RESEARCH ARTICLE

Expression of a Platelet-Derived Growth Factor–Like Protein in Simian Sarcoma Virus Transformed Cells

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Abstract. *The near identity of the partial amino acid sequence of human platelet-derived growth factor (PDGF) and that predicted for p28^{sis}, the putative transforming protein of the simian sarcoma virus (SSV), suggests expression of a growth factor activity may be central for transformation by SSV. It is now reported that SSV-transformed cells but not control cells contain a growth factor activity that is identical to PDGF in immunoassay, in mitogenic dose response, and in specific mitogenic activity. The protein immunoprecipitated by antiserum to human PDGF has an apparent molecular weight of 20,000, identical to that of p20^{sis}, the putative intracellular degradation product of p28^{sis}. The results support the concept that expression of a PDGF-like molecule, which appears to be the product of the viral-*sis *gene, is responsible for the abnormal regulation of growth is SSV-transformed cells.*

The platelet-derived growth factor (PDGF) is the major mitogenic protein in serum for cells of mesenchymal origin (*1–5*). It is also a potent chemoattractant agent for human neutrophils and monocytes (*6*), for fibroblasts, and for smooth muscle cells (*7–9*). Although its role in normal physiological states is not established, PDGF is well suited to mediate inflammatory and repair processes at sites of blood vessel injury and may play an important role in the genesis of atherosclerosis in humans (*5*) .

PDGF has been difficult to characterize. Initial purifications of the factor resulted in very small quantities of protein (*10, 11*), but more recently it has been purified in quantities sufficient to establish its purity and chemical composition (*12–16*). PDGF has been separated into two equally active mitogenic proteins of 31,000 (PDGF I) and 28,000 (PDGF II) daltons that have essentially identical amino acid compositions but different carbohydrate compositions (*12, 14*). PDGF I and II are highly basic [*p*I, 10.2 (*12, 14*)]; when PDGF is reduced, biologically inactive peptides ranging from 3,500 to 17,500 daltons are observed (*12–17*).

We recently reported (*17*) a partial amino acid sequence of PDGF that contained a region of 104 contiguous amino acids with virtual identity to the predicted amino acid sequence of p28^{sis} (*18*), the transforming protein of the simian sarcoma virus (SSV). During preparation of our manuscript (*17*), an amino-terminal sequence analysis of PDGF was described (*19*); subsequently, the near identity of the predicted amino acid sequence of p28^{sis} and that of PDGF was also reported (*17, 20*).

Cells transformed by retroviruses and cells stimulated by growth factors have been observed to have strikingly similar properties; these properties differ from those of nontransformed, nonstimulated control cells (*21–28*). The homology between the reported partial amino acid sequence of PDGF and that predicted for p28^{sis} and the phenotypic similarities of growth factor–stimulated cells and transformed cells suggests an important role for growth factor proteins in virally transformed cells and in cells transformed by other agents.

We now demonstrate that SSV-transformed cells but not control cells contain a growth factor that is identical to PDGF in immunoassay, in mitogenic dose response curve, and in specific mitogenic activity; this growth factor is different from PDGF because the apparent molecular weight in sodium dodecyl sulfate (SDS) gels after immunoprecipitation is 20,000. The product of the v-*sis* gene, p28^{sis}, is processed intracellularly to a molecule of 20,000 daltons, p20^{sis} (*29*). Our results provide direct evidence for expression of a PDGF-like molecule in SSV-transformed cells that is the same size as the v-*sis* expression product. It seems reasonable to consider that they may be identical.

Cells transformed by SSV contain a potent PDGF-like growth promoting activity. Evidence that SSV-transformed cells contained a growth promoting activity was obtained when lysates were tested for mitogenic activity and found to stimulate [^{3}H]thymidine incorporation into DNA in previously quiescent Swiss mouse 3T3 cells (Fig. 1). The increase in [^{3}H]thymidine incorporation was linear with amount of cell lysate added; the dose response of [^{3}H]thymidine incorporation in this assay system was essentially identical to that observed with purified PDGF but differered substantially from that of epidermal growth factor (EGF) and other growth factors (*30*). Cell lysates from control, non-SSV–transformed NIH 3T3 cells did not stimulate [^{3}H]thymidine incorporation above levels observed without added cell lysate. Similar growth factor activity was found in lysates of SSV-transformed NRK cells (data not shown).

