

Expression of *c-src* in Cultured Human Neuroblastoma and Small-Cell Lung Carcinoma Cell Lines Correlates with Neurocrine Differentiation

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Human cell lines with neuronal and neuroendocrine features were examined for their expression of pp60^{c-src}, the cellular homolog of the transforming gene product pp60^{v-src} of Rous sarcoma virus. Four neuroblastoma (LA-N-5, SH-SY5Y, Paju, and SK-N-MC) and three small-cell lung carcinoma (U-2020, U-1690, and U-1285) cell lines were selected on the basis of their stage of neurocrine differentiation, as determined by the expression of neuron-specific enolase. In an immune complex protein kinase assay, all seven cell lines displayed *c-src* kinase activity which was considerably higher than that found in nonneurocrine cells (human diploid fibroblasts, glioma, and non-small cell lung carcinoma cell lines). Furthermore, the *c-src* kinase activity, as determined by autophosphorylation or phosphorylation of an exogenous substrate, enolase, correlated with the stage of neurocrine differentiation. There was an approximately 30-fold difference in *c-src* kinase autophosphorylation activity between the cell lines representing the highest and lowest stages of neurocrine differentiation. A similar variation was found in the steady-state levels of the *c-src* protein of these cell lines. Highly differentiated neuroblastoma cells expressed two forms of the *src* protein. Digestion by *Staphylococcus aureus* V8 protease did reveal structural diversity in the amino-terminal ends of these *c-src* molecules. In summary, we found a clear correlation between *c-src* kinase activity and the stage of neuronal and neuroendocrine differentiation. Thus, the phenotypic similarity between neurons and neuroendocrine cells includes high *c-src* expression.

The oncogene of Rous sarcoma virus (RSV) and its cellular homolog code for phosphoproteins, pp60^{v-src} and pp60^{c-src}, respectively, which both have tyrosyl kinase activity (17, 18). The transforming capacity of the pp60^{v-src} protein is dependent on its tyrosyl kinase activity (48). The specific kinase activity of the *v-src* protein appears to be higher than that of pp60^{c-src} (27). However, increased expression of *c-src* in cells that become transformed by *v-src* does not lead to transformation (28, 30, 42). Furthermore, low levels of *v-src* kinase activity are sufficient for transformation (29), indicating that there are other differences between the *v-src* and *c-src* proteins that are essential for the transformation process. However, the association of middle tumor antigen of polyomavirus and pp60^{c-src} (21) activates the *c-src* kinase (14). This has been suggested as a necessary step for transformation by polyomavirus (22), which would imply that an increase in the *c-src* kinase activity in certain cells can mediate transformation.

Cotton and Brugge (20) demonstrated high levels of pp60^{c-src} in neural tissues from chicken embryos. Using primary cultures of rat brain, Brugge et al. (15) showed that astrocytes and postmitotic neurons express high and equal amounts of *c-src* protein. The specific kinase activity of the *c-src* protein from the neurons was 6 to 12 times higher than that of the astrocyte-derived *c-src* protein. Furthermore, the *c-src* protein in neurons was found to be modified at the amino terminus, resulting in a shift in electrophoretic mobility (15). These results, together with those of parallel studies by Sorge et al. (51) of pp60^{c-src} expression during development of the neural retina, demonstrate that neural differentiation is accompanied by high *c-src* kinase activity levels.

Cultured human neuroblastoma and glioma cells have similar *c-src* protein levels, but the specific pp60^{c-src} tyrosyl kinase activity is 20 to 40 times higher in neuroblastoma cells (13). The same authors (13) found that the electrophoretic mobility of a part of the neuroblastoma *c-src* protein of one tested cell line was similar to that of the *c-src* protein from neurons; in this neuroblastoma cell line, the *c-src* protein had an additional site of tyrosine phosphorylation in the amino-terminal part. These authors also discuss the possibility that the high specific pp60^{c-src} tyrosyl kinase activity may be linked to the malignant phenotype of neuroblastoma.

