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Increased pp60$^{c\text{-}src}$ tyrosyl kinase activity in human neuroblastomas is associated with amino-terminal tyrosine phosphorylation of the *src* gene product

(cellular oncogene/glioblastoma)

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ABSTRACT **We have observed a 20- to 40-fold increase in pp60$^{c\text{-}src}$ tyrosyl kinase activity in human neuroblastoma cell lines over that found in either human glioblastoma cells or human fibroblasts. The level of c-*src* gene transcripts and pp60$^{c\text{-}src}$ protein synthesis in the neuroblastoma cells was not significantly increased when compared to the levels found in glioblastoma cells. Approximately one-half of the pp60$^{c\text{-}src}$ molecules synthesized during a 4-hr [^{35}S]methionine or [^{32}P]orthophosphate labeling period in neuroblastoma cells were found to migrate more slowly on NaDodSO$_4$/polyacrylamide gels than pp60$^{c\text{-}src}$ molecules labeled in glioblastoma cells. Peptide and phosphoamino acid analysis of the *in vivo* phosphorylated c-*src* molecules from these two cell types revealed that pp60$^{c\text{-}src}$ molecules from the neuroblastoma cells possess in the amino-terminal portion of the protein at least one unique tyrosine phosphorylation site not found in pp60$^{c\text{-}src}$ derived from glioblastoma cells.**

Cellular proto-oncogenes, the homologs of retrovirus-transforming oncogenes, have been found in the genomes of a wide range of multicellular organisms (1). The DNA sequence conservation among related cellular proto-oncogenes of diverse species has led to the proposal that the proteins encoded by these genes play an essential role in normal cellular functions, including proliferation and differentiation (2). One such cellular proto-oncogene, c-*src*, is closely related to the transforming gene of Rous sarcoma virus. The c-*src* gene encodes a 60-kDa membrane-associated phosphoprotein (pp60$^{c\text{-}src}$), which possesses an intrinsic protein kinase activity that is specific for tyrosine (3–5).

Recent evidence has been presented which demonstrates that pp60$^{c\text{-}src}$ expression in brain and other neuronal tissues of both chicken and human is increased during embryogenesis (6–8). In addition, these studies show that the expression of pp60$^{c\text{-}src}$ and the level of pp60$^{c\text{-}src}$ kinase activity can remain high in brain and some other neuronal cells that are fully differentiated, raising the possibility that the pp60$^{c\text{-}src}$ kinase activity may be more closely associated with neuronal differentiation and function than with neuronal cell proliferation (6–8).

We have analyzed the c-*src* gene product in two human tumors derived from cells of neuroectodermal origin: neuroblastoma and glioblastoma. In this study, we report that the level of pp60$^{c\text{-}src}$ kinase activity in neuroblastoma cells is increased 20- to 40-fold over that found in either glioblastomas or normal human fibroblasts. However, the level of c-*src* transcripts and pp60$^{c\text{-}src}$ synthesis in neuroblastoma cells was found to be no greater than 2-fold higher than that observed in glioblastoma cells. Approximately one-half of the pp60$^{c\text{-}src}$ molecules in neuroblastoma cells were found to migrate slower in NaDodSO$_4$/polyacrylamide gels when compared to the pp60$^{c\text{-}src}$ molecules from glioblastoma cells. This slower migrating form of pp60$^{c\text{-}src}$ from neuroblastoma cells was found to contain *in vivo* amino-terminal phosphotyrosine. These results suggest that the increased pp60$^{c\text{-}src}$ tyrosyl kinase activity observed in human neuroblastoma cells may be a property of pp60$^{c\text{-}src}$ molecules that possess amino-terminal phosphorylation of tyrosine residues.

MATERIALS AND METHODS

Cells. The human neuroblastoma cell lines (KCN, KCNR, SKNSH, and IMR32), the human glioblastoma cell lines (A172, HTB14, HTB16, and HTB17), and normal human fibroblasts were maintained in RPMI medium supplemented with 10% or 15% (vol/vol) fetal bovine serum. The cell lines were obtained from either American Type Culture Collection or P. Reynolds (Naval Medical Research Institute).

Immune Complex Protein Kinase Assays. Immune complex protein kinase assays and casein kinase assays using monoclonal antibody (mAb) 327, a mouse mAb that recognizes mammalian pp60$^{c\text{-}src}$ (9), were conducted as described (10–14) by using cellular lysates adjusted to the same protein concentration (400 μg per reaction).