The growth promoting activities of SSV-transformed cell lysates and PDGF are antigenically similar. The striking homology between the partial amino acid sequence of PDGF and that predicted for p28^{sis}, the putative transforming protein of SSV, suggested strongly that the growth factor activity in SSV-transformed cells is attributable to p28^{sis}. Antiserum to PDGF was therefore tested as an inhibitor of the growth factor activity in SSV-transformed cell lysates. When added to Swiss mouse 3T3 cell cultures exposed to SSV-transformed cell lysates, antiserum to PDGF blocked the stimulation of [^{3}H]thymidine incorporation by 44 percent (Table 1). If the cell lysates were treated with antiserum to PDGF and adsorbed with protein A before the mitogenic assay, their growth stimulating activity was completely removed.

The finding that antiserum to PDGF blocked the growth promoting activity of lysates from SSV-transformed cells led us to test further antigenic similarities of

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the two activities. We compared the displacement of ^{125}I-labeled PDGF by increments of two different cell lysates from SSV-transformed cells with displacement by purified PDGF (Fig. 2). The displacement of ^{125}I-labeled PDGF by proportionate increases in two different preparations of SSV-transformed cell lysates and with purified PDGF was identical, indicating a high degree of antigenic relatedness between the two proteins.

The specific mitogenic activity of the growth promoting factor in SSV-transformed cell lysates is the same as purified PDGF. Radioimmunoassay and mitogenic activity ([^{3}H]thymidine incorporation) were measured in SSV-transformed cell lysates and in lysates from nontransformed NIH 3T3 cells (Table 2). Immunoreactive PDGF could not be detected in control 3T3 cell lysates (Table 2). PDGF immunoreactive protein in SSV-transformed cell lysates was 5.7 ng per milligram of cell protein; the radioactivity from [^{3}H]thymidine incorporated into the DNA of 3T3 cells was 0.4×10^5 disintegrations per minute per milligram of cell lysate protein. The specific mitogenic activity in SSV-transformed cell lysates was thus 7×10^5 units per milligram—almost identical to the specific mitogenic activity (6.0×10^5 U/mg) observed with purified PDGF (*14*).

Immunoprecipitation of SSV-transformed cell lysates. Immunoprecipitates of lysates from SSV-transformed cells were prepared with specific antiserum to PDGF and with control nonimmune serum after the cells were labeled with [^{35}S]methionine (Fig. 3). Autoradiograms of SDS gels revealed a labeled 20,000-dalton protein in immunoprecipitates prepared with the antiserum to PDGF but not in immunoprecipitates from cell lysates treated with the control serum. The 20,000-dalton protein is identical in molecular weight to the carboxyl-terminal 20,000-dalton fragment that results from the intracellular processing of p28^{sis} (*29*).

Discussion. The striking homology between a region of the observed amino acid sequence of PDGF and that predicted for p28^{sis}, the putative transforming protein of SSV (*17, 20*), suggests that the expression of PDGF or of a PDGF-like growth factor in SSV-transformed cells may mediate the unregulated growth that is characteristic of transformed cells. We have identified such growth factor activity in SSV-transformed cells but not in nontransformed cells. The properties of this growth factor activity are essentially the same as those of PDGF, as indicated by the blocking of SSV-transformed

