Expression of pp60c-src Protein Kinase in Adult and Fetal Human Tissue: High Activities in Some Sarcomas and Mammary Carcinomas

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ABSTRACT

An endogenous protein of human cells pp60c-src, which is closely related to the product of the transforming gene of Rous sarcoma virus, pp60v-src, has been quantitated by measuring its enzymatic activity in an immunoglobulin G protein kinase assay. The influence of normal developmental processes on pp60c-src expression was assessed by comparative analysis of various adult and fetal human tissues. The maximal difference detected was a 2- to 3-fold-enhanced activity in fetal muscle compared with adult muscle. Organ-specific variations in the enzyme level were observed. Highest activity was found in brain, followed by kidney, lung, muscle, and connective tissue.

Since overexpression of the cellular counterparts of viral-transforming genes may play a role in carcinogenesis, pp60c-src kinase was measured in nine spontaneous human sarcomas and 21 mammary carcinomas. Compared with the respective normal tissues and human diploid fibroblasts, 4- to 20-fold-enhanced activities were observed in one-third of the sarcomas and carcinomas. The remainder showed no or insignificantly elevated activity.

The enzymes from normal and malignant tissues were indistinguishable from the virus-coded enzyme with respect to specificity for divalent cations and a predominance of phosphorylation in tyrosine. Patients carrying tumors with high pp60c-src protein kinase activity did not develop kinase-reactive antibodies against pp60c-src or pp60v-src.

INTRODUCTION

pp60c-src was the first polypeptide identified as being coded for by a transforming RNA tumor virus gene (2, 6). It was also with this protein that the association of transforming gene products with an unusual, tyrosine-specific protein kinase activity was first demonstrated (5, 7, 11).

An endogenous protein of normal chicken cells, termed pp60-src, is closely related to the viral pp60v-src (1, 3, 12, 15, 16). This protein is highly conserved during evolution, and analogues have been found in other vertebrate cells, including human cells (15). Compared to the amount of the viral protein in the RSV-transformed chicken cell, pp60c-src is expressed in 50 to 100 times lower levels. Like the virus-coded protein, it is associated with a tyrosine-specific protein kinase activity (4), suggesting that the endogenous and the viral proteins may have similar or even identical functions. Functional similarity between these proteins is suggested by experiments demonstrating that transformation-defective mutants of the virus, which were deleted in src, regained their transforming capacity by recombination with the endogenous cellular gene (c-src) of chicken cells (9, 12).

Transformation of a cell therefore may be due to the overproduction of a normal, potentially regulatory protein (for review, see Ref. 13).

For this reason, spontaneous human tumors (sarcomas and mammary carcinomas) were analyzed in this study with respect to the expression of the c-src gene. Since the determination of gene activity on the transcriptional level does not necessarily reflect the amount and the activity of a gene product, the enzymatic activity of pp60c-src was measured. Taking advantage of the relatedness between virus-coded pp60v-src and the endogenous pp60c-src proteins of vertebrates, a protein kinase assay for human pp60c-src was established accordingly. In order to assess the influence of growth on pp60c-src expression and to evaluate a possible tissue specificity of this protein, the levels of pp60c-src protein kinase were also determined in several normal human tissues, in fetal organs, and in human embryonic fibroblasts grown in culture. The enzymatic activities from all tissues were characterized with respect to their requirements for divalent cations and for the phosphate-accepting amino acid. The sera from the tumor patients were screened for the presence of antibodies which could serve as substrates in a protein kinase reaction with pp60c-src or pp60v-src and thus be of potential diagnostic value.

MATERIALS AND METHODS

Cells and Viruses. Chicken embryo fibroblasts were prepared from 11-day-old embryos, infected, and transformed as described previously (17). The avian sarcoma virus strains used were: SR-D4; SR-D17; Pr-A; Pr-C; Pr-CXC; B-77; and RAV-50.

