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Characterization of the Molecular Genetic Mechanisms that Contribute to Pancreatic Cancer Carcinogenesis

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1. Introduction

Molecular genetic analyses have provided evidence that has helped characterize the carcinogenesis of pancreatic adenocarcinoma. Pancreatic carcinogenesis is a multistep process during which oncogenes are activated, and the function of tumor suppressor genes is lost. *K-ras* mutations, telomere shortening, loss of p16, loss of p53 and loss of smad4 are thought to contribute to pancreatic carcinogenesis. Recent studies have shown that some new signaling pathway contribute to pancreatic cancer development. Because the model of pancreatic cancer development suggests that several genetic alterations accumulate progressively, the molecular mechanisms underlying this disease should be investigated thoroughly. In addition, we have considered of the appearance of epigenetic and microRNA abnormalities in creating a profile of the molecular genetic mechanisms at work in pancreatic cancer carcinogenesis.

This chapter provides an overview of the most relevant molecular genetic alterations that have been implicated in pancreatic cancer development and includes the characterization of the development of precancerous lesions and invasive carcinoma.

2. Molecular genetics understanding of pathway in pancreatic cancer

2.1 Alterations in oncogenes

Many gene mutations have been implicated in the molecular mechanisms of pancreatic cancer formation. In this section, we focus on the oncogenic gene mutations that have been linked to pancreatic cancer.

2.1.1 K-ras

The most frequent genetic abnormality in invasive pancreatic cancer is mutation of the activating K-ras oncogene, which occurs in 75-90% of pancreatic cancers (Ji et al., 2009). K-

ras is a member of the Ras gene family, which is located on chromosome 12p and encodes a 21-kDa membrane-bound GTP-binding protein. This GTP-binding protein mediates various cellular functions, such as proliferation, cellular survival, motility, and cytoskeletal remodeling. The K-ras activating mutations abolish the regulated GTPase activity of the K-ras protein, which converts the Ras protein to the 'on' state and permanently activates downstream signaling events that may contribute to carcinogenesis. K-ras is activated by point mutations, most often in codon 12 but also in codons 13 and 61 (Jones et al., 2008). The role of H-ras, another member of the Ras family, in carcinogenesis is not as well characterized, but it has been reported that H-ras is responsible for mediating the growth-promoting effects in pancreatic cancer cells that possess K-ras mutations (Seufferlein et al., 1999).

The critical role of Ras signaling in pancreatic cancer has been confirmed by many experimental studies. The mutations in the K-ras gene are observed in the earliest form of pancreatic intraepithelial neoplasia (PanIN) lesions and are considered to be one of the earliest genetic events to take place during pancreatic tumorigenesis (Jones et al., 2008; Tada et al., 1996). However, the hyperactivation of the Ras signaling cascade alone is neither sufficient for the malignant transformation nor restricted to malignant pancreatic cells. Instead, Ras hyperactivation may be combined with many genetic abnormalities and signaling pathways to promote pancreatic cancer development. Moreover, K-ras mutations were also detected in nearly 25% of chronic pancreatitis patients and even in healthy elderly subjects (Guerra et al., 2007).

Until now, several studies have focused on K-ras as a therapeutic target and have worked to develop treatments, such as antisense therapy and RNA interference. In a phase II trial of patients with locally advanced and metastatic pancreatic cancers, the Ras family antisense inhibitor showed a response rate of 10.4% and a median survival of 6.6 months when the therapy was combined with gemcitabine treatment (Alberts et al., 2004). RNA interference technology is highly specific, but it has not yet entered the clinical trial stage. However, *in vitro* and *in vivo* studies have provided promising results for the use of RNAi as a pancreatic cancer therapy (Rejiba et al., 2007).

2.1.2 The PI3K/AKT pathway

The PI3K-AKT pathway is one of several signaling pathways that function downstream of K-ras, and it is also activated by mutations during carcinogenesis. AKT proteins are activated through PI3K in response to mitogenic stimulation, such as the activation of EGFR. Several downstream targets, including the mammalian target of rapamycin (mTOR) and the transcription factor NFκB, have a variety of roles in cell proliferation, survival, resistance to apoptosis, angiogenesis and invasion (Schneider & Wolf, 2009).

AKT is amplified and the PI3K–AKT pathway is activated in 20% and 59% of pancreatic cancers, respectively (Schlieman et al., 2003). The amplification of AKT2 genes are also observed in 10% to 20% of pancreatic cancers, and its suppression by antisense RNA results in the reduced growth and tumorigenicity of pancreatic cancer cell lines (Cheng et al., 1996).

Inhibition of this pathway through aberrant expression of PTEN (phosphatase and tensin homolog), which is a natural antagonist of PI3K, is frequently observed in pancreatic cancers (Asano et al., 2004). Furthermore, an architectural transcription factor, HMGA1, activates

PI3K–AKT signaling and appears to mediate resistance to gemcitabine. Together, these observations suggest that this gene is another potential target for inhibition therapy(Kim & Gallick, 2008; Liau & Whang, 2008). Other agents, including everolimus and sirolimus, are currently in phase II clinical trials (Azzariti et al., 2008). Furthermore, PTEN has also been described as a target for treating human pancreatic cancer.

2.1.3 EGF receptor

The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein receptor with an intracellular tyrosine kinase domain. Once bound to its ligands, the protein forms homodimers or heterodimers with other members of the ErbB family, which leads to the phosphorylation of tyrosine residues in its intracellular domain. Intracellular proteins were subsequently activated, which induce downstream signaling events through the PI3K–AKT family, STAT family, and, most notably, the MAPK signaling pathway. STAT proteins have roles in cell proliferation, survival, motility, invasion and adhesion. The mechanisms that lead to inappropriate activation of EGFR include receptor overexpression, activating mutations, overexpression of receptor ligands, and/or the loss of negative regulatory pathways. The overexpression of EGFR and its ligands (EGF and others) and/or the loss of the mechanisms that down-regulate the activity are frequently observed in pancreatic cancer (Bloomston et al., 2006; Preis & Korc, 2010).

