Interferon receptor alpha/beta is associated with improved survival after adjuvant therapy in resected pancreatic cancer

REZA F. SAIDI, STEPHEN G. REMINE & MICHAEL J. JACOBS

Department of Surgery, Providence Hospital and Medical Centers, Southfield, MI, USA

Abstract

Aim. Interferons (IFNs) are known to have antiproliferative and immunoregulatory activities that are modulated through specific cell surface ligands, known as IFN-α, -β, and -γ receptors. The presence of these receptors and their impact on response to adjuvant therapy in patients with pancreatic cancer has not been determined. Patients and methods. Slides were prepared from 46 patients with pancreatic adenocarcinoma. Immunohistochemistry (IHC) was subsequently used to determine the expression of IFN-α/β receptor-chain 2 (IFN-α/βR) and IFN-γ receptor-chain 1 (IFN-γR). The correlation between IFN receptor expression, tumor characteristics, and the overall patient response to adjuvant therapy were determined analytically. Results. The IHC performed for pancreatic adenocarcinoma demonstrated a high IFN-α/βR expression in 4% (2/46) of patients, moderate expression in 20% (9/46) of patients, and faint or no expression in 76% (35/46) of patients. IHC confirmed a high expression of IFN-γR in 52% (24/46) of patients, moderate expression in 35% (16/46) of patients, and faint or no expression in the remaining 13% (6/46) of patients. Thirty-two (69.7%) patients received adjuvant therapy. Clinicopathological survey did not demonstrate any significant correlation between IFN-α/βR and IFN-γR expression with regard to tumor size, vascular invasion, perineural invasion, lymph node metastases, or stage of disease. Use of adjuvant therapy was associated with increased survival in patients with IFN-α/βR-positive tumors compared with patients with IFN-α/βR-negative tumors (24 months versus 14.7 months in log rank test, p = 0.012). The expression of IFN-γR, however, had no impact on patient survival (20 months vs 17 months; p = 0.656, log rank test). Conclusion. IFN-α/βR is associated with improved survival for patients with resectable pancreatic cancer who received adjuvant therapy.

Key Words: pancreatic cancer, interferon receptor, interferon, adjuvant therapy

Introduction

Pancreatic carcinoma accounted for 30 700 new cases and 30 000 deaths in 2002 and represents the fourth leading cause of cancer-related death in the United States [1]. The diagnosis of pancreatic carcinoma carries a dismal prognosis, with a 5-year survival of 3% and median survivals of <6 months [2,3]. Operative intervention alone rarely achieves a curative endpoint [2] and therefore adjuvant therapies are required to facilitate survival and control disease. A desired strategy to improve care is to individualize cancer treatment by directing our patients towards those modalities that are more likely to offer a benefit according to their particular tumor or individual attributes. A cancer biomarker can be defined as a biological feature that provides diagnostic, prognostic, predictive, or therapeutic information about a particular disease or subject. Some fundamental aspects are worth considering when searching for a biomarker. The rapidly expanding knowledge of the pathogenesis of pancreatic cancer at the molecular level is providing new targets for disease characterization, early diagnosis, and drug discovery and development.

Recently, we were able to show that interferons (IFNs) have antitumoral and apoptotic effects on those pancreatic cancer cell lines that specifically express the corresponding IFN receptors [4]. Also, IFNs were found to augment the antitumoral effects of 5-fluorouracil (5-FU) and gemcitabine only in those cell lines that express the IFN receptor [4]. The apoptotic effects of IFNs are mediated through the caspase cascade [4]. We have previously reported that patients with pancreatic cancer who expressed
IFN receptor alpha/beta (IFN-α/βR) had better survival compared with patients who did not express this receptor [5]. Here, we correlate the expression of IFN receptors with survival after adjuvant therapy.

Patients and methods

The study was performed at the authors’ institution and included 46 patients with pancreatic ductal carcinoma who had undergone a curative operative resection from 1990 to 2002. In all patients, the specimens were fixed with formalin, embedded in paraffin, and cut into consecutive 4 μm thick sections. Standard staining techniques with hematoxylin and eosin (H&E) were performed. The slides that contained pancreatic tissue with invasive neoplasm were selected and reviewed. Also, slides that contained normal sections of pancreas were analyzed for IFN receptor and were confirmed to be tumor-free. A senior pathologist reviewed the histopathology blinded to the data collection. Patient data and characteristics of the neoplasm were collected from the tumor registry. None of the patients received IFN-based treatment. Since blood serum was not available, the measurement of serum IFN was not done.

