



Dan Namingha, *Hopi/I'ewa "Red Tailed-Hawk,"* 1986. Acrylic on canvas. Courtesy of the Heard Museum, Phoenix, Arizona.

*Several molecular alterations
may play a role in pancreatic
carcinogenesis.*

Molecular Prognostic Markers in Pancreatic Cancer

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Background: *Pancreatic cancer is one of the most aggressive human tumors and is virtually incurable. Its incidence in the United States has tripled in the past 50 years. The tumor is a frequent cause of cancer death in both men and women. The current treatment options are inadequate and probably reflect the fact that the etiologic factors and the pathogenesis of pancreatic cancer are unknown.*

Methods: *The author reviewed recent studies describing some of the molecular alterations that may play a role in pancreatic carcinogenesis.*

Results: *Most pancreatic tumors arise in the ductal epithelium. Cytogenetic abnormalities and alterations in proliferation, oncogenes and tumor suppressor genes, cell receptors, and growth factors are described.*

Conclusions: *Preliminary studies have implicated, among others, the insulin-like growth factor-1 receptor, Src, and Stat3 proteins in human pancreatic carcinogenesis. These molecules may represent important predictors of tumor behavior and targets of novel therapeutic modalities in human pancreatic cancer.*

Introduction

Pancreatic cancer is the fourth most common cause of cancer death in Western society and is a leading cause of cancer death worldwide. Its incidence and mortality rates are almost identical. The 5-year survival rate is approximately 1%-2%, and the median survival time after diagnosis is 4-6 months. The American Cancer Society estimates that 28,300 new cases of pancreatic cancer and 28,200 pancreatic cancer deaths will occur in 2000 in the United States.¹ These observations attest to the inefficacy of current treatment modalities for this form of human cancer and our lim-

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ited knowledge of the pathogenesis of pancreatic cancer. This article focuses on the molecular alterations identified to date in pancreatic carcinoma and their prognostic significance.

Clinico-Pathologic Characteristics

Histologically, the pancreatic parenchyma is divided in two components: the exocrine portion, which is composed of ducts and acini, and the endocrine component, which is composed of hormone-secreting cells arranged in islets (islets of Langerhans). Pancreatic cancer usually arises in the exocrine component of the gland, and almost all of these tumors exhibit ductal differentiation. However, the line of differentiation in a pancreatic tumor does not necessarily identify the "cell of origin" or histogenesis of that tumor. Recent data indicate that pancreatic cancer may originate not only from pancreatic ductal/ductular cells, but also from within the islets of Langerhans, probably from reserve cells (precursor, stem cells).² Tumors arising in the epithelium lining the pancreatic duct represent 85% of all pancreatic tumors, with the acinar cell tumors comprising less than 1% of them.³ Tumors arising from the islets of Langerhans are called islet cell tumors and comprise 1%-2% of all pancreatic cancers.⁴

Pancreatic cancer is most common in blacks, in men, and in patients with either diabetes mellitus or hereditary chronic pancreatitis. Most of these tumors occur after 60 years of age, and they involve the head of the pancreas.³ The incidence of pancreatic cancer has increased threefold in the last 50 years, especially in women.¹ This increase is probably related to changes in diet (high-fat diet associated with development of pancreatic carcinoma) or exposure to cigarette smoking and chemical carcinogens. Since this type of cancer grows rapidly and lacks symptoms, it is usually widespread and unresectable when diagnosed.⁴

Cytogenetic Abnormalities

Cytogenetic analysis of pancreatic carcinomas have identified alterations in the form of gene rearrangement or losses in chromosomes 1p, 3p, 6q, 8p, 12p, and 16q. Losses of chromosomes 17 and 18, which carry the p53 and DCC genes, are also common.⁵ Using fluorescent in situ hybridization on 10 pancreatic cancers, Adsay et al⁶ identified the frequent loss of chromosome 20, alterations of chromosome 8, and amplification of *c-myc* oncogene. To date, no diagnostic (specific) chromosomal changes have been identified for pancreatic carcinoma. Chung et al⁷ reported the allelic loss of a locus at chromosome

3p25, which may contain a novel pancreatic endocrine tumor suppressor gene. This may represent a molecular marker of prognosis.