Biopsies. Biopsies of tumors and normal tissues were obtained from the University Hospital in Cologne. Care was taken to use only nongeriatric tissue and to instantaneously add ice-cold lysis buffer (50 mm phosphate; 1 mm dithiothreitol; 10 mm EDTA; 10 mm NaF; 1 mm phenylmethylsulfonyl fluoride; 2 mm -aminocaproic acid; 0.2% Triton X-100, pH 7) to the sample after it had been removed from the patient. An aliquot of the tissue was routinely analyzed by the pathologist and tested for viability of the cells. The samples, generally 1 to 2 ml of tissue in 10 ml...
Preparation of Lysates. Thawed or fresh tumor samples in lysis buffer were manually ground on ice, using a mortar and pestle, until homogenous. The supernatants, containing 20% streptomycin sulfate (Serva, Heidelberg, Federal Republic of Germany) per ml of suspension, were added. The supernatants were vortexed until the streptomycin sulfate was dissolved and kept on ice for 30 min with occasional stirring. The samples were subsequently centrifuged for 1 hr at 20,000 rpm in a Sorvall SS 34 rotor. The supernatant was divided into aliquots of 300 to 500 μl. One aliquot was used for protein determination, and the others were either used immediately for protein kinase assays or frozen at −20°C until use. In the frozen state, the protein kinase activity of the extracts was stable for over 6 months. For protein determination, triplicate samples of the lysates were precipitated with 10% trichloroacetic acid and then assayed according to the method of Lowry et al. (14). Lysates from cultured cells were prepared by washing the plates with ice-cold phosphate-buffered saline (151 mM NaCl:10 mM sodium phosphate, pH 7.2), then adding 1 ml of lysis buffer per 15-cm dish, and scraping the cells off the plate using a rubber policeman. After this, streptomycin sulfate was added, and the extract was processed as described above for the biopsy material.

Antisera. Antisera recognizing human pp60^src were made by giving newborn rabbits injections of concentrated, mammalian-adapted avian sarcoma virus. Serum I used in this study was obtained from a rabbit which had received, in 3 different locations, SR-D17, Pr-CXC, and B-77 virus (19). At 4 weeks of age, the animal had developed tumors in all of the injected sites. The serum was from a bleed when the animal was 8 weeks old. Serum IV was a conventional serum from a tumor-bearing rabbit (2) using SR-D17 as the tumor-inducing material.

Protein Kinase Assay. Protein kinase activity associated with pp60^src or pp60^c-src was determined following a modification of the methods previously published (5, 17), which was designed to reduce the background of the assay; 50 μg of protein A Sepharose (Pharmacia) were equilibrated with 350 μl of lysis buffer on ice for 0.5 hr. For each sample to be analyzed, 5 μl of serum were reacted with 10 μl of the protein A Sepharose suspension for 1 hr at 0°C with occasional shaking. The lysates were diluted on ice so that each sample contained 50 μg of protein in a volume of 200 to 300 μl. The IgG-coated Sepharose beads were then washed 3 times with lysis buffer, taken up in 50 μl of lysis buffer per sample, and added to the lysates. The lysates were incubated with the beads for 1 hr on ice and mixed every 10 to 20 min. After this, the beads were washed in 400 μl each of Buffers 1 to 3 [Buffer 1, lysis buffer containing BSA (1 mg/ml); Buffer 2, lysis buffer containing BSA (1 mg/ml) and 1 mM NaCl; Buffer 3, lysis buffer containing BSA (1 mg/ml) and 1% Triton X-100] and in 1 ml of kinase buffer [20 mM 1,3-bis(trimethoxy)methylamino]propane (Sigma); 50 mM MgCl2, pH 5.9]. After centrifugation and removal of the supernatant, 10 μl of kinase buffer containing [γ-32P]ATP (2000 to 3000 Ci/mmol, Amersham) at a concentration of 0.1 μM were added. The samples were mixed and kept on ice for 3 min. The reaction was terminated by adding 200 μl of inhibition buffer (100 mM phosphate,40 mM NaF:10 mM EDTA, pH 7), the Sepharose beads were pelleted, and the supernatant was removed. The samples were then boiled for 2 min in 50 μl of sample buffer (0.06 M Tris, pH 6.8; 2% sodium dodecyl sulfate:5% β-mercaptoethanol:10% glycerol) and centrifuged, and the supernatant was applied to 12% sodium dodecyl sulfate/polyacrylamide gels (1.5 mm thick) which were run for 12 to 14 hr at 80 V. After staining with Coomassie Brilliant Blue, the gels were dried and exposed for 2 to 3 days to Kodak X-ray films using Cronex Lightning intensifying screens (DuPont). After autoradiograms had been obtained, the bands of IgG heavy chains were cut out from the gels. Since, particularly in the samples from normal human tissues, the radioactivity incorporated in the IgG band was low, similarly sized areas above and below the IgG band were cut out as well to be used for determination of the background in the gel and the counting procedure. To the gel pieces, 100 μl distilled water and 400 μl methylbenzenethionium hydroxide (Serva) were added. Following incubation at 60°C for 1 hr, 2 ml of liquid scintillation solution (Zinsser) were added, and the radioactivity in the samples was determined. Three times, 10 μl of the [γ-32P]ATP-containing kinase buffer were treated analogously and counted as well. After subtraction of the cpm determined for the background samples, the fmol of 32P incorporated into the heavy chain of IgG were calculated using the counts of the ATP-containing kinase buffer as reference. The protein kinase activity was expressed as fmol 32P incorporated into the IgG heavy chain per mg lystate protein used for the immunoprecipitation.

