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Pancreatic Cancer: Pathogenesis, Prevention and Treatment

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Abstract

Pancreatic cancer is the fourth leading cause of cancer death in the United States with a very low survival rate of 5 years. To better design new preventive and/or therapeutic strategies for the fight against pancreatic cancer, the knowledge of the pathogenesis of pancreatic cancer at the molecular level is very important. It has been known that the development and the progression of pancreatic cancer are caused by the activation of oncogenes, the inactivation of tumor suppressor genes, and the deregulation of many signaling pathways among which the EGFR, Akt, and NF- κ B pathways appear to be most relevant. Therefore, the strategies targeting EGFR, Akt, NF- κ B, and their downstream signaling could be promising for the prevention and/or treatment of pancreatic cancer. In this brief review, we will summarize the current knowledge regarding the pathogenesis, prevention, and treatment of pancreatic cancer.

Keywords

Pancreatic cancer; Molecular pathogenesis; EGFR; NF- κ B; COX-2; Akt

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States with a median survival of <6 months and a dismal 5-year survival rate of 4.6% (Jemal et al., 2006). Even for those patients diagnosed with local disease, the 5-year survival rate is only 16%. Approximately, 33,730 people are expected to develop pancreatic cancer, and 32,300 people will die from the disease in 2006 (Jemal et al., 2006). The lethal nature of pancreatic cancer stems from its propensity to rapidly disseminate to the lymphatic system and distant organs. The presence of occult or clinical metastases at the time of diagnosis together with the lack of effective chemotherapies contributes to the high mortality in patients with pancreatic cancer. Pancreatic cancer is one of the most intrinsically drug-resistant tumors and the cancer cell resistance to chemotherapeutic agents is a major cause of treatment failure in pancreatic cancer. Therefore, there is a dire need for designing new and targeted therapeutic strategies that can overcome the drug-resistance and improve the clinical outcome for patients diagnosed with pancreatic cancer. For this purpose, the knowledge on the molecular pathogenesis of pancreatic cancer is very important and is likely to be helpful in the design of newer drugs and the molecular selection of existing drugs for targeted therapy against pancreatic cancer. The following sections will summarize what we know regarding the molecular pathogenesis of

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pancreatic cancer and how some of these molecular pathways could be exploited for the prevention and/or treatment of pancreatic cancer.

Molecular pathogenesis of pancreatic cancer

Intensive investigation of molecular pathogenesis will aid in identifying useful molecules for diagnosis, treatment, and prognosis of pancreatic cancer. In the past several years, considerable research has focused on identifying molecular events in pancreatic carcinogenesis, and their correlation with clinicoathological status. It has been found that multiple subsets of genes undergo genetic changes, either activation or inactivation, during the development and progression of pancreatic cancer (Mimeault et al., 2005). The activation of oncogenes and the inactivation of tumor suppressor genes are partly responsible for the initiation and progression of pancreatic cancers (Figure 1) (Mimeault et al., 2005; Jimeno and Hidalgo, 2006; Maitra et al., 2006). Moreover, the deregulation of molecules in several cell signaling pathways, such as EGFR, Akt, NF- κ B, etc, and their molecular crosstalk (Figure 1) also play important roles in the molecular pathogenesis of pancreatic cancer (Jimeno and Hidalgo, 2006; Mimeault et al., 2005).

Activation of oncogenes

Oncogenes can be activated through different mechanisms including point mutation and amplification. The activation of the ras oncogene has been found in more than 90% of pancreatic cancers (Almoguera et al., 1988). The ras gene family encodes a 21-kDa membrane-bound protein involved in signal transduction and mediates pleiotropic effects including cell proliferation and migration. Activated ras is involved in growth factor-mediated signal transduction pathways. It has been found that approximately 80–90% of pancreatic cancers harbor point mutation at codons 12, 13 and 61 in K-ras (Almoguera et al., 1988). This is the highest fraction of K-ras alteration found in any human tumor type. The point mutation leads to the generation of a constitutively active form of ras. The constitutively activated ras binds to GTP and gives uncontrolled stimulation signals to downstream signaling cascades, promoting uncontrolled cell growth. K-ras mutation in pancreatic cancer typically develops during the early phase of carcinogenesis and patients with K-ras mutation have a shorter survival time than patients with the wild-type K-ras, suggesting that the mutation of K-ras is partly responsible for the initiation and progression of pancreatic cancer. In addition to point mutation, amplification of ras is also frequently observed in pancreatic cancers, suggesting that activation of ras oncogene is an important molecular event in pancreatic cancers. Moreover, recent studies have suggested the role of other genes such as Notch and COX-2 in pancreatic cancers as discussed below.

