Chronic Pancreatitis and Pancreatic Cancer

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Xiang-Yu Kong and Ke-Ping Xie

1 Introduction

Pancreatic cancer is a fast progressive and highly aggressive malignancy. Clinically, pancreatic cancer exhibits profound resistance to existing treatment modalities and surgical resection remains the only chance for cure of pancreatic cancer patients (Vincent et al. 2011). Development of efficient early detection modality is of highest priority. Epidemiological studies have shown that chronic pancreatitis is one of the major risk factors for pancreatic cancer, and patients with chronic pancreatitis have 2.3 to 18.5-fold increased risk than normal controls (Malka et al. 2002). Delineating the progression from chronic pancreatitis to pancreatic cancer and identifying the genetic and epigenetic events in this process will help detect early-stage pancreatic cancer in chronic pancreatitis cases and improve prognosis of these patients.

X.-Yu. Kong
Department of Gastroenterology, Shanghai Changhai Hospital, Shanghai, China

K.-P. Xie (✉)
Departments of Gastroenterology, Hepatology and Nutrition, Unit 1644, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA
e-mail: kepxie@mdanderson.org

Hereditary pancreatitis represents the best model to dissect the causal linkage between chronic pancreatitis and pancreatic cancer. Epidemiological analysis has shown that hereditary pancreatitis ranks the strongest known risk factor for pancreatic cancer (Schneider and Whitcomb 2002). With the advent of molecular techniques, several germ-line mutations within certain genes, *e.g.*, PRSS1 and CFTR, are identified as the major cause for hereditary pancreatitis (Keiles and Kammesheidt 2006). However, these mutations are rarely detected in common chronic pancreatitis cases, indicating that these genes are not directly associated with development of pancreatic cancer (Hengstler et al. 2000; Malats et al. 2001). It is widely believed that inflammation itself, but not those hereditary genes, promotes pancreatic cancer development and progression (Lu et al. 2006).

Once inflammation is initiated, inflammatory cells, especially macrophages, are recruited to the inflicted sites and release cytokines, growth factors, matrix-degrading enzymes and others (Jackson and Evers 2006). All those inflammatory factors, in combination with macrophages, constitute the extrinsic signals, which reprogram the microenvironment to facilitate pancreatic cancer development and progression (Jackson and Evers 2006). Evidently, the inflammatory milieu may disable surveillance mechanisms and destabilize the genome in normal pancreatic cells, which will enhance the generation and accumulation of genetic events within these cells

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and accelerate the process of pancreatic tumor formation. Mechanistically, a number of dysregulated signaling pathways are commonly identified in both chronic pancreatitis and pancreatic cancer tissues. Of clinical significance, most of those altered pathways, including cytokines, NF-κB, reactive oxygen species, PPARγ, and so on, have potential value in pancreatic cancer treatment (Uomo et al. 2010).

However, chronic pancreatitis and pancreatic cancer are two entirely different diseases with distinct prognosis. Under certain circumstances, it is clinically difficult to distinguish from each other (Sakorafas and Tsiotou 1999). Given that numerous signaling pathways are similarly altered in both chronic pancreatitis and pancreatic cancer; it is a daunting challenge to identify potential biomarkers for the differential diagnosis of the two diseases. Currently, all the global proteomic studies have failed to identify pancreatic cancer specific biomarkers, which may arguably be due to the low abundance of those biomarkers in human tissues. Nonetheless, recent studies have discovered a list of protein markers, a combination of which may enhance the diagnostic efficacy for pancreatic cancer (Chan et al. 2014). Moreover, the stability and abundance of DNA and microRNAs in human tissues have also been explored for their clinical utility as pancreatic cancer biomarkers (Schultz et al. 2014).