The expression of neuron-specific enolase (NSE; γ -subunit of enolase) is associated with late stages of neuronal differentiation (23, 47). The enzyme is also expressed in neuroendocrine cells (36), including tumor cells with neuroendocrine phenotypes, e.g., small-cell carcinoma of the lung (SCCL) (5, 53). We have investigated the expression of *c-src* in SCCL and neuroblastoma cell lines representing different stages of neurocrine (neuroendocrine and neuronal) differentiation. In these cell lines we found a 30-fold variation in *c-src* kinase activity. Highly differentiated cells, as determined by NSE expression, had high *c-src* kinase activity and protein levels. Thus, there was a correlation between *c-src* expression and stage of neurocrine differentiation.

MATERIALS AND METHODS

Cell culture conditions. All cell lines tested were of human origin and, except of U-266 and Corinna II, were grown routinely in medium supplemented with 10% fetal calf serum, penicillin (100 IU/ml), and streptomycin (50 μ g/ml). The medium used was either Eagle minimal essential medium (SH-SY5Y, SK-N-MC, U-251MG/AgCl1[U-251], U-343MGA/C12:6 [U-343], U-563, AG-1523) or RPMI 1640

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medium (LA-N-5, Paju, U-2020, U-1690, U-1285, H-125, U-1810, U-1752, U-2030). U-266 and Corinna II were grown in F-10 medium supplemented with 10% newborn calf serum and the antibiotics at the concentrations given above. In all experiments the cells were harvested 4 days after seeding. RSV- (Schmidt-Ruppin strain D)-infected BALB 3T3 cells were grown in Eagle minimal essential medium with 10% fetal calf serum.

Neuroblastoma cell lines. The morphology of LA-N-5, SH-SY5Y (11), and Paju (45) cells is neuroblastlike, whereas SK-N-MC cells have a fibroblastlike morphology (10). The SH-SY5Y and SK-N-MC cells express enzymes for neurotransmitter synthesis (11), and Paju, SH-SY5Y, and LA-N-5 cells can be induced to differentiate into cells that phenotypically resemble ganglion cells (41, 45, 50). All four neuroblastoma lines express NSE, although in varying amounts (40).

SCCL cell lines. The neurocrine properties of the three SCCL cell lines (U-2020, U-1690 [8], and U-1285 [6]) are exemplified by their expression of NSE (40) and neurofilament and by the fact that they possess neurosecretory granules (7).

Non-SCCL cell lines. The three non-SCCL cell lines represent squamous cell carcinoma (U-1752 [9]), large cell carcinoma (U-1810 [8]), and adenocarcinoma (H-125 [24]) of the lung. They express keratins but not neurofilament (7) and have low levels of NSE (40).

Glioma and fibroblast cell lines. The two glioma cell lines (U-343 and U-251) express the glial cell marker glial fibrillar acidic protein (44). AG-1523 cells are normal foreskin fibroblasts obtained from the Human Mutant Cell Repository Institute for Medical Research (Camden, N.J.).

Hematopoietic cell lines. U-266 (37) and U-2030 (29a) are myeloma cell lines, and Corinna II represents chronic lymphocytic leukemia cells infected by Epstein-Barr virus. These cell lines were selected because they are non-neuroendocrine cells with comparatively high (U-266 and Corinna II) and low (U-2030) expression of NSE (40).

Radioimmunoassay for NSE. Cells for NSE determination were washed twice with phosphate-buffered saline before being homogenized in 10 mM Tris hydrochloride buffer, pH 7.4, containing 5 mM MgSO₄. NSE was determined by radioimmunoassay with a commercially available kit (Pharmacia AB, Uppsala, Sweden), which is based on the antiserum and technique described by Pählman et al. (39). The NSE content was correlated to total protein, which was determined by a modified Lowry procedure (33).

Immunoprecipitation and determination of c-src kinase activity. Cells were washed twice in ice-cold phosphate-buffered saline and lysed at 0°C for 15 min in RIPA buffer (10 mM Tris hydrochloride, pH 7.2, 0.16 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) supplemented with 1 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid], and 1 mM EDTA. The lysates were clarified for 30 min at 30,000 × g, and the protein content of the supernatants was determined as described above. The samples were adjusted to equal protein concentrations (from 0.8 to 1 mg/ml in the different experiments), and 2 μl (ascites fluid) of monoclonal antibody (MAb) 327 (32) was added per mg of total protein. In parallel samples the specificity of the immunoprecipitation was checked by excluding the anti-src antibody. After 30 min of incubation on ice, Formalin-fixed *Staphylococcus aureus* cells preincubated with anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark) was added. The immunoprecipitates were washed four times in RIPA buffer. Prior to the last wash, the samples were split in two. One half of

the immunoprecipitate was used to assay c-src protein levels by the immunoblotting technique, and the other half was used to determine c-src kinase activity.