Notch gene has also been considered as an oncogene involved in the pathogenesis of pancreatic cancer (Miyamoto et al., 2003). So far, four Notch genes have been identified (Notch-1, Notch-2, Notch-3, and Notch-4) and five Notch ligands (Dll-1, Dll-3, Dll-4, Jagged-1, and Jagged-2) have been found in mammals. Notch protein can be activated by interacting with its ligands. Upon activation, Notch protein is cleaved, releasing intracellular Notch which translocates into the nucleus. The intracellular Notch associates with transcriptional factors, which regulate the expression of target genes, and thus plays an important role in both organ development and pancreatic carcinogenesis. Therefore, it is clear that alterations in Notch signaling are associated with carcinogenesis of the pancreas. We and others have found that Notch signaling is frequently deregulated in human pancreatic cancers (Wang et al., 2006e; Wang et al., 2006a; Buchler et al., 2005). High expression of Notch, which inhibits apoptosis, has been found in pancreatic cancers. Moreover, Notch-1 has been found to strongly induce the activity of NF- κ B and the activation of NF- κ B has been detected in the majority of pancreatic cancer, suggesting that the activation of Notch oncogene plays important roles in

the pathogenesis of pancreatic cancer by activation of NF- κ B and its downstream signaling pathways (Figure 1).

The cyclooxygenase (COX) enzymes promote the formation of prostaglandins, which leads to the induction of cell growth. There are two isoforms of the COX enzyme. COX-1 is produced at a constant rate and the prostaglandins formed are involved in several normal physiologic events. COX-2, in contrast, is an inducible enzyme, and is not usually present in most normal tissues. However, its synthesis is stimulated in inflammatory and carcinogenic processes by cytokines, growth factors, and other cancer promoters. COX-2 has been shown to be increased in a variety of cancers including pancreatic cancers. Several pancreatic cancer cell lines strongly express COX-2. Immunohistochemical studies have shown that 47–66% of human pancreatic cancers over-express COX-2 and that COX-2 mRNA expression is much higher in tumors than in normal surrounding tissue (Okami et al., 1999). Moreover, there is a positive association between ras mutation and COX-2 level because activated ras acts to increase the stability of COX-2 mRNA. Therefore, COX-2 appears to be of significance in pancreatic carcinogenesis and as such there appears to be a crosstalk between ras, NF- κ B, Notch and Cox-2 in cellular signaling that contributes to the molecular pathogenesis of pancreatic cancer (Figure 1).

Amplification of other oncogenes also plays a very important role in the development and progression of pancreatic cancer. It has been found that Akt-2 gene is amplified in 10–15% of pancreatic cancers while Myb gene is amplified in 10% of pancreatic cancers. The amplification of the oncogenes results in its activation and contributes to the stimulation of cell growth and the progression of pancreatic cancers. The experimental evidences also showed up-regulation of other oncogenes including Src, Bcl-6, S100P, Cyclin D1, etc (Mimeault et al., 2005). Up-regulation of cyclin D1 has been found in pancreatic cancers and over-expression of cyclin D1 is associated with poor prognosis. Inhibiting cyclin D1 in pancreatic cancer cell lines leads to growth inhibition and loss of tumorigenicity in nude mice (Kornmann et al., 1998). Recent studies by our laboratory have shown that overexpression of cyclin D1 promotes tumor cell growth and confers resistance to cisplatin-mediated apoptosis in an elastase-myc transgene-expressing pancreatic tumor cell line, suggesting the effect of cyclin D1 on the progression of pancreatic cancer (Biliran, Jr. et al., 2005).

In addition to these genes discussed above, no other oncogenes are known to play any significant or major role in pancreatic carcinogenesis; however, a group of tumor suppressor genes are also known to contribute in the molecular pathogenesis of pancreatic cancer as discussed below.

Inactivation of tumor suppressor genes

Inactivation of tumor suppressor genes is another important event for the initiation of pancreatic cancer. Tumor suppressor genes can be activated by mutation, deletion, or hypermethylation. The tumor suppressor genes targeted in pancreatic cancer include p16, p53, SMAD4, PTEN, etc.

It has been known that p16 inhibits the activity of cyclin D and CDK4/6 complex. CDK4 and CDK6 normally interact with cyclin D to phosphorylate the retinoblastoma (Rb) protein. The phosphorylation of Rb allows it to dissociate from a complex formed with elongation factor 2 (E2F), allowing E2F to activate genes required for DNA synthesis in cell cycle. p16 controls cell cycle progression through G1/S transition by inhibiting cyclin D and CDK4/6 mediated phosphorylation of Rb, inhibiting cell growth. Approximately 95% of pancreatic cancer patients have inactivated p16 (40% deletion; 40% mutation; 15% hypermethylation) in the tumors (Schutte et al., 1997). Experimental studies have demonstrated that transfection of wild-type p16 into human pancreatic cancer cells results in decreased tumor cell proliferation *in*

vitro and *in vivo*. Moreover, in pancreatic cancer patients the tumor size is significantly larger and the survival time is significantly shorter with p16 mutation compared to patients with wild type p16. These evidences demonstrate that p16 alterations participate in the aggressiveness of pancreatic cancer through its interaction with various cellular signaling pathways.

