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Malignant epithelial cells in primary human lung carcinomas coexpress *in vivo* platelet-derived growth factor (PDGF) and PDGF receptor mRNAs and their protein products

(lung cancer/gene expression/epithelium/chronic injury)

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ABSTRACT **Lung cancer represents one of the major human carcinomas with the highest degree of mortality. Epidemiologic studies have linked this disease to "chronic injury," largely induced by cigarette smoking. In the present studies, we demonstrate the *in vivo* expression of platelet-derived growth factor (PDGF) and PDGF receptor (PDGF-R) β mRNAs and their respective protein products in malignant epithelial cells of primary human lung carcinomas. In contrast, nonmalignant epithelial cells in control, normal lung tissue specimen did not express PDGF and PDGF-R mRNAs and did not produce their respective protein products. Epithelial cells in lung specimen from patients with idiopathic pulmonary fibrosis expressed only PDGF mRNA but not PDGF-R β mRNA. These findings of the inappropriate coexpression of a potent mitogen, PDGF, and its receptor in lung cancer epithelial cells suggest the presence of a powerful *in vivo* mechanism contributing to the self-stimulation and unregulated growth of lung cancer tumor cells.**

Lung cancer is the leading cause of cancer death. It affects >150,000 men and women in the United States, and most of these patients die within 1 year. The molecular events that underlie this disease are not fully understood. Amplification and point mutation of certain protooncogenes have been demonstrated in lung cancer cells. Amplification of the *MYC* protooncogene family has been shown to occur primarily in small cell lung cancer (1–3), and point mutations of the *RAS* protooncogene family have been reported in all types of lung cancer (4–7). Histologically, there are four major cell types of primary lung carcinomas as classified by the World Health Organization: the squamous or epidermoid carcinoma, small cell undifferentiated carcinoma, adenocarcinoma, and large cell anaplastic carcinoma. They arise from bronchial epithelial cells, which progress into cancer cells and carcinomas *in situ* (8).

Chronic injury induced by cigarette smoking is considered to be a leading cause of lung cancer (9, 10). However, there is no information on the nature of the mechanisms linking "chronic injury" to cancer. Recent *in vivo* studies provided a direct link between injury and activation of certain cellular protooncogenes and receptor genes. These studies have shown that acute cutaneous injury in swine induced *in vivo* expression of the c-sis/platelet-derived growth factor (PDGF)-2 and PDGF receptor (PDGF-R) β mRNAs and their respective protein products in the skin epithelial cells of the injured tissue (11). These findings were unexpected since epithelial cells do not normally express PDGF mitogen and PDGF-Rs. The expression levels in the epithelial cells of the injured site were correlated with the stage of tissue repair, being higher during the initial stages of the repair process and suppressed at the time of complete reepithelialization. This controlled, reversible coexpression of a potent mitogen and its receptor in the epithelial cells, induced by injury, appears to serve a physiologic function that contributes to normal tissue repair. In contrast, chronic injury may lead to irreversible induction of gene expression causing pathologic unregulated growth. For example, recent studies have shown the inappropriate expression of the c-*sis*/PDGF-2 protooncogene and its mitogenic protein product in pulmonary epithelial cells of human patients with idiopathic pulmonary fibrosis (IPF) (12). There was no detectable expression of c-sis mRNA in the lung epithelium of individuals without IPF. Injury is considered to be a leading cause of IPF. The inappropriate expression of PDGF by the lung epithelial cells of IPF patients seems to contribute to the excessive proliferation of lung fibroblasts and the abnormal production of collagens by the fibroblasts, events associated with IPF. We have extended these studies to include investigations on *in vivo* expression of PDGF and PDGF-R genes in primary human lung carcinomas. As reported here, the malignant epithelial cells of patients with primary lung cancer express *in vivo* both the c-sis/PDGF-2 mRNA and the PDGF-R β mRNA along with their respective protein products. Nonmalignant lung epithelial cells do not express c-sis/PDGF-2 mRNA and the PDGF-R β mRNA, while lung epithelial cells of patients with IPF express only c-sis/PDGF-2 mRNA but not the PDGF-R β mRNA.