2 Pancreatic Cancer Is Inflammatory Malignancy

Extensive histopathological studies have identified different precursor lesions as having the potential to evolve into highly malignant and invasive pancreatic cancer, including chronic pancreatitis, pancreatic intraepithelial neoplasia (PanIN), mucinous cystic neoplasms, and intraductal papillary mucinous neoplasms (Brugge et al. 2004; Maitra et al. 2005). PanINs are the most common precancerous lesions in pancreas, constituting the development course of pancreatic cancer (Costello et al. 2012), which is somewhat similar to that of adenoma-carcinoma sequence in the development of colon cancer (Vogelstein et al. 1988).

It have now well documented concerning the genetic and epigenetic alterations accompanying the sequential course of cellular transformation from normal pancreas to pancreatic cancer (Bardeesy and DePinho 2002). Multi-modality analyses with clinic-pathological parameters have further characterized the clinical significance of those alterations in detection and diagnosis, prognostic prediction, treatment selection, and etc. *K-ras* is the most notable and prevalent oncogene identified in pancreatic cancer cells. Although occasionally occurring in normal pancreatic tissue and only 30% of pancreatic lesions at the earliest stage of histopathological disturbance (Klimstra and Longnecker 1994), the frequency of K-ras activation increases as the disease progresses and is found in almost all PDAC cases, making this mutational activation virtually essential for PDAC pathogenesis (Rozenblum et al. 1997). Identification of K-ras mutation as the first notable genetic alteration led to much better understanding of pancreatic cancer genetics, which are including inactivation of tumor-suppressive genes, *e.g.*, p16/CDKN2A, TP53, and SMAD4 (Bardeesy and DePinho 2002). A recent landmark study of sequencing of 23,219 transcripts reveals 20,661 protein-coding genes in 24 PDAC cases. This detailed global genomic study has identified a large number of genetic alterations, among which a core set of 12 signaling pathways and processes are shown to have an altered gene expression in 67–100% of pancreatic cancer cases (Jones et al. 2008).

Apparently, pancreatic cancer takes 20 years to grow into a detectable tumor, and during this time course, an average of 63 genetic alterations happen in each case (Jones et al. 2008). Two prerequisites for pancreatic cancer are proposed: first, there must be immortal pancreatic cells to accumulate these genetic events; second, there must be harsh milieu to efficiently induce the genetic events to happen. Existence of cancer stem cells meets the first prerequisite, whereas the second prerequisite may largely be attributed to chronic inflammation surrounding pancreatic cells (Cooks et al. 2014). Lessons from ulcerative

colitis show that the inflammation process will lead to repeated cycles of epithelial cell damage and regeneration, which presumably increase the possibility of somatic mutations and favor cellular transformation and tumorigenesis (Itzkowitz and Yio 2004). Chronic pancreatitis may follow a similar path to pancreatic cancer.

On the other hand, the entire process of pancreatic cancer development and progression is full of inflammation. The inflammatory response is observed at the early stages of pancreatic cancer initiation. For example, we have recently found that active infiltration of inflammatory cells and immune cells is observed during acinar-to-ductal metaplasia phase and KLF4 plays a critical role in inflammatory response, which appears to be important for PanIN formation and progression to late-stage invasive pancreatic cancer. The inflammation is also important to pancreatic desmoplasia. Therefore, pancreatic inflammation could be a cause of pancreatic cellular transformation and cancer initiation, and also could be a driver force of pancreatic cancer promotion and progression.

3 Chronic Pancreatitis Is Malignant Driver

Chronic pancreatitis is a progressive inflammatory disease with irreversibly functional and morphological changes caused by various etiological factors (Liao et al. 2013). Recent investigation on 2008 patients with chronic pancreatitis shows that its incidence in China is increasing (13/100,000) (Wang et al. 2009). Given that inflammation is one of the major risk factor for carcinogenesis, chronic pancreatitis patients would be more susceptible to pancreatic cancer. However, it was Lowenfels and colleagues, who published an international cohort study on clarification of the nature of the risk in 1993 (Lowenfels et al. 1993). In their multicenter cohort study, 2015 chronic pancreatitis cases were enrolled and 56 cancers were identified during a mean follow-up of 7.4 years. The standardized incidence ratio was 14.4 and the risk of developing pancreatic cancer, 20 years after diagnosis, was as high as 4%. Similar studies further confirmed the increased cancer risk in chronic pancreatitis patients, which underlines the significance of differential diagnosis between chronic pancreatitis and pancreatic cancer (Bansal and Sonnenberg 1995).