Immunohistochemistry

Immunohistochemical staining was performed by the immunoperoxidase technique. Paraffin sections of the formalin-fixed tissues were dewaxed through five changes of xylene, rinsed in graded ethanol solutions, and finally rehydrated in three changes of phosphate-buffered saline (PBS). Antigen retrieval to enable IFN-α/βR staining was facilitated by microwave treatment to unmask tissue antigens. Endogenous peroxidase was blocked by incubating the sections in 0.3% hydrogen peroxidase in methanol for 30 min at room temperature. After rinsing three times with PBS for 5 min, nonspecific reactions were blocked by incubating the sections with PBS containing 5% normal rabbit or goat serum for 30 min at room temperature. The sections were then incubated with appropriate dilutions of the primary antibody at 4°C overnight.

To facilitate identification of the IFN-γR, a rabbit polyclonal IgG antibody (R&D Systems Inc., Minneapolis, MN, USA) at a 1:200 dilution was used, whereas for the IFN-α/βR, a goat polyclonal IgG antibody (R&D Systems Inc.) at a 1:200 dilution was used. The sections were rinsed three times with PBS for 5 min and incubated for 30 min with appropriate dilutions of the secondary antibody at room temperature. Each of the sections was then incubated for 30 min by the avidin biotin complex (ABC) method in accordance with the manufacturer’s instructions (Dako, Glostrup, Denmark). Additional rinsing with PBS was then performed three times for 5 min and the sections were incubated with diaminobenzidine substrate for 3 min. The sections were rinsed finally with distilled water and counterstained with Mayer’s hematoxylin solution.

Evaluation of immunostaining

The slides were evaluated to determine objectively the intensity of IHC staining using a standardized grading system. The immunoreactivity of the cancer cells for IFN-α/βR and IFN-γR was graded on a scale of 0–2 based on the number of cells stained and the intensity of the reaction within individual cells. The grades were defined as follows: 0 = 0–25% of the cells (weak staining); 1 = 26–50% (weak to moderate staining); 2 = 51–100% (moderate to strong staining). The slides were stained twice and examined randomly by independent, blinded investigators to facilitate accuracy and limit the inter-observer bias. Intra-observer variation was <5% in each of the three investigator grades.

Statistical analysis

The χ² test was used to evaluate the relationships between the expression of IFN receptors and the following clinicopathologic parameters: age, gender, size of neoplasm, tumor grade, vascular invasion, perineural invasion, depth of neoplasm, nodal status, resection margin status, and stage of disease. Kaplan–Meier analysis was used to assess the relationship between IFN receptor expression and overall survival (median).

Results

The IHC performed for pancreatic adenocarcinoma demonstrated high IFN-α/βR expression in 2/46 (4%) of patients, moderate expression in 9/46 (20%) (Figure 1), and faint or no expression in 35/46 (76%). IHC confirmed a high expression of IFN-γR in 24/46 (52%) of patients (Figure 1), moderate expression in 16/46 (35%), and faint or no expression in the remaining 6/46 (13%) of patients (Table I). By comparison, 27% and 45% of the corresponding
noncancerous normal pancreatic tissue in the same patients showed a high expression of IFN-α/βR and IFN-γR, respectively.

There was no correlation between the expression of IFN-α/βR or IFN-γR and the clinicopathologic features. These features included: age, gender, tumor size, vascular invasion, perineural invasion, tumor depth, nodal status, and stage of disease (Table II). Thirty-two patients (70%) received adjuvant therapy which was mainly based on 5-FU.

Use of adjuvant therapy was associated with increased survival in patients with IFN-α/βR-positive tumors compared with patients with IFN-α/βR-negative tumors (24 months vs 14.7 months in log rank test, \( p = 0.012 \)). As shown in Table III and Figure 1, patients who expressed IFN-α/βR and received adjuvant therapy had significantly better survival compared with other groups. As shown in Figure 2, the expression of IFN-γR had no impact on patient survival (20 months vs 17 months; \( p = 0.656 \), log rank test) regardless of whether they received adjuvant therapy or not.