Radioisotopic Labeling of Cells and Immunoprecipitation. KCNR and A172 cells were labeled in the presence of either [^{35}S]methionine (400 μCi/ml; 1 Ci = 37 GBq) or [^{32}P]orthophosphate (1 mCi/ml) for 4 hr as described (12). The cells were lysed in a modified RIPA buffer (12), and immunoprecipitation was carried out as described (9) by using mAb 327.

Peptide Analysis and Phosphoamino Acid Analysis. Peptide mapping by limited proteolysis using *Staphylococcus aureus* V8 protease and phosphoamino acid analysis have been described (10–14).

RESULTS

pp60$^{c\text{-}src}$ Kinase Activity in Human Neuroblastomas and Glioblastomas. We initially compared the level of pp60$^{c\text{-}src}$ kinase activity in four human neuroblastoma and four human glioblastoma cell lines. As shown in Fig. 1, the pp60$^{c\text{-}src}$ kinase activity, as measured by either autophosphorylation (Fig. 1 *A* and *B*) or casein phosphorylation (Fig. 1 *C* and *D*) in immune complex assays, was increased 20- to 40-fold in the neuroblastoma cell lysates. As shown in Fig. 1*E*, the level of pp60$^{c\text{-}src}$ autophosphorylation in immune complex kinase assays from the A172 glioblastoma cell line was approximately twice that observed for normal human fibroblasts.

Analysis of the 60-kDa *in vitro* phosphorylated bands in Fig. 1*A* lane 2 (KCNR) and lane 5 (A172) by limited proteolysis using *S. aureus* V8 protease is presented in Fig. 2*A*. As shown, both the KCNR- and A172-derived *in vitro* phosphorylated pp60$^{c\text{-}src}$ were found to have a phosphoryl-

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Abbreviation: mAb, monoclonal antibody.

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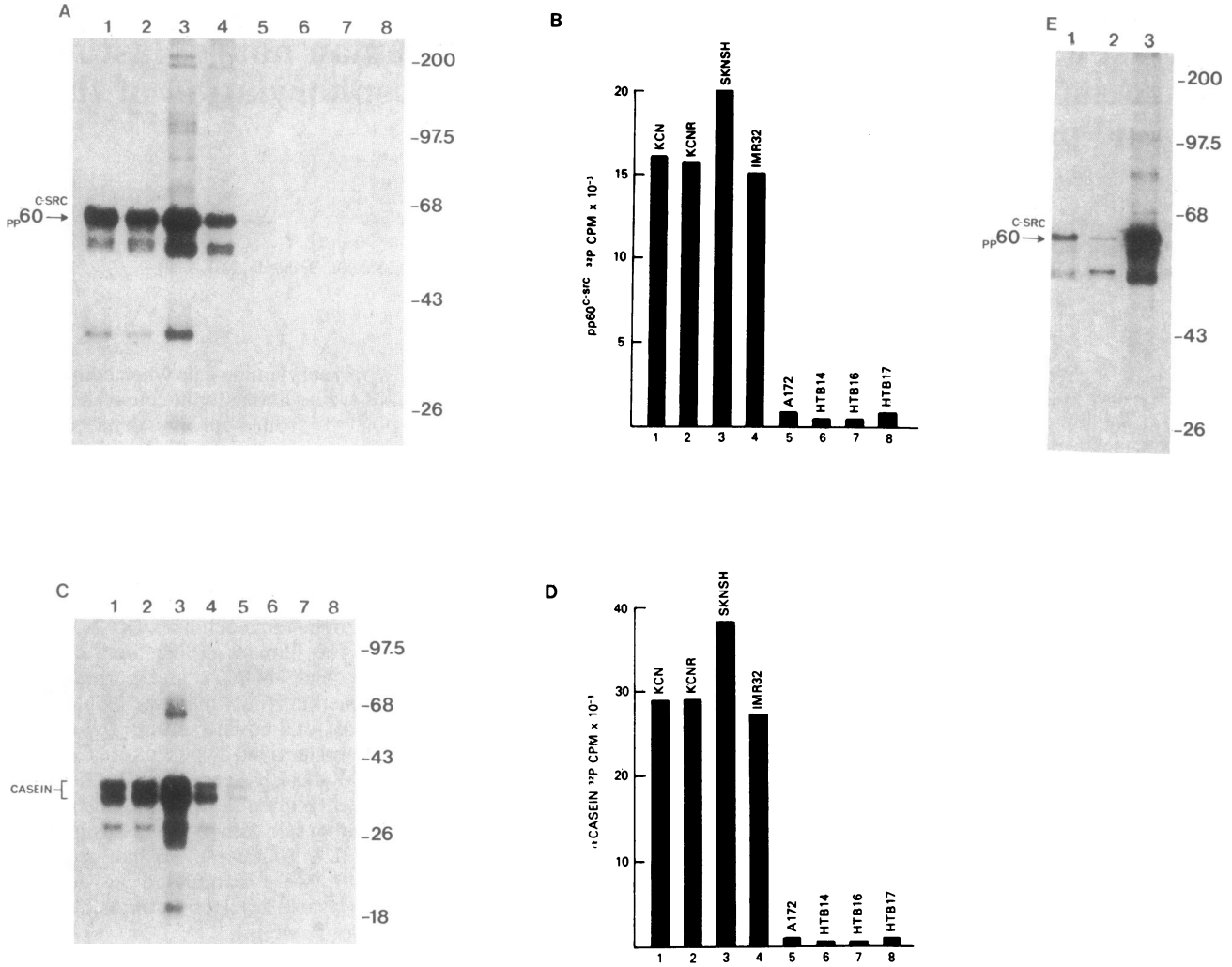