Hereditary pancreatitis represents the best model to dissect the causal link between chronic pancreatitis and pancreatic cancer. Epidemiological and experimental analyses show that hereditary pancreatitis ranks the strongest known risk factor for pancreatic cancer (Schneider and Whitcomb 2002). By the age of 70 years old, 40% of patients with hereditary pancreatitis will develop pancreatic tumors (Lowenfels et al. 1997). Genetic studies identified germ-line mutations in the PRSS1 gene and CFTR gene as leading causes to hereditary pancreatitis. One important question was whether these genes actually oncogenes for development of sporadic pancreatic carcinomas. To address this question, Hengstler et al. analyzed genomic DNA in pancreatic tissue for R122H mutations in the trypsinogen gene from 34 patients and corresponding normal tissue from 28 of these individuals. No mutations were found (Hengstler et al. 2000). Malats et al. have also shown that the incidence of mutations of CFTR gene in sporadic pancreatic cancers were similar to that in healthy controls (Malats et al. 2001). These studies suggest that PRSS1 and CFTR gene mutations are not directly associated with the development of pancreatic cancer. Interestingly, hereditary pancreatitis arises mainly during or soon after childhood, which means that those patients with hereditary pancreatitis will endure chronic inflammation with onset of an early age (Raimondi et al. 2009). Though mutations in PRSS1 and CFTR genes do not directly contribute to pancreatic cancer development and progression, the high-risk inflammation milieu caused by them may play important roles in this regard.

Histopathologically, pancreatic cancer contains two compartments as major components of pancreatic cancer, the malignant ductal cells and the surrounding stromal cells, with the latter accounts for 90% of total tumor mass (Neesse

et al. 2011). Previous studies establish that the stroma formation was initiated and sustained by the out-of-control inflammation, and recent studies from genetic mouse models indicate that inflammation induced by pancreatitis will significantly promote pancreatic tumor formation (Carriere et al. 2009; Gidekel Friedlander et al. 2009; Guerra et al. 2007; Morris et al. 2010). Though it is evident that chronic pancreatitis is a significant risk factor in inducing cellular transformation and pancreatic carcinogenesis, detailed mechanisms underlying the progression from chronic pancreatitis to pancreatic cancer remain to be explored (Kong et al. 2012).

4 Pathways Linking Chronic Pancreatitis to Pancreatic Cancer

The concept of the strong link between inflammation and cancer was first proposed in the nineteenth century by Virchow, who observed the presence of inflammation cells within neoplastic tissues (Balkwill and Mantovani 2001). Epidemiological and clinical researches support his hypothesis and reveal the causal link between chronic inflammation and cancer. For example, the ulcerative colitis, which is a common chronic inflammatory disease affecting the large bowel mucosa, may provide the strongest evidence to whether and how inflammation affect carcinogenesis progress. Patients with ulcerative colitis are more predisposed to colorectal cancer, which is in the order of tenfold greater than that in the general Western population (Itzkowitz and Yio 2004). Inflammation-cancer connection is not unique to a subset of tumors, but universally identified within different cancer types, including lung, bladder, gastrointestinal tract, skin and vulva. Use of anti-inflammatory medications, *e.g.*, aspirin, is usually associated with protection against various tumors, which to some extent substantiate that inflammation is a risk factor for many types of cancer (McKay et al. 2008). While the link between inflammation and cancer is clearly strong, detailed mechanisms underlying this connection warrant more studies.