For the kinase reactions, the immunoprecipitates were incubated with 15 μCi of [γ-³²P]ATP (New England Nuclear, Drieich, West Germany) in 10 mM Tris hydrochloride, pH 7.2, containing 5 mM MgCl₂ and 0.5 μM ATP for 20 min on ice. Acid-activated rabbit muscle enolase (10 μg per reaction) (Boehringer, Mannheim, West Germany) was used as the exogenous c-src substrate (19, 26). The reactions were stopped by the addition of SDS-containing sample buffer. The samples were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (31) with a 10% gel. Autophosphorylated c-src protein or enolase was identified by molecular weight on Kodak X-AR film exposed to the dried gel. The pp60^{c-src} kinase autophosphorylation activity was quantified by scanning the autoradiographs with a Beckman DU8 spectrophotometer.

Immunoblotting. For c-src protein level analyses, the immunoprecipitated material was subjected to SDS-PAGE, after which the proteins were electrophoretically blotted overnight at 4°C onto nitrocellulose filter paper (54). The filter was blocked for 24 h at room temperature in buffer A (50 mM Tris hydrochloride, pH 7.5, containing 0.15 M NaCl) supplemented with 5% human serum albumin (HSA) prior to incubation with MAb 327, diluted 1:300 in buffer A containing 0.5% Tween 20 and 3% HSA. After 4 h at room temperature, the filter was washed five times in buffer A containing 0.2% Nonidet P-40 and 0.5% HSA. Iodinated anti-mouse immunoglobulin (Radiochemical Centre, Amersham, England), diluted 1:100 in buffer A containing 0.5% Tween 20 and 3% HSA, was incubated with the filter for 2 h at room temperature. The filter was finally washed five times as described above, dried, and subjected to autoradiography. The c-src protein was identified by its molecular weight.

³⁵S labeling of cells. Routinely grown cells were labeled for 1 h in methionine-free F-10 medium supplemented with 0.5 mCi of [³⁵S]methionine (New England Nuclear) per ml. The cells were lysed, and the immunoprecipitation was performed as described above. In the SDS-PAGE analysis, 7.5% polyacrylamide gels were used. The fixed gels were soaked in a scintillation solution (Enlightning; New England Nuclear) prior to autoradiography.

V8 protease peptide mapping. Cells were grown for 4 days under routine conditions. The medium was changed to serum- and phosphate-free F-10 medium supplemented with 1 mCi of ³²P_i (Amersham) per ml. After 4 h of incubation, the cells were harvested and the c-src protein was immunoprecipitated and isolated by SDS-PAGE. The c-src protein was localized by autoradiography, excised, and reelectrophoresed on a 12.5% polyacrylamide gel in the presence of 200 ng of *Staphylococcus aureus* V8 protease (Boehringer-Mannheim, Bromma, Sweden) per well (16). The radioactive peptides were visualized by autoradiography.

RESULTS

NSE expression in the cell lines tested. To elucidate the possible role of pp60^{c-src} in neuronal and neuroendocrine differentiation of human cells, we looked for suitable in vitro models. Cultured human neuroblastoma and SCCL cells appear to be two systems in which established cell lines represent clonal expansions of cells at different stages of neuronal and neuroendocrine differentiation, respectively. From a panel of cell lines previously screened for NSE