FIG. 1. *In vitro* phosphorylation of pp60$^{c\text{-}src}$ and casein in immune complex kinase assays. (*A*) Autoradiogram of mAb 327 immune complex kinase assay reaction products from neuroblastoma (KCN, lane 1; KCNR, lane 2; SKNSH, lane 3; IMR 32, lane 4) and glioblastoma (A172, lane 5; HTB 14, lane 6; HTB 16, lane 7; HTB 17, lane 8) cell lines analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (8% gel). (*B*) Quantitation of the autophosphorylated pp60$^{c\text{-}src}$ shown in *A* by liquid scintillation spectroscopy of the excised 60-kDa bands. (*C*) Autoradiogram of the *in vitro* phosphorylated casein kinase reaction products from neuroblastoma and glioblastoma cell lines. Lanes and analysis are the same as in *A* except that a 10% gel was used. (*D*) Quantitation of the phosphorylated casein bands shown in *C* as described above. (*E*) Autoradiogram of mAb 327 immune complex kinase assay reaction products from glioblastoma, A172 (lane 1); human fibroblasts (lane 2); and neuroblastoma, IMR 32 (lane 3) analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. The positions of prestained molecular mass markers (Bethesda Research Laboratories) are shown in the right margins of the autoradiograms.

ated 26-kDa V8 peptide fragment representing the normally observed *in vitro* phosphorylation site in the carboxyl-terminal half of the molecule (15). The A172 and KCNR *in vitro* phosphorylated pp60$^{c\text{-}src}$ also were found to possess a diffuse doublet (noted by arrows) that migrated between the V2 and V3 V8 peptides. Two-dimensional V8 peptide mapping experiments suggest that both of these diffuse V8-generated peptides are derived from the carboxyl-terminal half of the molecule (data not shown). However, because these V8-derived peptides were not observed in *in vivo* phosphorylated pp60$^{c\text{-}src}$ from either KCNR or A172 cells (see below), the significance of these *in vitro* tyrosine phosphorylation sites is uncertain. More interestingly, the *in vitro* phosphorylated pp60$^{c\text{-}src}$ from KCNR, in clear contrast to that of A172, possessed a phosphorylated V1 peptide, which represents the amino-terminal half of the molecule. These results were also observed when the autoradiogram was exposed for longer periods (data not shown). The KCNR-derived *in vitro* phosphorylated pp60$^{c\text{-}src}$ also was found to have labeled V3 and V4 V8 peptides. These V8 peptides are generated through secondary V8 protease cleavage sites within the 34-kDa V1 peptide and represent the amino-terminal one-third of the src protein (15). Phosphoamino acid analysis of the 60-kDa *in vitro* phosphorylated src protein KCNR and A172 is shown in Fig. 2*B*. For these experiments, an approximately equal number of ^{32}P cpm from each sample were analyzed. The major product of these *in vitro* reactions was clearly phosphotyrosine.