MATERIALS AND METHODS

Tissue Specimens. Pulmonary specimens from seven patients with primary lung carcinomas were collected preoperatively and were immediately snap-frozen before being stored in a −80°C freezer. The specimens were obtained from patients with primary squamous, small cell, and large cell anaplastic carcinomas, as well as adenocarcinomas. All of these patients had a prior history of smoking. Lung specimens from four patients with IPF were collected by open lung biopsy. Control lung specimens were obtained from two victims of accidental death and from two patients undergoing lobectomy or wedge resection for removal of a primary lung tumor. The control lung specimens used in these studies were free of pathologic evidence of malignancy.

***In Situ* Hybridization.** Frozen tissues were embedded in OCT compound (Baxter) for cryostat serial sectioning (8 μm) and *in situ* hybridization with ^{35}S-labeled complementary antisense RNA (cRNA) probes as described (11). The spec-

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Abbreviations: PDGF, platelet-derived growth factor; PDGF-R; PDGF receptor; IPF, idiopathic pulmonary fibrosis.

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ificity of the cRNA probes was determined by hybridization of serial sections with noncomplementary sense RNA probes. Triplicate sections from each tissue were hybridized with either complementary antisense or noncomplementary sense probes and were developed at weekly intervals over a period of 3 weeks. In the examples described below, the hybridized tissues were developed at 2 weeks and were counterstained with eosin and hematoxylin. The probes used in these studies were the human c-*sis* cDNA (13) and the mouse PDGF-R β cDNA (14). PDGF-R β has a binding specificity for the c-sis/PDGF-2 homodimer (15, 16).

Detection of PDGF-Like and PDGF-R-Like Proteins. For identification of PDGF-like proteins, the tissues were stained with anti-PDGF antibody specific for the c-sis/PDGF-2 homodimer (Institute of Molecular Biology, Boston). For detection of PDGF-R-like protein, the tissues were stained with antiserum to a synthetic peptide corresponding to the kinase polypeptide region 958–980 of the sequence reported by Yarden *et al.* (14). This region has no homology to other known receptors. The specificity of the reaction with the antiserum was tested by parallel control studies based on preincubation of the PDGF antibody with excess (50 ng) purified recombinant c-sis/PDGF-2 homodimer and preincubation of the PDGF-R antiserum with excess (100 ng) synthetic peptide.

RESULTS

Fig. 1 demonstrates the strong expression of c-sis/PDGF-2 mRNA and PDGF-R β mRNA in the tumor cells of lung adenocarcinoma tissue sections obtained from two patients. Fig. 1 *A* and *C* shows the expression of c-sis/PDGF-2 mRNA, and Fig. 1 *B* and *D* shows the expression of PDGF-R β mRNA in the lung sections of the two patients. The examples in Fig. 1 are representative of those obtained in tissue sections of the seven individual lung carcinoma specimens examined. The specificity of the hybridization studies shown in Fig. 1 was controlled by parallel hybridization with noncomplementary sense RNA probes for c-sis and PDGF-R β. There was no significant expression of c-sis mRNA or PDGF-R β mRNA using their respective control noncomplementary, sense RNA probes. There is no evidence from these studies for rearrangement of the c-*sis* and PDGF-R β loci.

The expression of c-sis/PDGF-2 mRNA and PDGF-R β mRNA in tumor cells of the lung carcinoma specimens shown in Fig. 1 was accompanied by expression of their respective protein products. Immunostaining for PDGF-like proteins (Fig. 2*A*) and PDGF-R β-like proteins (Fig. 2*B*) produced strong staining of the respective proteins. The specificity of the immunostaining reaction was controlled by staining with PDGF antisera preincubated with excess PDGF-2 homodimer (50 ng) (Fig. 2*C*) and with PDGF-R β antiserum preincubated with excess synthetic receptor peptide (100 ng) (Fig. 2*D*). These control studies produced negative results. Fig. 2 indicates that ≈50% of the cells in the malignant tissue sections were stained with PDGF (Fig. 2*A*) and PDGF-R (Fig. 2*B*) antibodies. This is in contrast to the uniform mRNA expression seen across these sections by *in situ* hybridization (Fig. 1). This may be due to reduced sensitivity of the immunostaining process, compared to *in situ* hybridization, or to the fact that only part of the population of the malignant epithelial cells expresses the protein products at a given time.