Figure 3 shows the expression of IFN-α/βR and IFN-γR in pancreatic cancer.

### Discussion

In 2005, nearly 34,000 Americans were diagnosed with pancreatic cancer and 32,300 died of the disease [1]. Only a minority of newly diagnosed pancreatic cancer patients are considered eligible for resection. Five-year survival rates in those who undergo an attempted curative procedure are only 8–24% [2,3]. Adjuvant chemoradiation or chemotherapy is usually recommended to eligible patients after the resection procedure. Although not conclusive, recent data results showed a trend toward benefit of adjuvant therapy [2,3]. Opportunities for improvement in

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative or faint</th>
<th>Moderately positive</th>
<th>Strongly positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α/β receptor</td>
<td>76.08% (35/46)</td>
<td>19.56% (9/46)</td>
<td>2.17% (2/46)</td>
</tr>
<tr>
<td>IFN-γ receptor</td>
<td>13.04% (6/46)</td>
<td>34.78% (16/46)</td>
<td>52.12% (24/46)</td>
</tr>
</tbody>
</table>

Table I. Expression of IFN receptors in patients with pancreatic cancer.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IFN-α/β receptor</th>
<th>IFN-γ receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean)</td>
<td>68.8</td>
<td>67.7</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>7/4</td>
<td>14/10</td>
</tr>
<tr>
<td>Tumor extent (pT)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Nodal involvement (pN)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Positive margin</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Grading</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stage</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Adjuvant therapy</td>
<td>7</td>
<td>17</td>
</tr>
</tbody>
</table>

Table II. Relationship between expression of IFN-α/β receptor or IFN-γ receptor and clinicopathologic factors.

NS, not statistically significant.
*p value was calculated by \( \chi^2 \) test or Fisher’s exact test.
Based on AJCC, 6th edn.
outcome will likely come from improvements in early detection strategies, better identification of precursor lesions and high-risk groups, and from ongoing trials designed to identify active agents (chemotherapeutic, immunotherapeutic, and other) and implement their use in appropriate patient groups. Gene and protein expression profiling has advanced our understanding of pancreatic ductal adenocarcinoma, identifying genes that are highly expressed in pancreatic cancers, providing more insight into the clinicopathologic features of pancreatic cancer, and revealing novel features related to the process of tissue invasion by these tumors. The increasing knowledge of the pathway activation profile in pancreatic cancer is yielding new targets but also new markers to select patients and to guide and predict therapy efficacy. The discovery of genetic factors the presence of which predisposes pancreatic cancer to successful targeting, such as the association of BRCA2/Fanconi anemia gene defects and sensitivity to mitomycin C, will eventually lead to a more individualized treatment approach.

Adjuvant IFN therapy may hold promise for patients with pancreatic carcinoma. IFNs are known to bind to receptors on the cell surface that facilitate activation of Janus kinase/signal transduction pathway that result in activation of other transcription pathways [6–9]. The systemic effects in patients with pancreatic cancer have not been clearly elucidated. In fact, numerous phase II trials have been conducted that utilize IFN therapy in combination with other agents, such as 5-FU and leucovorin, to treat patients with metastatic or locally advanced pancreatic cancer [10–17]. However, the results from these early trials were not encouraging. More recently, investigators of a phase II trial that used IFN-α/cisplatin/5-FU-based adjuvant chemoradiation after pancreaticoduodenectomy have reported unsurpassed 2-year survival rates for patients with resected pancreatic cancer [18,19]. Recently, a trial from the Virginia Mason Medical Center showed encouraging survival data with a novel IFN-based chemoradiation regimen [20,21]. In their study, 43 patients underwent pancreaticoduodenectomy for pancreatic cancer. These patients then received external beam radiation at a dose of 4500–5400 cGy (25 fractions 1–21) and three-drug chemotherapy: continuous infusion 5-FU (200 mg/m² daily on days 1–35), weekly intravenous bolus cisplatin (30 mg/m² daily on days 1, 8, 15, 22, and 29), and subcutaneous IFN-α (3 × 106 U on days 1–35). This regimen was followed up by continuous infusion 5-FU (200 mg/m² daily on weeks 9–14 and 17–22). The treatment was generally initiated between 6 and 8 weeks after surgery. The mean follow-up time was 31.9 months, and 67% of the patients were alive at this time, although median survival had not been reached at the time of publication. The actuarial overall survival for the 1-, 2-, and 5-year periods was 95% (95% confidence interval [CI] 91–98%), 64% (95% CI 56–72%), and 55% (95% CI 46–65%), respectively. These results were obtained