A phase III trial that combines gemcitabine and erlotinib, an orally active small molecule that binds to the ATP-binding site of EGFR, has revealed a small but statistically significant increase in the survival of patients with advanced pancreatic cancer compared with gemcitabine treatment alone (Moore et al., 2007).

2.1.4 IGF

The insulin-like growth factor receptor (IGF-R) is structurally similar to the insulin receptor. Insulin-like growth factor-1 (IGF) exhibits structural homology to proinsulin and binds to IGF-R with high affinity and to the insulin receptor with a much lower affinity. Therefore, the insulin-receptor substrate is able to interact with many signaling molecules. These interactions facilitate the activation of multiple downstream signaling pathways, including the PI3K/AKT, MAPK, and JAK/STAT3 pathways, and result in anti-apoptosis and growth-stimulating effects. IGF and its receptors have been extensively studied in various cancers, such as colon, breast and prostate cancer (Moschos & Mantzoros, 2002).

A large portion of the exocrine pancreas is exposed to high levels of insulin, which may act on the exocrine cells via a proxicrine mechanism to provide the pancreatic cancer cells a growth advantage. These high insulin levels can activate both the insulin and IGF receptors. IGF-R is overexpressed in 64% of pancreatic cancers (Moschos & Mantzoros, 2002).

Together, these alterations may work in combination to further enhance cancer growth, indicating that IGF-R may be an important therapeutic target in pancreatic cancer. There are several IGF-1R-targeting agents that are currently being tested in clinical trials. The anti-IGF-R monoclonal antibodies, AMG-479 and IMC-A12, are in Phase I/II studies, which are currently enrolling patients. Moreover, small molecule inhibitors of IGF-R, such as BMS-754807, may provide an alternate approach for targeting this important pathway in pancreatic cancer treatment (Ma & Adjei, 2009).

2.1.5 VEGF

Tumor angiogenesis is essential for tumor growth and is largely mediated by the vascular endothelial growth factor (VEGF) family of proteins and receptors. VEGF is a glycoprotein that promotes endothelial cell survival, mitogenesis, migration, differentiation and vascular permeability. The upregulation of VEGF expression is stimulated by hypoxia and oncogenic proteins, such as Ras. In addition, growth factors, such as EGF, TGF-α, TGF-β, PDGF, and HIF, and cytokines, such as IL-1α and IL-6, can also upregulate the expression of VEGF. VEGF and its receptors are overexpressed in more than 90% of pancreatic cancers and are associated with increased microvessel density, tumor progression and poor prognosis (Seo et al., 2000).

The importance of VEGF and its receptor pathway for the growth of pancreatic tumors was demonstrated in several studies with animal models. These studies showed that VEGF and its receptors are the targets of numerous ongoing clinical trails that are evaluating the efficacy of these treatments in pancreatic cancer (Seo et al., 2000). Several other trials are being conducted to examine bevacizumab in combination with other agents or treatment modalities for pancreatic cancer; however, this agent seems unlikely to confer sufficient benefit to justify licensing for this condition. It has been suggested that angiogenic inhibitors that target other non-VEGF pathways may be better able to gain access to the tumor environment than an antibody (Whipple & Korc, 2008).

2.2 Tumor-suppressor genes and pathways

Tumor suppressor genes inhibit cell proliferation and signaling pathways and induce apoptosis and support DNA repair systems, which are thought to be key events that suppress transformation during tumor carcinogenesis. However, these genes are subjected genetic alterations that reduce or eliminate their normal function.

In pancreatic cancer, the frequently affected tumor suppressors include *p53*, *APC*, SMAD4/*DPC4*, *p16INK4A* and some additional candidate genes. The loss of these tumor-suppressor genes may participate and dominate the signaling pathways in pancreatic tissue carcinogenesis. A summary of these and other tumor-suppressor genes that are altered in pancreatic cancer are discussed below.

2.2.1 p16INK4A/retinoblastoma

The loss of function of the p16 gene, due to mutation, deletion or promoter hypermethylation, occurs in 80-95% of sporadic pancreatic cancers, which is a higher rate than that reported in any other tumor type (Caldas et al., 1994; Rozenblum et al., 1997). The p16 locus is located on chromosome 9q21, and it regulates cell cycle progression by limiting Rb phosphorylation through inhibition of the cyclin D/CDK4/6 complexes (Serrano et al., 1996). The inactivation of the pRb/p16 tumor-suppressor pathway may alter the activity of pRb, CDK4, and cyclin D to promote tumor development (Freeman et al., 2004).

The loss of p16 alone or in combination with the activity of other oncogenes has a significant role in the formation of pancreatic precursor lesions and the development of pancreatic cancer. Immunohistochemical analyses revealed that the loss of p16 protein expression occurred in approximately 30% of PanIN-1A lesions, 55% of PanIN-1B lesions and PanIN-2

lesions, 71% of PanIN-3 lesions and 100% of PDAC (Real et al., 2008). Recently, Aguirre et al. found that p16 limits the malignant conversion of these PanIN lesions to ductal adenocarcinoma in activated *KRAS*-initiated PanIN formation, which suggested that p16 is not the earliest event but is an important event in the progression of pancreatic carcinogenesis (Aguirre et al., 2003).

Clinical research has focused on the contribution of p16 in pancreatic cancer. It appears that p16 plays a significant role in pancreatic carcinogenesis and is an important diagnostic or therapeutic target. Rosty et al. proposed that the loss of the expression of the suppressor gene p16 was a major risk factor for the development of pancreatic cancer in patients with chronic pancreatitis (Rosty et al., 2003). DNA hypermethylation of p16 in pancreatic juice was demonstrated to be a valuable diagnostic marker to predict pancreatic cancer progression. However, further studies are needed to provide evidence for the clinical applications that target the p16 gene (Matsubayashi et al., 2006; Yan et al., 2005).