In contrast to the results described in Figs. 1 and 2, epithelial cells in control nonmalignant lung specimens did not express c-sis/PDGF-2 and PDGF-R β mRNAs. These results are shown in Fig. 3, demonstrating the lack of significant expression of c-sis/PDGF-2 mRNA (Fig. 3*A*) and PDGF-R β mRNA (Fig. 3*B*) in the epithelium of the nonmalignant, control lung tissues. Expression of PDGF-R β mRNA can be seen only in connective tissue cells (Fig. 3*B*). This is consistent with previous reports demonstrating the presence of cell-surface receptors in connective tissue cells

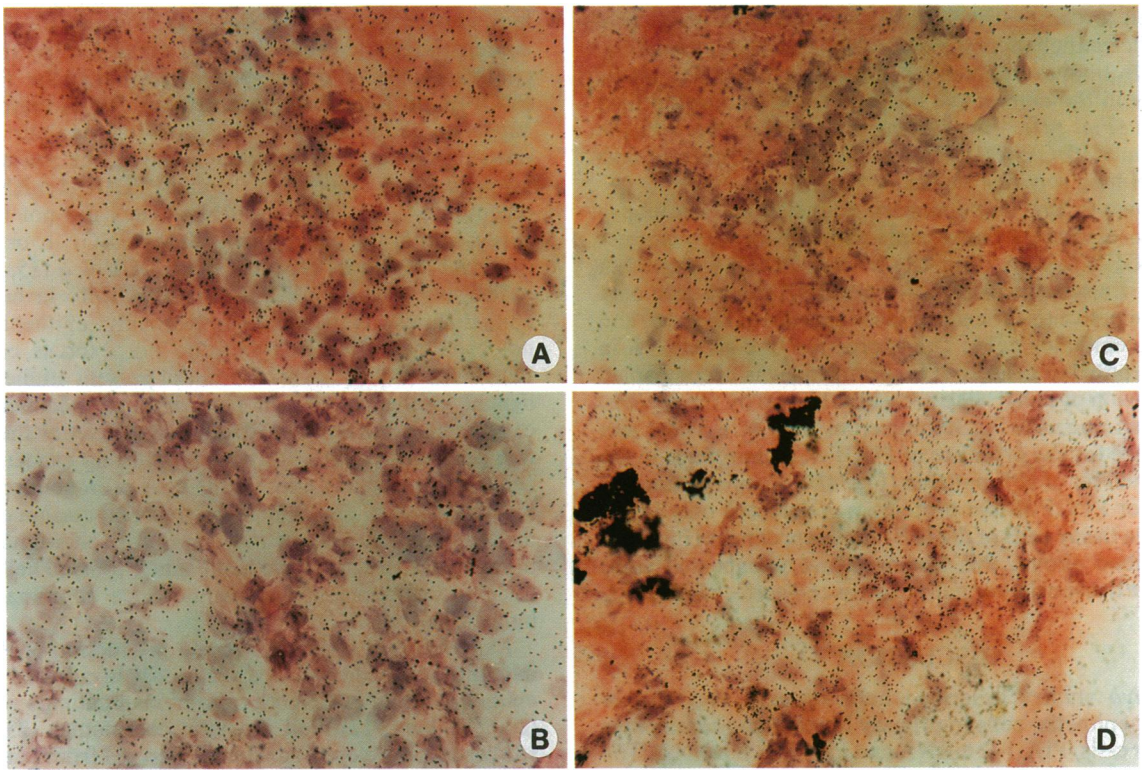

FIG. 1. *In situ* hybridization shows a strong expression of c-sis/PDGF-2 mRNA and PDGF-R β mRNA in tumor cells of two primary human lung adenocarcinomas. Strong expression of c-sis/PDGF-2 mRNA in the two tumor sections is shown in *A* and *C*. Expression of their respective PDGF-R β mRNA is shown in *B* and *D*. (×390.)