DNA Ploidy and Cell Proliferation

Studies using image cytometry and/or flow cytometry have shown that a nondiploid or aneuploid DNA content is usually associated with advanced tumor stage and shorter survival.⁸ Ohta et al⁹ observed that patients with pancreatic cancers expressing a low AgNORs (argyrophilic nucleolar organizer regions) count per tumor cell (less than 3.25) had a better prognosis than those with a high AgNORs count per tumor cell. Pancreatic tumor cells also express high proliferating cell nuclear antigen (PCNA) compared with chronic pancreatitis tissues, a finding that may be useful in supporting the diagnosis of malignancy when only a small biopsy specimen is available for pathologic interpretation.¹⁰ Similarly, high Ki-67 stain, a marker of proliferating tumor cells, correlated with liver metastases and short survival.¹¹

Oncogenes and Tumor Suppressor Genes

Mutations with or without overexpression of p53 have been detected in 37% to 63% of human pancreatic carcinomas and have been associated with poor prognosis.¹²⁻¹⁷ Mutations of p53 in pancreatic cells may be caused by smoking, which explains the predisposing role of tobacco in pancreatic cancer.¹⁸ However, this association has not been confirmed. It is thought that wild-type p53 has the capability of inducing p21WAF1, a cyclin-dependent kinase inhibitor able to arrest cell proliferation.¹⁹ A mutated p53 would be unable to provide this function. We and others observed a lack of correlation between p53 alterations and p21WAF1 expression in human pancreatic carcinomas,^{19,20} a finding that is consistent with the reported TGF- β 1 induction of p21 WAF1 through a p53-independent mechanism.^{21,22}

Mutations of the *K-ras* oncogene have also been identified in approximately 80% of pancreatic cancers.²³ It seems that patients with *K-ras*-negative tumors have improved survival after radiation therapy compared with patients with *K-ras*-positive tumors.²⁴ Similarly, patients with tumors carrying a mutated p53 have shorter survival after radiation and/or chemotherapy compared with patients with wild-type p53.²⁴ This observation probably reflects the fact that tumors containing a mutated p53 are usually radioresistant and/or chemoresistant.

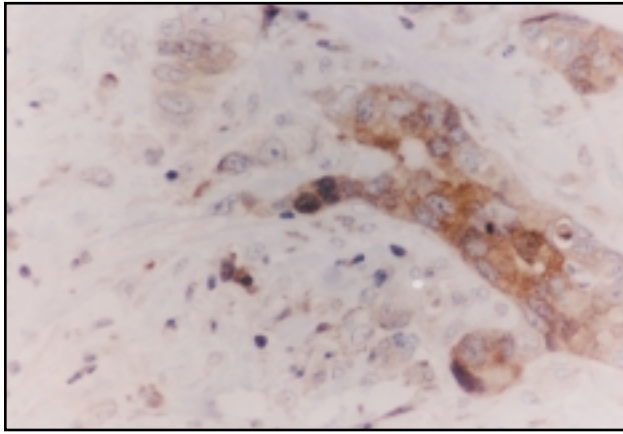


Fig 1. — Pancreatic tumor overexpressing c-Src protein. Immunohistochemistry was carried out using an anti-c-Src mouse monoclonal antibody. The stain has the expected cellular localization (Immunostain, original magnification $\times 400$).