2.2.2 p53

The p53 locus, which is on the 17p13 chromosome, regulates the cell cycle by integrating numerous signals to control cell death (Rozenblum et al., 1997). The abrogation of p53 activity through mutation occurs in more than 50% of sporadic pancreatic cancers. Wild-type p53 maintains a G2-M arrest and regulates the G1-S checkpoint to facilitate normal cell cycle progression (Vogelstein & Kinzler, 2004). The inactivation of p53 affects PTEN, which inhibits the AKT signaling pathway and induces apoptosis in pancreatic cancers. p53 is short-lived and expressed at very low levels in normal cells, but p53 becomes stable and accumulates if the cell has DNA damage. Pinho AV et al. found that p53 controls both growth and epithelial cell differentiation in the pancreas, which indicates that p53 inactivation in tumors is associated with aggressive biological behavior (Pinho et al., 2011).

Because p53 mutations accumulate relatively late in carcinogenesis, clinical research has focused on the therapeutic contribution of p53 in pancreatic cancer. Patients with pancreatic cancer that carry a p53 mutation have shorter survival rates than patients with wild-type p53. Moreover, tumors that contain a mutated p53 are typically radioresistant and/or chemoresistant, indicating that p53 may serve as treatment indicator in pancreatic cancer (Dergham et al., 1998). In addition, p53 gene therapy strategies can induce tumor regression in patients with advanced NSCLC and with recurrent head and neck cancer (Roth et al., 1999).

2.2.3 SMAD4/DPC4

The SMAD4/DPC4 locus on 18q21 is the critical component of the TGFβ signaling pathway and negatively regulates the growth of epithelial cells (Massague et al., 2000). SMAD4 (DPC) is another commonly mutated gene in PDAC, and it is activated in approximately 50% of pancreatic cancers as a result of homozygous deletion mutations. Wilentz et al. revealed that expression of the SMAD4 protein is associated with the histopathological grades of pancreatic cancer (Hahn et al., 1996). In addition, immunohistochemical assays revealed that the smad4 protein was not expressed in 31% (9/29) of the high-grade lesions (PanIN-3).

Conversely, the loss of SMAD4 expression did not occur in PanIN-1 and -2, indicating that the loss of SMAD4 typically occurs late in PanIN progression to PDAC, similarly to p53 (Miyaki & Kuroki, 2003; Wilentz et al., 2000).

SMAD4 is an integral member of the TGF-β signaling cascade, which plays an integral role in tumor initiation and progression (Bierie & Moses, 2006; Massague, 2008). There are three TGF-β ligands (TGF-β1, TGF-β2 and TGF-β3), which bind to TbRII, TbRI and phosphorylate the downstream mediators SMAD2 and SMAD3. The phosphorylated SMAD2 and SMAD3 for a complex with SMAD4 and enter the nucleus to modulate gene transcription (Derynck & Zhang, 2003).

Clinical research has focused on the therapeutic contribution of smad4 in pancreatic cancer. Melisi D et al. found that the TGF-β/Smad-independent pathway can increase apoptosis inhibitors to produce pancreatic cancer cells that are resistant to the pro-apoptotic effects of gemcitabine (Melisi et al., 2011). Some ongoing clinical trials are employing different TGF-β inhibitors to inhibit the TGF-β signaling pathway in advanced pancreatic carcinoma (Korpal & Kang, 2010; Nagaraj & Datta, 2010). The loss of SMAD4 plays a crucial role in abrogating the TGFβ-mediated cancer cell growth and metastasis. However, further studies are needed to investigate and improve the effectiveness of combined TGFβ inhibitor treatment and SMAD4 gene therapy.

2.2.4 Candidate tumor suppressor genes

2.2.4.1 ARHI gene

The maternally imprinted gene Aplesia Ras homolog member I (ARHI, *DIRAS3*) is a member of the Ras superfamily locus on chromosome 1q. It is a small 26-kDa GTPase that inhibits anchorage-dependent and independent growth, motility, invasion and angiogenesis, despite sharing 54-62% amino acid homology with *Ras* and *Rap* (Yu et al., 1999). Artificially induced expression of ARHI in mice leads to small body size, infertility and decreased lactation (Xu et al., 2000) . Ectopic overexpression of ARHI in cancer cells that express low levels of ARHI triggers apoptosis through a caspase-independent, calpain-dependent mechanism (Bao et al., 2002). Recent studies suggest that the return of ARHI to normal physiological expression levels also induces a G2/M cell cycle arrest, autophagy and tumor dormancy in ovarian cancer (Lu et al., 2008) . The expression and function of ARHI in pancreatic cancer has received relatively little attention. Because ARHI appears to oppose Ras function, and *K-ras* is frequently activated in pancreatic cancers, it is possible that the loss of ARHI contributes to pancreatic carcinogenesis. In the present study, we measured the expression of ARHI in normal and cancerous pancreatic tissue. Yang et al. found that ARHI is widely expressed in the ductal and acinar cells of normal pancreatic tissue but is down-regulated or lost in approximately 50% of pancreatic cancers (Yang et al., 2010). This study also examined the methylation status of ARHI in pancreatic cancer cell lines with low ARHI expression and found that hypermethylation was the main mechanism for the loss of function of ARHI. Stable transfections of ARHI can inhibit cell cycle progression and induce cell apoptosis in pancreatic cancer cells through the inhibition of PI3K/AKT signaling (Lu et al., 2009). The role of ARHI in regulating growth and its loss in half of pancreatic cancers suggest that the loss of ARHI could be an important event in the pathogenesis of pancreatic cancer. However, the identification of clinical applications of ARHI requires further studies.

2.2.4.2 KLF4 gene

The KLF4 gene, which locus on chromosome 9q31.1-3, negatively regulates G protein-coupled mitogenic signal transduction, cell proliferation, transformation, and oncogenesis. Zammarchi F et al. used immunohistochemical analysis to show that the KLF4 protein is expressed in 86.8% cases of DPC (33/38). The overexpression of KLF4 in a human pancreatic carcinoma cell line induced the up-regulation of p21 and the down-regulation of cyclin D1. It appears that the KLF4 gene may be a key suppressor in pancreatic tumorigenesis (Zammarchi et al., 2011).