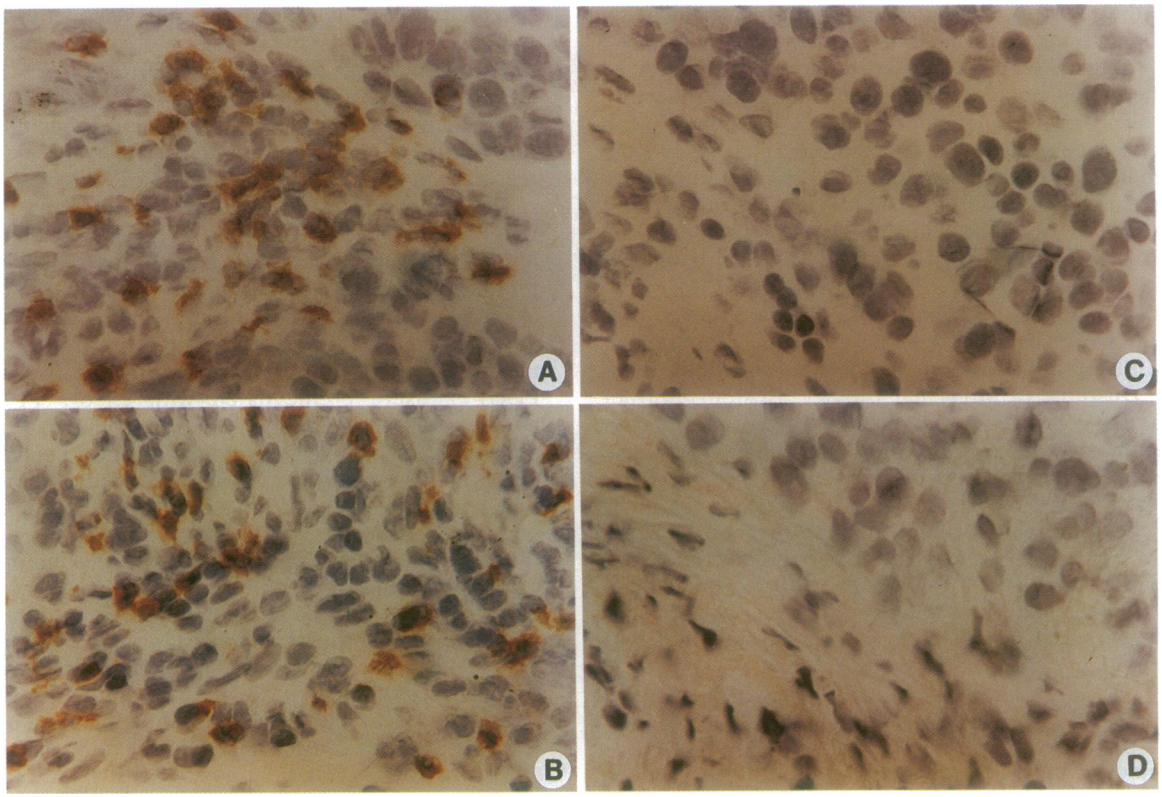

FIG. 2. Expression of PDGF-like and PDGF-R-like proteins in lung tissue sections derived from patients with primary lung adenocarcinoma. (*A*) Expression of PDGF-like proteins immunostained with specific PDGF antibody. (*B*) Expression of PDGF-R-like proteins immunostained with specific PDGF-R antiserum. (*C*) Control immunostaining for PDGF-like proteins in the presence of excess c-sis/PDGF-2 homodimer (50 ng). (*D*) Control immunostaining for PDGF-R-like proteins in the presence of excess synthetic receptor polypeptide (100 ng). (×390.)

such as fibroblasts and arterial smooth muscle cells (17–19). Immunostaining for PDGF-like and PDGF-R-like proteins also failed to demonstrate significant staining of these proteins in the epithelium of the control lung tissue sections (Fig. 3 *C* and *D*). Immunostaining for PDGF-R-like proteins can be seen only in the connective tissue cells of the control lung specimen (Fig. 3*D*). This finding is in agreement with the expression of PDGF-R β mRNA in the connective tissue cells of control lung sections shown in Fig. 3*B*.

As shown in Fig. 4, pulmonary epithelial cells in biopsy specimens obtained from patients with IPF strongly express c-sis/PDGF-2 mRNA (Fig. 4*A*) but not PDGF-R β mRNA (Fig. 4*B*). Expression of PDGF-R β mRNA can be seen only in the connective tissue cells of these specimens (Fig. 4*B*) and not in the epithelial cells. The expression of c-sis mRNA in the lung epithelial cells of IPF specimens was accompanied by expression of PDGF-like protein as shown by the positive immunostaining with PDGF antiserum (Fig. 4*C*). There was no evidence for the presence of PDGF-R-like proteins in the lung epithelium of IPF patients (Fig. 4*D*). Positive immunostaining for PDGF-R-like proteins was detected only in the connective tissue cells of the IPF biopsy specimens (Fig. 4*D*). As described above, this finding is not unexpected since connective tissue cells normally express cell-surface PDGF-Rs.

