Presence of Elevated Levels of Platelet-Derived Growth Factor (PDGF) in Lung Adenocarcinoma Pleural Effusions*

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Significant tumor stroma development is a specific feature of adenocarcinoma of the lung in comparison to small-cell lung cancer (SCLC). The fibrotic component of tumor stroma is thought to result from the migration and local replication of mesenchymal cells in response to the presence of cytokines. One of them, platelet-derived growth factor (PDGF), is a chemotactic and growth factor for mesenchymal cells. Since several lung adenocarcinoma cell lines, but not SCLC cell lines, have been shown in vitro to express PDGF genes, we evaluated pleural effusions for the presence of PDGF in patients with adenocarcinoma of the lung, SCLC, or nonmalignant pleural effusions. In adenocarcinoma of the lung, PDGF levels in pleural effusions were higher than in SCLC and in nonmalignant pleural effusions and were associated with the presence of a growth-promoting activity for fibroblasts due, in part, to the presence of PDGF. This observation suggests the role of PDGF in tumor stroma formation in adenocarcinoma of the lung.

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DMEM = Dulbecco’s modified Eagle’s medium; FCS = fetal calf serum; kDa = kilodalton; mAb = monoclonal antibody; PDGF = platelet-derived growth factor; SCLC = small-cell lung cancer

Solid tumors progressively develop in parallel with a tumor stroma composed of blood neovessels, an inflammatory cell infiltration, and a fibrotic reaction.1-3 In the lung, while small-cell lung cancer (SCLC) is characterized by a poor stroma reaction, in contrast, non-SCLC (adenocarcinoma and squamous cell carcinoma) development induces the production of a marked tumor stroma in their vicinity.4-6

It is currently thought that tumor cells, by the release of chemotactic or growth factor (or both), for mesenchymal cells, can participate in the formation of the fibrotic component of tumor stroma.1,3-7 One of these factors is platelet-derived growth factor (PDGF), a 31-kDa glycoprotein composed of two chains, A and B, bound by disulfide bridges.5-11 PDGF is a potent chemotactic factor and growth factor for mesenchymal cells, and PDGF is involved in wound healing and several fibrotic processes including lung fibrosis12,13 and atherosclerosis.10 Interestingly, several non-SCLC cell lines, but not SCLC cell lines, have been shown to express PDGF genes.14,16

Most evidence, if not all, for the role of PDGF comes from in vitro data obtained with cell lines. Thus, in order to specify if PDGF is present in vitro, we evaluated malignant pleural effusions (defined by the presence of malignant cells) for the presence of PDGF and compared these effusions to benign pleural effusions. Interestingly, the levels of PDGF were parallel to the presence of growth-promoting activity for fibroblasts and were higher in adenocarcinoma of the lung, a tumor with important tumor stroma,4,6 than in SCLC, a tumor with very limited tumor stroma, enforcing the concept of the role of the local presence of PDGF in tumor stroma formation.

**Materials and Methods**

**Study Populations**

Pleural fluids were obtained from patients with malignant pleural effusion (n = 15; mean age, 60 ± 5 yr; nine male and 6 female patients) and from patients with nonmalignant pleural effusion (n = 10; mean age, 68 ± 6 yr; seven male and three female patients). No macroscopically hemorrhagic pleural effusion was studied. For all patients with malignant effusion, diagnoses were obtained by cytology or pleural biopsy under thoracoscopy (or both). The diagnoses were, according to WHO criteria,17 adenocarcinoma of the lung (n = 11) and SCLC (n = 4); while nonmalignant pleural effusions corresponded to tuberculosis (n = 2), parapneumonic pleural effusion (n = 4), chronic benign pleural effusion (n = 2), and congestive heart failure (n = 2). Pleural fluid was recovered on heparin (Sigma, St Louis, Mo) before centrifugation (800 × g for 10 min at 4°C) to remove cells and insoluble material. The fluids were then kept frozen (−70°C) until use.