More recently, we and others observed the overexpression and activation of tyrosine kinase Src in human pancreatic ductal adenocarcinoma.^{25,26} Src is a cytoplasmic membrane-associated protein tyrosine kinase involved in the regulation of cell growth and differentiation and cell adhesion.²⁷ The activation of Src appears to induce the insulin-like growth factor-1 (IGF-1)-dependent proliferation of pancreatic tumor cells by increasing the number of IGF-1 receptors per tumor cell.²⁸ In preliminary studies using immunohistochemical techniques, we observed strong, diffuse cytoplasmic c-Src staining in 33 (70%) of 47 human pancreatic tumors (Fig 1). In only 5 cases, c-Src was either negative or weak and focal. These results were mirrored by strong and diffuse membranous IGF-1R staining in 30 (64%) of the 47 tumors. Normal pancreatic tissue, when present, was negative for both stains. Areas of chronic pancreatitis usually revealed weak to moderate c-Src stain.²⁵ These data support the role of c-Src and IGF-1R in human pancreatic carcinogenesis. It seems that constitutive activation of Stat3 may participate to the oncogenic transformation mediated by activated c-Src kinases.²⁹

In the case of multiple myeloma, constitutive Stat3 activation induces the transcription of the antiapoptotic regulatory protein Bcl-x_L, thus preventing programmed cell death.³⁰ Bcl-x_L expression has been described in human pan-

creatic ductal carcinoma³¹ and could reflect the possible role of STAT signaling in pancreatic ductal carcinoma (Fig 2). At our institute, we are in the process of analyzing the expression of activated Stat3 in human pancreatic carcinomas overexpressing Src and Bcl-x_L proteins compared with tumors negative for these proteins and with normal pancreatic tissues. If significant levels of STAT activation are identified in a subset of human pancreatic cancers, it may represent a possible mechanism against which future therapy may be directed.

Growth Factors and Cell Receptors

Human pancreatic cells express a variety of growth factor receptors and their ligands, suggesting that these may be important to the pancreatic tumor cells for achieving selective growth advantage. For example, it has been shown that pancreatic cell lines produce large amounts of TGF- α and - β , IGF-1, and the beta chain of platelet-derived growth factor. The epidermal growth factor receptor is expressed in normal pancreatic cells, but it is overexpressed in 30%-50% of pancreatic tumors and plays an important role in tumor growth.³² In fact, peptide hormone analogs have recently been shown to induce growth inhibition of pancreatic cancer cells by decreasing the number of epidermal growth factor receptors on the tumor cells.³³ The *c-erbB2* protooncogene and IGF-1 receptor are also overexpressed by pancreatic cancer cells.^{34,35} In vitro studies support the hypothesis that IGF-1 may be involved in the