Phosphoamino Acid Analysis. After electrophoresis on slab gels following the kinase reaction, the wet gels were covered with Saran Wrap, and X-ray films were exposed to the gels for 1 to 2 days at 4°C in order to localize the IgG bands to be excised. The radioactively labeled bands were cut out, and the proteins were eluted electrophoretically, extensively dialyzed against double-distilled water, and lyophilized in Eppendorf vials. The samples were digested for 3 hr at 110°C with 300 μl of 6.5 N HCl under nitrogen, lyophilized, taken up in 300 μl of water, and lyophilized again. The residue was dissolved in 5 μl of a mixture containing PSer, PTyr, and PThr (1 mg/ml) in 10-3 M H3PO4 spotted on Merck (Darmstadt, Federal Republic of Germany) 5577 cellulose plates; and separated by electrophoresis for 2 hr at 500 V in acetic acid:pyridine:H2O (50:5:945, pH 3.5). The phosphoamino acid markers were visualized using ninhydrin spray (Merck), and the radioactivity was detected by autoradiography.

RESULTS

The aim of this study was to determine the levels of pp60^src protein kinase in human tumors, differentiated human tissue, and fetal tissue in order to assess a possible function of this protein in carcinogenesis and/or differentiation. In analogy to the assay for the viral enzyme, the reaction of the cellular pp60^src with the heavy chain of immune IgG was determined. Antibodies raised against the viral polypeptide, which cross-reacted with the endogenous protein of human origin (see "Materials and Methods"), were bound to protein A-conjugated Sepharose, followed by incubation with the lysate to be tested. The presence of cellular or viral protein on the beads carrying the immune IgG was subsequently detected by its ability to phosphorylate the heavy chain of IgG when [γ-32P]ATP and a suitable divalent cation were added. We established that the amount of radioactive phosphate incorporated into the IgG of a pp60^src-reactive serum was a quantitative measure for the amount of enzyme in the lystate using the conditions described in "Materials and Methods": (a) the antibody was always in excess; (b) exogenously added IgG was not phosphorylated, suggesting that the enzyme phosphorylated only the IgG molecule to which it was bound; and (c) maximum incorporation had occurred after 3 min on ice, and freshly added ATP did not enhance the phosphorylation of IgG beyond the value reached after 3 min, again suggesting that, also for the nucleotide substrate, only one round of reaction was possible (data not shown).

While for a given serum the assay should therefore allow quantitative comparisons of enzyme activity in lysters from various human sources, a different system may provide other values for protein kinase activity, since its ability to cross-react with the endogenous human protein may be different. Furthermore, variations between sera in the ability to accept the phosphate group have been observed, even if they precipitated the same amount of pp60^src protein (10, 17).

In order to establish the assay system for human pp60^src, 4 different tumor-bearing rabbit sera were characterized for their
reactivity with pp60vsrc from different strains of avian sarcoma virus and with pp60csrc from chicken embryo fibroblasts and from human embryonic fibroblasts. The characterization of the 2 sera offering the best cross-reaction over a wide range of pp60csrc relatives, Sera I and IV, is given in Table 1.