**Growth-Promoting Activity Quantification**

This activity was measured by incubating normal human fetal lung fibroblasts with pleural fluid samples. Lung fibroblasts were grown from fetal lung kindly provided by Dr. Leheup (Laboratoire...
de Biologie Sexuelle et du Développement, CHRU, Nancy). After allowing the fibroblasts to replicate from explants in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10 percent fetal calf serum (FCS; Institut Jacques Boy SA, Reims, France) at 37°C in 5 percent CO2 over a period of 7 to 10 days (with media change every 3 days), the culture vessels were covered with confluent cells, the explants were discarded, and the cells were subcultured, characterized, and used. The tests were carried out by incubating preconfluent fibroblasts (6 x 10^6 cells per milliliter; 1 ml per well) in six-well Falcon plates (Becton-Dickinson Labware, Lincoln Park, NJ) for 48 h in the presence of DMEM alone as a negative control, or with the addition of 10 percent decomplemented FCS as positive control, or with the addition of the pleural sample (10 percent v/v or 100 μl of pleural fluid per well) to be tested.

After incubation (48 h at 37°C in 5 percent CO2), the supernatants were removed, and the fibroblasts were trypsinized and counted. Each sample was tested in triplicate, and the results are expressed as fibroblasts counted in percentage of fibroblasts counted after incubation in the presence of FCS.

In some experiments, pleural fluids from patients with adenocarcinoma of the lung were incubated for 60 min with a goat antihuman PDGF polyclonal antibody (Collaborative Research; Bedford, Mass) or with goat serum, as a control, before being tested for the presence of growth-promoting activity.

Quantification of PDGF

For all patients, PDGF was quantified on the pleural fluid extracted at the first thoracentesis. Since PDGF can be present in biologic samples under three different forms (AA, AB, and BB dimers), each dimer was quantified by a specific ELISA.

For determination of PDGF AA dimers, 96-well flat-bottom polystyrene microtiter plates were coated with 200 μl per well of a mouse monoclonal antibody against human PDGF AA, at 5 μg/ml in 100 mM NaHCO3, pH 9.5, at room temperature for 16 to 24 h. Plates were washed three times with water and then blocked with 200 mM TRIS/HCl, pH 7.5, 1 percent bovine serum albumin, and 0.025 percent thimerosal for 24 h at room temperature. Following removal of the blocking buffer, samples and human PDGF AA as a standard were titrated in doubling dilutions in a final volume of 200 μl per well in 250 mM phosphate buffer, pH 8.0, containing 0.5 percent bovine serum albumin. To each well, 50 μl of the mouse mAb conjugated with horseradish peroxidase (Boehringer, Mannheim, Germany) was added and incubated for 16 to 24 h at room temperature, followed by washing with water; and peroxidase activity was determined by incubation with substrate buffer (200 μl of 2.5 mM H2O2 with 1 mM 3,3',5,5'-tetramethylbenzidine in 30 mM citrate buffer, pH 4.1) for 10 min before the reaction was stopped with 100 μl of 1 M H2SO4. Extinction was measured at 450 nm.

For determination of PDGF AB dimers, microtiter plates were coated with a mouse monoclonal antibody against human PDGF BB, and as a second antibody, peroxidase-conjugated anti-PDGF AA was used.

For determination of PDGF BB dimers, first and second antibodies were anti-PDGF BB mouse monoclonal antibodies.

The results are expressed as nanograms of each dimer present in 1 ml of pleural fluid, and total PDGF corresponds to the total amount of PDGF (AA + AB + BB dimers) present in 1 ml of pleural fluid.

Statistical Analysis

All data are presented as the mean ± SEM. All statistical comparisons were made using a two-tailed Student's t-test.

RESULTS

As an initial step, pleural fluids were tested for the
presence of a growth-promoting activity for human lung fibroblasts (Fig 1), demonstrating that pleural effusions from patients with adenocarcinoma of the lung were characterized by a growth-promoting activity (mitotic index, 125 ± 34 percent) higher than in benign effusions (mitotic index 82 ± 33 percent; p<0.02 vs adenocarcinoma) and in effusions complicating SCLC (83 ± 17 percent; p<0.05 vs adenocarcinoma, and p>0.3 vs benign effusions). Furthermore, when pleural effusions from patients with adenocarcinoma of the lung were tested after incubation with an anti-PDGF antibody, the growth-promoting activity was reduced to 64 ± 13 percent, suggesting the importance of the local presence of PDGF in the growth-promoting activity observed.