The chronic pancreatitis-pancreatic cancer link has also been established in different ethnic cohorts independently by different groups. Nonetheless, "chronic pancreatitis-pancreatic cancer" connection holds certain traits different from other cancer types. For example, COX-2 levels were significantly elevated in chronic pancreatitis patients, and targeted therapy against COX-2 appears be a good therapeutic strategy to treat chronic pancreatitis (Reding et al. 2006). However, aspirin, the most commonly used COX-2 inhibitor, exhibits controversial effects to pancreatic cancer (Jacobs et al. 2004). Extended periods of regular aspirin use may likely increase risk of pancreatic cancer among women (Schernhammer et al. 2004). These inconsistent results suggested that there exist a complex network among pancreatic cells and inflammatory milieu, and a list of comprehensive studies is needed to explore or validate current knowledge framework concerning "inflammation-cancer" connection in pancreatic cancer. To this end, multiple animal models have been established for dissecting the effects of inflammatory mediators and anti-inflammatory drugs on "chronic pancreatitis-pancreatic cancer" progression (Mazur et al. 2015). Currently used animal models are primarily developed based on the genetic activation of resident *K-ras* oncogenes knocked-in within the endogenous *K-ras* locus (Olive et al. 2009). These models faithfully recapitulate the histological lesions that characterize many aspects of human pancreatic tumors, including a desmoplastic stroma and inflammatory responses that closely resemble those observed in human patients. Of particular interest, these animals will not develop into pancreatic cancer, unless undergo pancreatic damage in the form of pancreatitis (Hingorani et al. 2003). These results further confirm the indispensable functions of inflammation in pancreatic carcinogenesis. Many insightful mechanisms have been identified linking pancreatitis and pancreatic cancer by using the genetic animal models and can be arguably grouped into intrinsic pathway and extrinsic pathway.

4.1 Extrinsic Pathways Linking Chronic Pancreatitis and Pancreatic Cancer

Besides neoplastic cells, the pancreatic cancer mass is composed of a stroma constituting of fibroblasts, vessels and leukocytes. Inflammation is the primary insult causing the robust stroma reaction surrounding pancreatic cancer cells (Elinav et al. 2013). Significantly, inflammatory cells, especially those leukocytes, are the major players in initiating and sustaining the inflammation reaction. The leukocytes within tumor stroma, as well as those cytokines and growth factors derived from leukocytes, constitute the complex extrinsic pathways, which play a critical role in cellular transformation and tumorigenesis process (Hidalgo 2010).

Tumor associated macrophages (TAMs) are the principal leukocytes driving an amplification of the inflammatory response in the tumor milieu. TAMs belong to the myeloid cell lineage and derive from myeloid progenitor cells. These precursor cells are located in the bone marrow; upon maturation, monocytes are released into the bloodstream. At the recruitment by certain chemokines, *e.g.*, CCL2 and CCL5, TAMs accumulate in tumor stroma, where TAMs are educated to facilitate cancer progression. TAMs assists tumor cell malignant behavior in many ways by releasing cytokines, growth factors and matrix-degrading enzymes and many angiogenic factors (Colotta et al. 2009). Numerous molecular alterations are involved in "TAM-pancreatic cancer" connection. For examples, administration of agonist CD40 antibody will activate macrophages, which help macrophages infiltrate tumors, became tumoricidal, and facilitated the depletion of tumor stroma (Beatty et al. 2011). TAMs secrete MIP3α to increase migration ability of pancreatic cancer cells by binding to the transmembrane receptor CCR6 (Campbell et al. 2005). TAMs could convey proangiogenetic effects to pancreatic cancer cells. Blocking angiopoietin-2 (ANG2), a TIE2 ligand and angiogenic factor, could impede the upregulation of Tie2 in TAMs, and decrease tumor angiogenesis of pancreatic cancer (Mazzieri et al. 2011). Furthermore, TAMs could help maintain the cancer stem cells, which have been linked to chemoresistance, metastatic dissemination, and the induction of immune suppression (Mitchem et al. 2013). Blockage the connection between TAMs and cancer stem cells could eliminate the cancer stem cells, improve chemotherapeutic response, and generate promising therapeutic efficacy. Therefore, TAMs and the complex cytokines network greatly enhance the tumorigenesis of pancreatic cancer. Targeted treatment against TAM may be promising in future pancreatic cancer therapeutics.

