Expression of EGF and EGFR Strongly Correlates with Metastasis of Pancreatic Ductal Carcinoma

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Abstract. Background: The epidermal growth factor family members: EGF, EGFR and the c-erbB-2(HER-2/neu) gene product have been found to play a role in carcinomas of the stomach, liver, breast, ovary and lungs. Recent reports have indicated that they are also involved in the growth of pancreatic ductal carcinoma, its invasiveness and metastasis. Patients and Methods: Thirty-six patients with pancreatic ductal carcinoma were analysed with respect to sex, age, histological type, malignancy grade (G), pTN status (pTN), local lymph node involvement and distant metastasis. The tumor levels of EGF, EGFR and c-erbB-2 expression were determined immunohistochemically. Results: Expression of c-erbB-2 was observed in 24/36 cases, EGF in 13/36 cases and EGFR in 18/36 cases. Overexpression of EGF and EGFR was associated with metastasis to lymph nodes and other organs. A correlation was also found between EGF expression and the presence of EGFR in the tumour. The expression of c-erbB-2 protein was not found to correlate with any parameters. Conclusion: EGF and EGFR play a key role in neoplastic spread through lymph node involvement and metastasis to other organs.

Pancreatic ductal carcinoma is associated with a poor prognosis and high mortality rate. Recent literature reports have indicated that many regulatory factors are implicated in the growth of pancreatic carcinoma, its invasiveness and metastasis. Molecular studies have revealed that the development of this neoplasm is frequently related to disturbances in the tyrosine kinase receptors: EGFR, c-erbB-2 (HER-2), c-erbB-3 and c-erbB-4, i.e. those belonging to a family of the epidermal growth factor (EGF) receptors (1).

The epidermal growth factor (EGF) is a single polypeptide built up of 53 amino acids, with a major role in intercellular interactions. Interaction between the EGF of one cell and the receptor (EGFR) on an adjacent cell has a number of biological effects, e.g. migration, growth or morphological changes in the cell. EGF stimulates the growth or exerts a trophic effect in many tissues, including the pancreas (2) and fibroblasts (3), and plays an essential role in proliferation, differentiation and maturation of the embryonic intestine (4). However, apart from its key role in normal development, EGF receptors are also involved in neoplastic transformation. Overexpression of EGF and EGFR has been observed in various malignancies, including carcinomas of the pancreas (5), stomach (6) and liver (7), as well as tumours of the brain (8). Moreover, the HER-2/neu oncogene (erbB-2) that encodes the transmembrane glycoprotein closely associated with EGFR has been widely described. Overexpression of c-erbB-2 has been found in adenomas of the breast (9), ovary, (9), lungs (10), stomach (11) and in over 50% of pancreatic adenomas (12).

The aim of the current study was to assess the expression of EGF, including EGFR and c-erbB-2 (HER-2/neu), in pancreatic ductal adenocarcinomas in correlation with chosen anatomoclinical parameters.

Patients and Methods

Patients. The study was performed using archive material. The study group included 36 patients treated surgically for pancreatic ductal carcinomas in the years 2001-2003 at the Department of General Surgery and Gastroenterology, Medical University of Białystok. Sections, 5 Bm-thick, were cut from paraffin blocks and stained with haematoxylin and eosin (H+E). The routine histopathological assessment of the sections assessed the histological type, malignancy grade (G), clinicopathological pTN status, regional lymph node involvement and the presence of distant metastases.
Immunohistochemistry. Immunohistochemical examination was performed according to the following protocol. Formalin-fixed, paraffin-embedded tissue specimens were cut on a microtome into 5 µm sections, which were then deparaffinized in xylene and hydrated in alcohol. To expose the antigen, the slides were heated in a microwave oven for 15 min in citric acid buffer (pH=6.0). The activity of endogenous peroxidase was blocked by incubating the sections in 0.5% hydrogen peroxide in methanol. The slides were then incubated overnight with monoclonal c-erbB-2 antibodies (NCL-c-erbB-2-316, Novocastra, Poland), with EGF (EGF-Sigma, clone EGF-10; Sigma-Aldrich, Poland) and EGFR (Clone: H11, Dako, Poland) for 60 min at room temperature. The reaction was performed using the ABC technique with Novostatin Super ABC Universal Kit (Novocastra Laboratories Ltd.). The colour reaction of peroxidase was carried out with diaminobenzidine as chromogen (DAKO S3000; Dako, Poland). A semiquantitative method was used to evaluate protein expression, which was defined as positive (reaction present in >30% of tumour cells) or negative (lack of reaction or reaction present in <30% of cells). The protein-positive cells were calculated in at least 500 neoplastic cells per each sample using an Olympus CX41 light microscope (x400).