In addition to p16, another tumor suppressor gene p53 is well known to be involved in the control of cell cycle. p53 binds to the p21^{WAF1} promoter and stimulates the production of p21^{WAF1}, which negatively regulates the complex consisting of cyclin D1 and CDK2, thereby arresting the cell at the G1 phase and inhibiting cell growth. The p53 also plays important roles in the induction of apoptotic cell death. Inactivation of p53 during carcinogenesis can lead to uncontrolled cell growth and increased cell survival. The p53 gene is inactivated in about 50% of pancreatic cancers through gene mutation and deletion. We have shown that p53 mutation results in alteration of the 3D structure of the p53 protein (Li et al., 1998). The p53 mutation and the alteration of the protein 3D structure have been associated with shorter survival in patients with pancreatic cancer. In addition, alterations in the p53 gene are associated with K-ras mutations, suggesting a crosstalk and cooperative activity between p53 and K-ras in the molecular pathogenesis of pancreatic cancers. Moreover, the status of the p53 is important in mediating the cancer cell specific effects of chemotherapeutic agents. Loss of p53 function could result in decreased sensitivity to certain types of chemotherapeutic agents. Therefore, the status of p53 could be a useful guide for those patients who are likely to respond to adjuvant chemotherapy.

The inactivation of DPC4 (deleted in pancreatic cancer locus 4, Smad4) tumor suppressor gene is another common genetic alteration identified in pancreatic cancer. The DPC4 gene encodes for a 64 kDa protein, Smad 4, which plays roles in the inhibition of cell growth and angiogenesis. The inactivation of DPC4 is relatively specific to pancreatic cancer although it occurs with low incidence in other cancers. It has been found that DPC4 tumor suppressor gene is deleted in approximately 50% of pancreatic cancers (Cowgill and Muscarella, 2003). Up to 90% of pancreatic adenocarcinomas could harbor loss of heterozygosity. DPC4 alterations occur relatively late in pancreatic carcinogenesis. The frequency of loss of DPC4 expression is significantly higher in poorly differentiated pancreatic adenocarcinoma and the pancreatic cancer patients with intact DPC4 gene have significantly longer survival after resection compared to the patients with mutant DPC4 gene. Furthermore, DPC4 inactivation is always accompanied by inactivation of p16, suggesting its importance in the pathogenesis of pancreatic cancer.

As indicated earlier, p21^{WAF1} is an inhibitor of CDK. It forms complexes with cyclinA/CDK2 or cyclinD1/CDK4 and inhibits their activity, causing cell cycle arrest in G1 phase. Loss of p21^{WAF1} activity has been observed in approximately 30–60% of pancreatic cancers (Garcea et al., 2005). p27^{CIP1} is another CDK inhibitor which regulates cell cycle progression from G1 to S phase. The loss of p27^{CIP1} expression has also been observed in pancreatic cancers (Garcea et al., 2005). Another tumor suppressor gene, BRCA2, has been found to participate in DNA damage repair and mutations in BRCA2 have been linked to a significantly increased risk of pancreatic cancer. These evidences demonstrate that inactivation of p21^{WAF1}, p27^{CIP1}, and BRCA2 tumor suppressor genes is involved in the pathogenesis of pancreatic cancer. Although these genes as discussed above play important roles in pancreatic cancer, the role of these genes in cellular signaling is still poorly understood. Below we will discuss several important signaling pathways that are known to play significant roles and as such became important target for pancreatic cancer therapy.

Deregulation of EGFR signaling

EGFR consists of an extracellular ligand-binding domain, a hydrophobic transmembrane region, and an intracellular tyrosine kinase domain. EGFR is a member of the ErbB family of

receptor tyrosine kinases, which include ErbB-1 (EGFR), ErbB-2 (HER-2), ErbB-3, and ErbB-4. The principal ligands of EGFR are EGF and TGF- α . Binding of a ligand to EGFR induces receptor dimerization, which results in intracellular transphosphorylation of tyrosine residues. Phosphorylation of EGFR activates molecules in different cell signaling pathways including PI3K, Src, MAPK, STAT, etc., inducing cell cycle progression, cell division, survival, motility, invasion, and metastasis.

The genomic alterations of EGFR that occur in cancers include over-expression, mutation, deletion, and rearrangement. These alterations of EGFR induce the activity of receptor tyrosine kinases and may promote the development and progression of pancreatic cancer. Experimental studies have shown that EGFR activation plays important roles in proliferation, apoptosis inhibition, angiogenesis, metastasis, and resistance to chemotherapy or radiation therapy. Overexpression of EGF and EGFR is a common feature of human pancreatic cancer (Talar-Wojnarowska and Malecka-Panas, 2006). HER-2/neu amplification and p185 overexpression have been observed in 60% of pancreatic cancers. Moreover, EGFR overexpression has been found significantly more often in advanced clinical stages of pancreatic cancer and thus is associated with shorter survival in pancreatic cancer patients (Talar-Wojnarowska and Malecka-Panas, 2006), suggesting that deregulation of the EGFR pathway participates in the development and progression of pancreatic cancer. The EGFR signaling and its downstream signaling is therefore an important signaling pathway targeted for pancreatic cancer therapy.