4.2 Intrinsic Pathways Within Pancreatic Cancer Cells Modulated by Chronic Pancreatitis

Previous studies have reported that each pancreatic cancer case contain an average of 63 genetic alterations, most of which are point mutations. These alterations define a core set of 12 cellular signaling pathways (Jones et al. 2008). However, the rates of spontaneous mutations within normal pancreatic cells are very low. Compared with normal pancreatic cells, the widespread destabilization of gene copy number and nucleotide sequence assure us that instability of the genome is universally inherent within pancreatic cancer cells (Hanahan and Weinberg 2011). In normal cells, there is extraordinary ability of genome maintenance systems to detect and resolve defects in the DNA, whereas in cancer cells, the protection mechanisms are defected and enable these cells to accumulate more genetic alterations and advantageously develop into a tumor (Hanahan and Weinberg 2011).

Evidently, inflammatory microenvironment participates in genome instability of pancreatic cancer cells. For example, Bielas et al. have reported that the mutation rate in the inflamed microenvironment is higher than in normal tissues, with a mutation frequency of 4×10^{-8} and $<1 \times 10^{8}$ per base pair, respectively (Bielas et al. 2006). The hallmark suppressor p53, critical in protecting genomes from instability, shows high

frequency of mutations in chronic pancreatitis (Gansauge et al. 1998). Higher incidence of mutations within pancreatic cancer cells is largely attributed to the deregulate DNA repair systems and altered cell cycle checkpoints, whereas derivatives generated by inflammatory cells are also responsible for the destruction against these genome maintainers. For example, inflammatory cytokines, *e.g.*, TNF and IL-1β, could induce HIF-α in pancreatic cancer cells, which may destruct the mismatch repair mechanisms and leads to instability of genomes (Akakura et al. 2001). Nitrogen oxide (NO), another important mediator derived from inflammation microenvironment, could induce upregulation of DNA methyltransferase and result in promoter silencing and loss of gene expression of the mismatch repair member hMLH1 (Fleisher et al. 2000). NO and its derivatives could also inhibit the function of p53 and are associated with p53 mutations (Jaiswal et al. 2001), which will significantly attenuate its detection and repair ability against genome instability. Other inflammatory elements, including COX-2, reactive oxygen species, and MMPs can also tamper the genome surveillance machinery (Hanahan and Weinberg 2011). Through these different mechanisms, inflammatory elements render the cancer genomes instable, which leads to a genomically heterogeneous population of expanding cells naturally selected for their ability to proliferate, invade and metastasize to distant tissues, and evading host defenses (Hanahan and Weinberg 2011).

5 Common Alterations Involved in Pancreatic Cancer and Chronic Pancreatitis

Epidemiologic data identified inflammation as a significant risk factor for solid tumors. Both hereditary and sporadic forms of chronic pancreatitis are associated with increased risk of pancreatic cancer. In such context, the focus of cancer research have recently shifted from pancreatic cancer cells to the inflammatory milieu surrounding them, and identified a list of notable targets involved in inflammation-associated carcinogenesis. Targeted therapy against certain molecules generates promising effects in clinic. Novel therapeutics targeting the extrinsic and intrinsic pathways linking chronic pancreatitis and pancreatic cancer would decrease the levels of tumor-promoting properties of the inflammatory cells, and ultimately balance the inflammatory network to regain a normal homeostasis.

