EPIDERMAL GROWTH FACTOR RECEPTOR: IS A NOVEL THERAPEUTIC TARGET FOR PANCREATIC CANCER?

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1. ABSTRACT

Expression of epidermal growth factor receptors (EGFR) is exaggerated in pancreatic adenocarcinoma and activation of EGFR appears to have an important role in the growth and differentiation of this and in other tumors. Therefore, blockade or inactivation of EGFR by monoclonal antibodies or by tyrosine kinase inhibitors has significant potential as an effective anti-cancer therapy. One of the very recent significant developments in the field of molecular biology involves the use of antisense of EGFR or EGFR gene silencing in pancreatic cancer cells as a potential targeted therapy for patients with pancreatic adenocarcinoma.

2. INTRODUCTION

EGFR is a member of the protein tyrosine kinase (PTK) family. EGFR is a 170kda glycoprotein on the cell surface of a variety of cell types and is characterized by its ligand dependent tyrosine kinase activity. These kinases have important roles in regulating cellular functions such as growth, differentiation and apoptosis or programmed cell death (1). When the ligands, EGF or TGFβ, bind to EGFR, it undergoes receptor dimerization, which, in turn, leads to autophosphorylation and a conformational change. EGFR then recruits and phosphorylates cytoplasmic signal transduction molecules. These signal transduction molecules include phosphoinoside kinase (PI3K), Grb-2, She, Src kinases and others. PI3K activates protein kinase B / Akt kinases, protein kinase C (PKC) and nuclear factor kappa B (NFκB) (2). All of these molecules are adaptor molecules that interact with SOS (son of sevenless), a guanosine triphosphate (GTP) exchange enzyme that leads to recruitment and activation of Ras (3). Ras then activates Raf, mitogen activated protein kinase kinase (MAPKK) and mitogen activated protein kinase (MAPK), which then transmit signals to the nucleus that regulate gene expression (4). EGFR simultaneously activates transcription factors, which activate a number of signal transducers and activators. Upon activation, the transcription signal transducers and activators undergo translocation from the cytoplasm to the nucleus where these transducers and activators regulate genes (Figure 1).

Multiple investigators hypothesize that EGFR plays an important role in tumor development, growth and survival. Under normal conditions, expression of EGFR is tightly regulated whereas altered expression and activation of EGFR are frequently detected in human cancer (5). Elevated expression of EGFR and its ligand binding has been reported in a variety of epithelial cancers, including head and neck, breast, ovarian, colorectal and pancreatic cancer (4, 6 and 7).

3. PANCREATIC CANCER AND EGFR

Adenocarcinoma of the pancreas, with an annual incidence of 9-10 cases per 100,000 per year, accounts for approximately 2% of all malignancies (excluding basal and squamous cell cancers) (8-10). However, although relatively uncommon, pancreatic adenocarcinoma is the fourth leading cause of cancer death in the United States (11), it has a very poor prognosis, and the two years survival rate is less than 10% (10). Surgery is the only potentially curative treatment but, unfortunately, less than 20% of patients are candidates for curative resection at the time of presentation/diagnosis, due to the advanced stage of the disease at presentation. The majority of patients with pancreatic cancer are therefore managed with non-surgical therapies including chemotherapy and/or radiation therapy. Additionally, strategies for early detection of pancreatic cancer have not been successful and most patients with pancreatic carcinoma still present with metastatic or locally advanced disease at the time of diagnosis. New therapeutic alternatives are needed for patients with pancreatic adenocarcinoma, because non-surgical therapies (chemotherapy, immunotherapy, radiation therapy etc.) are largely ineffective, and metastatic disease frequently develops even after potentially curative surgery. Although
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**Figure 1.** Diagrammatic representation EGFR mediated signaling pathways.

**Figure 2.** Immunohistochemical detection of EGFR in human pancreas using EGFR monoclonal antibody. A: normal, B: pancreatitis and C: adenocarcinoma.

Currently available radiotherapy and chemotherapy regimens have not demonstrated improved survival in advanced disease, studies of the biology of pancreatic adenocarcinoma have revealed that activation of specific oncogenes, growth factors and transcription factors that regulate inflammatory gene expression play a critical role in pancreatic cancer. Therefore, these basic cellular processes may be used to develop prognostic markers to identify subgroups of patients who may benefit from aggressive therapy or innovative therapeutics, in an attempt to improve survival.

Pancreatic cancer develops through a cascade or series of molecular and genetic events. These events may cause progression of an adenomatous or proliferative phenotype to dysplasia and finally progression to invasive cancer. One of the most frequent and early events in development of pancreatic cancer is mutation of K-ras oncogene (4). This leads to activation of a K-ras protein that in turn provides constant signals for cell proliferation (4). Previous studies from our laboratory demonstrated that K-ras mutation is associated with increased tumor angiogenesis in human pancreatic adenocarcinoma (12). Mutation of p-53 and other tumor suppressor genes, such as DPC 4 (SMAD-4) and p16, has also been implicated in the development of pancreatic cancer (13). In addition, mutation of p53, NF-kB and multidrug resistance associated protein contributes to the resistance to chemotherapy for this disease (14).