TABLE 1. Protein levels of NSE and *c-src* kinase activity in human cell lines^a

Cell line	Derivation	Mean NSE of ($\mu\text{g}/\text{mg}$ of protein) \pm SD	<i>c-src</i> activity (area units)
LA-N-5	Neuroblastoma	1.4 \pm 0.2	6.0
SH-SY5Y	Neuroblastoma	0.65 \pm 0.10	4.8
Paju	Neuroblastoma	0.35 \pm 0.04	1.4
SK-N-MC	Neuroblastoma	0.18 \pm 0.02	0.2
Lung carcinomas			
U-2020	SCCL	1.5 \pm 0.1	4.0
U-1690	SCCL	1.2 \pm 0.1	5.2
U-1285	SCCL	0.28 \pm 0.02	0.3
H-125	Adenocarcinoma	0.010 \pm 0.003	1.4
U-1810	Large cell carcinoma	0.02 \pm 0.01	Not detectable
U-1752	Squamous cell carcinoma	0.007 \pm 0.001	Not detectable
U-251	Glioma	0.15 \pm 0.01	Detectable
U-343	Glioma	0.09 \pm 0.01	Not detectable
AG-1523	Foreskin fibroblasts	0.04 \pm 0.01	Detectable
U-266	Myeloma	0.32 \pm 0.05	Not detectable
U-2030	Myeloma	<0.002	Not detectable
Corinna II	Epstein-Barr virus-transformed chronic lymphocytic leukemia	0.43 \pm 0.13	Detectable

^a NSE was determined by radioimmunoassay, and the level is expressed in relation to total cell protein. *c-src* kinase activity was determined after immunoprecipitation and analysis of the autophosphorylated pp60^{c-src} by SDS-PAGE. The amount of autophosphorylated *c-src* protein was estimated by densitometry, and kinase activity is expressed as area units. The U-1690-derived *c-src* kinase activity was used to normalize for differences in experimental conditions between the different experiments.

expression (40), four neuroblastoma and three SCCL cell lines which varied in their expression of NSE were selected. NSE expression was determined in the passages used in this study (Table 1). The results were in good accordance with those obtained previously (40). Thus, these cell lines provided us with cultured human cells at different stages of neurocrine differentiation.

pp60^{c-src} kinase activity and *c-src* protein levels in cultured neuroblastoma and SCCL cells. The level of *c-src* kinase activity in four neuroblastoma cell lines (LA-N-5, SH-SY5Y, Paju, and SK-N-MC) was determined in cell lysates after normalization of the protein concentration. In a kinase assay in which immunoprecipitated *c-src* protein was autophosphorylated, pp60^{c-src} was identified by its molecular weight on the autoradiographs of polyacrylamide gels. The autophosphorylated *v-src* protein of SRD3T3 cells was used as a marker for pp60. In all four neuroblastoma cell lines tested, *c-src* kinase activity was detected, although the level varied considerably (Fig. 1A). This activity was estimated by scanning the autoradiographs (Table 1). There was an approximately 30-fold difference in kinase activity between LA-N-5 and SK-N-MC cells (Table 1). Compared with that found in two human glioma cell lines (U-251 and U-343) and human diploid fibroblasts (AG-1523), the *c-src* kinase activity in all neuroblastoma cell lines was high (Fig. 1A and Table 1). In Table 1 the levels of NSE protein in the different cell lines are also listed. Apparently there is a clear correlation between *c-src* kinase autophosphorylation activity and NSE expression in neuroblastoma cell lines. With an exogenous substrate for *c-src*, rabbit muscle enolase, qualitatively the same correlation between *c-src* kinase activity levels and NSE expression was obtained (Fig. 2). With another anti-*src* antiserum, MA b 273 (32), similar results were obtained (not shown).

The immunoprecipitates used in the *c-src* kinase activity determinations were also analyzed for *c-src* protein levels by the immunoblotting technique. As shown in Fig. 1, the steady-state protein level and the kinase activity of the *c-src* protein correlated in the neuroblastoma cell lines. The *c-src*

protein level in the glioma cell line U-251 was low and not detectable in this experiment (Fig. 1C).

Three SCCL cell lines (U-2020, U-1690, and U-1285) were analyzed for *c-src* kinase activity and protein expression. As in the neuroblastoma cell lines, both the *c-src* kinase activity (autophosphorylation and phosphorylation of enolase) and protein level correlated with NSE expression (Fig. 1, 2, and 3; Table 1). An analysis was also made of three cell lines representing the other major forms of lung carcinoma with nonneurocrine features (7), adenocarcinoma (H-125), squamous cell carcinoma (U-1752), and large cell carcinoma (U-1810), for *c-src* and NSE expression. Very low levels of NSE were observed in all three non-SCCL cell lines (Table 1). In this experiment, two of the cell lines (U-1752 and U-1810) had undetectable levels of *c-src* kinase activity and protein (Fig. 3 and Table 1). The H-125 cell line, however, had comparatively high *c-src* kinase activity and protein levels (Fig. 3).

