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Mini-review

Road to early detection of pancreatic cancer: Attempts to utilize epigenetic biomarkers

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ABSTRACT

The prognosis of pancreatic cancer is extremely poor, mainly because of its aggressive biological behavior and late onset of symptoms for clinical diagnosis; these impose limitations on therapeutic intervention. Deeper genomic sequencing analyses of pancreatic cancers revealed 12 core pathways and a long duration, nearly 20 years from initiation to distant metastases. This evidence will offer a broader aspect and time window of opportunity for early detection, thus preventing deaths from this cruel cancer. Epigenetic biomarkers can be utilized for assessing cancer risk, early detection, and predicting prognosis and therapeutic responses. In this review, we briefly summarize relevant issues associated with pancreatic cancer progression and recent advances in epigenetic biomarkers such as DNA methylation, miRNAs, satellite repeats, and histone modifications for early diagnosis.

1. Introduction

Pancreatic cancer is an aggressive malignancy with a 5-year mortality of 97–98%. Although surgical resection is the only possible curative method for pancreatic cancer in the early stages, only a minority (10–15%) of patients can undergo a curative operation at the time of diagnosis, mainly because most of them only show symptoms at the later stages, and these symptoms are mostly unspecific in nature [1]. Both the timely detection and the accurate differential diagnosis of pancreatic cancer are exceedingly difficult with currently available diagnostic means. Approximately 10% of pancreatic cancers are familial, and the poor survival among patients with pancreatic cancer is particularly of concern to individuals with an extensive family history [2,3]. Although little is known about the genetic and epigenetic alterations that contribute to familial pancreatic cancers, patients' family histories are useful in assessment of risk for developing pancreatic cancer [4,5]. As an example, the individual risk of developing pancreatic cancer rises in parallel with the number of affected first-degree relatives [5]. Persons suffering from long-standing chronic pancreatitis, particularly those with hereditary pancreatitis, also have an increased risk of developing pancreatic cancer [6–8].

Clinical screening methods for pancreatic cancer in asymptomatic individuals enables detection of a number of preinvasive pancreatic neoplasms within the time frame of a curative operation [9,10]. The variety of imaging techniques available includes ultrasonography (US), computed tomography (CT) scanning, magnetic resonance imaging (MRI), endoscopic ultrasonography (EUS), endoscopic retrograde cholangiopancreatography (ERCP), and magnetic resonance cholangiopancreatography (MRCP). Normally, combinations of different imaging modalities are employed in the preoperative diagnosis and staging of patients with suspected pancreatic carcinoma, because no single method provides sufficient sensitivity and specificity by itself. Individuals with pancreatic neoplasms, as well as some highly suspicious cases, undergo surgical resection; even a total pancreatectomy can be a therapeutic choice for some selected pancreatic cancer patients in order to prevent cancer death from development of new neoplasms in the remnant pancreas [10,11]. Because of the obvious limitations of imaging techniques, samples for histopathological or cytopathological assessment are often obtained to help confirm the diagnosis in possible cases. However, pancreatic juice contains proteolytic enzymes, so intact cells are not always obtained for accurate cytopathological diagnosis [12]. Considering all these factors, DNA-based diagnosis has clear advantages [13].

The obvious limitations of conventional diagnostic procedures in the detection and classification of pancreatic lesions, especially, small ones, has facilitated the search for additional molecular biomarkers to increase the sensitivity and specificity of diagnosis. Such indicators can be based on genomic, epigenomic, proteomic, or metabolomic changes. Because it is well known that many genetic and epigenetic alterations occur during pancreatic tumorigenesis, many researchers have focused on these possibilities.

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Among the genetic alterations, *KRAS* mutations are most readily detectable, but they are not specific for invasive pancreatic cancer [14]. Although they are found in the pancreatic juice and the feces of patients with pancreatic cancer, irrespective of their clinical stage, these mutations are also found in some chronic pancreatitis patients [15]. An alternative and promising strategy for diagnosing pancreatic neoplasia is the detection of aberrant DNA methylation. Promoter CpG island methylation, a common mechanism for silencing genes during tumorigenesis [16], is readily detected using methylation-specific PCR (MSP) analysis.