Cytokines The progressive and irreversible desmoplasia within chronic pancreatitis are largely attributed to the cytokines existing in stroma (Cavestro et al. 2003). Many cytokines have been identified within chronic pancreatitis, including TNF-α, IL-1, IL-6, IL-8, PDGF, TGF-β, and etc. A similar expression pattern of chemokines is found in pancreatic cancer (Farrow and Evers 2002). As pancreatic inflammation represents an early step in the development of pancreatic cancer (McKay et al. 2008), it is logical to believe that these cytokines are engaged in pancreatic carcinogenesis. Numerous studies have demonstrated the tumor-promoting roles of these cytokines in pancreatic carcinogenesis (Yu and Kim 2014). For example, TNF-α is normally expressed under the context of pancreatic acinar cell injury, whereas in chronic pancreatitis, TNF-α could upregulate PDGF expression, which is known to strongly stimulate fibrogenesis (Friess et al. 1999). PDGF, as well as other TNF-α downstream targets, *e.g.*, EGFR and TGF-α, are all well-known oncogenes in pancreatic carcinogenesis (Kalthoff et al. 1993). Through NF-κB activation, TNF-α may also inhibit apoptosis of pancreatic cancer cells (McDade et al. 1999). These data suggest that abnormal cytokine expressions in chronic pancreatitis are significantly associated with pancreatic carcinogenesis. A new generation of vaccines directed against cytokine activity could be beneficial in future treatment of cancer (Zagury et al. 2001).

Nuclear factor kappa B Nuclear factor kappa B (NF-κB) is an important transcription factor proved to be involved in multiple cellular activities (Suzuki et al. 2011). The functional NF-κB in

pancreas is a p65/p50 heterodimer. Under normal conditions, NF-κB dimers are bound to inhibitory proteins, IκBs, which block nuclear localization sequences and thus trap the dimers within the cytoplasm where they were inactive (DiDonato et al. 1997). However, in inflammatory response, *e.g.*, pancreatitis, IκBs are degraded and NF-κB subsequently translocate into the nucleus, where it interacts with other transcription factors and binds to its consensus sequence on promoters of target genes (Huang et al. 2013). Enhanced NF-κB activity is associated with increased severity of acute and chronic pancreatitis (Huang et al. 2013). NF-κB activity is ubiquitously unregulated in many cancer types. It has been suggested that NF-κB plays its role in carcinogenesis through its inhibition of apoptosis of preneoplastic cells and the maintenance of a pro-neoplastic microenvironment rich in proinflammatory mediators (McKay et al. 2008). Targeted therapy against NF-κB could induce apoptosis and increase gemcitabine effectiveness in a subset of pancreatic cancer cells (Pan et al. 2008). Due to the critical role that NF-κB plays in linking chronic pancreatitis and pancreatic cancer, restoration of its expression and function may decrease the tumor-promoting effects of inflammatory cells, with hope to orchestrate the homeostatic relationship between inflammation and pancreatic cells.

Peroxisome proliferator-activated receptor-γ Peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear receptor and transcription factor, which can repress inflammatory genes and orchestrate inflammation homeostasis (Glass and Saijo 2010). Ligands of PPARγ play important roles in preventing the out-of-control inflammation in various tissues. For examples, different PPARγ agonists could reduce the severity of cerulein-induced acute pancreatitis (Hashimoto et al. 2003; Cuzzocrea et al. 2004). PPARγ agonists could inhibit the proinflammatory cytokine gene expression within macrophages to prevent the development of chronic pancreatitis (Shimizu et al. 2002). Also, PPARγ overexpression could inhibit pro-fibrogenic activities of immortalized rat pancreatic stellate cells to suppress chronic inflammation process (Jaster et al. 2005). Furthermore, the impact of PPARγ on pancreatic cancer development and progression has support the notion that chronic pancreatitis is strongly associated pancreatic cancer. For examples, PPARγ ligand could suppress cancer growth (Elnemr et al. 2000). A list of targets, including cyclin D1, p27KiP1, and PTEN are implicated in its anti-tumor effects (Diao and Chen 2007). Troglitazone is a well-known PPARγ ligand, and clinical studies have shown that troglitazone could significantly lower PSA levels in prostate cancer patients (Mueller et al. 2000). However, its therapeutic efficacy against pancreatic cancer have not been evaluated in clinics.