2.3 Telomere length abnormalities

2.3.1 The definition and function of telomeres

A telomere is a region of repetitive DNA sequences at the end of a chromosome. This region protects the end of the chromosome from deterioration and from fusion with neighboring chromosomes. Human telomeres are nucleoprotein complexes consisting of 8–15 kb of hexameric DNA repeat sequences (TTAGGG) and specifically bound proteins at chromosomes ends (Blackburn, 1991). These structures prevent the chromosome termini from being recognized as double-stranded DNA breaks and are essential for genomic stability (Artandi et al., 2000). During DNA replication, the DNA polymerase protein complex cannot replicate the sequences that are present at the ends. In somatic cells, telomeres become progressively shorter during each round of cell division through replication-dependent loss of the DNA termini (Harley et al., 1990). Over time, due to each cell division, the telomere ends become shorter. This is the reason why telomeres are so important in context of successful cell division; they "cap" the end sequences and are lost in the process of DNA replication. The cell has an enzyme termed telomerase, which carries out the task of adding repetitive nucleotide sequences to the ends of the DNA. Telomerase is the natural enzyme that promotes telomere repair. Its expression is low or absent in somatic cells, but it is active in stem cells, germ cells, hair follicles, and 90 percent of cancer cells (Blackburn, 1991).

The consecutive shortening of telomeres ultimately leads to excessive telomere erosion, loss of telomere capping function, and eventually genetic instability and cellular senescence when telomeres become critically short (Counter et al., 1992). Consequently, epithelial cells with excessive telomere shortening are largely eliminated by protective mechanisms (Artandi et al., 2000). Therefore, telomere shortening has been suggested to be an important biological factor in aging and cellular senescence, which could limit the over-growth of cells and prevent them from transforming into cancer cells.

2.3.2 The relationship between telomeres and human cancer

It is clear that telomeres could function as protectors of chromosome stability and prevent uncontrolled cellular growth. In cancer progression, telomeres help to maintain genomic integrity, similar to the role played by caretaker genes. It is assumed that the loss of telomere function might permit subsequent accumulation of additional genomic changes at the chromosomal level, which may facilitate the progression toward a fully malignant phenotype (Hackett & Greider, 2002).

Telomeres can be maintained through recombination or by telomerase activation. Telomerase is an RNA-dependent DNA polymerase that is generally inactivated in normal human somatic cells. Introduction of telomerase into normal human somatic cells may facilitate unlimited cellular growth and extend the cellular lifespan (Bodnar et al., 1998).

In most human cancers, telomerase was activated through the accumulation of multiple genomic and epigenetic aberrations, and these changes help the cells restore the minimal length of telomeres required to maintain cell function and escape from cellular senescence (O'Hagan et al., 2002). Therefore, the reactivation of telomerase has become an additional hallmark of some human cancers, including pancreatic cancer (Hiyama et al., 1997).

Telomeric fusion is mechanism of telomere dysfunction and leads to uncontrolled mitosis of cancer cells. Telomeric fusions between chromosomal arms may occur in the presence of critically shortened telomere repeat sequences; these fusions lead to ring and dicentric chromosomes that form anaphase bridges during mitosis (Gisselsson et al., 2001).

Highly recombinogenic free DNA ends are generated when anaphase bridges are broken, and fusion of the broken ends results in novel chromosomal rearrangements. Some of these abnormal chromosomes may then form bridges during the next cell division, setting in motion a self-perpetuating breakage-fusion-bridge cycle. The presence of unbalanced chromosomal rearrangements is an essential feature of most human epithelial cancers (Gisselsson et al., 2001) .

2.3.3 The relationship between telomeres and pancreatic cancer

Pancreatic adenocarcinomas, which are remarkable for their highly complex karyotypes, numerous chromosomal abnormalities, and multiple deletions, often possess chromosome ends that lack telomeric repeat sequences (Griffin et al., 1995). The evidence for up-regulated human telomerase reverse transcriptase expression has been demonstrated in invasive pancreatic cancer (Hiyama et al., 1997) and in the intraductal papillary mucinous neoplasms (IPMN) of the pancreas (Hashimoto et al., 2008). Telomere dysfunction was also found to play a role in the multistep progression model for the development of pancreatic cancer. In this multistep model of pancreatic cancer development, noninvasive precursor lesions in the pancreatic ductules accumulate genetic alterations in cancer-associated genes that ultimately lead to the development of an invasive cancer. In the pancreas, the noninvasive precursor lesions are called pancreatic intraepithelial neoplasia or PanIN. PanINs are believed to progress from a flat and papillary appearance without dysplasia to a papillary appearance with dysplasia to carcinoma in situ (van et al., 2002). Telomere fluorescence in situ hybridization and immunostaining was used to assess the telomere length in tissue microarrays containing a variety of noninvasive pancreatic ductal lesions (van et al., 2002) found that the telomere signals were strikingly reduced in 79 of 82 (96%) of PanINs compared with adjacent normal structures. The 82 PanIN lesions that were examined included all histological grades (PanIN-1A, PanIN-1B, PanIN-2, and PanIN-3). Thus, this study reveals that telomere shortening is the most common early genetic abnormality in the progression of pancreatic adenocarcinomas. Telomeres may be an essential gatekeeper for maintaining chromosomal integrity and normal cellular physiology in pancreatic ductal epithelium. A critical shortening of telomere length in PanINs may predispose these noninvasive ductal lesions to accumulate progressive chromosomal abnormalities and to

progress toward the stage of invasive carcinoma. Another research group also found that the telomeres were significantly shortened (97.3%) in 37 intraductal papillary mucinous neoplasm (IPMN) loci of the pancreas, which has been increasingly identified as a precursor to infiltrating ductal adenocarcinoma (Hashimoto et al., 2008) .

Therefore, telomere abnormalities may function as a cancer marker in invasive pancreatic cancer and may also function as the earliest known event in the cascade of pancreatic cancer development.

Telomere shortening has been suggested to be an important biological factor in aging, cellular senescence, cell immortality, and transformation to cancer. Cellular immortality and transformation are associated with the reactivation of telomerase and with telomere dysfunction in cells with critically shortened telomeres and may play an important role in the development of pancreatic cancers.