Deregulation of Akt signaling

It has been known that EGF binding and subsequent EGFR, ras, or Src activation lead to the activation of the PI3K pathway. Activated PI3K phosphorylates phosphatidylinositides (PIP3), which then phosphorylates and activates Akt. Phosphorylated Akt (p-Akt) promotes cell survival by inhibiting apoptosis and activating NF- κ B. It has been known that p-Akt inhibits apoptosis through its ability to phosphorylate and inactivate several targets including Bad, Forkhead transcription factors, and caspase-9, all of which are involved in the apoptotic pathway. Akt also regulates the NF- κ B pathway via phosphorylation and activation of molecules in the NF- κ B pathway, suggesting that there is a cross talk between these two signaling pathways.

It has been found that a significant proportion (46–70%) of pancreatic cancer has high levels of p-Akt, which is correlated with high tumor grade and poor prognosis. Inhibition of PI3K in pancreatic cell lines results in a decreased p-Akt, G1 cell cycle arrest, and reduced cell proliferation, suggesting that PI3K/Akt pathway plays important roles in the survival of pancreatic cancer cells. Moreover, the inhibition of Akt decreases the function of NF- κ B, and has been shown to sensitize Mia-PaCa-2 pancreatic cancer cells to chemotherapy (Fahy et al., 2004), suggesting that both Akt and NF- κ B are targets for the treatment of pancreatic cancers.

Deregulation of NF- κ B signaling

The NF- κ B signaling pathway plays important roles in the control of cell growth, differentiation, apoptosis, inflammation, stress response, and many other physiological processes in cellular signaling (Karin, 2006). In human cells without specific signal, NF- κ B is sequestered in the cytoplasm through tight association with its inhibitors: I κ B α which acts as a NF- κ B inhibitor and p100 proteins which serves as both an inhibitor and precursor of NF- κ B DNA-binding subunits. NF- κ B can be activated through phosphorylation of I κ B α by IKK β and/or phosphorylation of p100 by IKK α , leading to degradation of I κ B α and/or the processing of p100 into small form (p52). This process allows two forms of NF- κ B (p50–p65 and p52-RelB) to become free, resulting in the translocation of active NF- κ B into the nucleus for binding to NF- κ B-specific DNA-binding sites and, in turn, regulating gene transcription. By binding to the promoters of target genes, NF- κ B controls the expression of many genes (i.e.

survivin, MMP-9, uPA, VEGF, etc.) that are involved in cell survival, apoptosis, invasion, metastasis, and angiogenesis.

NF- κ B is constitutively activated in most human pancreatic cancer tissues and cell lines but not in normal pancreatic tissues and cells, suggesting that the activation of NF- κ B is involved in the carcinogenesis of pancreatic cancers. Inhibition of NF- κ B by a super-inhibitor of NF- κ B results in impaired proliferation and induction of apoptosis (Liptay et al., 2003), suggesting an important role of NF- κ B in pancreatic tumorigenesis. Moreover, it has been found that the inhibition of constitutive NF- κ B activity completely suppresses the liver metastasis of the pancreatic cancer cell line ASPC-1 (Fujioka et al., 2003). An experimental study also demonstrates that urokinase-type plasminogen activator (uPA), one of the critical proteases involved in tumor invasion and metastasis, is over-expressed in pancreatic cancer cells, and its over-expression is induced by constitutive NF- κ B activity (Wang et al., 1999). These results suggest that constitutively activated NF- κ B is tightly related to the invasion and metastasis frequently observed in pancreatic cancers. Moreover, the deregulation of NF- κ B could also be due to Notch signaling as discussed earlier and as such the crosstalk between Notch and NF- κ B appears to be an important signaling event that regulates the processes of tumor invasion and angiogenesis in pancreatic cancer.

Deregulation of Hedgehog and other signaling

Hedgehog (Hh) signaling is an essential pathway for embryonic pancreatic development. It has been known that Hh signaling plays important roles in proper tissue morphogenesis and organ formation during the developing gastrointestinal tract. Hedgehog ligands including sonic hedgehog (Shh) are expressed throughout the endodermal epithelium at early embryonic stages but excluded from the region that forms the pancreas. Deregulation of the Hh pathway has been implicated in a variety of cancers including pancreatic cancer. It has been reported that overexpression of Shh may contribute to pancreatic tumorigenesis. Thayer et al found that no Shh was detected in the islets, acini, or ductal epithelium of normal pancreas while shh was aberrantly expressed in 70% of specimens from the patients with pancreatic adenocarcinoma, suggesting that Shh is a mediator of pancreatic cancer tumorigenesis (Thayer et al., 2003). Importantly, the down-regulation of Shh by cyclopamine, a specific inhibitor of Shh, can reduce the growth and viability of pancreatic cancer cells, suggesting that targeting Shh signaling may be an effective novel approach for the treatment of pancreatic cancer (Berman et al., 2003). A recent report shows that NF- κ B contributes to Hh signaling pathway activation through Shh induction in pancreatic cancer (Nakashima et al., 2006), demonstrating the crosstalk between NF- κ B and Hh signaling pathways in pancreatic cancer.