DISCUSSION

The *in vivo* studies described here strongly suggest that malignant epithelial cells in primary human lung carcinomas coexpress c-sis/PDGF-2 mRNA and PDGF-R β mRNA. The c-sis mRNA encodes a potent mitogen, the c-sis/PDGF-2 homodimer (17). The PDGF-R β mRNA encodes the PDGF-R β, which serves as a receptor for the c-sis/PDGF-2 mitogen. Immunostaining studies with PDGF antiserum specific for the c-sis/PDGF-2 homodimer demonstrated expression of PDGF-like proteins in the malignant epithelial cells of lung cancer specimens. Similarly, antisera to PDGF-R β demonstrated the presence of PDGF-R-like proteins in the epithelial cells of these specimens. Thus, the PDGF and PDGF-R mRNAs expressed in lung cancer epithelial cells appear to be functional mRNAs expressing their respective protein products. As mentioned above, the present studies investigated expression of the c-sis/PDGF-2 mRNA and its receptor, the PDGF-R β mRNA. They did not investigate expression of the genes encoding the PDGF-1, or PDGF-A, isoform (18) and its receptor, the PDGF-R α (15, 16, 19).

The expression of PDGF and PDGF-Rs in lung cancer epithelial cells is unexpected. Normally, epithelial cells do not produce PDGF and they do not express cell-surface PDGF-Rs. Indeed, as shown in the present studies, nonmalignant epithelial cells in control lung specimen did not express c-sis mRNA and PDGF-R β mRNA or their respective protein products. It appears that lung cancer epithelial cells inappropriately express the c-sis protooncogene and its receptor β gene. This inappropriate coexpression of a potent mitogen and its receptor may serve for self-stimulation and unregulated growth of the malignant epithelial cells contributing to development and maintenance of primary human lung carcinomas.

Expression of c-sis mRNA and production of PDGF-like mitogen have been reported previously in cultured malignant epithelial cell lines derived from human patients with breast (20, 21), lung (22), and prostatic carcinomas (23). *In vivo* studies in lung biopsy specimens derived from patients with IPF demonstrated the strong expression of c-sis mRNA and production of PDGF-like proteins in the pulmonary epithelial cells of the fibrotic specimens (12). The present studies confirmed these earlier findings and also demonstrated that