2.4 Epigenetic abnormalities

Both epigenetic abnormalities and genetic alterations contribute greatly to cancer development at all stages and may drive the initial steps of cancer progression. DNA methylation and chromatin configurations underlie the abnormal patterns in cancer, and cumulative epigenetic abnormalities of the host genes without accompanying changes in the DNA sequences are critical contributors to oncogenesis. Interestingly, cancer-specific epigenetic alterations can be reversed by pharmacological targeting, and increasing attention has been given to this field as a means to treat cancer.

In the United States, it is estimated that 44,030 new cases of pancreatic cancer were diagnosed and 37,660 deaths occurred in 2011(Siegel et al., 2011), which indicates that pancreatic ductal adenocarcinoma is an extremely aggressive and devastating neoplasm. Therefore, a better understanding of pancreatic cancer molecular genetics is important and can provide the basis for the development of valuable biomarkers and targets for therapeutic intervention.

Over the past two decades, extensive interest has revealed many advances in the understanding of genetic alterations that are important in pancreatic cancer. The mutations and deletions of oncogenes and tumor suppressor genes, such as k-ras,p53 CDKN1A/p16, SMAD4/DPC4, etc., appear to play an important role in pancreatic carcinogenesis. In addition, by understanding of the progression of pancreatic cancer, a model of pancreatic carcinogenesis, from precursor lesions to invasive cancers with genetic alterations, was proposed.

Recently, the epigenetic abnormalities found in pancreatic cancers were also of considerable interest among researchers and clinicians. This interest was especially piqued after demethylating drugs, 5-azacytidine (5-aza-CR) and 5-aza-29-deoxycytidine (5-aza-dC), were shown to be effective in treating myelodysplastic syndrome and were approved by the Food and Drug Administration (FDA) (Venturelli et al., 2011). The key epigenetic mechanisms that may affect gene expression include DNA methylation, histone modification, and microRNA expression (Hong et al., 2011). Epigenetic abnormalities may be functionally involved in precursor lesions, tumor growth, invasion and metastasis in pancreatic cancer. In the following section, we will review recent advances in our understanding of the

epigenetic features associated with pancreatic neoplastic progression, specifically focusing on their role in precursor lesions and their potential clinical benefits.

2.4.1 DNA methylation

DNA methylation is a biochemical process where a methyl group is added to the fifth positon of the cytosine pyrimidine ring or the sixth nitrogen of the adenine purine ring. DNA methylation stably alters the gene expression pattern to provide cellular memory or decrease gene expression. DNA methylation also plays a crucial role in the development of nearly all types of cancer. Both hypermethylation and hypomethylation distinguish normal tissue from tissue associated with pancreatic cancer (Jaenisch & Bird, 2003). Hypermethylation is one of the major epigenetic modifications that repress transcription via the promoter region of tumor suppressor genes. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with gene inactivation. Global hypomethylation has also been implicated in the development and progression of cancer through alternative mechanisms (Jeffrey & Nicholas, 2011).

2.4.2 DNA methylation and precursor lesions

It has been shown that PDAC develops through a stepwise progression from preinvasive lesions, including PanINs, IPMNs, and MCNs, to invasive neoplasms (Haugk, 2010). The discovery of abnormal methylation in pancreatic cancer has been followed by the investigation of methylation in precursor lesions. Many genes that are epigenetically silenced in pancreatic cancers also are silenced or have reduced expression in precursor lesions of pancreatic cancer. The molecular genesis of precursor lesions may lay the foundation for our understanding of pancreatic carcinogenesis and the identification of valuable tumor markers and therapeutic targets.

Many genes showed epigenetic abnormalities in precursor lesions of pancreatic cancer, including Reprimo, SPARC, SAPR2, NPTX2, LHX1, CLDN5, CDH3, and ST14 for PanIN and 119 CDKN1C/p57KIP2 and CyclinD2 for IPMN (Fukushima et al., 2002, 2003; Gerdes et al., 2003; Matsubayashi et al., 2003; Sato et al., 2008). Using methylation-specific PCR analysis (Singh & Maitra, 2007), eight genes (Reprimo, SPARC, SAPR2, NPTX2, LHX1, CLDN5, CDH3, and ST14) were tested in 65 PanIN lesions. The results revealed that these eight genes may be detected in more than 70% of the earliest lesions (PanIN-1A). In addition, aberrant DNA methylation can be detected in PanIN-2 and PanIN-3 lesions, which suggests that DNA methylation alterations may begin in the early stages of precursor lesions, such as in PanINs, IPMNs, and MCNs. Moreover, their prevalence was shown to progressively increase during pancreatic carcinogenesis. Because DNA methylation of particular genes can occur in the precursor lesions, the methylation targets may be valuable tumor markers and treatment strategies.

2.4.3 DNA methylation and pancreatic cancer

Changes in the DNA methylation program are closely associated with pancreatic carcinogenesis, including CpG island hypermethylation and hypomethylation (Sato & Goggins, 2006). Recently, high-throughput screening technologies and single gene methylation technologies have identified several genes that are affected by aberrant DNA

methylation in pancreatic cancer. Tan AC et al. detected 1505 CpG sites across 807 genes to identify DNA methylation patterns in the pancreatic cancer genome and found that 289 CpG sites show different patterns in the normal pancreas, pancreatic tumors and cancer cell lines (Tan et al., 2009). The promoter and CpG island array was used to compare the Panc-1 cell lines with a non-neoplastic pancreatic duct line, and 1,010 of 87,922 probes on the 88 K promoter array (606 genes) had higher signals (log2 > 2) in the pancreatic cancer line.