The deregulation of other signaling pathways (i.e. STAT3, MAPK, VEGF, IGF, etc) also plays roles in the development and progression of pancreatic cancer. Therefore, oncogenes, tumor suppressor genes, and the complex interactions between many cellular signaling pathways make up a network whose crosstalk contributes to the molecular pathogenesis of pancreatic cancer, suggesting that targeted inactivation of these important signaling pathways could be a novel and newer approach for the prevention and/or treatment of pancreatic cancer.

Prevention of pancreatic cancer progression by dietary chemopreventive agents

It has been estimated that more than two-thirds of human cancers could be prevented by modification of lifestyle including dietary modification. The dietary factors which are associated with increased risk of pancreatic cancer are meat, red meat in particular, and energy. Protection is mainly provided by fruit, vegetables, and vitamins. In recent years, more dietary compounds have been recognized as cancer chemopreventive agents because of their anti-

carcinogenic activity. Therefore, early invasion and metastasis of pancreatic cancer could be preventable by these dietary compounds.

Soy isoflavone, Genistein

Genistein is the main isoflavone found in a relatively high concentration in soybeans and most soy-protein products. It has been found that genistein inhibits cell growth and induces apoptosis in various cancers including pancreatic cancer. Our laboratory has investigated whether genistein treatment could modulate NF- κ B DNA binding activity in pancreatic cancer cells (Li et al., 2004b; Li et al., 2005a; Banerjee et al., 2005). We have found that genistein treatment significantly inhibited NF- κ B DNA-binding activity in pancreatic cancers *in vitro* and *in vivo*. We have also investigated the effect of genistein on the Akt signaling pathway. We have found that genistein also inhibited Akt activity in pancreatic cancer cells (Banerjee et al., 2005; Li et al., 2005a). Furthermore, we found that genistein significantly down-regulated Notch signaling, leading to the inhibition of NF- κ B and induction of apoptosis in pancreatic cancer cells (Wang et al., 2006e; Wang et al., 2006d). Our data together with others have revealed that genistein inhibits carcinogenesis and cancer progression through inhibition of Akt, Notch, and NF- κ B activation, resulting in the inhibition of their downstream genes as depicted in Figure 1. These results suggest that genistein could be a potent agent for the prevention and/or treatment of pancreatic cancer progression. In addition to genistein, there are other attracting agents that could also be useful for pancreatic cancer as discussed below.

I3C and DIM

Indole-3-carbinol (I3C) is produced from naturally occurring glucosinolates contained in a wide variety of plants including members of the family Cruciferae. Vegetables of the genus Brassica in the family Cruciferae contribute to most of our intake of glucosinolates and include all kinds of cabbages, broccoli, cauliflower, and brussels sprouts. I3C is biologically active and it is easily converted *in vivo* to its dimeric product 3,3'-diindolylmethane (DIM), which is also biologically active. It has been found that both I3C and DIM inhibit cell proliferation and induces apoptotic cell death in a variety of cancers including pancreatic cancer (Abdelrahim et al., 2006). It has been reported that I3C induces apoptosis in pancreatic cancer cells through the inhibition of STAT3 whose activation has been observed in human pancreatic carcinoma specimens and pancreatic cell line but not in normal pancreatic tissues (Lian et al., 2004), suggesting that I3C and DIM could have some beneficial effects on pancreatic cancer.

Curcumin

Curcumin is a compound from *Curcuma longa* (turmeric). *Curcuma longa* is a plant widely cultivated in tropical regions of Asia and Central America. Curcumin has recently received considerable attention due to its pronounced anti-inflammatory, anti-oxidative, immunomodulating, anti-atherogenic, and anti-carcinogenic activities. It has been found that curcumin suppress the activation of NF- κ B through inhibition of IKK activity in pancreatic cancer cells (Li et al., 2004a). By inhibition of NF- κ B, curcumin also down-regulates the expression of COX-2, resulting in increased PGE₂ and activation of several cytokines and chemokines especially IL-8. We have also found that curcumin inhibits cell growth and induces apoptosis through down-regulation of Notch and NF- κ B signaling in pancreatic cancer (Wang et al., 2006c), suggesting that curcumin could be useful for the prevention and/or treatment of pancreatic cancer similar to soy isoflavone genistein.

