better characterize the Mono Q peak IV kinase and its physical association with human EGF-class receptors.

The observed cross-reactivity of the 40-kDa protein in Mono Q peak IV with the anti-p44 MAPK antibody involves epitopes other than phosphotyrosine, since the presence of 1 mM phosphotyrosine was demonstrated to have no effect on the resulting immunoreactivity (not shown). Remarkably, the putative 40-kDa protein kinase in Mono Q peak IV did not cross-react with several monospecific antibodies selective for the identified...
mammalian MAPK/ERKs. However, this 40-kDa protein was recognized by an affinity purified antibody (Upstate Biotechnologies Inc., anti-Cdc2-CT) generated against the carboxyl-terminal 34 residues of the murine cyclin-dependent protein kinase p34cdc2 (Fig. 1e), a sequence that spans kinase subdomain XI (30). To a lesser extent, p42mapk, but not p44erk1, in Mono Q kinase peak II was also detected with this anti-p34cdc2 antibody. The consistent and specific cross-reactivity of the novel 40-kDa MAPK with anti-Cdc2-CT, but not with a second antibody raised against the seven most carboxyl-terminal residues of murine-human p34cdc2 (DNNIKKKM), demonstrated that the epitopes exhibiting cross-reactivity can be mapped to p34cdc2 subdomain XI. The comparative immunoreactivities of p42mapk, p44erk1, and the novel 40-kDa putative MAPK were further examined in side-by-side Western blots of Mono Q peak II fractions versus Mono Q peak IV fractions from non-stimulated cells. As shown in Fig. 2, both p42mapk and p44erk1 were readily detected with the mpk-I Ab (24), anti-ERK-I Ab, and anti-p44mapk and p44erk1 antibodies, whereas the 40-kDa protein in Mono Q peak IV reacted only with the latter of these three antibodies (also see Ref. 23). To further establish that p40 is a novel member of the MAPK/ERK family, we developed an additional Pan-ERK antibody directed against the highly conserved subdomain VIII, which includes the dual (TEY) regulatory phosphorylation sites (1). In addition to recognizing the p42mapk and p44erk1 proteins in Mono Q peak II, appreciable immunoreactivity toward the 40-kDa putative MAPK in Mono Q peak IV was observed with this Pan-ERK antibody, which is interesting in that this anti-peptide antibody (generated against a dephosphopeptide) would be expected to recognize only the inactive, nonphosphorylated species of these enzymes. The observed immunoreactivity of p40 with the Pan-ERK antibody is substantially less than that of the p42mapk and p44erk1 proteins, which suggests that the primary structure of p40 is either somewhat divergent or that one or both of the (TEY) regulatory sites may be phosphorylated. In these comparative Western analyses, strong immunoreactivity of the novel p40 MAPK with anti-Cdc2-CT antibodies was consistently observed, whereas the immunoreactivity of p42mapk was comparatively weak and cross-reactivity with p44erk1 was not observed. The detection of p40 in Mono Q Peak IV with anti-p44mapk and anti-Cdc2-CT antibodies, as well as Pan-ERK antibodies, in the absence of EGF pretreatment (compare with Fig. 1) further suggests that enzyme activation may not be a prerequisite for EGF receptor binding (also see below).