Level of pp60$^{c\text{-}src}$ Expression Is Not Significantly Increased in Neuroblastomas. As noted above, the level of pp60$^{c\text{-}src}$ in brain and other neuronal tissues from several species is increased during embryogenesis (6–8). Thus, the increased level of pp60$^{c\text{-}src}$ kinase activity observed in neuroblastoma cells could be the result of high levels of *src* gene expression. To evaluate this possibility, the level of c-*src* mRNA present in KCNR and A172 cells was determined by dot-blot analysis of poly(A)-selected cytoplasmic RNA from these two cell lines by using a ^{32}P-labeled nick-translated v-*src* DNA probe. A significant difference in the level of c-*src* RNA was not observed (data not shown).

We next compared the levels of pp60$^{c\text{-}src}$ in KCNR and A172 cells by incubating these cells in the presence of either [^{35}S]methionine or [^{32}P]orthophosphate and immunoprecipitating the radioactively labeled pp60$^{c\text{-}src}$ in the lysates of these

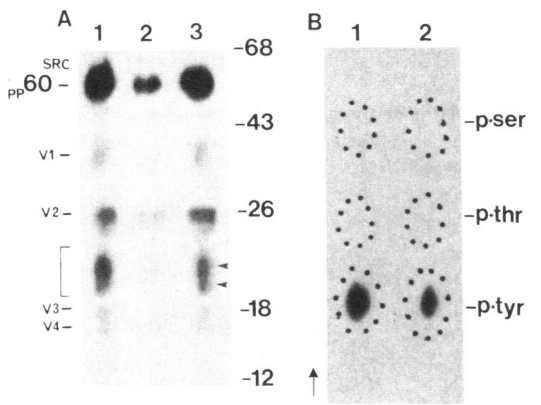

FIG. 2. (*A*) Peptide mapping of *in vitro* phosphorylated pp60$^{c\text{-}src}$ by limited V8 proteolysis. Lanes: 1, pp60$^{c\text{-}src}$ from KCNR cells; 2, pp60$^{c\text{-}src}$ from A172 cells; 3, pp60$^{c\text{-}src}$ from IMR 32 cells. The gel slices were digested with 100 ng of V8 protease per lane (Pierce) and the digestion products were separated on a NaDodSO$_4$/10% polyacrylamide gel. (*B*) Phosphoamino acid analysis of *in vitro* phosphorylated pp60$^{c\text{-}src}$. Lane 1, pp60$^{c\text{-}src}$ from KCNR cells; lane 2, pp60$^{c\text{-}src}$ from A172 cells.

cells with mAb 327. As shown in Fig. 3*A*, the amount of [^{35}S]methionine-labeled pp60$^{c\text{-}src}$ from KCNR cell lysates was found to be no more than twice that observed in the A172 cell lysates. Comparable results were also found with the *in vivo* phosphorylated pp60$^{c\text{-}src}$ from these two cell lines (Fig. 3*B*). However, evident in the autoradiograms shown in Fig. 3 is the altered mobility in the NaDodSO$_4$/polyacrylamide gels of approximately one-half of the pp60$^{c\text{-}src}$ from the KCNR cells. The slightly decreased migration of the pp60$^{c\text{-}src}$ from KCNR cells on these gels was similar to that observed with the "activated" forms of pp60$^{c\text{-}src}$ from rodent cells oncogenically transformed by polyoma virus that are physically associated with virus-encoded middle tumor antigen (16).

