

Immunohistochemical Expression and Prognostic Significance of Fatty Acid Synthase in Pancreatic Carcinoma

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Abstract. *Background:* The aim of this study was to evaluate immunohistochemical markers in pancreatic cancer and to determine the association of their expression with clinicopathological features and prognosis. *Patients and Methods:* Thirty pancreatic adenocarcinoma patients were followed-up for an average period of 5 years. FAS, bcl-2, p53 and Ki-67 expression were detected immunohistochemically to determine their prognostic value. *Results:* FAS was statistically associated with p53 ($p=0.002$), Ki-67 ($p=0.003$), higher histological grade ($p=0.001$) and recurrence and overall survival ($p=0.001$). *Conclusion:* The newly found overexpression of FAS in highly aggressive pancreatic carcinomas may help us stratify patients into different prognostic groups and indicate new therapeutic strategies.

In the United States there are 28,000 to 30,000 newly diagnosed cases of pancreatic cancer and approximately an equal number of deaths per year from pancreatic cancer (1,2). Surgery, radiotherapy and chemotherapy have improved the life quality and survival rate in only 10% of cancer cases so the search for reliable prognostic markers to apply to a larger group of patients is imperative (3). It is proposed that the best way to reduce mortality is to improve early detection of this deadly cancer (4). The biological characteristics underlying the aggressive behavior of these tumors are not completely understood. Currently, the hypothesis of multistep carcinogenesis that states cumulative genetic mutations occur in the normal pancreas therefore progressing into pancreatic cancer is the most accepted (5). However, a detailed explanation for

each step of carcinogenesis is necessary. Recent studies (6) have demonstrated that neoplastic cells need high levels of energy to fulfil their metabolic functions. Fatty acid synthase (FAS) is a multifunctional enzyme with two identical subunits of 260,000 kDa containing seven catalytic domains and a 4'-phosphopantetheine prosthetic group. FAS catalyzes the synthesis of long-chain fatty acids from acetyl-CoA, NADPH and ATP (7). The main product of FAS is palmitate (80%); the enzyme also produces stearate (10%) and myristate (10%). FAS expression in lipogenic tissues is mainly regulated by sterol regulatory element-binding proteins (SREBPs) at a transcriptional level (8). Long-chain fatty acids are essential constituents of membrane lipids and are important substrates for energy metabolisms of the cells. In adult tissues, high FAS expression is found in cells with active lipid metabolism and/or hormone sensitive cells, such as hepatocytes, adipocytes, type II alveolar lung cells and cells of mammary and endometrial glands during the proliferative phase. FAS has specific physiological functions such as energy storage, production of surfactant in lungs, and lipids in lactating breast tissue (9).

FAS expression in differentiated tissues is regulated by hormones, such as insulin, glucagon, glucocorticoids and thyroxine, and also by nutrients (glucose and fatty acids) (10). FAS levels in most normal tissues are minimal due to their down-regulation by dietary lipids, but the enzyme is highly expressed in many types of human cancer, including breast (11), prostate (12), colon (13), lung (14) and stomach (15), insensitive to circulating lipids and producing high levels of fatty acids. Insensitivity to nutritional signals facilitates disease progression in malnourished patients (16). As FAS has a role in the metabolic activity of normal and pathological tissues, the aim of our study was to evaluate by means of immunohistochemistry if FAS is overexpressed in pancreatic carcinomas and if its overexpression is associated with oncogenes (p53 and bcl-2), proliferative markers (Ki-67), clinicopathological features or prognosis.

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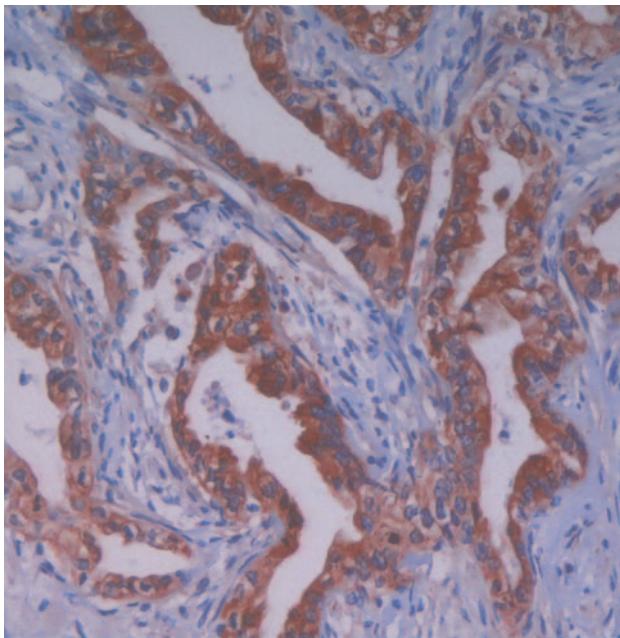


Figure 1. Cytoplasmic overexpression of fatty acid synthase in pancreatic carcinoma. (DAB 40x).