Statistical analysis. Spearman’s correlation coefficient was used for statistical analysis. A p-value of <0.0001 was considered statistically significant.

Results

Membrane expression was found for EGF, EGFR and c-erbB-2 in all cases (Figures 1-3). In pancreatic ductal carcinomas, a positive reaction for c-erbB-2 was observed in 24/36 cases, for EGF protein in 13/36 cases, and for EGFR protein in 18/36 cases. Statistical analysis of the results revealed no statistically significant correlations of c-erbB-2, EGF and EGFR expression with sex or age of patients, or the histological type or grade of malignancy (G) (Tables I-III). No correlation was found between c-erbB-2 expression and lymph node involvement (pN) or the presence of distant metastases (pM). However, a correlation of EGF and EGFR with metastasis to lymph nodes and other organs was detected. Moreover, EGF expression statistically significantly correlated with EGFR expression in the tumour.

Discussion

The role of the EGF family members in such malignant neoplasms as carcinoma of the stomach, liver, breast, ovary or lungs has been the focus of recent cancer research. In the current study, we assessed the expression of the EGF, EGFR and c-erbB-2(HER-2/neu) gene

Figure 1. Expression of c-erbB-2 in the main tumor mass (magnification x400).
Figure 2. Immunostaining of EGF shows strong membrane staining in the main tumor mass. Magnification x200.

Figure 3. EGFR-positive reaction in the membrane of cancer cells. Magnification x200.
product in pancreatic ductal carcinomas in correlation with chosen anatomoclinical parameters using an immunohistochemical method.

EGF is a protein that plays a major role in the regulation of cell growth, proliferation and differentiation. Human EGF is composed of 53 amino acids, has a molecular weight of 6,045 Da, has a high affinity for EGF R located at the cell surface and stimulates intrinsic activity of tyrosine kinase (13). Many scientists suggest that a rise in EGF expression may be partly responsible for carcinogenesis in certain carcinomas (14). In our study, the expression of EGF was found to be absent from normal pancreas and enhanced in pancreatic ductal carcinoma cells. However, no correlation was observed between EGF expression in pancreatic carcinoma and such anatomoclinical parameters as age, sex, histological type and malignancy grade. Similar results have been reported by Hall et al. (15), who also found no correlation between EGF expression and histological type or differentiation of tumours. However, some researchers have observed EGF expression more frequently in advanced pancreatic carcinomas (16). In our study, EGF expression was found to correlate with lymph node involvement and the presence of distant metastases, thus we can suggest that EGF may play a key role in neoplastic spread.

EGFR belongs to a family of ErbB receptors that consists of four closely related tyrosine kinase receptors: EGFR (ErbB-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3) and HER-4 (ErbB-4). EGFR can bind and be activated by different ligands, including the EGF, transforming growth factor alpha (TGFα) and certain growth factors encoded by viruses. Ligand-induced receptor stimulation causes activation of tyrosine kinase, which in turn initiates intracellular signalling cascades. This results in a variety of biochemical changes, including elevated intracellular calcium levels, increased glycolysis, protein synthesis and expression of certain genes, eventually leading to increased DNA synthesis in the cell, stimulation of cell proliferation and inhibition of programmed cell death (13, 17).

Many authors have described EGFR expression in organs such as the lungs, prostate, vulva and pancreas (12, 17-19). Yamanaka et al. have revealed that in pancreatic carcinoma EGFR expression correlated with the disease stage and mean survival time reduction (20). In addition Zhang and Yuan reported that the assessment of EGFR expression may help evaluate malignancy, stage and metastasis of pancreatic ductal carcinoma (21). We noted a correlation of EGFR expression with such anatomoclinical parameters as lymph

Table I. Correlation between EGF and other parameters.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Hp</th>
<th>G</th>
<th>pN</th>
<th>pM</th>
<th>EGF</th>
<th>c-erbB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.209</td>
<td>-0.050</td>
<td>0.135</td>
<td>0.040</td>
<td><strong>0.791</strong></td>
<td><strong>0.645</strong></td>
<td><strong>0.610</strong></td>
</tr>
<tr>
<td>p-value</td>
<td>0.221</td>
<td>0.773</td>
<td>0.433</td>
<td>0.819</td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
</tr>
</tbody>
</table>

N=36. Significant correlation between EGF expression and other parameters is marked in bold. Correlation is significant at the level of p<0.0001 (two-tailed). Hp, histological type; G, malignancy grade; pN, lymph node metastasis; pM, distant metastasis.