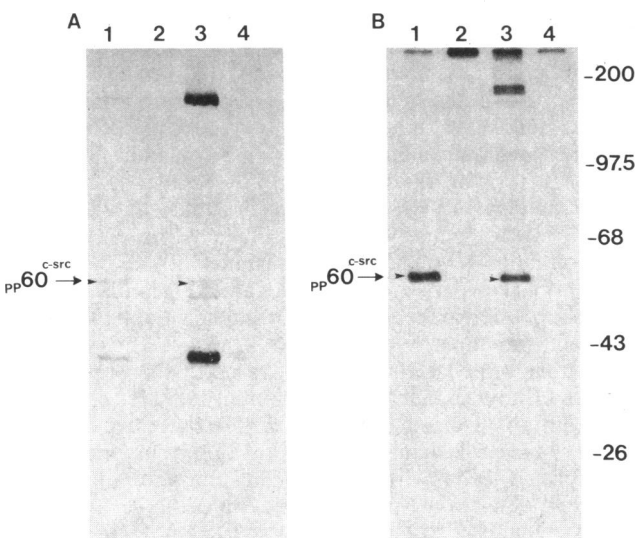

FIG. 3. (*A*) Immunoprecipitation of [^{32}S]methionine-labeled pp60$^{c\text{-}src}$. KCNR (lanes 1 and 2) and A172 (lanes 3 and 4) were labeled for 4 hr in the presence of [^{35}S]methionine, lysed in RIPA buffer, and immunoprecipitated with either mAb 327 (lanes 1 and 3) or normal mouse serum (lanes 2 and 4). (*B*) Immunoprecipitation of [^{32}P]orthophosphate-labeled pp60$^{c\text{-}src}$. KCNR (lanes 1 and 2) and A172 (lanes 3 and 4) were labeled for 4 hr in the presence of [^{32}P]orthophosphate, lysed in RIPA buffer, and immunoprecipitated with either mAb 327 (lanes 1 and 3) or normal mouse serum (lanes 2 and 4) *in vivo*. The labeled reaction products were separated on NaDodSO$_4$/8% polyacrylamide gels.

pp60$^{c\text{-}src}$ from Neuroblastoma Cells Contains Amino-Terminal Phosphotyrosine. To examine the phosphorylation pattern of the pp60$^{c\text{-}src}$ immunoprecipitated by mAb 327 from KCNR and A172 cells, the bands designated as pp60$^{c\text{-}src}$ in Fig. 3*B* (lanes 1 and 3) were analyzed by partial proteolysis using V8 protease. When these proteins were digested with 5 ng of V8 protease per lane (Fig. 4*A*), the major cleavage product observed was the 34-kDa V1 peptide derived from the amino-terminal portion of the molecule (15). The altered mobility of the V1 peptide from the neuroblastoma cells in this figure is clearly evident. Interestingly, little of the 26-kDa V2 peptide from the carboxyl-terminal portion of the ^{32}P-labeled *src* molecules was found in either the KCNR- or A172-derived pp60$^{c\text{-}src}$, although this peptide was easily observed after V8 protease cleavage of [^{35}S]methionine-labeled pp60$^{c\text{-}src}$ from both cell lines (data not shown). Partial proteolytic cleavage of parallel ^{32}P-labeled *src* molecules with 50 ng of V8 protease per lane resulted in further cleavage of the V1 peptide (Fig. 4*B*). As shown, the V1 peptide from A172 pp60$^{c\text{-}src}$ yielded the characteristic 18-kDa (V3) and 16-kDa (V4) V8 peptides (lanes 2 and 4). Cleavage of the KCNR-derived V1 peptide also resulted in the appearance of V3 and V4 peptides, which comigrated with those derived from the A172 cells. However, the ^{32}P-labeled V1 peptide from the KCNR pp60$^{c\text{-}src}$ molecules yielded two additional peptides that migrated slightly slower than the normal V3 and V4 peptides (lanes 1 and 3, noted by arrows). This result is most easily observed in lane 3 of this figure. Fig. 4*B* also demonstrates that the phosphorylated V2 peptide can be observed in the *src* molecules from these cells (see lane 1) although, as previously noted, the apparent extent of detectable carboxyl-terminal phosphorylation under the conditions used to label the molecules in these experiments was less than that previously observed for *in vivo* phosphorylated pp60$^{c\text{-}src}$ from rodent cells using the same labeling protocol (10, 12–14). Cleavage of the ^{32}P-labeled *src* molecules from KCNR and A172 cells with 50 ng of V8 protease per lane also resulted in the appearance of additional phosphorylated peptides that migrated on the NaDodSO$_4$/polyacrylamide gels between the 16-kDa V4 peptide and the 12-kDa cytochrome *c* molecular weight marker. The more rapidly migrating ^{32}P-labeled peptide appeared to be common in pp60$^{c\text{-}src}$ molecules from both KCNR and A172 cells, while the more slowly migrating phosphopeptide was observed only in *src* molecules from the neuroblastoma cells.