**pp60csrc Activity in Nonmalignant Tissues.** Since the amount of normal human biopsy material was limited, an attempt was made to assess the genetic variation in pp60csrc expression between human individuals by measuring the kinase activity in cultured cells prepared from 24 separate fetuses, each measurement being repeated 3 to 6 times. As shown in Table 1, the mean kinase activity determined with Sera I and IV was 0.4 and 0.21 fmol 32P incorporated per mg lysate protein, the S.D. being 0.06 and 0.05. Between cultures derived from separate fetuses, the values did not vary by more than a factor of 2. These results were interpreted as indicating only a modest genetic variability in the expression of pp60csrc, thus allowing the calculation of mean values from data on small numbers of individual biopsies.

The determination of pp60csrc activity in adult human tissues from various organs and in fetal tissues is shown in Table 2. Muscle, connective tissue, and breast biopsies (selected to contain as much mammary gland tissue and as little fat as possible) expressed comparable values of enzyme activity, while in kidney, 2- to 3-fold-higher activities were observed. When growing fetal tissues were compared to the corresponding adult tissues, either no difference in pp60csrc expression was observed (kidney), or there was a change by a factor of 2 to 3 (muscle). Together with the data on growing human embryo fibroblasts in culture (Table 1), these results suggested that the expression of pp60csrc is not strongly dependent on the growth state of cells. Tissue-specific differences in pp60csrc levels were, however, indicated by the high activity measured in extracts from fetal brain.

Because of the limited amount of material from different human individuals, the tissue specificity of pp60csrc expression was also tested in organs pooled from six 11-day-old chicken embryos. In agreement with the observations on human fetal organs, by far the highest enzyme activity was again found in brain. The activities measured were 6.6, 1.5, and 1 fmol/mg protein for embryonic chicken brain, kidney, and muscle, respectively (Serum I).

**pp60csrc Protein Kinase Activity in Tumors.** For the analysis of pp60csrc expression, mainly 2 types of tumor were chosen: sarcomas, because RSV induces this disease in the natural host; and mammary carcinomas, because it is one of the most frequent neoplasias in humans. A typical autoradiograph of IgG protein kinase assays on the various malignant and control tissues is shown in Fig. 1.

Compared with the normal tissues of the same patients (O, □), the sarcomas (◆) of patients 13 and 14 and the mammary carcinoma of Patient 9 (●) contained considerably higher levels of the enzyme than did the normal tissues. While the tumors from Patients 5, 7, and 12 also expressed increased amounts of the enzyme, no substantial elevation in pp60csrc activity was observed in Cases 8 and 10. As a control, the reaction of tumors containing high enzyme levels with normal rabbit serum is shown to be negative (Fig. 1, right). The finding that certain tumors contained elevated enzyme levels while others did not was substantiated by the analysis of a total of 9 human sarcomas, shown in Chart 1. When analyzed using Serum I, 3 tumors (Tumors 4, 23, and 101) were characterized by enzyme activities close to normal tissues (Chart 1, dotted horizontal line; see also Table 2). Another set (Tumors 13, 21, and 43) displayed 2 to 3 times enhanced values, comparable to the difference observed between adult and fetal muscle. In the remaining samples, the enzyme was expressed at levels which were 4- to 10-fold higher than those in normal muscle and connective tissue. It should be noted that Serum IV detected a very similar pattern of high and low activity. Again, the highest activity was found in Tumor 81, the activity being in this case 20-fold higher than the value determined for normal muscle (not shown).

Because experimental data could only be obtained on normal tissue from 3 patients, a statistical analysis, comparing values between the same number of normal and tumor tissues, was not possible. To assess the variation among individual tissue samples, a randomization test (18) was carried out, which compared the values from the tumors and the normal biopsies to the

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**Table 2**

<table>
<thead>
<tr>
<th>Protein kinase activity (fmol/mg lysate protein) determined using</th>
<th>Serum I</th>
<th>Serum IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle and connective tissue</td>
<td>0.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Foreskin</td>
<td>0.41</td>
<td>0.19</td>
</tr>
<tr>
<td>Mammary gland tissue</td>
<td>0.21</td>
<td>0.15</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.2</td>
<td>0.51</td>
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<tr>
<td>Fetal kidney</td>
<td>1</td>
<td>0.86</td>
</tr>
<tr>
<td>Fetal lung</td>
<td>1</td>
<td>0.71</td>
</tr>
<tr>
<td>Fetal muscle</td>
<td>0.7</td>
<td>0.63</td>
</tr>
<tr>
<td>Fetal brain</td>
<td>3.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