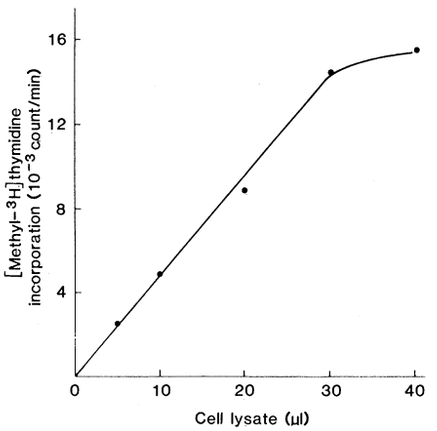

Fig. 1. Stimulation of [methyl-^{3}H]thymidine incorporation into DNA of Swiss mouse 3T3 fibroblasts by lysates of SSV-transformed NIH 3T3 cells. The assays were performed as described (*30*). Purified PDGF II (*12, 14*) was used as standard. SSV-transformed NIH 3T3 cells (courtesy of S. A. Aaronson) or control NIH 3T3 cells were harvested (1500 cm^2), sonicated in 1 ml of phosphate-buffered saline, and centrifuged at 15,000*g*. The supernatants (cell lysates) were immediately frozen at −20°C. Control NIH 3T3 cell lysates contained essentially no mitogenic activity.

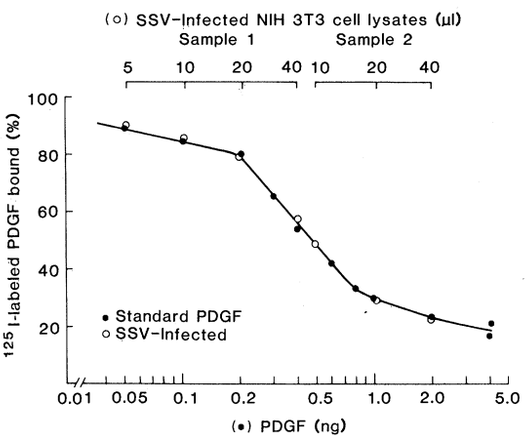

Fig. 2. Radioimmunoassay of SSV-transformed NIH 3T3 cell lysates with antiserum to human PDGF. Immunoassays were carried out as previously described (*32*). Up to 40 μl of cell lysate (sample 1 or sample 2) did not influence the immunoreactivity of the exogenous PDGF standard (0.2 ng). The protein concentrations of cell lysates were 4 mg to 10 mg/ml.

Table 1. Effect of antiserum to PDGF on the mitogenic activity of lysates of SSV-transformed NIH 3T3 cells after fractionation with Sulfadex gels. Cell lysates (0.5 ml) of SSV-transformed NIH 3T3 cells were mixed with 100 mg (wet weight) of Sulfadex G-50 (*14*) overnight. The gels were then washed with 1 ml of phosphate-buffered saline three times. PDGF-like activity was eluted with 0.5 ml of 10 m*M* phosphate buffer containing 1*M* NaCl (Sulfadex-retained fraction). Ten microliters of the Sulfadex-retained fraction was tested for mitogenic activity. More than 85 percent of the mitogenic activity in the cell lysates was recovered in the Sulfadex-retained fraction. The antiserum to human PDGF was purified by DEAE-blue Sepharose column chromatography (*32*). No endogenous growth factor activity could be detected in this purified antiserum.

Sample	[Methyl-^{3}H] thymidine incorporation (dpm/well)	Activity (%)
Sulfadex-retained fraction	22,588	100
Sulfadex-retained fraction plus antiserum to human PDGF (5 μl)	12,706	56

Table 2. Antigenic and mitogenic activity of cell lysates from SSV-transformed cells and control NIH 3T3 cells. The antigenic activity was measured by radioimmunoassay as described (*32*). The mitogenic activity was measured by [methyl-^{3}H]thymidine incorporation into trichloroacetic acid precipitable substances as described (*30*).

Cell lysates	Antigenic activity (A) (ng/mg protein)	Mitogenic activity (M) (dpm/mg protein)	Specific mitogenic activity (M/A) (U/mg)*
PDGF			6×10^5
NIH-3T3	< 0.3	0†	
SSV-transformed NIH 3T3	5.7	0.4×10^5‡	7×10^5

*One unit of PDGF activity is that activity with a net incorporation of 10,000 dpm in excess of the control culture. †No PDGF-like mitogenic activity was found. ‡The mitogenic activity of SSV-transformed NIH 3T3 cell lysates has been corrected from the assays in the presence of exogenous PDGF standard (correction factor is 3).