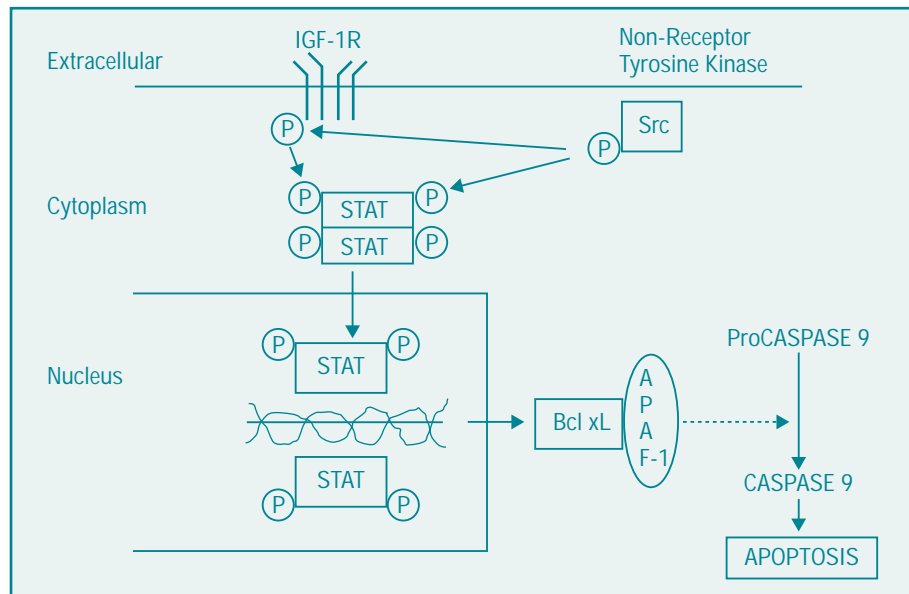


Fig 2. — The IGF-1R/Src/STAT pathway. Src and/or IGF-1R phosphorylates activating Stat3, inducing its dimerization and translocation to the nucleus. It has been shown that Stat3 may upregulate the expression of Bcl-x_L. This protein is critical in sequestering the protease-activating factor-1 (APAF-1) and inhibiting apoptosis, as the activation of caspase 9 requires its binding to the APAF-1 to complete the apoptotic signaling cascade.

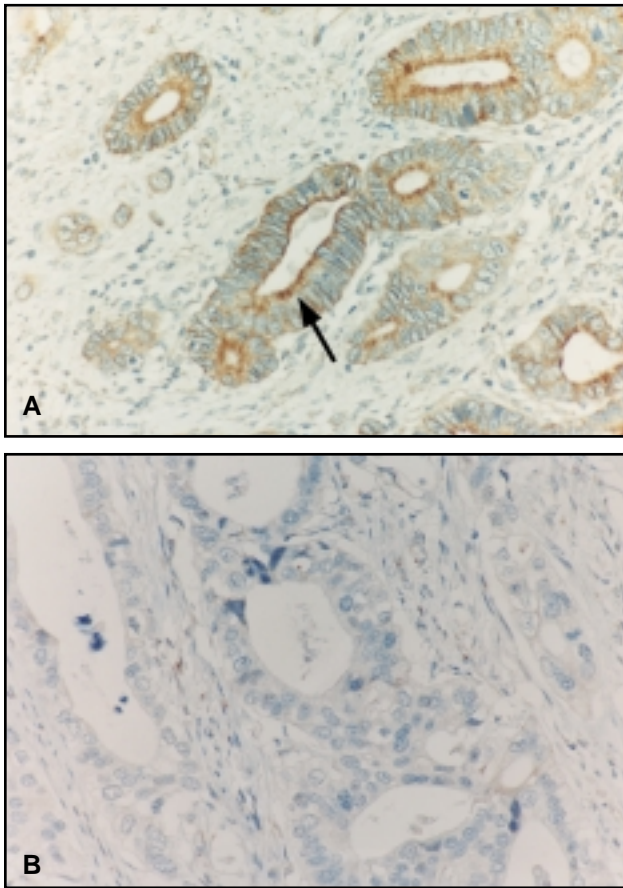


Fig 3. — Pancreatic tumor overexpressing the IGF-1 receptor (IGF-1R). (A) We used an antibody recognizing the beta chain of the IGF-1R. Therefore, the stain has the characteristic submembranous localization (arrow) (Immunostain, original magnification $\times 200$). (B) The same tumor cells are deprived of the transforming growth factor receptor beta type RII (TGF- β -RII). The lack of TGF- β -RII seems to potentiate the tumorigenic effect of the IGF-1R (Immunostain, original magnification $\times 250$).

autocrine and paracrine activation of the IGF-1R during pancreatic carcinogenesis. This hypothesis is based on the fact that pancreatic tumor tissues have a 32-fold increase in IGF-1 mRNA compared with normal human pancreatic tissues.³⁵ It has been shown that the *src* oncogene may contribute to the proliferation of pancreatic tumor cells by increasing the expression of IGF-1R per tumor cell.²⁷ Ohmura et al³⁶ have reported that both IGF-1 and TGF- α stimulate pancreatic cell growth in vitro through a postulated autocrine mechanism. Similarly, Freeman et al³⁷ have shown that the increased tumorigenicity of human pancreatic cells is associated with aberrant regulation of IGF-1 autocrine loop. This effect seems to be potentiated by the loss of response to TGF- β in tumor cells lacking the TGF- β receptor type RII (Fig 3A-B). Transforming growth factors of the beta type (TGF- β 1, TGF- β 2, and TGF- β 3) bind to specific cell receptors, decreasing phosphorylation of targeted proteins involved in cell cycle regulation and inhibiting cell proliferation.³⁸ Baldwin and Korc³⁹ have shown that TGF- β 1 arrests the proliferation of pancreatic adenocarcinoma