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**Table 1**

Characterization of the sera used for measurement of human pp60vsrc kinase

<table>
<thead>
<tr>
<th>Serum</th>
<th>SR-D</th>
<th>Pr-A</th>
<th>NRS</th>
<th>B-77</th>
<th>Untransformed CEF (R-50 infected)</th>
<th>Normal HEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>332</td>
<td>± 34</td>
<td>25</td>
<td>± 3</td>
<td>19 ± 2</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>IV</td>
<td>440</td>
<td>± 50</td>
<td>14</td>
<td>± 2</td>
<td>11 ± 2</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>NRS</td>
<td>1.6</td>
<td>± 0.4</td>
<td>0.05</td>
<td>± 0.04</td>
<td>0.08 ± 0.04</td>
<td>0.02 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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*The determination of protein kinase activity was carried out as described in "Materials and Methods."  
CEF, chicken embryo fibroblasts; HEF, human embryo fibroblasts; NRS, normal rabbit serum (control).  
*Values obtained from 24 cultures prepared from 24 separate fetuses.  
*Serum was made by simultaneous injection of SR-D17, Pr-CXC (see "Materials and Methods."), and B-77 virus into newborn rabbits.  
*Mean ± S.D. from at least 3 independent determinations on 3 to 6 different cultures of each cell type.  
*Serum was made by injecting only SR-D17 virus into newborn rabbits.
pp60src Protein Kinase in Human Tumors

pp60src activities determined in the same number (i.e., 12) of independent, low-passage human fibroblast cultures which, according to the data in Tables 1 and 2, expressed similar pp60src values as did normal muscle. Since each of these cultures was derived from a different individual and since the cells were growing actively, this method should reflect a variation between individuals and an influence of growth on the enzyme levels. The probability (p) of the assumption was tested to determine if any of the fibroblast cultures contained higher enzyme activity than the tissue samples. p < 0.05 was taken to indicate a statistically significant increase in the biopsy material. Using this test, the high enzyme levels in the samples of Tumors 14, 21, 43, 45, and 81 were classified as significant. The Sarcomas 4, 13, 23, and 101 and all normal biopsies were found to contain pp60src activities, which did not differ significantly from normal human fibroblasts.

Sarcomas are a rare disease. It seemed important to determine if an enhanced enzyme level was typical for some sarcomas only, or if it also occurred in other cancers. For this reason, 21 biopsies from mammary carcinomas were subjected to the same analysis of pp60src kinase (Chart 2). In analogy to the observation made with the sarcomas, normal enzyme levels were detected in one-third of the samples, and another 30% contained moderately elevated activity. In the remaining samples, the activity was 3- to 6-fold higher than the mean value from 3 normal breast biopsies (Chart 2, dotted horizontal line). When the randomization test as described for the sarcomas was applied, using the data from the 24 independent fibroblast cultures as reference, Carcinomas 5, 7, 12, 16, 31, 33, 35, 53, 79, and 80 were found to contain significantly enhanced levels (p < 0.05). None of the normal biopsies fell into this category.

Analysis of the Sera from Tumor Patients for Antibodies to Be Detected in a Protein Kinase Test. Most animals produce antibodies against pp60src when carrying a RSV-induced tumor, and in some cases, at least, these antibodies also react with the endogenous pp60src (1). Since the identification of such antibodies could be of diagnostic value, the sera of patients whose tumors contained high pp60src activity were subjected to a protein kinase test with lysates from their own tumors, from those of other patients, and from RSV-transformed chicken cells. In no case could a substantial phosphorylation in the human IgG be detected.

Characterization of pp60src Protein Kinase from Normal and Malignant Human Tissue. To date, no enzymatic difference between the virus-coded pp60src and the cellular pp60src of chicken has been reported, although it is known that the phosphorylated peptide containing PTyr is different in the 2 proteins (3). It is therefore still an unanswered question as to whether RSV exerts its function by simply overproducing a normal, potentially regulatory protein, or if the viral enzyme and the cellular enzyme do, in fact, catalyze different reactions in the cell. Since high enzymatic activities in the tumors could also be due to mutations in the c-src gene and not only to an increased expres-

Fig. 1: pp60src protein kinase assay in human tumors in normal tissue and in cultured cells. Biopsy specimens and cultured cells were lysed and processed as described in "Materials and Methods." For 50 μg of lysate protein, 5 μl of Serum I bound to protein A Sepharose were used. Fig. 1 shows an autoradiograph of the region of the gel containing the heavy chain of IgG. The numbers below the autoradiogram spots identify the patients (see also Chart 1). The symbols above each sample characterize the tissue (•, normal tissue from the same patient).