EGFR also has an important role in the development of pancreatic adenocarcinoma. Increased expression of EGFR is detected in human pancreatic cancer (15). Co-expression of EGFR and its ligands following activation of receptor mediated signaling molecules is commonly linked with pancreatic cancer cell growth (6). Clinical studies have demonstrated that co-expression of EGFR and EGF or TGFα is correlated with increased tumor size and decreased patient survival (16). EGFR has also been implicated in tumor angiogenesis, an important step in the progression of pancreatic cancer (17). Recent data suggested both EGF and TGFβ stimulate malignancy-associated angiogenesis through vascular endothelial growth factor (VEGF) (18, 19). Our laboratory also demonstrated increased EGFR expression in human pancreatic cancer tissues using immunohistochemical techniques, whereas very low EGFR expression was detected in chronic pancreatitis, and no EGFR expression was detected in control (normal) pancreatic tissue (Figure 2).

In summary, EGFR appears to have an important role in cell growth regulation, tumorigenesis and angiogenesis in pancreatic cancer. Therefore, agents capable of inhibiting EGFR activity with resultant inhibition of cell proliferation and angiogenesis, have significant potential as chemotherapeutic agents for the treatment of pancreatic adenocarcinomas as well as multiple other malignancies.

4. TARGETED THERAPY DIRECTED AGAINST EGFR

Two types of EGFR inhibitors are currently available. One is a small molecule, that inhibits tyrosine kinase activity (TK inhibitor) and the other type of inhibitor inhibits ligand binding to EGFR (monoclonal antibody). TK inhibitors are generally quinolone derivatives that inhibit EGFR tyrosine kinase. Several TK inhibitors have already been developed for treatment of pancreatic cancer including ZD1839 or Iressa (20), PKI-166 (21), OSI 774 (22) and EKB 569 (23). These drugs appear to block...
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Figure 3. Diagrammatic representation of therapy targeted towards EGFR in Pancreatic Cancer.

tyrosine kinase activity by attaching to the ATP binding site on the intracellular portion of receptor.

One of most promising and most studied anti-EGFR monoclonal antibodies is IMC-C225. In addition to competing effectively with EGF (24), IMC-C225 also inhibits cell growth (4, 25). Another EGFR-specific monoclonal antibody, ABX-EGF, also binds to EGFR and inhibits EGFR mediated signal transduction and cell proliferation (26). Interestingly, the antitumor activity of EGFR inhibitors does not correlate with the level of EGFR expression. Unlike most of the TK inhibitors, ABX-EGF and IMC-225 alone do not eliminate tumor so they must be combined with cytotoxic agents for tumor eradication.

The mechanism of action of EGFR targeted therapies has been investigated in an orthotropic pancreatic cancer model. IMC-225 and PKI-166 effectively inhibited EGFR autophosphorylation both in vitro and in vivo (21, 26). Both of these agents modestly inhibit cell proliferation. IMC-225 alone inhibits cell growth by only 20%, whereas an additive inhibitory effect was observed when both agents were combined with a cytotoxic chemotherapeutic agent, gercitabine. IMC-225 and PKI-166 showed profound inhibition of growth of orthotopically implanted pancreatic tumors. Recent studies also suggested that EGFR-targeted therapies stimulate apoptosis and inhibit tumor cell proliferation. It has also been demonstrated that EGFR targeted therapies diminished micro-vascular densities and also the production of VEGF and IL-8 in the tumors (4). These observations suggest that EGFR inhibitors not only inhibit proliferative and angiogenic activities but also induced an antitumor effect in pancreatic cancer (27).

Two other monoclonal antibodies that are targeted against the EGFR family, tratuzumab and centuximab, have demonstrated very encouraging clinical responses in breast cancer and colorectal cancer (8, 28). Several clinical trials evaluating these agents in patients with pancreatic cancer have reported encouraging early evidence of anticancer activity with acceptable toxicities (8).

Another approach could be taken in pancreatic cancer using antisense or gene therapy engineered in viral vectors including gene deletion mutants, tissue specific promoter-regulated virus, and tumor-selective viruses. Antisense approach involves targeting specific RNA sequences to reduce the translation of the mRNA message into protein, in turn, inhibiting gene and ultimately, protein expression (29). The recent preliminary study from our laboratory demonstrated that EGFR siRNA silencing in MIA PaCa2, pancreatic cancer cells, reduces the protein expression of EGFR (30). Future studies will be designed to evaluate the down-stream signaling pathways in EGFR silenced cells.

5. CONCLUSIONS

During last decade, molecular biology and technology have contributed significantly to the development of therapeutic agents in medicine, and especially in oncology. Important areas have included inhibition of tumor growth, inhibition of metastatic invasion, and inhibition of intercellular signal transduction and compensation of gene expression. EGFR is one of the first molecules, with an identified and important role in the regulation of cell proliferation, differentiation and tumorigenesis. Therefore, a molecular based-approach targeting EGFR in pancreatic cancer would be a novel approach for treatment and it is summarized in Figure 3.

6. ACKNOWLEDGEMENTS

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7. REFERENCES


27. Mendelsohn, J: The epidermal growth factor receptor as a target for cancer therapy. Endocrine-Related Cancer 8, 3-9, (2001)

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