EGCG

Consumption of green tea has been implicated in better human health including the prevention of cancers. Green tea contains several catechins including epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG). However,

EGCG has been believed to be the most potent for the inhibition of oncogenesis and reduction of oxidative stress among these catechins (Mukhtar and Ahmad, 1999). EGCG has been shown to inhibit the formation and the growth of solid tumors in laboratory animals. By targeting multiple signaling pathways including MAPK, EGFR and NF- κ B, EGCG is able to inhibit the malignant transformation of epidermal cell lines, to inhibit cell growth, and to induce apoptosis in a number of cancer cells including pancreatic cancer (Mukhtar and Ahmad, 1999; Khan et al., 2006; Qanungo et al., 2005). EGCG also selectively inhibits COX-2 without affecting COX-1 expression (Hussain et al., 2005) and down-regulates K-ras (Lyn-Cook et al., 1999), suggesting its effects on the inactivation of oncogenes. Furthermore, the treatment of PANC1, Mia-PaCa-2, and BxPC-3 pancreatic cell lines with EGCG caused significant suppression of the invasive ability of the pancreatic cancer cells (Takada et al., 2002). These reports provide strong evidence in support of the roles of EGCG in chemoprevention and/or treatment of pancreatic cancer, especially because EGCG targets important cell signaling molecules as depicted in Figure 1.

Resveratrol

Resveratrol is a phytoalexin present in a wide variety of plant species including grapes, mulberries, and peanuts. Relatively high quantities of resveratrol are found in grape juice and red wine. Resveratrol has been shown to have beneficial effects on the reduction of oxidative stress and the prevention cancers. Resveratrol was first noted to be a cancer chemopreventive agent having antioxidant and anti-tumorigenic properties (Jang et al., 1997). Like EGCG, it is a polyphenol which can cause G1 cell cycle arrest in various tumor cell lines including pancreatic cancer (Ding and Adrian, 2002). Resveratrol can activate protein kinases such as Jun N-terminal kinase, resulting in phosphorylation and activation of p53. Resveratrol can also induce apoptosis independent of p53 status. Moreover, resveratrol-induced apoptosis was found to be associated with the inhibition of NF- κ B activity in pancreatic cell lines, suggesting its potential chemopreventive activity against pancreatic cancer.

Lycopene and vitamins

Tomato products including ketchup, tomato juice, and pizza sauce, are the richest sources of lycopene in the US diet. It has been reported that dietary intake of lycopene is associated with reduced pancreatic cancer risk, suggesting its role in the prevention of pancreatic cancer (Nkondjock et al., 2005). However, the role of lycopene in pancreatic cancer has been very limited.

The sources of vitamin C are fruits and vegetables, particularly orange, strawberry, citrus, kiwi, and cauliflower. Studies using rats in which pancreatic lesions have been induced with azaserine have shown that a diet high in vitamin C results in reduced tumor formation (Woutersen et al., 1999). It has been reported that a reduced risk for pancreatic cancer is associated with higher intake of vitamin C and D (Skinner et al., 2006; Lin et al., 2005). These limited studies provide little evidence in support of their roles in pancreatic cancer prevention.

The above results especially soy isoflavones, I3C, curcumin, EGCG, and resveratrol appear to target similar signaling pathways all of which are known to be involved in the development and progression of pancreatic cancers and are important targets for pancreatic cancer prevention and/or treatment. It is important to note that primary prevention of pancreatic cancer is not feasible due to lack of identifiable risk factors for the development of pancreatic cancer. However, existing knowledge provides sufficient information as to the novel application of several dietary or nutritional agents for the prevention of pancreatic cancer progression. In addition, these agents could be also useful for the treatment of pancreatic cancer either as single agents or in combination, especially in combination with existing therapeutic agents as discussed in the following paragraphs.

New strategies for the treatment of pancreatic cancers

Targeting EGFR pathway for enhancing cancer therapeutic efficacy

Two classes of EGFR inhibitors, monoclonal antibodies and small molecule tyrosine kinase inhibitors have been considered for the treatment of pancreatic cancer. Cetuximab, a monoclonal antibody targeting EGFR, binds to EGFR competitively with high affinity, preventing activation of EGFR by its ligands. By binding to EGFR, cetuximab inhibits cell proliferation, enhances apoptosis, and reduces angiogenesis and invasion (Marshall, 2006). Cetuximab is currently under investigation in pancreatic cancer. The combination of cetuximab and gemcitabine showed promising activity against advanced pancreatic cancer, and improved survival in animal study (Bruns et al., 2000) and clinical trial (Xiong et al., 2004). Trastuzumab, an anti-HER-2/neu antibody, also showed cell growth inhibitory activity against human pancreatic cancer cell lines and anti-tumor activity in orthotopic mouse model.