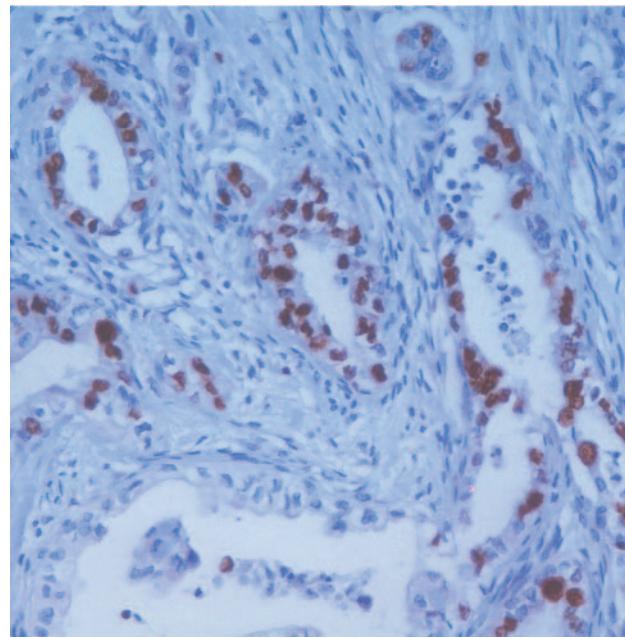


Figure 2. Nuclear expression of Ki-67 in pancreatic carcinoma. (DABx40).

Patients and Methods

We studied 30 patients surgically treated for Stage I pancreatic carcinoma at the Azienda Ospedaliera S. Giovanni Rome, Italy, between January 1998 and January 2001. All patients were followed-up for a minimum of five years. Clinical information was obtained from the medical records. The clinical data collected included the patient's name, race, age, the family and patient's pancreatic cancer history and type of surgery, post-operative treatment, date and site of the eventual recurrence and the patient's current status (alive or deceased). Disease-free months of survival were calculated from the date of diagnosis to the date of the first recurrence. Patients were stratified into two groups. The first group consisted of patients deceased from cancer within four months after surgery. The second group consisted of patients whose cancer recurred or patients who had died of cancer later than four months from surgery. Ethical approval and informed consent from eligible patients were appropriately acquired. Histopathological data included tumor size, histological subtype (17) and grade (18), evidence of necrosis and stage of disease according to TNM classification (19). Control specimens were obtained from 5 patients negative for cancer who underwent surgery for pancreatitis. Representative blocks of the tumor were chosen for immunohistochemical stainings.

Immunohistochemical procedures and evaluation of FAS, p53, bcl-2 and Ki-67 expression. We used 3-μ tissue sections cut from specimens formalin-fixed and paraffin-embedded at the S. Giovanni hospital of Rome, Italy following the guidelines for the use of discarded human tissue. Initially, haematoxylin and eosin-stained sections were prepared from each tissue block. Afterwards,

immunoperoxidase assays were performed using a commercially available DAKO ABC kit (Carpinteria, CA, USA). Sections were incubated with non-immune rabbit serum 1:100 dilution in TRIS buffer saline (pH 7.6 at 37°C) for 30 min and, after intervening washes in TRIS buffer saline, incubated with each primary antibody at concentrations ranging from 50 to 3000 µg/ml for 60 min at 25°C in a moist chamber. To block endogenous peroxidase activity, sections were subsequently incubated with 3% hydrogen peroxidase in methanol for 30 min. After incubation with biotinylated rabbit antimouse antibody for 1 h at 25°C, immunoperoxidase reaction was developed using 3'3-diaminobenzidine (DAB) as chromogen substrate. After extensive washing, nuclear counterstaining was obtained using Meyer's haematoxylin. Anti-p53, bcl-2 and Ki-67 antibodies were obtained from Dako. Fatty acid synthase was a gift from FASgen (FASgen, Inc. UMB, Baltimore, Maryland, USA).

Scoring of immunoreactivity. Slides were considered to overexpress FAS when strong granular cytoplasmic staining was observed at least in 10% of the neoplastic cells at low power (x2). Slides were considered to overexpress p53 and bcl-2 when more than 10% of the neoplastic cells nuclei expressed the two oncogenes. Pancreatic carcinomas were considered highly proliferative when more than 10% of the neoplastic cells nuclei expressed Ki-67.

Statistical analysis. Cut-off end-points were determined according to the positive and negative immunohistochemical expressions observed. FAS, bcl-2, p53 and Ki-67 immunostainings were tested for association with clinicopathological features and prognosis using the Chi-square test or the Fisher's exact test when appropriate. The primary statistical outcomes were disease-free survival and overall survival from the date of surgery. Univariate

and multivariate analysis were conducted using the Cox regression model. All the analysis were conducted using the SYSTAT® software statistic package (SPSS Inc., Chicago, IL, USA).

Results

Histotype. All 30 cases were pancreatic adenocarcinomas.

Histological grade. Five cases were well-differentiated pancreatic carcinomas (16%), 3 cases were moderately-differentiated carcinomas (10%) and 22 cases were poorly-differentiated carcinomas (74%).