The immunoreactivity of the putative 40-kDa MAP kinase identified in Mono Q peak IV with an antibody based upon p34cdc2 sequences is not surprising, since MAP kinases are highly related to p34cdc2 in primary structure (1). Furthermore, kinase subdomain XI, which is encompassed by the peptide used to generate the anti-Cdc2-CT antibody, is a region of particularly high amino acid identity. Within this region, p42mapk/erk2 is somewhat more closely related to murine p34cdc2 than is p44erk1, which may account for the appreciable difference in immunoreactivity of p42mapk/erk2 versus p44erk1 in Mono Q peak II observed with this anti-Cdc2-CT antibody (see Fig. 1e). The strong cross-reactivity of the 40-kDa putative MAPK in Mono Q peak IV with antibodies recognizing two conserved protein kinase subdomains (subdomains VIII and XI) is meaningful in that this finding provides additional confirmation that p40 is a protein kinase. Perhaps significantly, of all the known MAP kinases, p40iso3 from Saccharomyces cerevisiae displays the greatest amino acid sequence identity to murine p34cdc2 in kinase subdomain XI (31). Furthermore, p40iso3 is the least conserved of the known MAP kinases to rat p44erk1 at the carboxyl terminus. Accordingly, antibodies directed against the carboxyl-terminal 35 residues of rat p44erk1 (anti-erk1-CT) immunoblot both p42mapk and p44erk1 in Mono Q peak II, but not the 40-kDa protein in Mono Q peak IV (23). Taken together with the biochemical data, these immunohistochemical findings indicate that the 40-kDa protein in Mono Q peak IV is a protein kinase. It appears to be a MAP kinase, perhaps most closely related to the yeast p40iso3 based upon its apparent molecular size and profile of immunoreactivity.

To test the hypothesis that one or more of these putative EGF receptor Thr-669 kinases identified in human A431 epithelial carcinoma cells may be physically associated with this growth factor receptor (23), we performed Western analysis of each of the respective FPLC Mono Q peak fractions. As shown in Fig. 1c, the majority of EGF receptor immunoreactivity co-eluted with Mono Q peak II, although a substantial portion of this receptor also co-eluted with Thr-669 kinase activity in peak IV. Although an interaction between PDPK and the EGF receptor would be in accord with the concept that the multifunctional PDPK may serve a triggering function at the mammalian R point (i.e. during the G1 to S phase transition) (28, 32), only traces of the receptor were detected in those fractions corresponding to peak III. FPLC Superose 6 gel filtration analysis of Mono Q peak II revealed quantitative recovery of MBP kinase activity at an apparent molecular mass of approximately 44,000, which precludes a physical association between the EGF receptor and either p42mapk/erk2 or p44erk1 under these conditions. By contrast, the major MBP kinase in Mono Q peak IV displayed an apparent molecular mass of approximately 420,000 upon gel filtration, which taken together with the detection of a 40-kDa anti-p44mapk reactive protein by SDS-PAGE and Western blotting of the high molecular weight fractions (23) indicates that this kinase does indeed reside in a multi-protein complex. This conclusion is further supported by the finding that most of the MBP kinase activity in the Mono Q peak IV fractions can be adsorbed by wheat germ agglutinin (WGA)-agarose (data not shown).

In an effort to isolate a larger amount of this Mono Q peak IV kinase and to further characterize the relationship with the EGF-class of receptors, we undertook to purify this enzyme...
from Wilm's tumors, utilizing preparative (Pharmacia Fast Q) rather than analytical (FPLC Mono Q) anion exchange chromatography followed by FPLC Superose 12 gel permeations chromatography. After eliminating the identified MAPKs by a series of ion exchange steps (see "Experimental Procedures"), the respective Wilm's tumor Thr-669 peptide kinase was eluted by a high salt gradient (350–800 mM NaCl) and an aliquot of the peak kinase activity was applied to a calibrated Superose 12 sizing column (Fig. 3a). The resulting Thr-669 kinase activity was determined to reside in a high molecular mass complex with an apparent molecular mass greater than 400 kDa. The peak activity fractions contained several proteins in the 120–200-kDa range that were phosphorylated in vitro upon the addition of [γ-32P]ATP (Fig. 3b). Western blotting of Superose 12 fractions, as well as peak anion exchange column fractions, revealed traces of EGF receptor; however, HER2 was readily identified as a prominent protein that co-fractionated with both Thr-669 kinase activity and anti-p44αMAPK Ab immunoreactivity (Fig. 3, b and c). This is an interesting finding in that fetal kidney is exceptionally rich in HER2 (33). As in A431 cells (23), the 40-kDa protein from Wilm's tumors did not cross-react with the anti-mpk-I Ab (Fig. 3c), which readily detects p42mpk/erk2 and p44erk1. These results indicate that a similar if not identical MAPK is present in a high molecular weight complex with the EGF receptor in A431 cells and with HER2 in Wilm's tumors. Based on this finding, as well as the ability to phosphorylate the EGF receptor Thr-669 phosphorylation site, this novel 40-kDa protein kinase is operationally designated Human EGF Receptor-Associated Kinase (p40HERAK), until microsequence information and/or molecular cloning can place this enzyme more specifically within a subfamily of the proline-directed protein kinases (1, 18, 28). Precedent for the observed physical association between a growth factor receptor/tyrosine kinase and a distinct serine/threonine protein kinase has recently been established in the case of the PDGF receptor and the cytoplasmic oncoprotein kinase p74arf (34).