***c-src* activity and NSE levels do not correlate in non-neurocrine cells.** NSE is also expressed in some non-neurocrine cells (40). To ascertain whether high *c-src* kinase activity levels correlate with high NSE expression as such, the *c-src* kinase activity of three hematopoietic cell lines was assayed, two with high (U-266 and Corinna II) and one with low (U-2030) NSE expression. Compared with the cell lines of neurocrine derivation, the kinase levels of the hematopoietic cells were low (Table 1).

Highly differentiated neuroblastoma cell lines express two forms of pp60^{c-src}. Neurons express a distinct *c-src* product with an altered, slightly slower, electrophoretic mobility than the *c-src* product in, e.g., astrocytes (15). The cell lines were therefore screened for the expression of the two forms of *c-src* protein by immunoprecipitation of lysates from [³⁵S]methionine-labeled cells and analysis by SDS-PAGE. Only the two most highly differentiated neuroblastoma cell lines expressed both forms of *c-src* protein (Fig. 4). In SH-SY5Y cells, approximately 50% of the *src* protein was in the slower-migrating form, while the corresponding figure for the LA-N-5 cells was 90%. V8 protease mapping con-

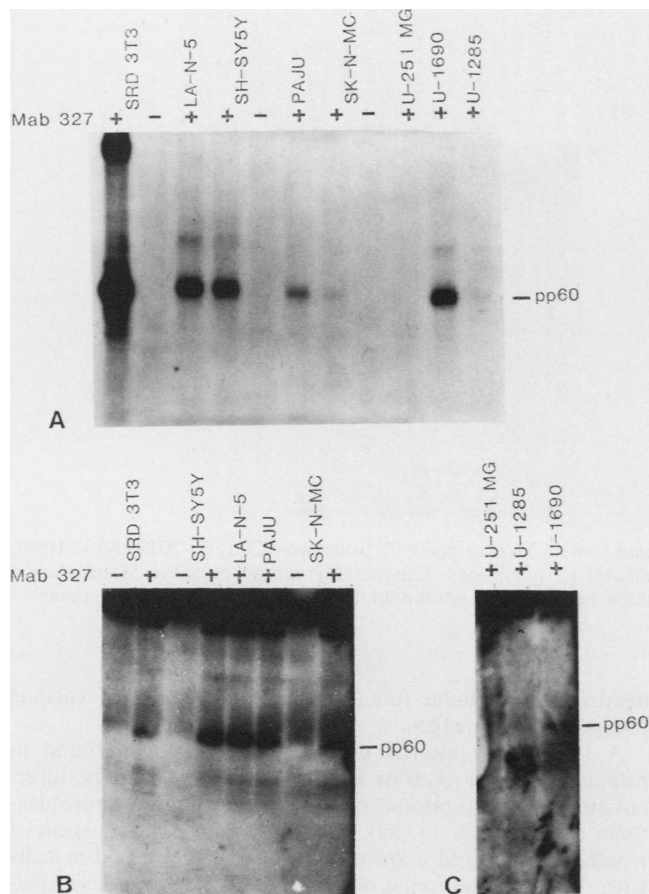


FIG. 1. Comparison of the *c-src* kinase autophosphorylation activity and *c-src* protein level in four neuroblastoma cell lines (LA-N-5, SH-SY5Y, Paju, and SK-N-MC), two SCCL cell lines (U-1690 and U-1285), and one glioma cell line (U-251). pp60^{c-src} was immunoprecipitated from cell lysates as described in Materials and Methods. After the last wash, the immunoprecipitates were split into two parts. (A) Kinase assay (see Materials and Methods) performed on one part of the immunoprecipitate. The proteins were separated by SDS-PAGE, and autophosphorylated pp60^{c-src} was visualized by autoradiography and identified by its molecular weight and comigration with the autophosphorylated *v-src* protein of SRD3T3 cells. (B and C) Other half of the immunoprecipitate analyzed for the pp60^{c-src} protein level by the immunoblotting technique (see Materials and Methods). After the *c-src* protein was isolated by SDS-PAGE, it was electrophoretically blotted onto nitrocellulose filter paper. The filter was subjected to sequential incubation with MAb 327 and iodinated anti-mouse immunoglobulin antiserum. The *c-src* protein was identified by its molecular weight. The film in panel C was exposed for a shorter time than that in panel B. Control immunoprecipitations without MAb 327 were also performed as indicated.