To determine which amino acids were phosphorylated in the *src* molecules derived from the KCNR and A172 cell lines, the *in vivo* phosphorylated pp60$^{c\text{-}src}$ from these two cell lines was isolated from preparative gels and cleaved with 5 ng of V8 protease per lane to generate principally the V1 and V2 peptides. The phosphorylated V1 and V2 peptides and uncleaved *in vivo* phosphorylated 60-kDa pp60$^{c\text{-}src}$ were then hydrolyzed, and the products were separated by high-voltage paper electrophoresis. As shown in Fig. 4*C*, *in vivo* phosphorylated pp60$^{c\text{-}src}$ from the A172 glioblastoma cell line contained phosphoserine as well as phosphotyrosine. The 34-kDa V1 peptide from the A172 *src* molecules was found to contain exclusively phosphoserine, while the 26-kDa V2 peptide from these molecules contained only phosphotyrosine. As shown in Fig. 4*D*, *in vivo* phosphorylated 60-kDa *src* molecules from the KCNR neuroblastoma cell line also contained both phosphoserine and phosphotyrosine, although the ratio of the two was apparently reversed when compared to the A172 glioblastoma-derived pp60$^{c\text{-}src}$. While the 26-kDa V2 peptide from the KCNR *src* molecules, like that of the V2 peptide from the A172 src protein, contained only phosphotyrosine, the 34-kDa V1 peptide from the KCNR *src* molecules was found to contain phosphotyrosine in addition to phosphoserine. These results demonstrate that pp60$^{c\text{-}src}$ from the KCNR neuroblastoma cell line possesses

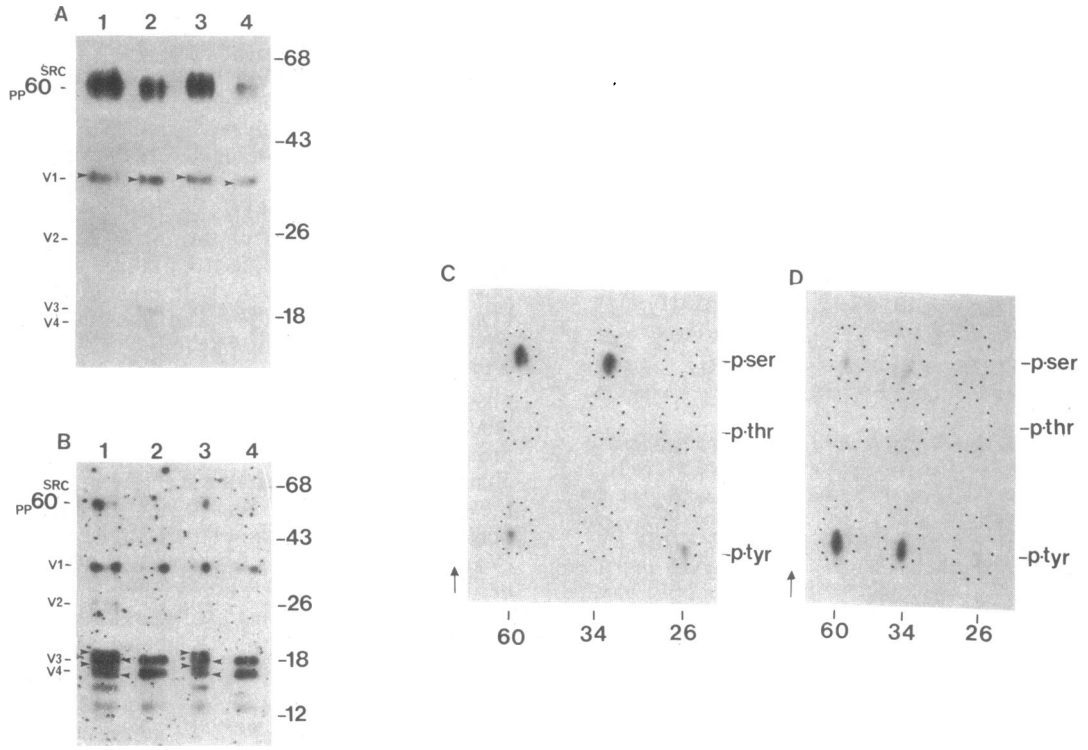

FIG. 4. Peptide mapping and phosphoamino acid analysis of *in vivo* phosphorylated pp60$^{c\text{-}src}$. (*A*) KCNR (lanes 1 and 3) and A172 (lanes 2 and 4) *in vivo* phosphorylated pp60$^{c\text{-}src}$ digested with 5 ng of V8 protease per lane. (*B*) KCNR (lanes 1 and 3) and A172 (lanes 2 and 4) *in vivo* phosphorylated pp60$^{c\text{-}src}$ digested with 50 ng of V8 protease per lane. (*C*) Phosphoamino acid analysis of A172-derived *in vivo* phosphorylated pp60$^{c\text{-}src}$ (*60*), 34-kDa V1-peptide (*34*), and 26-kDa V2-peptide (*26*). (*D*) Phosphoamino acid analysis of KCNR-derived *in vivo* phosphorylated pp60$^{c\text{-}src}$ (*60*), ≈34-kDa V1-peptide (*34*), and 26-kDa V2-peptide (*26*).

at least one phosphorylated tyrosine residue in the amino-terminal half of the *src* molecule, which is not detectable in pp60$^{c\text{-}src}$ from A172 glioblastoma cells.