Chart 1. pp60src protein kinase activity in human sarcomas. Extracts from fresh biopsies were prepared and subjected to the protein kinase assay. Vertical Bars, data from an analysis using Serum I, representing the mean values from 3 to 6 determinations on each of the lysates. In the cases where high activities were observed (Extracts 14, 21, 43, 46, and 81), it was verified by titration experiments that the antibody had been in excess, when the conditions of the assay were as indicated in "Materials and Methods." The experimental error (horizontal bars) was 30 to 50% of the value (between the horizontal bars). Dotted horizontal line, mean of the enzyme activities in normal muscle and connective tissue from Patients 13, 14, and 101 (Table 2).

Chart 2. pp60src-related protein kinase activity in mammary carcinomas. Twenty-one biopsies were analyzed as described in "Materials and Methods." Bars, mean values from 3 to 6 determinations on each of the lysates using Serum I, dotted horizontal line, value for normal breast tissue (Table 2). The experimental error is indicated by the horizontal bars (upper bar, mean + S.D.; lower bar, mean – S.D.).
demonstrated in Fig. 2, its phosphorylation occurred exclusively in serine. Growing tissues (prostate adenoma, fetal kidney, and bladder papilloma) contained a protein kinase with a predominant specificity for tyrosine as did stationary tissues (muscle and foreskin). As shown in Fig. 2c, the pp60⁰⁰ src protein kinase in malignant tissues was also found to mainly phosphorylate tyrosine. Upon longer exposure of the films, PSer could be detected in all samples from protein kinase assays with the human pp60⁰⁰ src. The PSer:PTyr ratio was somewhat variable from one experiment to another and between the tissues. In some cases, the PSer content reached 10 to 15% of the incorporation of phosphate into tyrosine. In contrast, for the phosphorylation of IgG by viral pp60⁰⁰ src protein kinases, the amount of PSer and/or PThr never exceeded 1% (data not shown). This result, however, taken by itself, cannot be interpreted as indicating an additional specificity on the endogenous human enzyme for serine, which was not displayed by the viral kinase. Since the reaction of the human enzyme is much weaker, a contribution by an unspecifically adsorbed serine-specific kinase could reach a higher percentage.

**DISCUSSION**

While in a recent investigation (8) the expression of oncogenes in human tumor cells was studied in terms of transcription, the present study was concerned with the determination of the gene dosage of one such oncogene, c-src, at the posttranslational level. Because the amount of mRNA made for a certain protein does not necessarily reflect the amount of a functional gene product, we have measured the enzymatic activity of pp60⁰⁰ src, which is a direct and sensitive assay for the presence and most probably also for the functional activity of the protein.

As mentioned before, the assay did not allow the measurement of absolute enzyme levels but was a method for screening a variety of samples for relative activities. Since the maximum incorporation of phosphate into IgG was determined, but not the initial velocity of the reactions, it quantitated the amount of active enzyme molecules. The assay would not, however, have distinguished between enzymes catalyzing the reaction at different rates, as long as they reached the same maximum incorporation within the given reaction time. About one-third of the sarcomas and mammary carcinomas analyzed contained enzyme levels, which were between 4- and 20-fold higher than the activity in the respective normal tissues or in human diploid fibroblasts. Since the genetic variation among 24 cultures of human embryonic fibroblasts, which were derived from individual fetuses, did not exceed a factor of 2, it is unlikely that this result is due to random variation between human individuals, as was also evidenced by the statistical analysis. Two other possibilities should, however, be discussed, which could result in elevated pp60⁰⁰ src activities that were only indirectly related to transformation. The first is that the expression of pp60⁰⁰ src could be changed as a result of increased growth of the tumor cells, compared with their normal counterparts. A correlation with tumor growth was clearly not given in the few cases where growth rates could be assessed, because the tumor could not be removed surgically for a certain amount of time after its clinical manifestation. For example, Sarcoma 4, which displayed normal levels of pp60⁰⁰ src kinase, grew extremely fast and totally destroyed the surrounding thigh muscle within 3 months. In contrast, a tumor with high enzyme levels, Sarcoma 45, was present for 9 months and did not vary in size very much. Only upon surgical removal and subsequent pathological examination it was recognized as malignant.