Erlotinib (Tarceva) is a small molecule inhibitor of the EGFR tyrosine kinase. Experimental studies have shown that erlotinib inhibits EGFR tyrosine kinase activity and cell growth in pancreatic cancer cell lines and in an animal model (Marshall, 2006). In a phase III trial for patients with advanced pancreatic cancer, erlotinib plus gemcitabine has shown a statistically significant survival benefit compared with gemcitabine alone (Tang et al., 2006). In another study combining erlotinib with gemcitabine and irradiation for locally advanced unresectable pancreatic cancer, most of the evaluable patients showed disease stabilization (Iannitti et al., 2005). When combined with capecitabine, erlotinib has shown considerable anti-tumor activity and better tumor control in a phase II trial for advanced pancreatic cancer. These results suggest that targeting EGFR signaling could be useful for the treatment of pancreatic cancer. Because of the importance of EGFR in pancreatic cancer, we were also interested in finding newer ways to block EGFR signaling and our efforts resulted in the identification and characterization of a novel negative regulator of EGFR, termed EGFR-Related Protein (ERRP), whose expression was found to attenuate EGFR activation.

We have also found that ERRP significantly inhibits cell proliferation and induces apoptosis in BxPC-3, HPAC, and PANC-1 pancreatic cancer cells (Zhang et al., 2005; Zhang et al., 2006). ERRP also inhibits ligand-induced activation of EGFR, HER-2, and HER-3. Most importantly, ERRP was found to inhibit pancreatic tumor growth in a SCID mouse xenograft model. The anti-tumor activity of ERRP correlated well with down-regulation of NF- κ B, MAPK, Akt, and Notch-1 (Wang et al., 2006b; Zhang et al., 2005; Zhang et al., 2006). ERRP also down-regulates NF- κ B downstream genes such as VEGF and MMP-9, and inhibits cancer cell invasion, suggesting that ERRP could be a very potent agent for the treatment of pancreatic cancer by inhibiting cell survival signaling, tumor growth, invasion, and angiogenesis. These results suggest that further development of ERRP for human application is warranted.

Targeting COX-2 for enhancing cancer therapeutic efficacy

As indicated earlier, COX-2 and its metabolic product (PGE₂) play important roles in pancreatic cancer, suggesting that targeting COX-2 could have a therapeutic benefit against pancreatic cancer. Several COX-2 inhibitors have shown their activity in reducing tumor growth with different mechanisms. Indomethacin, one of the COX-2 inhibitors, inhibits both isoforms of the COX enzyme while newer agents, such as celecoxib, inhibit only COX-2 and are more desirable for clinical use. In an orthotopic pancreatic cancer animal model, celecoxib treatment showed inhibition of tumor growth, angiogenesis, and metastasis (Wei et al., 2004). Nonsteroidal anti-inflammatory drugs (NSAIDs) also show their inhibitory effect on COX-2 and PGE₂, leading to a reduced incidence of tumor formation and a reduced number of tumors per animal. Experiments using two NSAIDs, sulindac and NS398, in pancreatic

cancer cell lines, have shown that both agents cause a dose-dependent inhibition of cancer cell growth.

A combination of the agents, celecoxib and Zylflo (a 5-lipoxygenase inhibitor), has shown a reduction in the incidence and size of pancreatic tumors, and a reduced number of liver metastases in animal model. We have previously reported that celecoxib potentiates gemcitabine-induced growth inhibition through the down-regulation of NF- κ B activation and induction of apoptosis in pancreatic cancer cells (El-Rayes et al., 2004). In a clinical trial, the patients with advanced pancreatic adenocarcinoma were given celecoxib in combination with 5-fluorouracil. The results showed that celecoxib with 5-fluorouracil was capable of inducing durable and objective responses, even in gemcitabine-resistant pancreatic cancer (Milella et al., 2004). These results collectively suggest that COX-2 inhibition with other novel agents could be useful in future clinical trials for pancreatic cancer. The concept of extracellular signaling leading to the activation of internal signaling in the pathogenesis of pancreatic cancer has been well recognized. These signaling cascades that lead to the activation of membrane signaling, such as Akt signaling, could be a novel therapeutic target for pancreatic cancer treatment as discussed below.

Targeting Akt to enhance cancer therapeutic efficacy

Akt pathway is another important cell signaling pathway involved in drug resistance. It has been found that genistein enhanced necrotic-like cell death due to the significant inhibition of Akt activity in cancer cells treated with genistein and adriamycin, suggesting that the enhanced growth inhibition of combination treatment is through the inactivation of the Akt pathway (Satoh et al., 2003). Reports from our laboratory and others have also showed that activated Akt is inhibited by genistein combined with gemcitabine or radiation in pancreatic and other cancer cells, suggesting that enhancement of chemotherapeutic or radiation effects by genistein may be partially mediated by the Akt pathway (Yashar et al., 2005; Banerjee et al., 2005). Bava et al reported that curcumin down-regulated Taxol induced phosphorylation of Akt, which interacts with NF- κ B, suggesting that enhanced anti-tumor activity by curcumin is through the inactivation of Akt and NF- κ B pathways (Bava et al., 2005). These results clearly suggest that many chemopreventive agents as discussed earlier are potent inhibitors of Akt and NF- κ B signaling pathways. In addition, it has been reported that a small molecule inhibitor, QLT0254, inhibits tumor growth through the PI3K/Akt pathway and enhances gemcitabine-induced apoptosis in human orthotopic primary pancreatic cancer xenografts (Yau et al., 2005). These results suggest that down-regulation of Akt can enhance cancer therapeutic efficacy. The studies from our laboratory and others have shown that Akt is directly linked with the activation of NF- κ B and as such may represent a novel approach for targeting both Akt and NF- κ B for the prevention and/or treatment of pancreatic cancer.