Reactive oxygen species Reactive oxygen species are generated by activated neutrophils and macrophages during inflammation. Reactive oxygen species has been implicated in the pathogenesis of acute and chronic pancreatitis, and antioxidants could be potentially effective against the development of pancreatic fibrosis in patients with chronic pancreatitis (Asaumi et al. 2007). Furthermore, highly reactive oxygen species could promote repeated tissue damage and regeneration during chronic pancreatitis. In this process, reactive oxygen species can induce genotoxic effects, including DNA strand breaks, sister chromatid exchanges, and formation of adducts with DNA (Jackson and Loeb 2001). Repeated damage-regeneration stimuli could impose permanent genomic alterations into pancreatic cells, which further accelerate the mutation accumulation, and subsequently the carcinogenesis process (Campisi and d'Adda di Fagagna 2007). Though the anti-tumor effects of those compounds with anti-oxidant activity have not been validated in clinic, epidemiologic studies show that intake of fresh fruit and vegetables appears to be inversely correlated with pancreatic cancer (Wiseman and Halliwell 1996). Eradication of reactive oxygen species from chronic inflammatory milieu will protect pancreatic cells DNA from genotoxic damage, and could perhaps lower the incident of transformation of these cells.

6 Differential Diagnosis Between Pancreatic Cancer and Chronic Pancreatitis

Chronic pancreatitis and pancreatic cancer are two distinct diseases with entirely different prognosis. However, it is a great challenge to discriminate them from each other in many cases. Several reasons contribute to this challenge. First, just like chronic pancreatitis tissues which are mostly made of fibrosis, more than 90% of pancreatic tumor mass is also made of the stroma element. Fine needle aspiration, the most commonly used technique to sample a pancreatic mass, usually could not obtain the tissue containing pancreatic cells. Second, there are too many common pathways dysregulated among chronic pancreatitis and pancreatic cancer tissues, such as reactive oxygen species, Hedgehog, NF-κB, and etc. At the present time, none of the global proteomic studies have identified cancer-specific proteins (Goggins 2005). Third, as chronic pancreatitis patients are more susceptible to develop into pancreatic cancer, the chronic pancreatitis patients are frequently followed up. However, even using the most advanced techniques, one cannot exclude the existence of malignant cells hidden within a chronic inflammatory mass (Cote et al. 2013).

It is generally accepted that stroma formation is a critical hallmark characteristic for pancreatic cancer, and the transformation of pancreatic cells usually occur in inflamed tissues (Xie and Xie 2015). Most previous cancer biomarker studies using modern technologies are methodologically flawed as they compare samples from cancer patients with those of healthy, inflammation-free people (Morcos et al. 2013). An accurate and non-invasive test to differentiate pancreatic cancer from chronic pancreatitis would be extremely helpful to detect pancreatic cancer at an early stage and help physicians to make the right treatment decisions and prolong patients' survival.

Protein Biomarkers CA19-9 is the most widely used tumor maker for pancreatic cancer, and its sensitivity is ~80%, while only about 55% for small and resectable cancers (<3 cm) (Ballehaninna and Chamberlain 2011). Disappointingly, in high-risk, asymptomatic individuals harboring IPMNs or high-grade PanINs, serum CA19-9 is often normal (Maitra and Hruban 2008). Furthermore, chronic pancreatitis tissues also exhibit high positive rates of CA19-9 expression (Shi et al. 2014). These data showed that CA19-9 is not an optimal biomarker to detect those non-malignant pancreatic tumors, nor is suitable to distinguish pancreatic cancer from chronic pancreatitis and other nonneoplastic pancreatic diseases. Recent efforts have been devoted to those markers with capacity to distinguish pancreatic cancer from chronic pancreatitis cases, and have identified a list of markers with potential clinical application (Crnogorac-Jurcevic et al. 2005). For example, using quantitative RT-PCR and immunohistochemistry to analyze the expression of UHRF1, ATP7A and aldehyde oxidase 1 in combination could potentially provide an additional useful diagnostic tool for fine-needle aspirated or cytological specimens obtained during endoscopic procedures (Crnogorac-Jurcevic et al. 2005). PAM4 is a monoclonal antibody expressed by 90% of PDAC, as well as the precursor lesions PanIN and intraductal papillary mucinous neoplasm, and shows high specificity for PDAC and precursor lesions versus benign, nonneoplastic pancreatic tissues. Interestingly, approximately 80% of chronic pancreatitis patients are negative for circulating PAM4 antigen, which highlights PAM4's potential use in differential diagnosis between chronic pancreatitis and pancreatic cancer (Shi et al. 2014).