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6 A Mr 42,000 phosphoprotein was consistently found to be associated with immunoprecipitates of lysates from normal or malignant prostate biopsies. As demonstrated in Fig. 2, its phosphorylation occurred exclusively in serine.
Another observation, which argues against a strong influence of growth on pp60^src expression, is the data given in Table 2. The comparison of the same tissue in the growing, fetal stage and in the stationary, adult stage either did not reveal any difference in enzyme activity (kidney) or revealed only a difference of a factor of 2 to 3 (muscle). Thus, the cases in which only 2- to 3-fold-enhanced activities were observed in the tumor could be due to increased growth. A 4- to 20-fold increase in pp60^src kinase activity, however, is difficult to explain on this basis. The 24 independent cultures of human diploid fibroblasts also did not display enzyme levels which were higher than the levels determined for normal muscle and connective tissue (Tables 1 and 2). Since these cultures were serum stimulated and growing actively, high enzyme levels would have to be expected were pp60^src expression strongly correlated with growth.

Another possible reason unrelated to transformation which might explain the high enzyme levels in the tumor tissues could be given by the monoclonal nature of the tumors. If the cell from which the tumor was derived, already in its normal state, contained high enzyme activity but was only a small percentage among other cell types in the tissue, the average activity in the normal tissue could be low. In contrast, the tumor tissue, which consisted mainly of this particular cell, would display high enzyme levels, even if the expression of the enzyme was not changed upon transformation of the cell. The high activities observed in a certain percentage of cases in this study, however, are most likely not due to the monoclonality of the tumors, since two-thirds of the malignant tissues analyzed contained normal or only 2- to 3-fold-enhanced activities. It should be justified to regard these as internal controls.

An alternative to the study of biopsy material, particularly the human system, where consistent long-term observations on tumor growth or other experimental parameters cannot be made, is the use of cultured tumor cells. In this case, it is possible to compare cultures which are pure and the growth of which is controlled, although adaptation to tissue culture may remove the cell populations from their in vivo state. We have analyzed a set of 10 human tumor cell lines (kindly provided by Dr. H. zur Hausen, Freiburg, Federal Republic of Germany). The cells were derived from melanomas, larynx carcinomas, hepatomas, and a vulva carcinoma. Compared with a fibroblastic cell line derived from a benign skin tumor and the human embryo fibroblast cultures already discussed, half of the cell lines contained levels of pp60^src kinase, which were elevated by factors of 5 to 30, while the others showed no significant elevation.

A certain percentage of spontaneous tumors thus seems to be characterized by a transformation-specific increase in pp60^src protein kinase activity. It remains unclear, though, if this enhanced activity is a cause or a consequence of malignant transformation. The increase in enzyme expression was clearly not specific for a particular type of tumor, e.g., sarcomas, but occurred in carcinomas as well, and based on the results with cultured cells, such expression may also apply to melanomas and hepatomas. These results are in concordance with the report by Eva et al. (8). No correlation could be made between an increased level of RNA, which was specific for a given oncogene, and the type of tumor in which it was detected. Also, in analogy to the findings described in the present study, elevated oncogene-specific RNA levels were not observed in all tumors, but a considerable percentage contained normal amounts.

Natural carcinogenesis is a multistep process and may require several, independent, rate-limiting events. The provision of an active oncogene, whether by rearrangement, mutation, or viral insertion, may only be one of these steps. Furthermore, each oncogene does not appear to be important in every tissue, and there may be a hierarchy among them which is defined by their functions. Further analysis of spontaneous tumors for oncogene expression at the transcriptional and translational levels should eventually lead to understanding their differential expression in some tumors and the regulatory changes induced by them.

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C. Jacobs and H. Rubsamenn


Expression of pp60\textsuperscript{c-src} Protein Kinase in Adult and Fetal Human Tissue: High Activities in Some Sarcomas and Mammary Carcinomas

Christiane Jacobs and Helga Rübsamen


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