DISCUSSION

The results presented in this report demonstrate that pp60$^{c\text{-}src}$ tyrosyl kinase activity in human neuroblastoma cell is significantly increased (20- to 40-fold) over that observed in either human glioblastoma cells or normal human fibroblasts (Fig. 1). Since the degree of the increase in pp60$^{c\text{-}src}$ protein kinase activity in neuroblastoma cells was not paralleled by an increase in the level of c-*src* transcription or protein synthesis (Fig. 3), our results indicate that the increased pp60$^{c\text{-}src}$ kinase activity in neuroblastoma cells reflects an increase in the specific activity of the c-src phosphotransferase. While the molecular basis for the increased pp60$^{c\text{-}src}$ kinase activity is not known, our finding that c-*src* molecules in neuroblastoma cells possess an amino-terminal tyrosine phosphorylation site *in vivo* not found in c-*src* molecules from glioblastoma cells (Fig. 4) suggests that this posttranslational modification may be an important feature of c-*src* molecules with increased protein kinase activity.

The potential importance of tyrosine phosphorylation in the amino-terminal half of the *src* molecules for the regulation of *src*-associated protein kinase activity was first proposed by Collett and co-workers (17, 18) using pp60$^{v\text{-}src}$ encoded by Rous sarcoma virus. In these studies, this group demonstrated that incubation of partially purified pp60$^{v\text{-}src}$ with Mg^{2+} and ATP at concentrations above the v-src kinase K_m for ATP (20 μM) resulted in phosphorylation of the v-src molecules at tyrosine sites within the amino-terminal half of the protein. The phosphorylation of these new sites in the amino-terminal portion of the enzyme resulted in a 5- to 8-fold increase in the protein kinase specific activity and the appearance of v-src molecules, which migrated slower than unmodified v-src molecules in NaDodSO$_4$/polyacrylamide gels. We have recently demonstrated that pp60$^{c\text{-}src}$ tyrosyl kinase activity is increased after polyoma virus infection and oncogenic transformation of rodent cells (10, 14). Only c-src molecules physically associated with the polyoma virus-encoded middle tumor antigen were found to possess increased protein kinase activity, and Yonemoto *et al.* (16) have shown that at least one site of amino-terminal tyrosine phosphorylation is found only in c-src molecules that are associated with the middle tumor antigen as measured in *in vitro* kinase assays. Interestingly, these c-src molecules were found to migrate in NaDodSO$_4$/polyacrylamide gels more slowly than pp60$^{c\text{-}src}$ molecules not associated with middle tumor antigen. These results suggest that, like pp60$^{v\text{-}src}$, pp60$^{c\text{-}src}$ kinase activity may be influenced by phosphorylation of amino-terminal tyrosine residues.

Additional evidence for the role of amino-terminal tyrosine phosphorylation in the regulation of src kinase activity *in vivo* has been provided by experiments in which Rous sarcoma virus transformed cells were incubated in the presence of sodium orthovanadate (19, 20), a potent inhibitor of cellular phosphotyrosyl phosphatases (21, 22). In these experiments, it was observed that treatment of Rous sarcoma virus-transformed cells with micromolar amounts of sodium orthovanadate resulted in the detection of slower-migrating forms of pp60$^{v\text{-}src}$ when analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis compared to pp60$^{v\text{-}src}$ from parallel cultures not incubated with sodium orthovanadate. The slower-migrating forms of pp60$^{v\text{-}src}$ from the vanadate-treated cells were found to be uniquely phosphorylated on tyrosine residues within the amino-terminal portion of the molecule and to possess enhanced tyrosine kinase activity when assayed in *in vitro* kinase assays. Similar treatment of polyoma virus-transformed cells also resulted in the detection of an *in vivo* phosphorylated form of pp60$^{c\text{-}src}$ associated with middle tumor antigen, which migrated in NaDodSO$_4$/polyacrylamide gels slower than pp60$^{c\text{-}src}$ molecules not