firmly that LA-N-5 and SH-SY5Y cells expressed a modified *c-src* protein and that the modification was located at the N-terminal part of the molecule (Fig. 5A). These experiments confirmed that the SCCL cell lines did not express the slowly migrating *c-src* protein (Fig. 5B).

DISCUSSION

Neuroblastoma and SCCL cells have many morphological and biochemical characteristics in common. Neuroblastoma cells frequently express genes and functions that are associ-

ated with neurocrine differentiation. NSE is one such gene, which is predominantly expressed in neurons and neuroendocrine cells (23, 36). In neurons, NSE expression is associated with a late stage of differentiation (47). In neuroblastoma there is an association between NSE expression and stage of neuronal differentiation, i.e., the differentiated forms of the tumor, namely ganglioneuroblastoma and ganglioneuroma (a benign form), have higher NSE levels than neuroblastomas of lower stages of differentiation (38). Furthermore, the NSE level in neuroblastoma cells differentiated *in vitro* increases together with other markers of neuronal differentiation, e.g., neurotransmitter synthesis, neurosecretory granules, neurite formation (41), and the electromembrane potential (1). In SCCL cells the correlation between neuroendocrine differentiation and NSE expression is not well established.

In this study a clear correlation was found between high *c-src* kinase activity and neurocrine differentiation as defined by NSE expression in neuroblastoma and SCCL cells. However, NSE is expressed in some nonneurocrine cells, including blood platelets, which also express high *c-src* levels (26, 35). We therefore investigated whether nonneurocrine cells with high NSE levels generally display high *c-src* expression. The results show that this is not the case; the *c-src* activities in the two hematopoietic cell lines with high NSE levels (Corinna II and U-266) tested were much lower than those found in neurocrine cell lines (SK-N-MC, Paju, and U-1285) with similar or lower NSE levels.

There is a discrepancy between the high *c-src* kinase activity levels and the malignant properties of the neuroblastoma cells which we studied. For instance, SK-N-MC cells have a low *c-src* kinase activity level (although comparatively high *c-src* kinase levels have been reported for this cell line [46]) and a karyotype with frequent homogeneously staining regions and double minutes (12), karyotypic abnormalities that are found in highly malignant neuroblastoma tumors. In contrast, SH-SY5Y cells have a high *c-src* kinase activity level and an almost normal karyotype (12). We therefore consider it less likely that high *c-src* activity is related to the malignant phenotype of neuroblastoma cells, as discussed by Bolen et al. (13).

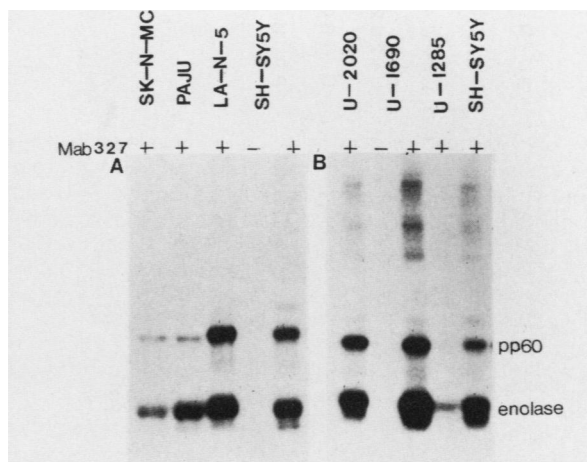


FIG. 2. Analyses of the *c-src* kinase activity with acid-activated rabbit muscle enolase (10 μ g per reaction) as an exogenous substrate. Control immunoprecipitations without MAb 327 were performed as indicated. (A) *c-src* kinase activity in four neuroblastoma cell lines. (B) *c-src* kinase activity in one neuroblastoma (SH-SY5Y) and three SCCL (U-2020, U-1690, and U-1285) cell lines.