The aberrant hypermethylation of CpG islands is an important cause of altered tumor suppressor gene function in pancreatic cancers. Several of the classic tumor suppressor genes, such as p16, p53, and SMAD4/DPC4, showed DNA hypermethylation, which suggests that DNA hypermethylation is an important mechanism in pancreatic carcinogenesis. DNA hypermethylation has also been observed in many other genes that are implicated in pancreatic carcinogenesis, including TNFRSF10C, NPTX2, SPARC, FOXA1/2, RUNX3, GATA-4, GATA-5, ppENK, CDKN1C/p57KIP2, HHIP, DUSP6, CXCR4, TFPI-2, HIN-1, SOCS-1, WWOX, RASSF1A, CACNA1G, TIMP-3, E-cad, THBS1, hMLH1, DAP kinase, and ARHI (Cai et al., 2011; Dammann et al., 2003; Fendrich et al., 2005; Fu et al., 2007; Gao et al., 2010; Komazaki et al., 2004; Krop et al., 2004; Kuroki et al., 2004; Martin et al., 2005; Nakayama et al., 2009; Nomoto et al., 2008; Ohtsubo et al., 2006; Park et al., 2007, 2011; Sato et al., 2003, 2005, 2005, 2005, 2005; Song et al., 2010; Ueki et al., 2000; Xu et al., 2005).

DNA hypomethylation an additional type of epigenetic alteration that is found in pancreatic cancer (Ehrlich, 2002). Global DNA hypomethylation and hypomethylation of specific genes have been observed. Global DNA hypomethylation is associated with folate metabolism, indicating that essential nutrients are helpful for preventing cancer progression (Gaudet et al., 2003; Kim, 2004). DNA hypomethylation of many oncogenes, such as claudin4, lipocalin2, 14-3-3 sigma, trefoil factor 2, S100A4, mesothelin, PSA, has also been shown to be important for facilitating their over-expression during pancreatic carcinogenesis.

2.4.4 DNA methylation and clinical applications

Does targeting DNA methylation in pancreatic cancer show a clinical benefit as an early detection method or an effective treatment strategy? Initially, the serum level of the hypermethylation of specific genes appeared to hold potential diagnostic value. Gotoh M found that the methylation status of twelve bacterial artificial chromosome (BAC) clones could predict pancreatic tumors with 100% sensitivity and specificity and could also identify patients that would show early relapse with 100% specificity (Gotoh et al., 2011). Park JK found that the level of serum NPTX2 hypermethylation was a valuable diagnostic marker for identifying pancreatic cancers with 80% sensitivity and 76% specificity (Park et al., 2011). Gerdes B et al. found that p16(INK4a) alterations can be observed in a significant number of PanIN1 in chronic pancreatitis tissues, and methylation of the p16(INK4a) promoter may indicate a high-risk for progression from chronic pancreatitis to cancer (Gerdes et al., 2001). In addition, DNA methylation of p16, ppENK, SARP2 and some additional genes was demonstrated to be a valuable diagnostic tool to predict pancreatic cancer (Yan et al., 2005). Overall, the detection of DNA methylation, either alone or in combination with other tumor markers, will be helpful for screening and diagnosing pancreatic cancer.

Importantly, DNA methylation, unlike genetic changes, are considered to be reversible biological alterations, so pharmacological agents that target this change are attractive potential

strategies for treating cancer. Drugs that target the DNA methyltransferase are promising chemotherapeutic agents because this enzyme is a limiting factor for DNA methylation.

Yang et al (Yang et al., 2010). demonstrated that the inhibitor decitabine (5-aza-dC, 2'-deoxy-5-azacytidine DNMT inhibitor) could inhibit pancreatic cancer cell growth, induce apoptosis, induce ARHI gene demethylation and induce ARHI re-expression. Many studies have demonstrated that tumor-suppressor gene expression can be restored by DNMT inhibitors to induce pancreatic cancer apoptosis, including NPTX2, BNIP3, SOCS-1, WWOX, and cyclin D2. Although demethylating drugs have been approved by the FDA to treat MDS, these demethylating drugs must be further investigated to understand the mechanism that prevents pancreatic cancer progression and to predict potential side effects (Matsubayashi et al., 2006; Sato & Goggins, 2006).

2.4.5 Histone modifications and pancreatic cancer

Histone proteins influence chromatin accessibility and gene activity through post-translational modifications (Bernstein et al., 2007; Gaudet et al., 2003; Ting et al., 2006). Histone acetylases/deacetylases, the polycomb group proteins, and HP1 are the key histone protein complexes that influence chromatin accessibility and gene activity. Histone modifications have been linked to the altered expression of several critical genes in pancreatic cancer, including the IL-13 receptor, MUC17, MUC4, MUC1 and MUC2 (Esteller, 2007; Fujisawa et al., 2011; Kitamoto et al., 2011; Vincent et al., 2008; Yamada et al., 2008).

The importance of histone modifications lies in their potential use as a diagnostic and therapeutic intervention. For instance, it has been shown that histone deacetylase inhibitors induce apoptosis of human pancreatic cancer cells. Donadelli M found that histone deacetylase inhibitors, in combination with conventional chemotherapeutic drugs, such as gemcitabine, leads to a synergistic inhibition of pancreatic adenocarcinoma cell growth. In addition, targeting the Polycomb members and HP1 has also been shown to be effective in inhibiting pancreatic cancer cells. Furthermore, Manuyakorn A et al. showed that the pattern of H3K4ME2, H3K9me2 and H3K18ac can predict the prognosis and treatment response of patients (Donadelli et al., 2007; Garcia-Morales et al., 2005; Haefner et al., 2008; Manuyakorn et al., 2010; Yamada et al., 2006).

Recently, many studies have focused only on somatic genetics; however, these areas represent only a small portion of mechanisms that contribute to gene alteration in pancreatic cancer. Epigenetic changes, including CpG island hypermethylation, hypomethylation, and histone modifications, comprise a new arena for pancreatic cancer research, which may provide new diagnostic and therapeutic tools to combat pancreatic cancer. However, many fundamental questions about the biological and clinical significance of epigenetic changes have yet to be answered, and further studies are needed to do to create effective clinical applications for pancreatic cancer.