cells in vitro. We found that TGF- β 1 was expressed in 31% of 42 human pancreatic adenocarcinomas. The TGF- β 1-positive tumors were usually of low grade and low stage compared with the TGF- β 1-negative tumors. Patients with TGF- β 1-positive tumors had longer survival than those with TGF- β 1-negative tumors.²⁰ In another study, however, Wagner et al⁴⁰ observed that patients with tumors overexpressing the TGF- β 1 receptor type II had decreased survival compared with TGF- β 1 receptor type II-negative tumors. These conflicting results are explained by new findings describing the interaction between TGF- β 1, TGF- β 1 receptor, and cyclin D1. It seems that TGF- β 1 is capable of inhibiting tumor cell growth by interacting with cyclin D1, a protein kinase controlling cell cycle progression, and that the suppression of cyclin D1 is associated with down-regulation of the TGF- β 1 receptor.⁴¹

The researcher's attention has recently been focused on SMAD proteins. These molecules play an important role in the TGF- β signaling pathway.

It seems that TGF- β signals, from the cellular membrane to the nucleus, via activation of the TGF- β receptor, and phosphorylation of TGF- β intracellular mediators Smad2 and Smad3. When phosphorylated, Smad2 and Smad3 complex with Smad4 protein and undergo nuclear translocation. On the other hand, Smad6 and Smad7 can prevent TGF- β signaling by inhibiting either the receptor or Smad2 and Smad3. Jonson et al⁴² have recently shown that alterations in the expression of Smad2, Smad3, Smad6, and Smad7 are rare in pancreatic cancer and that the inactivation of Smad4 (through losses of 15q and 18q genetic material) is of importance in pancreatic carcinogenesis.

Finally, overexpression of vascular endothelial growth factor (VEGF) and its receptors has also been described in pancreatic cancer, which further underlies the importance of vascularization in tumor growth.⁴³

Factors Involved in Tumor/Stromal Interaction

The poor prognosis of pancreatic cancer is dependent on its invasive and metastatic capabilities. Pancreatic ductal adenocarcinoma is especially prone to invasion of the surrounding tissues and to metastasis. It has been reported that the expression of CD44, a transmembrane glycoprotein involved in cell-to-cell and cell-to-matrix interactions, is increased in pancreatic cancer. A variant isoform of CD44 promotes metastatic potential of pancreatic carcinoma cells,⁴⁴ and CD44 variants 6 and 2, only expressed in pancreatic tumor cells, correlate with decreased overall survival.^{45,46}

However, Gansauge et al⁴⁷ found that low serum levels of soluble CD44 variant 6 predict poor prognosis in patients with pancreatic cancer.

Lysosomal cathepsins B, D, and L may promote carcinogenesis and tumor progression. In particular, cathepsin B catalyzes the degradation of laminin, with consequent rupture of the basement membrane and facilitation of tumor invasion and metastasis.⁴⁸ Therefore, the finding that increased serum levels of cathepsin can predict malignant progression in pancreatic cancer is not surprising.⁴⁹ Interestingly, the expression of laminin receptor identifies pancreatic endocrine tumors with a high proliferative index, large size, and metastatic potential, and it usually correlates with poor clinical outcome.⁵⁰

Urokinase plasminogen activator (uPA), a serine proteinase implicated in cancer invasion and metastasis, and its receptor (uPAR) have also been found to be overexpressed in pancreatic cancers, especially in the areas of tumor invasion. It appears that patients with uPA- and uPAR-positive tumors have shorter postoperative survival as compared to patients with uPA- and uPAR-negative tumors.⁵¹