associated with middle tumor antigen. Partial proteolytic mapping of this altered form of $pp60^{c\text{-}src}$ with V8 protease yielded peptide maps consistent with the idea that the slower-migrating forms of $pp60^{c\text{-}src}$ contained an additional phosphorylation site within the amino-terminal half of the molecule, although sufficient ^{32}P counts were not present in the V8 protease amino-terminal peptide to allow for phosphoamino acid analysis (16). These results were interpreted to suggest that amino-terminal tyrosine phosphorylation within $pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$ molecules associated with middle tumor antigen exists *in vivo*. The failure to detect these modifications in the absence of vanadate suggests that phosphate groups at these sites may rapidly turn over *in vivo*, presumably due to the actions of cellular phosphotyrosyl phosphatases (16, 19, 20). Our ability to detect the amino-terminal tyrosine phosphorylation site(s) in $pp60^{c\text{-}src}$ in neuroblastoma cells suggests that this modification may be more stable in this cell type. This stability is apparently not, however, the result of $pp60^{c\text{-}src}$ complex formation with other proteins in neuroblastoma cells analogous to polyoma virus middle tumor antigen (23, 24). Support for this view was provided through analysis of $pp60^{c\text{-}src}$ from neuroblastoma and glioblastoma cell lysates by sedimentation in glycerol gradients. These studies demonstrated that $pp60^{c\text{-}src}$ from both cell types sedimented at indistinguishable rates in the 50- to 75-kDa region of the gradients (unpublished results). Perhaps alterations (e.g., conformational) in neuroblastoma-derived $pp60^{c\text{-}src}$ and/or alterations in the interaction between $pp60^{c\text{-}src}$ and phosphatases in these cells contribute to the apparent stability of the phosphorylated tyrosine residues within the amino-terminal portion of these *src* molecules. These results also imply, based on the observations from other laboratories discussed above, that the increased $pp60^{c\text{-}src}$ protein kinase activity found in neuroblastoma cells may be directly related to the apparent absence of phosphate turnover from tyrosine residues in the amino-terminal half of the molecule. The observation that the insulin receptor tyrosyl kinase activity can also be increased after autophosphorylation of the receptor on tyrosine residues (25) suggests that several tyrosine-specific protein kinases may be regulated by this mechanism.

Recent evidence suggests, however, that all c-*src* molecules may not require phosphorylation of tyrosine residues within the amino-terminal portion of the molecule to possess elevated protein kinase activity. Brugge *et al.* (26) have found that c-*src* molecules in primary neuronal cells derived from rat central nervous system tissue have a 6- to 12-fold increase in specific activity when compared to c-*src* molecules in astrocytes derived from the same source. The c-*src* molecules from neuronal cultures were found to migrate more slowly on $NaDodSO_4$/polyacrylamide gels than $pp60^{c\text{-}src}$ from astrocytes and to possess some structural alteration(s) within their amino-terminal region that contributed to the observed mobility changes. While the nature of the amino-terminal alterations was not determined, phosphoamino acid analysis of these molecules did not reveal the presence of phosphotyrosine.

The elevated tyrosyl kinase activity observed in the four neuroblastoma cell lines analyzed in this report does not allow for a determination of whether or not the increased levels of this enzyme activity contribute to the oncogenic phenotype of these neuronal tumor cell lines, because we do not have the normal embryonic tissue from which neuroblastoma is thought to arise. We have compared $pp60^{c\text{-}src}$ kinase activity from normal muscle, kidney, and brain tissue to neuroblastoma tumor tissue from the same patient. Our results indicate that $pp60^{c\text{-}src}$ kinase activity from brain tissue is not significantly different than c-*src* kinase activity in tumor tissue, although both brain and neuroblastoma tissues have $pp60^{c\text{-}src}$ kinase activity levels greatly elevated over normal muscle and kidney tissue (unpublished results). However, the neuroblastoma cell lines used in this study, like most other human neuroblastoma cell lines, express an additional oncogene: N-*myc* (27–29). Thus, it is possible that the combined effects of the products of these two proto-oncogenes could contribute to the transformed phenotype of these cells. We can test this possibility by screening a variety of human tumor cell lines and primary tumor tissues to identify cancers in which $pp60^{c\text{-}src}$ kinase activity is increased and the alterations in this activity might be more easily determined in the cell type from which the tumor is thought to arise.

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