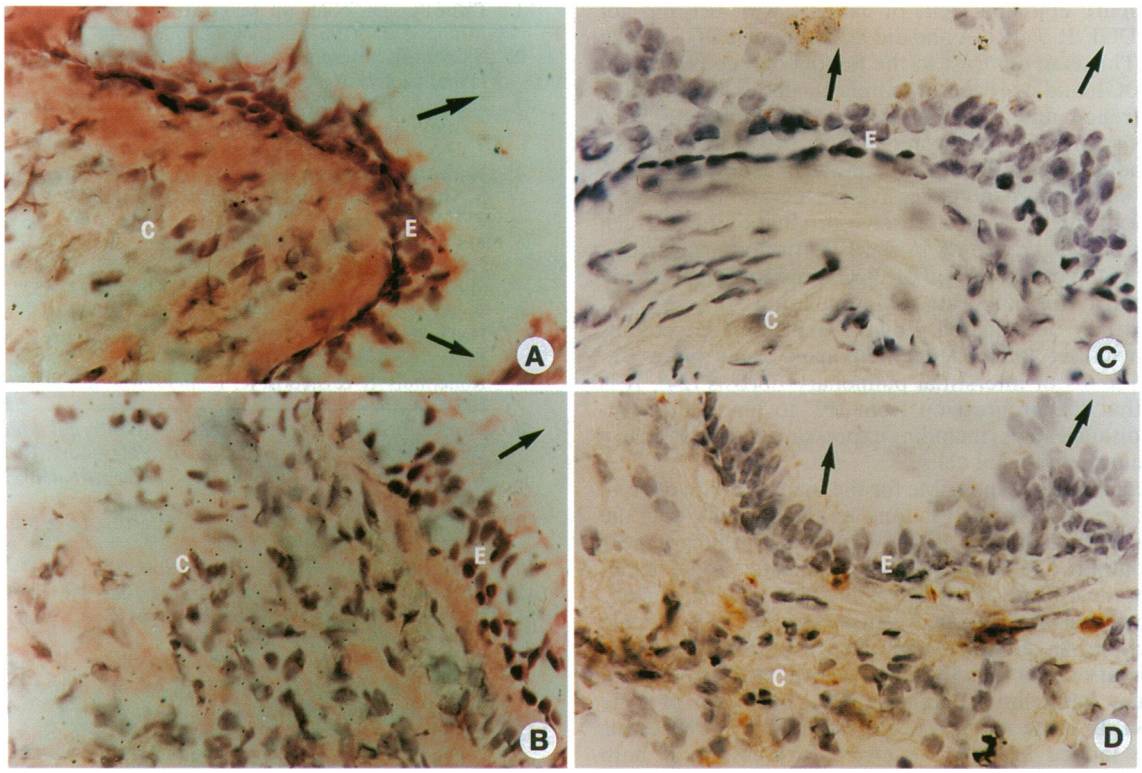

FIG. 3. *In situ* hybridization for c-sis mRNA and PDGF-R β mRNAs (*A* and *B*) and immunostaining for their respective protein products (*C* and *D*) in a control lung specimen obtained from a subject without IPF or cancer. (*A*) Note the lack of expression of c-sis/PDGF-2 mRNA in epithelial cells of control lung tissue. (*B*) Lung epithelial cells of the control specimen do not express PDGF-R β mRNA. PDGF-R β mRNA is localized in the connective tissue cells of the control specimen. (*C*) There is no detectable immunostaining for PDGF-like proteins in the control lung specimen. (*D*) Immunostaining for PDGF-R-like proteins in the control specimen is localized only in connective tissue cells. There is no detectable immunostaining in the epithelium. Bronchial lumen (arrows), epithelium (E), and connective tissue (C) are indicated. (×390.)