The role of tissue transglutaminase (TG) in pancreatic cancer has also been studied.⁵² TG is a calcium-dependent enzyme that binds to proteins of the extracellular matrix and renders them more stable and resistant to proteolysis. It seems that TG, synthesized by the host endothelial cells and macrophages, is able to inhibit tumor growth.⁵³

Pancreatic Cancer and Apoptosis

The importance of apoptosis (programmed cell death) during fetal development and in adults as a regulator of tissue homeostasis is now evident. It is thought that damaged cells in normal tissues are eliminated by apoptosis, which also provides the balance between cell proliferation and cell death under physiologic conditions.⁵⁴ This view is supported by the observation that transgenic mice overexpressing Bcl-2, an inhibitor of apoptosis, develop spontaneous malignant tumors.⁵⁵

Proapoptotic (Bcl-2, Bcl-x_L, and Mcl-1) and antiapoptotic (Bax, Bcl-x_s) proteins have been detected in pancreatic cancer.⁵⁶ Specifically, either Bax expression or concomitant expression of p53 and Bcl-2 has been found to be strong predictors of longer survival in patients with pancreatic cancer.^{57,58} Conversely, the enhanced expression of Bcl-x_L in pancreatic cancer has been found to be associated with shorter patient sur-

vival.³¹ As previously noted, constitutive Stat3 activation may induce the transcription of the antiapoptotic regulatory protein Bcl-x_L, thus preventing programmed cell death. A similar interaction could explain the limited sensitivity of pancreatic cancer to anticancer treatment.

Pancreatic cancer cells are usually resistant to apoptosis induced by cytotoxic drugs that activate surface receptors such as Fas and tumor necrosis factor (TNF) receptors. It appears that pancreatic cancer cells can evade Fas-mediated immune surveillance in two ways: (1) a nonfunctional Fas receptor may render tumor cells resistant to Fas-mediated apoptosis and (2) the pancreatic tumor cells may express aberrant Fas ligand and allowing them to induce apoptosis in activated Fas-sensitive T cells.⁵⁹ TNF- α -induced apoptosis is limited by its coactivation of nuclear factor-kappa B (NF- κ B)-dependent antiapoptotic genes. McDade et al⁶⁰ recently showed that the treatment of pancreatic cancer cells with sodium salicylate enhances TNF- α -induced apoptosis by inhibiting NF- κ B activation via underphosphorylation of its bound inhibitor protein I κ B- α . Interestingly, Kleeff et al⁶¹ have also shown that actinomycin D induces apoptosis of pancreatic cancer cells (PANC-1) by activating the c-Jun-N-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathway and by increasing the expression of Bax but not Bad or p53.

Cyclooxygenase-2 Expression in Human Pancreatic Cancer

Recent studies have underlined the potential role of cyclooxygenase-2 in human pancreatic carcinogenesis. Cyclooxygenases COX-1 and COX-2 are enzymes necessary for the conversion of arachidonic acid to prostaglandin H₂, a precursor of prostacyclin, thromboxanes, and other prostaglandins.⁶² Surprisingly, it has been noted that COX-2 expression is induced by growth factors, cytokines, and oncogenes and that COX-2 but not COX-1 is overexpressed in a variety of epithelial neoplasms including pancreatic carcinoma.⁶³⁻⁶⁷ It is becoming evident that specific COX-2 inhibitors can prevent carcinogenesis and induce apoptosis of tumor cells.^{68,69} The use of COX-2 inhibitors is being tested as a new form of cancer prevention and therapy.^{70,71}

Conclusions

Our understanding of pancreatic tumor biology depends on our ability to uncover the biochemical/molecular mechanisms underlying the progression from normal to neoplastic pancreas. We recently learned about the role of DPC4 tumor suppressor gene inactivation during the progression from an

intraductal precursor of pancreatic cancer (PanIN [pancreatic intraepithelial neoplasia]) to overt cancer.⁷² Researchers are continuing their search to reveal the molecular steps involved in pancreatic carcinogenesis. Identifying these steps is essential to prevent pancreatic cancer and to design alternative therapeutic approaches for this disease.

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