Table II. Correlation between EGFR and other parameters.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Hp</th>
<th>G</th>
<th>pN</th>
<th>pM</th>
<th>EGF</th>
<th>c-erbB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.104</td>
<td>0.115</td>
<td>0.117</td>
<td>0.020</td>
<td><strong>0.748</strong></td>
<td><strong>0.610</strong></td>
<td><strong>0.610</strong></td>
</tr>
<tr>
<td>p-value</td>
<td>0.506</td>
<td>0.506</td>
<td>0.495</td>
<td>0.909</td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
<td><strong>&lt;0.0001</strong></td>
</tr>
</tbody>
</table>

N=36. Significant correlation between EGFR expression and other parameters is marked in bold. Correlation is significant at the level of p<0.0001 (two-tailed).

Table III. Correlation between c-erbB-2 and other parameters.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Hp</th>
<th>G</th>
<th>pN</th>
<th>pM</th>
<th>EGFR</th>
<th>EGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.090</td>
<td>-0.122</td>
<td>0.051</td>
<td>-0.060</td>
<td>0.000</td>
<td>-0.163</td>
<td>-0.103</td>
</tr>
<tr>
<td>p-value</td>
<td>0.600</td>
<td>0.477</td>
<td>0.768</td>
<td>0.729</td>
<td>1.000</td>
<td>0.343</td>
<td>0.548</td>
</tr>
</tbody>
</table>

N=36. Correlation is significant at the level of p<0.0001 (two-tailed).
node involvement and distant metastasis, as well as with EGF expression. In every case of pancreatic ductal carcinoma, the elevated EGF expression was accompanied by a rise in EGFR in the tumour. Increased expression of EGF, by stimulating EGFR receptors, triggers numerous processes that involve enhanced gene expression e.g. the EGFR gene, which eventually causes increased expression of the EGFR. This has been confirmed by other researchers. Orth et al. (22) showed that cells can be stimulated to produce EGFR receptors through EGF-induced stimulation.

Her-2/neu (also known as c-erbB-2) is a proto-oncogene belonging to the EGF family, located in the long arm of chromosome 17. It was identified as the result of DNA transportation from chemically induced rat neuroblastoma. This gene encodes a new tyrosine kinase family marker, a 185 kDa transmembrane glycoprotein closely related in sequence and structure to the EGFR (23). c-erbB-2 is involved in neoplastic transformation and its overexpression associated with a poor prognosis has been observed in 20-30\% of adenocarcinomas of the breast, ovary, lungs and stomach (24), and in over 50\% of pancreatic carcinomas (25). However, literature data concerning a prognostic role of Her-2 are not uniform. We found no correlation between the expression of c-erbB-2 and anatomoclinical parameters. Novotny et al. (26) and Safran (27) found no correlation between tumour differentiation grade and c-erbB-2 expression. Satoh et al. (28) and Yamao et al. (29) revealed no correlation between c-erbB-2 expression and clinical parameters. However, some authors observed more frequent expression of c-erbB-2 in highly-differentiated tumours (30). Williams et al. (31) found that c-erbB-2 overexpression may correlate with a more malignant phenotype of pancreatic carcinoma and thus can facilitate immunodiagnostics and immunotherapy. The c-erbB-2 proto-oncogene structurally resembles EGFR. In particular, the intracellular tyrosine kinase domain shows the highest homology with EGFR (82\%). Activation of the Her-2 gene, and then overexpression of its product, p185 neu protein, is followed by EGFR activation to accelerate cell proliferation and mitosis. Zhang and Yuan (21) observed overexpression of these two receptors in 41.7\% of their cases of pancreatic ductal adenocarcinoma, thus indicating that c-erbB-2 and EGFR play an equal role in pancreatic ductal carcinoma. The ErbB receptors are known to form homo- and heterodimers. The c-erbB-2 receptor does not show high affinity for ligand and is activated only via heterodimerization with another receptor e.g. EGFR (32). However, we found no correlation of EGF or EGFR with Her-2 expression in the tumour.

Concluding, in our study group of 36 patients, both EGF and EGFR were found to strongly correlate with lymph node involvement and distant metastasis.