Interestingly, when pleural effusions were evaluated for the presence of PDGF (Fig 2), in a similar fashion to growth-promoting activity, adenocarcinoma effusions were characterized by the presence of elevated levels of PDGF (4.2 ± 1.3 ng/ml) in comparison to benign effusions (1.0 ± 0.3 ng/ml; p<0.05 vs adenocarcinoma) and to SCLC effusions (0.1 ± 0.1 ng/ml; p<0.05 vs adenocarcinoma and vs benign effusions).

Finally, in respect to PDGF dimers (Table 1), it was shown that (1) while no significant difference was found among PDGF BB dimer low levels in all types of effusions, (2) AB dimer levels were significantly higher in adenocarcinoma than in benign effusions and SCLC, and (3) AA dimer levels were higher, but not significantly, in adenocarcinoma than in benign effusions (p<0.09) and SCLC (p<0.08).

**DISCUSSION**

The observation of the presence of high levels of PDGF in pleural fluid obtained from patients with adenocarcinoma of the lung is of interest in regard to PDGF biologic activities and to the pathogenesis of tumor stroma.

Platelet-derived growth factor (PDGF) is a glycoprotein composed of two chains, A and B, sharing 66 percent homology; PDGF can be present under three forms: AA, AB, and BB dimers. Platelet-derived growth factor is a potent chemotactic and growth factor for mesenchymal cells and is biologically active only as a dimer. This biologic activity is modulated by the presence of two types of PDGF receptor subunits on target cells: the α-subunit binds PDGF A-chain, and the β-subunit binds only PDGF B-chain. As a result, the effects of PDGF are modulated by the differential and specific expression of both types of PDGF receptor subunits by each target cell.

The fibrotic component of tumor stroma results from the local accumulation of mesenchymal cells (fibroblasts, smooth muscle cells, and myofibroblasts) and their extracellular matrix, in parallel with the development of neovessels and an inflammatory cell accumulation. While tumor stroma is likely to modulate the prognosis of other cancers, its place in lung cancer is not yet definitively understood. Mesenchymal cell accumulation can be due to their recruitment by migration or to their local replication induced by the local presence of chemotactic or growth factors for these cells. In this respect, PDGF genes have been shown to be expressed by some non-SCLC cell lines and not by SCLC cell lines, and furthermore, only lung cancer cells expressing PDGF genes induced tumor stroma formation when injected into nude mice.

Malignant pleural effusion, a fluid characterized by the presence of malignant cells and from which several cancer cell lines have been established, corresponds to the tumor direct environment. High levels of PDGF observed in adenocarcinoma, a cancer with important tumor stroma, are relevant, since the PDGF levels observed are sufficient to induce, at least in vitro, mesenchymal cell migration and replication. Interestingly, high levels of growth-promoting activity for fibroblasts were also observed in pleural effusions of patients with adenocarcinoma of the lungs, and a major part of this biologic activity was suppressed by incubation with a specific antibody, suggesting that local PDGF plays an important role in the growth-promoting activity measured.

Platelet-derived growth factor is known to be produced by platelets, blood monocytes, alveolar macrophages, endothelial cells, some mesenchymal cells, and several human tumor cell lines, including adenocarcinoma of the lung. Thus, due to the difficulties in establishing a direct proof of it, the production of pleural PDGF mainly by cancer cells can only be stated as possible. Furthermore, malignant effusions being frequently hemorrhagic, platelet contamination could be a source of growth-promoting activity; however, the differences observed between adenocarcinoma and SCLC effusions suggest a limited place of platelets in the release of growth-promoting activity. In respect to PDGF dimers, establishing the specific biologic activity of each dimer in a clinical setting is complex and depends upon the expression of α-subunit and β-subunit of PDGF receptors by target cells. Thus, the evaluation of lung

**Table 1—Concentrations of PDGF Dimers in Pleural Effusions**

<table>
<thead>
<tr>
<th>Group</th>
<th>AA Dimers</th>
<th>AB Dimers</th>
<th>BB Dimers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign effusions</td>
<td>0.6 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Adenocarcinomas</td>
<td>2.5 ± 1.8</td>
<td>1.1 ± 0.4</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>SCLC</td>
<td>0.1 ± 0.1</td>
<td>0.2</td>
<td>0</td>
</tr>
</tbody>
</table>

*p<0.05.
†p<0.05.
mesenchymal cell expression of the genes coding for PDGF receptor subunits would be necessary to definitively ascertain PDGF's role in tumor stroma formation.

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