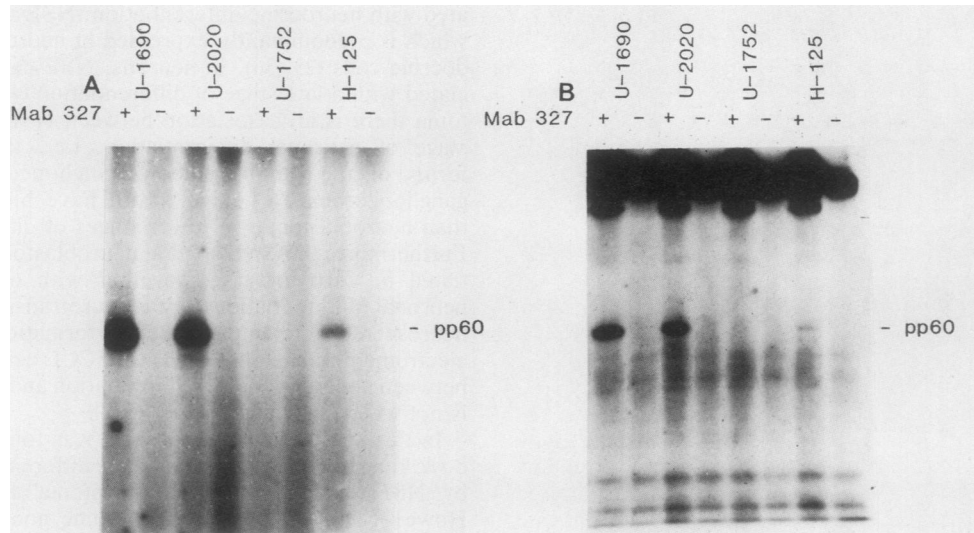


FIG. 3. Comparison of the *c-src* kinase autophosphorylation activity and protein level of pp60^{*c-src*} from two SCCL (U-2020 and U-1690), one squamous cell lung carcinoma (U-1752), and one lung adenocarcinoma (H-125) cell lines. The methods are described in Materials and Methods and in the legend to Fig. 1. (A) pp60^{*c-src*} kinase activity levels in these cell lines. (B) Immunoblot showing the corresponding pp60^{*c-src*} protein levels.

The fact that blood platelets (26), myeloid cells (4, 25), certain tumor tissues and cultured cells (46), and adenocarcinoma cells of the lung (H-125 cells in this report) have comparatively high *c-src* kinase activities shows that high *c-src* expression is not restricted to neurocrine cells. Furthermore, Sorge et al. (52) demonstrated *c-src* kinase expression in a variety of human fetal and adult tissues, including brain, kidney, liver, and skeletal muscle. A general finding was that the fetal tissues contained more pp60^{*c-src*} kinase activity than the corresponding adult tissues. This could imply that pp60^{*c-src*} plays a role in many cell systems during the late stages of differentiation or that *c-src* expression is

needed for a cellular function common to cells of various differentiation lineages.

A structurally altered *c-src* protein has been found in primary cultured neurons from rat brain, neuronally differentiated mouse teratoma cells, and one human neuroblastoma cell line (13, 15, 34). We approached the question of whether the altered *c-src* protein is also expressed in cells with the neuroendocrine phenotype. The V8 protease anal-

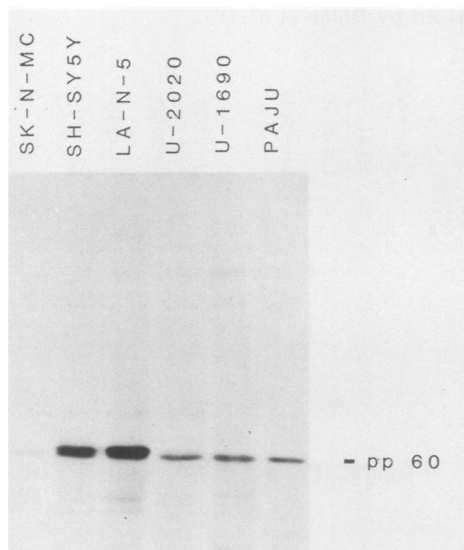


FIG. 4. Immunoprecipitation of *c-src* protein from [³⁵S]methionine-labeled neuroblastoma cells (SK-N-MC, Paju, SH-SY5Y, and LA-N-5) and SCCL cells (U-2020 and U-1690). The immunoprecipitated proteins were analyzed by SDS-PAGE with a 7.5% polyacrylamide gel.