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α/βR+/AT+(n=6)</td>
<td>24.4* (16.2–36.8)</td>
</tr>
<tr>
<td>IFN-α/βR+/AT−(n=5)</td>
<td>14.7 (9.2–18.7)</td>
</tr>
<tr>
<td>IFN-α/βR−/AT+(n=26)</td>
<td>16.2 (8.2–22.6)</td>
</tr>
<tr>
<td>IFN-α/βR−/AT−(n=9)</td>
<td>13.6 (7.8–17.5)</td>
</tr>
</tbody>
</table>

CI, confidence interval.

*p < 0.01.
despite a high incidence of lymph node involvement and advanced tumor stage. One major drawback to this regimen, however, was the associated toxicity. In all, 42% of patients were hospitalized during the treatment, mostly because of gastrointestinal toxicity. From this limited patient series, however, the actuarial 2- and 5-year overall survival rates suggest a significant potential for improved long-term survival. Because these results showed a remarkable improvement compared with accepted regimens, the American College of Surgeons carefully audited the study data and reconfirmed their accuracy. The American College of Surgeons Oncology group has since supported a large multicenter, phase II study (Z05031A2) to further evaluate IFN-α-based adjuvant therapy for resectable pancreatic cancer [22].

We determined the presence of IFN receptors in pancreatic cancer and the impact that each type had on patient survival after adjuvant therapy. We found that IFN-γR was expressed in >85% of patients with pancreatic cancer. However, expression of this receptor had no significant impact on patient survival regardless of whether or not adjuvant treatment was given. IFN-α/βR was expressed in only 25% of patients in the pancreatic cancer tissue compared with 27% in non-cancerous pancreatic parenchyma. These patients had a more favorable survival, if they received adjuvant therapy. In fact, the mean survival for patients with IFN-α/βR-positive neoplasm who received adjuvant therapy was 24.4 months. Furthermore, expression of the IFN-α/βR did not correlate with lymphovascular or perineural invasion, lymph node metastases, size, or stage of neoplasm. Therefore, the data would suggest that IFN-α/βR can predict response to adjuvant therapy after pancreatic resection.

Adjuvant therapy may improve long-term survival in patients with resected pancreatic cancer [23–25], but its routine use is not universal [23]. The European Study Group for Pancreatic Cancer (ESPAC) recently published their results for 73 patients with resected pancreatic ductal adenocarcinoma who were treated with chemoradiotherapy alone (20 Gy over a 2-week period plus fluorouracil), 75 patients who received chemotherapy alone (fluorouracil), 72 patients with both chemoradiotherapy and chemotherapy, and 69 patients without treatment (observation only) [25]. The estimated 5-year survival rate was 10% among patients assigned to receive chemoradiotherapy and 20% among patients who did not receive chemoradiotherapy (p = 0.05). The 5-year survival rate was 21% among patients who received chemotherapy and 8% among patients who did not receive chemotherapy (p = 0.009). Based on the results it seems that adjuvant chemotherapy has a significant survival benefit in patients with resected pancreatic cancer, whereas adjuvant chemoradiotherapy has a deleterious effect on survival.

Although the information provided by our research is unique, the study itself has several shortcomings. First, the number of patients in our series was small. Second, it may have been helpful to know the serum IFN levels, which may have explained why the patients with IFN-α/βR expression have better survival. It was not possible, however, to assay these levels secondary to the retrospective collection of patient data. Lastly, the results of our study should prompt further validation, perhaps by other investigators with larger series of patients.

In summary, our study showed that expression of IFN-α/βR was associated with improved survival for patients with resectable pancreatic cancer who received adjuvant therapy. Interestingly, we have determined recently that IFNs enable apoptosis in human pancreatic cancer cell lines that express the IFN receptor [4]. We believe that these data may influence future targeted therapies for those patients with receptor-positive neoplasms. Further clinical trials are necessary to determine the utility of such treatments.

Acknowledgements and disclosures
No disclosures.

References