The utilization of large scale Wilm's tumor preparations enabled additional FPLC sizing runs, providing sufficient material to visualize the p40HERAK upon Coomassie Blue staining of SDS-PAGE gels (Fig. 4a). The identity of this protein as the novel MAPK was confirmed by subsequent immunoblotting of peak fractions. The Wilm's tumor preparations also enabled the assessment of additional affinity interactions with receptor proteins. As shown in Fig. 4b, the p40HERAK was absorbed by wheat germ agglutinin-Sepharose, which is generally selective for membrane glycoproteins. Moreover, the p40HERAK was detected in HER2 immunoprecipitates only in the presence of the primary antibody (Fig. 4c). These results confirm the physical association, if not the physiological relationship, between p40HERAK and HER2, providing additional routes for future purifications, subsequent microsequencing, and eventual cloning of this potentially important enzyme.

The growth factor sensitivity of p40HERAK observed in human A431 cells was further investigated by comparative immunoblotting. As shown in Fig. 5a, the levels of p40 immunoreactivity detected with anti-p44αMAPK Ab were not significantly altered by EGF pretreatment, which suggests that the levels of this protein in Mono Q peak IV do not depend on prior receptor

![Fig. 3. Identification of the novel 40-kDa MAPK isoform in a high molecular weight complex containing HER2. Panel a, application of 0.5 ml of Wilm's tumor Fast Q peak IV kinase to a calibrated FPLC Superose 12 column (see Refs. 25 and 32) revealed a prominent peak of Thr-669 kinase activity at Mₐ ~ 440,000. The elution profile of molecular weight standards are as indicated; the void volume is depicted by the elution of blue dextran (Mₐ = 2,000,000). Panel b, co-fractionation of HER2 with the major peak of Thr-669 kinase activity is demonstrated by SDS-PAGE and Western blotting of peak fractions with a monoclonal antibody (Oncogene Science, Ab-2) specific for c-neu/HER2 (36). The identity of the immunoreactive doublet as HER2 protein was confirmed by including control (c) and anti-HER2 (a) antibodies. Panel c, Western analysis of Superose 12 peak Thr-669 kinase fractions with affinity purified polyclonal antibodies directed against the purified sea star p44αMAPK (anti-p44αMAPK) readily recognize the 40-kDa MAPK isoform present in these high molecular weight complexes (arrow). However, rabbit anti-peptide antibodies generated against the p44αMAPK sequence GLAYIGEGAYG-MVC (anti-mpk-1), which recognize p44αMAPK, p42mpk/erk2, and p44erk1 isomers on Western blots (23, 24), failed to detect this protein. In this experiment, a MAPK preparation that reacts with both antibodies was utilized as a positive control (c) in the development of the Western blots.](image-url)