2.5 Aberrant microRNA expression in pancreatic cancer

2.5.1 Introduction to microRNA

MicroRNAs(miRNAs) are non-protein-coding RNA molecules that are approximately 22 nucleotides and regulate gene function in various silencing pathways. These molecules are

also encoded by genes and are transcribed by RNA polymerase II. miRNAs are phylogenetically conserved and play an important role in cell survival, proliferation, differentiation, apoptosis and angiogenesis (Ambros, 2004; Farh et al., 2005). miRNAs expression patterns differ, depending upon the cell, tissue, and disease type.

miRNAs regulate their targets by direct mRNA cleavage or translational inhibition and each miRNA can regulate multiple target genes.In the most recent database (miRBase release 15), over 21,643 mature miRNAs have been identified in 168 species (Kozomara & Griffiths-Jones, 2011).

2.5.2 miRNAs and pancreatic cancer

The overexpression and deregulation of several miRNAs has been observed in human cancers (Lu et al., 2005; Metzler et al., 2004; Takamizawa et al., 2004). These studies have also shown that miRNA expression signatures correlate well with specific cancer clinical characteristics and could be used to differentiate normal and cancerous tissues, as well as subtypes of malignancy (Calin & Croce, 2006; Cummins & Velculescu, 2006; Dalmay & Edwards, 2006).Deregulation of miRNAs in cancer may be caused by several changes: (1) chromosomal regional gain, loss or translocation, (2) aberrant expression and activation of transcriptional factors, (3) epigenetic alterations, or (4) changes in miRNA processing (Deng et al., 2008).

The miRNA expression profiles in pancreatic tumor tissues are different from those observed in the normal pancreas or in patients with chronic pancreatitis. Most miRNA expression profile analyses show that miRNAs are deregulated in tumor tissues compared with normal pancreatic tissue, and the expression pattern is tissue specific.

Szafranska *et al.* (Szafranska et al., 2007) demonstrated that two miRNAs, miR-216 and miR-217, are pancreas specific, which was in agreement with two previous studies (Sood et al., 2006). Furthermore, both miR-216 and miR-217 are absent or only minimally expressed in pancreatic carcinoma tissues and cell lines. Therefore, miR-216 and miR-217 are potential biomarkers. Based on clustering analysis, the three pancreatic tissue types (normal pancreas, chronic pancreatitis and pancreatic cancer) can be classified according to their respective miRNA expression profiles. Among 26 miRNAs that have been identified as most prominently deregulated in PDAC, only miR-217 and miR-196a have been found to discriminate between normal pancreas, chronic pancreatitis and tumor tissues. These miRNAs are also potential biomarkers.

Zhang *et al.* (Zhang et al., 2009) evaluated 95 miRNAs, which were selected from pancreatic cancer profiling, and correlated them with their potential biological functions, such as cancer biology, cell development, and apoptosis. Among them, eight miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b and miR-95) are differentially expressed in most pancreatic cancer tissues and cell lines. These eight genes are all significantly up-regulated, from 3- to 2018-fold, in pancreatic tumors compared with normal control samples.

miRNAs are functionally classified as oncogenes or tumor suppressors based on whether their targets are oncogenes or tumor suppressor genes. Therefore, oncogenic miRNAs are upregulated in tumors, whereas tumor suppressor miRNAs are downregulated. Torrisani *et*

al. (Torrisani et al., 2009) have reported that the tumor suppressor let-7 miRNA is expressed in normal acinar pancreatic cells but is extensively downregulated in PDAC samples compared with adjacent unaffected tissues.

2.5.3 miRNAs and clinical applications

2.5.3.1 miRNAs as biomarkers for pancreatic cancer diagnosis

Recent studies indicate that aberrant miRNA expression occurs early in the precursor lesions during the multiple stages of pancreatic cancer development. In addition, miRNA profiles may be assessed in more clinically accessible samples, such as pancreatic juice, and may be used as a diagnostic tool.

Szafranska *et al.* (Szafranska et al., 2008) identified potential miRNA markers in EUS-FNA biopsies of pancreatic tissue. The results show that the combined expression pattern of miR-196a and miR-217 can differentiate PDAC cases from healthy controls and chronic pancreatitis in the FNA samples. Furthermore, miR-196a expression is likely to be specific to PDAC cells and is positively associated with the progression of PDAC.

The potential use of these miRNAs as biomarkers has been evaluated in pancreatic juices. Habbe *et al.* (Habbe et al., 2009) have observed significant overexpression of 10 miRNAs in IPMNs (n = 15). miR-155 and miR-21 show the highest relative fold-changes in the precursor lesions. The upregulation of both miR-155 and miR-21 in the subset of IPMN-associated pancreatic juices was observed.

Wang *et al.* (Wang et al., 2009) have studied plasma samples from patients with PDAC and found that four miRNAs (miR-21, miR-210, miR-155 and miR-196a) are able to differentiate pancreatic cancer patients from healthy controls with moderate accuracy (64% sensitivity and 89% specificity).

2.5.3.2 miRNAs as therapeutic targets in pancreatic cancer

Several studies have shown that the events leading to EMT are regulated by miRNAs (Gregory et al., 2008; Korpal & Kang, 2008; Wellner et al., 2009). Li *et al.* (Li et al., 2009) investigated the effects of let-7 and miR-200 on the morphological changes of EMT in gemcitabine-resistant pancreatic cancer cells (GRPCCs). They noted several observations: (1) the expression of miR-200 and let-7 is significantly downregulated in GRPCCs, which have EMT characteristics; and (2) transfection of GRPCCs with miR-200 rescues the epithelial phenotype by upregulating the epithelial marker E-cadherin and downregulating the mesenchymal markers ZEB1 and vimentin.

Oh *et al.* (Oh et al., 2010) have shown that upregulation of let-7a results in the attenuated expression of Kras and increased radiosensitization of pancreatic cancer cells. This suggests that miRNA could be used as a valuable therapeutic option in radioresistant tumors that have K-ras mutations.

Weiss *et al.* (Weiss et al., 2009) have shown that miR-10a expression promotes metastasis, and repression of miR-10a inhibits invasion and metastasis in xenotransplantation experiments using zebrafish embryos. These data also suggest new therapeutic applications for miRNA in patients with metastatic pancreatic cancer.

Moriyama *et al.* (Moriyama et al., 2009) showed that miR-21 could be a target for a therapeutic strategy for patients with chemoresistant pancreatic cancer. Ji *et al.* (Ji et al., 2009) showed that miRNAs, such as miR-34, can be a novel molecular therapy for human pancreatic cancer *via* inhibiting pancreatic cancer stem cell differentiation.