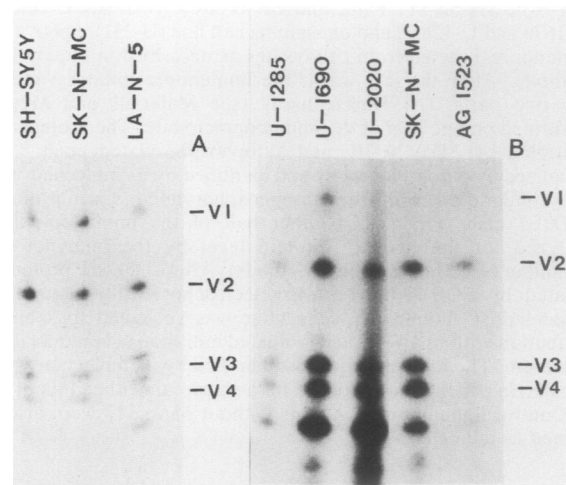


FIG. 5. V8 protease cleavage pattern of *c-src* proteins from neuroblastoma and SCCL cells. pp60^{*c-src*} was immunoprecipitated from ³²P-labeled cells and separated by SDS-PAGE. The 60-kilodalton protein band was excised and reelectrophoresed in the presence of 200 ng of V8 protease. (A) V8 cleavage pattern of the *c-src* protein from three neuroblastoma (SH-SY5Y, SK-N-MC, and LA-N-5) cell lines. The peptides termed V1, V3, and V4 represent the amino-terminal part of the *c-src* protein, and the V2 fragment originates from the carboxyl end (15). (B) V8 cleavage pattern of the *c-src* protein from three SCCL (U-1285, U-1690, and U-2020) cell lines, one neuroblastoma (SK-N-MC) cell line, and human diploid fibroblasts (AG-1523).

yses of the *c-src* proteins from three neuroblastoma, three SCCL cell lines, and human diploid fibroblasts revealed structural modifications only in the *c-src* protein from highly differentiated neuroblastoma cell lines (LA-N-5 and SH-SY5Y). The cleavage pattern from these cells closely resembles that seen in primary cultured neurons and neuronally differentiated teratoma cells. The cell line representing the highest stage of differentiation, LA-N-5, had 90% of the *c-src* molecules in the altered form. Thus, in addition to high *c-src* kinase activity and protein levels, cells of a high stage of neuronal differentiation express an altered *c-src* form. The expression of both *c-src* forms in LA-N-5 and SH-SY5Y cells could be explained by the presence of at least two populations of cells expressing one form each. Alternatively, all cells could express both forms in a ratio that depends on the stage of neuronal differentiation. In SCCL cell lines, the slower-migrating form of *c-src* was never found, despite their high NSE and *c-src* expression. In addition, a cell line derived from a colon carcinoid did not express the altered *c-src* product (unpublished results). These findings indicate that expression of the altered *c-src* protein is not a common feature of all differentiated neurocrine cells.

We conclude that the expression of *c-src* in cultured human neuroblastoma and SCCL cells is high and correlates positively with neurocrine differentiation. This may seem paradoxical in view of the tumorigenic effect of RSV. However, RSV infection of chicken fibroblasts *in vivo* or *in vitro* will not give rise to infinitely growing heteroploid lines (43), a feature commonly associated with malignant tumors. Alema et al. (3) observed that transformation by pp60^{v-src} is more similar to disturbed differentiation than to malignant cell conversion. In spite of certain differences in the biology of the *c-src* and *v-src* proteins, the possibility remains that both essentially affect regulation of differentiation. An increase in *c-src* expression is found during neuronal differentiation of mouse teratoma cells (34, 49), and rat PC12 pheochromocytoma cells differentiate after RSV infection (2).

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