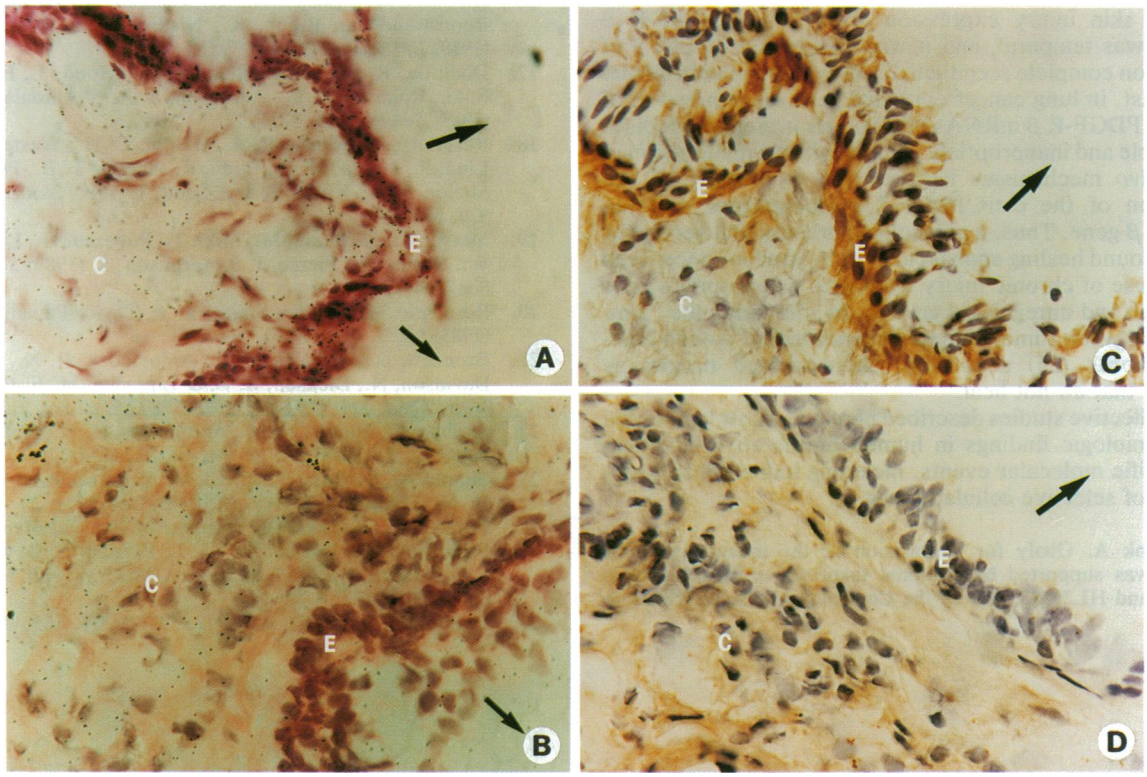

FIG. 4. Expression of c-sis/PDGF-2 and PDGF-R β mRNAs and their protein products in lung specimens from patients with IPF. Note the strong focal expression of c-sis/PDGF-2 mRNA (*A*) and of PDGF-like proteins (*C*) in lung epithelial cells of the IPF specimen. There is no significant expression of PDGF-R β mRNA (*B*) or of its protein product (*D*) in the lung epithelium of these patients. Expression of PDGF-R mRNA and immunostaining for PDGF-R-like proteins is localized only in connective tissue cells of the IPF specimen. Bronchial lumen (arrows), epithelium (E), and connective tissue (C) are indicated. (×390.)

the pulmonary epithelial cells of patients with IPF did not express PDGF-R β mRNA and did not produce PDGF-R-like proteins. This is in contrast to the finding of a coexpression of both PDGF and its receptor in lung cancer epithelial cells. This difference may account for the different pathologic influences seen in IPF and lung cancer. In IPF, the constitutive production of PDGF by epithelial cells may cause paracrine stimulation and excessive proliferation of connective tissue fibroblasts and excessive production of collagen by the fibroblasts, resulting in fibrosis. In lung cancer epithelial cells, the coexpression of PDGF and its receptor may serve primarily for autocrine self-stimulation of these cells, leading to their unregulated growth. Epidemiologic studies have shown that lung adenocarcinomas occur more frequently in areas of interstitial pulmonary fibrosis (24). It is possible that the progression from IPF to lung cancer involves additional steps, including expression of PDGF-R β by the pulmonary epithelial cells.

The mechanisms responsible for the inappropriate expression of c-sis mRNA in IPF lung epithelial cells and the coexpression of c-sis and PDGF-R mRNAs in lung cancer cells are unknown. Chronic injury is implicated in the development of several proliferative disorders including pulmonary fibrosis and lung cancer. Agents associated with chronic injury include tar, nicotine, coal dust, asbestos, beryllium, toxins, dietary factors, and viruses. Recent studies provided a molecular basis linking injury to the induction of gene expression. In these studies, acute skin injury in swine was shown to induce *in vivo* the strong expression of both c-sis/PDGF-2 mRNA and PDGF-R β mRNA in the skin epithelial cells of the injured tissue. Epithelial cells in control, unwounded skin did not express c-sis and PDGF-R β mRNAs (11). The coexpression of c-sis mRNA and PDGF-R β mRNA in wounded skin epithelial cells is similar to coexpression of these mRNAs in the lung cancer epithelial cells shown in the present studies. The important difference is that in the case of acute skin injury expression of c-sis and PDGF-R β mRNAs was temporal, and it was suppressed by day 9 of injury upon complete reepithelialization of the injured tissue. In contrast, in lung cancer epithelial cells the expression of c-sis and PDGF-R β mRNAs and of their protein products is irreversible and inappropriate. This signifies a malfunction in the *in vivo* mechanisms required for suppression of the expression of the c-sis/PDGF-2 protooncogene and the PDGF-R β gene. Thus, a physiologic process that serves for normal wound healing appears to be subverted in cancer cells by an abuse of chronic injury leading to inappropriate gene expression and unregulated growth. In comparing the "similarities between tumor stroma generation and wound healing," Dvorak (25) accurately characterized tumors as "wounds that do not heal."

The collective studies described here may allow translation of epidemiologic findings in human proliferative disorders into specific molecular events, including induction and suppression of selective cellular genes.

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