Overall, many reasearchers suggest that miRNA play an important role in pancreatic carcinogenesis. However, many questions about the function and clinical application need to be further answered for pancreatic cancer.

2.6 A multistep model that involves the accumulation of genetic alterations during the development of pancreatic cancer

We now know that the development of pancreatic cancer, like other malignant diseases, is a multistep process involving the accumulation of genetic and epigenetic mutations. Furthermore, it has been shown that some genetic alterations occur early in the disease and can be designated disease-promoting mutations, whereas others occur later and enhance the oncogenic potential of earlier mutations. Three different types of preneoplastic lesions have been identified in the pancreas: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasia (IPMN) and mucinous cystic neoplasms (MCN). Of these, PanIN lesions are the best characterized, both genetically and pathologically. A well-known progression model of pancreatic cancer development explains that normal pancreatic ductal cells progress from flat (PanIN-1A) and papillary lesions (PanIN-1B) without dysplasia to papillary lesions with dysplasia (PanIN-2) to carcinoma in situ (PanIN-3) and finally to invasive pancreatic cancer (Hruban et al., 2008).

There are two distinct genetic events that occur in the early stages of pancreatic cancer PanIN -1 lesions: telomere shortening and K-ras mutations (Hruban et al., 2000). Activating point mutations of K-ras occur in approximately 45% of PanIN-1 lesions (Hingorani et al., 2003). Telomere shortening is found in approximately 90% of PanIN-1 lesions and may contribute to global chromosomal abnormalities in PanINs (van et al., 2002). Inactivating mutations of CDKN2A/p16 begin to occur in PanIN-2 lesions, whereas inactivation of TP53, SMAD4/DPC4, and BRCA2 are generally associated with higher-grade PanIN lesions (PanIN-3) (Schonleben et al., 2008).

Furthermore, a recent study described a cell surface marker-mediated system for identifying pancreatic cancer stem cells. Pancreatic cancer cells share several features with embryonic pancreatic cells, including activation of the Notch and Hedgehog signaling pathways, which regulate the growth of many organs during embryogenesis and is aberrantly activated in pancreatic cancer cells (Hong et al., 2011; Wong & Lemoine, 2009). The Notch pathway is a critical regulator of pancreatic development and appears to be active in the early stages of pancreatic cancer initiation as well as in invasive cancers. Activation of this pathway leads to the proteolytic intramembrane cleavage of Notch receptors, which results in the release and translocation of their active intracellular domain to the nucleus. Moreover, the upregulation of several Notch target genes in invasive pancreatic cancer as well as preneoplastic lesions suggests that this pathway is an important contributing factor in the development of pancreatic cancer (Maitra & Hruban, 2008).

The activity of the Hedgehog pathway is another important pathway in the development of the gastrointestinal tract and has been implicated in the development and maintenance of

the pancreatic cancer phenotype. The Hedgehog family is composed of Sonic Hedgehog (Shh), Indian Hedgehog (Ihh), and Desert Hedgehog (Dhh). Many studies have shown that many of the components of the Hedgehog family show abnormal expression in pancreatic cancer and precursor lesions (Dosch et al., 2010). These studies indicate that Hedgehog signaling plays a role in the initiation and growth of pancreatic cancer (Kayed et al., 2006). Overall, multistep changes and pathway involves the development of pancreatic cancer.

3. Conclusion

As in colorectal cancer, two distinct tumor categories exist in pancreatic cancer, which are distinguishable by the predominant mutagenic mechanism. Most pancreatic cancers exhibit chromosomal instability (CIN), which causes numerous gross chromosomal changes that result in aneuploidy. A second category is characterized by microsatellite instability (MSI) (Vogelstein & Kinzler, 2004), which results in a drastically decreased fidelity of DNA replication and repair due to defects in the DNA mismatch-repair pathway. Therefore, MSI tumors exhibit frequent errors during DNA replication, which are particularly pronounced at repetitive sequences termed microsatellites.

In the past decade, major advances have been made in understanding the earliest histological and molecular changes that occur in precursor lesions and cancers of the pancreas (Hruban & Adsay, 2009). In addition, the identification of molecular signatures that mark the earliest changes of carcinogenesis may lead to the earlier detection of pancreatic cancer. Understanding the signature of molecular alterations that occur before the development of invasive pancreatic cancer may lead to improved detection and survival of pancreatic cancer patients.

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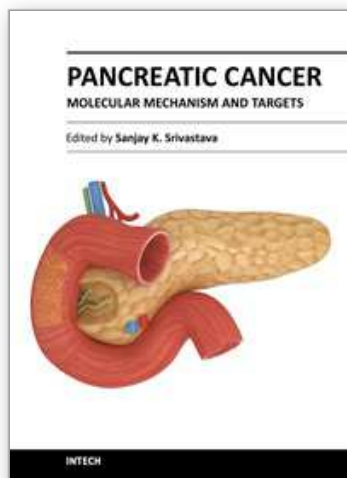
Pancreatic Cancer - Molecular Mechanism and Targets

Edited by Prof. Sanjay Srivastava

This book provides the reader with an overall understanding of the biology of pancreatic cancer, hereditary, complex signaling pathways and alternative therapies. The book explains nutrigenomics and epigenetics mechanisms such as DNA methylation, which may explain the etiology or progression of pancreatic cancer. Book also summarizes the molecular control of oncogenic pathways such as K-Ras and KLF4. Since pancreatic cancer metastasizes to vital organs resulting in poor prognosis, special emphasis is given to the mechanism of tumor cell invasion and metastasis. Role of nitric oxide and Syk kinase in tumor metastasis is discussed in detail. Prevention strategies for pancreatic cancer are also described. The molecular mechanisms of the anti-cancer effects of curcumin, benzyl isothiocyante and vitamin D are discussed in detail. Furthermore, this book covers the basic mechanisms of resistance of pancreatic cancer to chemotherapy drugs such as gemcitabine and 5-flourouracil.

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