Targeting NF- κ B for enhancing cancer therapeutic efficacy

De novo resistance to chemotherapeutic agents is a major cause of treatment failure in pancreatic cancer, which could be due to the constitutive activation of NF- κ B among others. Moreover, chemotherapeutic agents can activate NF- κ B in pancreatic and other cancer cells, leading to cancer cell resistance to chemotherapy (acquired resistance). Therefore, the strategies by which NF- κ B could be inactivated represent a novel approach for the treatment of pancreatic cancer.

The *in vitro* and *in vivo* studies from our laboratory and others have demonstrated that the anti-tumor effects of chemotherapeutic agents can be enhanced by combination treatment with genistein through inhibition of NF- κ B. By *in vitro* and *in vivo* studies, we have found that NF- κ B activity was significantly increased by cisplatin, docetaxel, doxorubicin, and gemcitabine treatment and that the NF- κ B inducing activity of these agents was completely abrogated by

genistein pre-treatment in pancreatic cancer cells, suggesting that genistein pre-treatment inactivates NF- κ B and may contribute to increased growth inhibition and apoptosis induced by these agents (Banerjee et al., 2005; Li et al., 2004b; Li et al., 2005a; Mohammad et al., 2006). Recently, we found that anti-tumor and anti-metastatic activities of docetaxel could be enhanced by genistein through regulation of osteoprotegerin/receptor activator of NF- κ B (RANK)/RANK ligand/MMP-9 signaling *in vitro* and *in vivo* (Li et al., 2006). Other investigators have also reported similar results showing that the inhibition of NF- κ B contributes to the sensitization of pancreatic cancer cells to chemotherapeutic agents (Muerkoster et al., 2003; Zhang et al., 2003).

We have found that I3C and DIM inhibit cancer cell growth and induce apoptosis through inhibition of NF- κ B (Rahman and Sarkar, 2005; Li et al., 2005b). We and others have also found that combinations of I3C and cisplatin or tamoxifen inhibit the growth of cancer cells more effectively than either agent alone (Sarkar and Li, 2004; Cover et al., 1999), suggesting that I3C and DIM could potentiate the activity of chemotherapeutic agents by down-regulation of NF- κ B, which appears to be a central and important target for the treatment of pancreatic cancer.

Using gene therapy to enhance cancer chemotherapeutic efficacy

In recent years, gene therapy based on the knowledge of molecular pathogenesis has been actively developed as a novel therapeutic strategy to be used alone or in combination with conventional chemotherapy in pancreatic cancer. Strategies for gene therapy in pancreatic cancer include antisense and RNA interference strategies whereby the function of activated oncogenes (K-ras, H-ras, Notch, LSM1, etc) is inhibited, and strategies to restore the function of tumor suppressor genes (p53, p16, p21, Smad4, etc). These therapeutic strategies have shown their promising effects on the inhibition of pancreatic cancer *in vitro* and *in vivo* (Bhattacharyya and Lemoine, 2006). In addition, gene-directed pro-drug activation therapy is another gene therapy system in which a gene that encodes an enzyme is delivered to tumor cell. Then, a pro-drug administered as chemotherapy is metabolized by the enzyme to release cytotoxic drug at the site of the tumor. A recent report has shown that introduction of a fusion gene combining deoxycytidine kinase and uridine monophosphate kinase, which convert gemcitabine into its toxic phosphorylated metabolite, sensitizes pancreatic cancer cells to gemcitabine by reducing dramatically both *in vitro* cell viability and *in vivo* tumor volume (Vernejoul et al., 2006). Moreover, the combinations of gemcitabine treatment with introduction of IFN- α , NK4, or p53 significantly suppress the growth and the metastasis of human pancreatic cancer cells, suggesting the enhancement of chemotherapy by gene therapy approach in pancreatic cancer (Bhattacharyya and Lemoine, 2006; Ogura et al., 2006). Collectively, the brief information presented in this article provides state-of-the art knowledge toward new and novel therapies for pancreatic cancer and as such must be tested in human patients.

Conclusion

The development and progression of pancreatic cancer are closely associated with the activation of oncogenes, the inactivation of tumor suppressor genes, the deregulation of EGFR, Akt, NF- κ B, and their downstream signaling pathways. Therefore, novel and newer strategies targeting EGFR, NF- κ B, COX-2, and Akt signaling pathways to interrupt their molecular crosstalk (Figure 1) could be promising for the prevention and/or treatment of pancreatic cancer.

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Figure 1.
The major cell signaling pathways involved in the pathogenesis of pancreatic cancer.