Growth Factors in Human Pancreatic Cancer: Update on the Role of the Epidermal Growth Factor Receptor

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Introduction

Carcinoma of the pancreas is the fifth leading cause of cancer death in the United States. The reasons for the aggressiveness of this cancer are not known, and the molecular mechanisms that govern the growth of pancreatic cancer cells are still not clearly defined. Previous work has established that human pancreatic cancer cell lines overexpress the epidermal growth factor (EGF) receptor and produce transforming growth factor alpha (TGF- α), and that EGF is recycled by these cells whereas TGF- α is extensively degraded [1-5].

Overexpression of the EGF receptor occurs in a variety of tumors and has been correlated with enhanced meta-static potential and tumor invasiveness [6, 7]. Furthermore, overexpression of TGF- α confers onto cells the ability to proliferate in an anchorage-independent manner and to form tumors in nude mice [8]. Therefore, the observation that human pancreatic cancer cell lines over-express the EGF receptor and produce TGF- α , and avidly bind and internalize both EGF and TGF- α , suggested that there may be an important EGF receptor autocrine loop in these cells that provides them with a growth advantage. The present review will focus on recent findings concerning the role of the EGF receptor and its ligands in human pancreatic cancer.

EGF Receptor

The EGF receptor is a 170-kilodalton glycosylated phosphoprotein that is encoded by a gene located on the short arm of chromosome 7 [9,10]. It consists of an extracellular domain that contains the ligand binding region, a transmembrane domain, and an intracellular domain that contains the tyrosine kinase region that catalyzes the auto-phosphorylation process [10, 11]. The extracellular domain of the EGF receptor is structurally subdivided into subdomain I at the amino-terminal end, a cysteine-rich subdomain II, the critically important subdomain III that contains the EGF binding site, and a relatively understudied subdomain IV [10,11]. Following

ligand binding, the EGF receptor undergoes dimerization, and auto- and transphosphorylation on tyrosine residues 992, 1068, 1086, 1148, and 1173 located within the intracellular domain [11]. These residues then become the sites of association of proteins containing src homology 2 (SH2) motifs [12], resulting of biochemical reactions that culminate in the biological responses to EGF. The normal human pancreas expresses low levels of the EGF receptor [13], raising the possibility that it may have a role in modulating normal pancreatic exocrine function. By immunohistochemistry, the EGF receptor is especially prominent at the apical surface of ductal cells, and is also evident in the cytoplasm of ductal and acinar cells [13]. Hu-

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Human pancreatic ductal adenocarcinoma exhibits increased EGF receptor immunostaining [13, 14]. By Northern blot analysis and in situ hybridization, EGF receptor mRNA levels are increased in these carcinomas, and this overexpression is localized to the duct-like cancer cells [13].

Family of EGF Receptor Ligands

In addition to binding EGF and TGF- α , the EGF receptor can be activated by heparin-binding EGF-like growth factor (HB-EGF), betacellulin and amphiregulin [15-17]. All five growth factors possess six cysteine residues in the same relative position. Ostensibly, this results in the formation of disulfide bonds that confer similar three-dimensional configurations to these polypeptides. Previously, it was shown that human pancreatic cancers exhibit increased EGF and TGF- α immunostaining [18]. Immunohistochemical and in situ hybridization studies in conjunction with serial sectioning of tissue samples revealed that the EGF receptor often colocalizes with EGF and TGF- α in these tumors [13], indicating that the ligands may exert autocrine and paracrine effects on the cancer cells. In support of this hypothesis, it was found that the concomitant presence of the EGF receptor with either EGF or TGF- α is associated with a significantly shorter postoperative survival period [19].

Recently, additional studies have produced findings that underscore the importance of the EGF receptor in human pancreatic cancer. Thus, we found that these cancers also overexpress amphiregulin and HB-EGF [20, 21]. Amphiregulin expression was detected in both cancer cell lines and in normal and cancerous pancreatic tissues by use of the polymerase chain reaction (PCR). Following induction with either TGF- α or tetradecanoyl phorbol-acetate (TPA), there was a marked increase in the levels of the amphiregulin transcript, which was now readily visible by Northern blot analysis of total RNA extracted from the cell lines [20]. In the normal pancreas, immunohistochemical analysis revealed the presence of amphiregulin in the nuclei of ductal cells [20]. In contrast, in some carcinomas, amphiregulin immunoreactivity was observed in both the nuclei and the cytoplasm of the duct-like cancer cells, and in other carcinomas it was only seen in the cytoplasm of the cells [20]. The reasons for this differential localization are not known. HB-EGF mRNA transcripts were also present in the cultured pancreatic cancer cells [21]. Furthermore, HB-EGF mRNA levels were enhanced by HB-EGF, TGF- α and TPA [21], indicating that HB-EGF was also capable of causing its own induction. As in the case of EGF, TGF- α , and amphiregulin, HB-EGF mRNA levels were increased in the pancreatic cancers by comparison with the levels expressed in the normal pancreas. Together, these findings suggest that all the members of the EGF family of ligands may participate in aberrant autocrine and paracrine activation of the EGF receptor, thereby contributing to pancreatic cancer cell growth.

Receptors Related to the EGF Receptor

There are three receptors which are closely related to the EGF receptor. They are characterized by the presence of an extracellular ligand binding domain with two cysteine-rich regions, a transmembrane domain, and an intracellular domain whose tyrosine kinase activity resides within a continuous region of amino acids [22]. They have been variably named as the human EGF receptor type 2 (HER-2) or c-erbB-2, the human EGF receptor-type 3 (HER-3) or c-erbB-3, and the human EGF receptor type 4 (HER-4) or c-erbB-4 [22]. Several ligands which bind to HER-2 have been described to date. These include the neu differentiation factor also known as heregulin, and glial growth factors [23, 24]. The ligands that bind to HER-3 and HER-4 are yet to be isolated. While the role of these ligands in pancreatic cancer is not known, it has been established that these cancers overexpress HER-2 and HER-3 [25, 26]. Interestingly, HER-2 overexpression was not associated with a worse prognosis [25]. The relationship between HER-3 expression and the survival of patients with pancreatic cancer is not known.

Conclusion

The excessive expression of ligands and receptors in pancreatic cancer suggests that their normal function has been usurped by the cancer cells, leading to aberrant activation of growth-promoting pathways. However, we have recently determined that the expression of transmembrane tyrosine kinase receptors and growth factors is also increased in the pancreas of patients with chronic pancreatitis [27, 29]. Therefore, it is likely that this overexpression is not sufficient for pancreatic malignant transformation. Nonetheless, in combination with mutations of the K-ras oncogene [30, 31] and the p53 tumor suppressor gene [32, 33], as well as other genetic perturbations, the overexpression of growth factors and their receptors may give pancreatic cancer cells a distinct growth advantage, which may explain the extreme aggressiveness of this malignancy.

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