![Fig. 4. Physical association of p40HERAK with the HER2 receptor in Wilm's tumor preparations. Panel a, column fractions corresponding to the high molecular weight peak of Thr-669 kinase activity obtained from FPLC Superose 12 chromatography of Wilm's tumor preparations (see Fig. 3) were subjected to SDS-PAGE on a 4–15% gradient gel and stained with Coomassie Blue. The identity of the 40-kDa protein, which was present in this high molecular weight complex (arrow) and which correlated precisely with Thr-669 phosphorylation, was confirmed by alignment of bands following Western analysis with anti-p44αMAPK antibodies. Panel b, absorption of p40HERAK to WGA was confirmed by incubating 120 µl of the Wilm's tumor Fast Q peak IV fractions with 30 µl of WGA-Sepharose (Pharmacia) on ice for 5 min followed by two washes with Fast Q buffer, addition of 2 x SDS buffer to the resulting pellet, SDS-PAGE, and Western blotting. The detection of p40HERAK (arrow) in the resulting precipitates was confirmed by immunoreactivity with anti-p44αMAPK antibodies, utilizing horseradish peroxidase-labeled secondary antibodies and 3,3'-diaminobenzidine tetrahydrochloride. Panel c, the physical association of p40HERAK with the HER2 was confirmed by incubating 100-µl aliquots of the Fast Q peak fractions in the presence (+) or absence (−) of a monoclonal antibody specific for c-neu/HER2 (Oncogene Science, Ab-2), followed by precipitation with protein G-agarose, washing two times with Fast Q buffer, and Western analysis with anti-p44αMAPK antibodies utilizing an enhanced chemiluminescence (ECL, Amersham) detection system.](image-url)
activation. In contrast, Western blotting with anti-phosphotyrosine antibodies detected phosphorylated p40HERAK only after EGF pretreatment (Fig. 5b). Under the present experimental conditions, the tyrosine phosphorylation of p40HERAK resides at the limits of detection, which precludes a more quantitative analysis at this time. Nonetheless, the detection of phosphotyrosine on p40HERAK is remarkable, for this is a common regulatory feature ascribed to members of the mammalian MAPK/ERK family, as well as the yeast enzyme p40PUS3 (1). In addition to EGF-mediated tyrosine phosphorylation, the observed growth factor sensitivity, profile of immunoreactivity, and deduced substrate specificity (23) are all consistent with the assignment of p40HERAK as a novel member of the MAP kinase family.

In summary, we have identified a new member of the MAP kinase family of serine/threonine protein kinases that is physically associated with EGF-class receptors. This receptor-associated kinase phosphorylates the EGF receptor Thr-669 phosphorylation site motif with relatively high affinity. While the regulatory significance of this receptor phosphorylation has yet to be fully elucidated (20, 35), this site-specific phosphorylation is predominant in vivo (19, 20), and the corresponding site is also conserved in both HER2 (36) and HER3 (37). This particular domain within these growth factor receptors is in close proximity to the intrinsic tyrosine kinase, ATP binding site, where covalent modification may well have subtle but important kinetic effects (35, 38). The finding that a similar and immunologically related protein kinase is associated with the EGF receptor and with HER2 is particularly interesting in that these receptors have been found to associate as heterodimers in cells which co-express both receptors (39). Alternatively, this novel EGF receptor-associated kinase may be representative of a new subfamily of receptor-associated MAPKs that are specific for receptor subtype. Since both the EGF receptor and HER2 are characterized as proto-oncogenes, the abnormal expression and biological activity of which are closely correlated with a number of human neoplasms, determination of the functional role of the receptor-associated serine/threonine kinase in modulating receptor function and/or mitogenic signaling may provide new insights into the mechanisms of oncogenesis as well as a strategic locus for potential therapeutic intervention.

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Addendum—While this study was near completion, we became aware of evidence for the association of p44ERK with the nerve growth factor receptor (encoded by c-erbB-2) (D. M. Loeb, H. Tsao, M. H. Cobb, and L. A. Greene, personal communication). Thus, the association of specific MAP kinases with specific growth factor receptors may indeed be a general phenomenon.

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