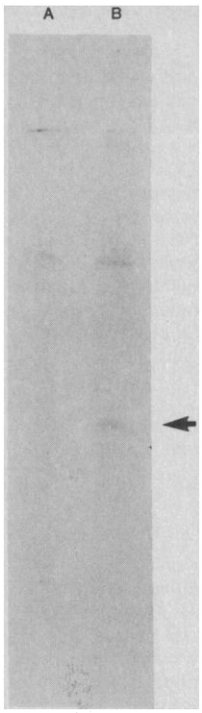

Fig. 3. Immunoprecipitation of lysates of [^{35}S]methionine-labeled SSV-transformed NIH 3T3 cells with antiserum to human PDGF. SSV-transformed NIH 3T3 cells, and control NIH 3T3 cells were grown to confluence in 100 cm^2 petri dishes. After washing with methionine-deficient Dulbecco's modified Eagle's medium, the monolayer cells were labeled with [^{35}S]methionine (100 μCi/ml) in the same Dulbecco's modified Eagle's medium for 4 to 5 hours. The cells were lysed in 1 ml of 10 m*M* sodium phosphate, 100 m*M* NaCl, 1 percent Triton X-100, and 0.5 percent sodium deoxycholate, and centrifuged. Two-tenths of a milliter of labeled cell lysates was reacted with 5 μl of rabbit antiserum to human PDGF or nonimmune serum (overnight, 4°C). Fifty microliters of 10 percent IgGsorb was added and the mixture was incubated (4°C, 2 hours) and then washed three times with 1 ml of 10 m*M* sodium phosphate buffer containing 0.1 percent Tween 80, 0.5*M* NaCl, and 0.01 percent sodium azide and subjected to SDS gel electrophoresis (15 percent gel) and fluorography. The protein markers used included ovalbumin (molecular weight 43,000, R_F 0.14), carbonic anhydrase (30,000, R_F 0.32), soybean trypsin inhibitor (21,500, R_F 0.46), and lysozyme (14,500, R_F 0.64). The arrow indicates the labeled protein immunoprecipitated by the antiserum to human PDGF (20,000, R_F 0.5).

growth factor activity by a specific antiserum to PDGF, by the displacement of ^{125}I-labeled PDGF with proportionate increments of SSV-transformed cell lysates and purified PDGF in immunoassay, and by the similarities of the activities of PDGF and the growth factor in lysates of SSV-transformed cells.

The transforming gene of SSV could encode the synthesis of a protein with 226 amino acids (p28^{sis}). A glycoprotein of this apparent molecular weight has been identified by labeling SSV-transformed cells with [^{3}H]mannose. An antiserum against a chemically synthesized carboxyl-terminal p28^{sis} peptide (residues 212–226) has been used in pulse chase analysis to identify a 20,000-dalton processed product, p20^{sis} (*29*). Our antiserum to human PDGF precipitates a 20,000-dalton protein from SSV-transformed cells (SDS gel electrophoresis). The homology between the amino acid sequences of PDGF and the transforming protein of SSV coupled with the identification of a labeled 20,000-dalton protein in immunoprecipitates from SSV-transformed cell lysates treated with antibody to PDGF provides compelling evidence that the growth factor is in fact the transforming protein of SSV.

It is conceptually difficult to reconcile the fact that PDGF is a secreted glycoprotein while p20^{sis} is not known to be secreted. The mechanisms whereby growth factors may mediate transformation-like events in target cells are, however, unknown. Whether p28^{sis} mediates transformation through intracellular receptors, through interactions with extracellular receptors on p28^{sis} producer cells, or through interactions with receptors on contiguous but non-SSV–transformed cells are among questions that need to be resolved to further establish mechanisms for virus- and carcinogen-induced cell transformation. These questions should have relevance for other tumor systems since cells producing increased amounts of the product of the human v-*sis* homolog (c-*sis*) may also undergo cellular transformation by a similar mechanism. Human tumor cell lines have been identified with elevated transcripts hybridizing with v-*sis* probes (*31*).

References and Notes

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33. We thank Dr. Stuart A. Aaronson for the gift of NIH 3T3 cells and NRK cells transformed by SSV.

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