DNA and RNA biomarkers Early studies indicate that human plasma are rich in nuclease, so that DNA and RNA fragments could not be stably detected as markers for cancer detection and diagnosis (Kong et al. 2011). However, recent studies show that DNA and RNA molecules could also serve as markers with potential clinical applications. For example, KRAS2 mutations in codon 12 could stably be detected in circulating deoxyribo nucleic acid, and its positive rate is

significantly higher in pancreatic cancer than in chronic pancreatitis patients (47% *vs*. 13%). A combined normal serum CA19-9 and absence of circulating KRAS2 mutations may significantly increase the differential diagnosis efficacy between chronic pancreatitis and pancreatic cancer (Maire et al. 2002).

microRNAs microRNAs (miRNAs) are endogenous, small, non-coding RNAs that repress the expression of target mRNAs. More than 1500 mature human miRNA sequences are listed in the miRNA database (Kozomara and Griffiths-Jones 2014). These sequences play important roles in virtually all biological pathways in mammals and other multicellular organisms (Berezikov 2011). Despite their subtle effects on individual targets, miRNAs are responsible for the modulation of multiple signaling pathways involved in cell growth, proliferation, differentiation, motility, and apoptosis (Kang et al. 2016). In fact, a number of miRNAs are located in fragile regions of the human genome that are associated with cancer development, and dysregulated miRNAs play crucial roles in tumor initiation, progression, and metastasis and are often associated with diagnosis, prognosis, and response to therapy (Rachagani et al. 2015).

There are many miRNAs that play important roles in pancreatic cancer development and progression, including miR-494 (Li et al. 2014). Our recent study has suggested that miRNAs could be stably detected in plasma and may serve as good markers for pancreatic cancer diagnosis (Kong et al. 2011). Among seven miRNAs evaluated in our study, miR-21 is able to distinguish pancreatic cancer from chronic pancreatitis and healthy patients; whereas miR-155 and miR-196a are able to differentiate sera with diseased pancreas (pancreatic cancer/chronic pancreatitis) from normal pancreas. Further quantitation of miRNAs in whole blood from pancreatic cancer, chronic pancreatitis and healthy controls has identified two panels of microRNAs with potential to distinguish pancreatic cancer patients from chronic pancreatitis and healthy controls (Schultz et al. 2014).

7 Summary

Pancreatic cancer is a lethal disease with unknown etiology. Chronic pancreatitis is a common chronic inflammatory disease, which affects the structural integrity and functions of pancreas. Clinical and experimental studies have suggested that chronic pancreatitis is a major risk factor for pancreatic cancer, whereas numerous extrinsic and intrinsic pathways have been linked to chronic pancreatitis to pancreatic cancer pathogenesis. Intriguingly, many genetic and epigenetic alterations are commonly detected in both chronic pancreatitis and pancreatic cancer tissues, while therapy targeting some alterations produces certain efficacy in pancreatic cancer treatment. However, chronic pancreatitis and pancreatic cancer patients have entirely distinct prognosis and require different management, differential diagnosis between them in clinic is extremely important. Most cancer biomarker studies using modern technologies are methodologically flawed as they compare samples from cancer patients with those from healthy, inflammation-free people. Future studies regarding pancreatic cancer biomarker discovery should exclude those molecules that are dysregulated in chronic pancreatitis tissues, thus uncovering pancreatic cancer specific biomarkers for diagnosis and treatment.

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