Immunohistochemical expression. FAS was overexpressed in 12 patients (40%) (Figure 1), Ki-67 was expressed in 8 cases (26%) (Figure 2), p53 oncogene was expressed in 11 cases (36%). No case overexpressed bcl-2. Eighteen out of 22 poorly-differentiated carcinoma and 1 out of 3 moderately-differentiated carcinoma patients died from cancer within four months from the initial diagnosis. All other patients died later than four months but within two years from the initial diagnosis of carcinoma.

Statistical analysis. Statistical analysis revealed that FAS overexpression was associated with p53 ($p=0.002$) and Ki-67 ($p=0.003$) overexpression with the overall survival ($p=0.001$).

Discussion

Pancreatic cancer is one of the most deadly types of cancer in the world. The mortality over morbidity ratio is 0.99:1. Only 10% patients have cancer cells confined only to the pancreas at the time of diagnosis, 40% have local invasion and 50% have distal metastasis (20). Although surgery, radiotherapy and chemotherapy have improved the quality of life and survival rate in cancer patients, only 10% of patients show significant improvement; most patients die from the cancer 4 to 6 months from diagnosis. Janes et al (21) reported that the 1-year survival rate was less than 20%, decreasing to 7% for 3-year and 3% for 5-year. It is proposed that the best way to reduce the mortality is to improve early detection of this deadly cancer. The 5-year survival rate among pancreatic carcinoma patients is less than 20% despite surgery and/or chemotherapy. This very poor prognosis is mainly due to the propensity of this tumor to invade the adjacent structures and metastasize to distant organs early in the course of disease. Despite intensive efforts to improve therapy for this advanced disease, treatment remains unsatisfactory and most patients die within a few months as a result of rapid local spread of the tumor or metastatic dissemination.

The biological characteristics underlying the aggressive behavior of these tumors are incompletely understood.

Pancreatic carcinomas are heterogeneous diseases; clinicopathological features seem unable to define prognosis, while biological markers evaluated separately have revealed controversial results for therapeutic strategies. In an effort to improve our knowledge on pancreatic carcinoma prognosis and therapeutic approach we determined a group of markers in order to reveal statistical associations between their expression, and clinicopathological features and prognosis.

Most tissues with high cellular turn-over appear to utilize circulating lipids for the synthesis of new structural lipids (21), but hyperplastic as well as neoplastic tissues seem to require alternative sources for energy storage. A minor pathway of metabolic accumulation of energy involves the biosynthesis of fatty acids. In mammalian and birds, the de novo synthesis of fatty acids is consolidated into a single protein that is the product of a single gene. This multifunctional enzyme is FAS. FAS is the key enzyme in fatty acid biosynthesis, involved in the conversion of dietary carbohydrates and fatty acids. FAS synthesizes long-chain fatty acids using acetyl-CoA as a primer, malonyl-CoA as a two-carbon donor and NADPH as reductant of the intermediates (22), and mainly synthesizes palmitate (80%), myristate (10%) and stearate (10%). FAS expression in normal tissues is regulated by several hormonal signals and related to dietary fat intake and metabolism while FAS expression in tumor tissues occurs at very high rates; in fact it has been shown that FAS expression parallels increased malignant potential during neoplastic progression. It seems that FAS overexpression confers a selective growth advantage on neoplastic cells (23).

Our data revealed that FAS was overexpressed in poorly-differentiated pancreatic adenocarcinomas and its overexpression was associated with p53 and Ki-67 overexpression and with the patient's outcome. This is not surprising, since FAS overexpression has been demonstrated in many human carcinomas with aggressive features and poor outcome, such as ovary (24), prostate (25), vulva (26), colon (27), bladder (28), esophagus (29), endometrium carcinomas (30), some pediatric tumors (31), mesotheliomas (32), melanomas (33) and soft tissue sarcomas (34), using conventional immunohistochemistry.

p53 is a tumor suppressor gene coding for a transcription factor present at minute levels in any normal cell. Although the number of genes activated by p53 is rather large, the outcome of p53 activation is either cell arrest in G1, in G2 or apoptosis. The cell growth arrest activity of p53 allows the activation of the DNA repair system of the cell. p53 is mutated in 50%-75% of pancreatic cancer cases, preferentially observed in advanced and aggressive forms (35). Statistical analysis revealed no associations between p53 overexpression and prognosis in pancreatic carcinomas.

The bcl-2 family of proteins are important regulators of apoptosis. Bcl-2 inhibits cell death. The bcl-2 family of

proteins are widely expressed in human cancer cells and are induced in response to diverse survival signals; they are expressed at significant levels in cell lines derived from ovarian, colon and breast carcinomas. In pancreatic carcinomas, bcl-2 overexpression has been shown in 25%-30% of cases (36). We observed that bcl-2 had no association with FAS, p53, Ki-67 expression or with patient's outcome emphasizing its lack of significance, as a marker for this lesion.

The search for new indicators for recurrence and survival in pancreatic carcinoma patients is a challenge. Our study revealed that FAS is a reliable marker of tumor aggressiveness in pancreatic carcinoma. The knowledge of the significance FAS in pancreatic carcinoma could allow stratification of patients into appropriate prognostic groups and suggest new therapeutic approaches.

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