2. Implications of early detection in pancreatic cancer

In the 20th century, many investigators tried to identify genetic alterations in human cancers, including pancreatic cancer. We also characterized genome-wide alterations in pancreatic cancer using microsatellite markers and CGH [17,18] and further attempted to identify early detection using pancreatic juice [13]. Currently, next-generation sequencing technology can provide deeper insights into the pathogeneses and progression of human malignancies. The first efforts were directed toward breast and colon cancer [19], and the time of genetic progression to develop cancer was calculated [20]. Pancreatic cancers have also been analyzed in the same way [21–23]. In these series of studies on pancreatic cancer, 12 core pathways and processes were identified [21], followed by characterization of chromosomal rearrangements. Frequent fold-back inversions were observed as one of the early and crucial driver alterations [22]. Deeper genomic sequencing of primary and metastatic pancreatic cancers allowed estimation of the time scales of the progression of pancreatic cancer: an average of 11.7 years from the tumor-initiating mutation to the birth of the parental clone that results in pancreatic cancer (T_1), and a further average of 6.8 years to the development of metastatic subclones (T_2), soon followed by the patient's death at an average of 2.7 years (T_3) [23].

Meza et al. performed quantitative analyses of the age-specific incidence of pancreatic cancer in the general population on the basis of a general mathematical model that recognizes the random nature of both mutation accumulation and clonal expansion [24]. They estimated that mean sojourn time from the tumor-initiating mutation to clinical diagnosis may be as much as five to six decades, much longer than Yachida's estimation [23]; it is probable that the mean sojourn time for pancreatic cancer is somewhat between or around these estimations. In any case, there still remains a possibility for early diagnosis within the curative stage to prevent pancreatic cancer deaths.

3. Advantages of epigenetic biomarkers for early diagnosis

Pancreatic cancer is a disease of epigenetic, as well as genetic, abnormalities. The best-known epigenetic marker is DNA methylation. The initial finding of global hypomethylation of DNA in human tumors was soon followed by the identification of hypermethylated tumor-suppressor genes and then, more recently, by the discovery of inactivation of microRNA (miRNA) genes by DNA methylation. Detection of DNA methylation offers several advantages over genetic and serum markers [25–28]. First, incidences of aberrant DNA methylation of specific CpG islands are higher than those of genetic defects. For example, by a genome-wide sequencing of 13,023 genes, a typical colorectal cancer has been estimated to have an average of only 14 significant mutations [19]. On the other hand, by screening cell lines and validating tumor-specific hypermethylation in a panel of primary human colorectal cancer, Schuebel et al. estimated that nearly 5% or more of all known genes may be promoter methylated in an individual colorectal cancer [28]. Second, the aberrant DNA methylation seen in cancer cells can be sensitively detected, even when it is embedded in substantial amounts of contaminating normal DNA. Third, detection of aberrant DNA methylation is technically simple; it can be detected using MSP. Fourth, aberrant DNA methylation seems to occur in early-stage tumors, causing loss- and/or gain-of-function of key processes and signalling properties. Therefore, detection of aberrant DNA methylation is potentially a good early indicator of existing cancer and even of risk assessment for the future development of cancer.

4. Aberrant DNA methylation in pancreatic cancer

Inactivation of tumor suppressor genes caused by aberrant methylation was first suggested in *RB* [29], and aberrant methylation-mediated functional loss has been found in all sorts of cancers including pancreatic cancer; these genes are rarely hypermethylated in non-neoplastic tissues. Several important pancreatic cancer-related genes, including *CDKN2A* [30,31], *MLH1* [31,32], and *CDH1* [31], were the first ones analyzed in detail, followed by identification of many other cancer-related genes undergoing aberrant methylation that play roles in pancreatic carcinogeneses; these include *SPARC* [33,34], *DUSP6* [35], *RELN* [36], *RASSF1A* [37], *CCND2* [38], *TFPI2* [39], *RUNX3* [40,41], *SOCS1* [42], and *TSLC1* [43].

Understanding global methylation patterns has long been limited by technological concerns. However, genome-wide screening has made it possible to identify epigenetic alterations in novel genes within the setting of pancreatic cancer. Ueki et al. used methylated CpG island amplification (MCA) coupled with representational difference analysis to identify CpG islands differentially methylated in pancreatic cancer [44]. *PENK* was identified by this method and was aberrantly methylated in more than 90% of pancreatic cancers [44,45]. Sato et al. analyzed global changes in gene expression profiles of four pancreatic cancer cell lines after treatment with the demethylating agent 5-aza-2′-deoxycytidine (5Aza-dC) [46]; they identified a total of 475 candidate genes that were induced by 5Aza-dC in pancreatic cancer cell lines but not in a non-neoplastic pancreatic ductal epithelial cell line. Of these 475 genes, *UCHL1*, *CLDN5*, *NPTX2*, and *SFRP1* genes were highly methylated in the vast majority of primary pancreatic cancers [46]. *RPRM*, a gene involved in the TP53-induced G2 cell cycle arrest, was methylated in 60% of pancreatic cancers, and was associated with genetic instability and unfavorable outcome after surgical resection [47]. Omura et al. used MCA coupled with promoter and CpG island microarrays to identify differential DNA methylation patterns in pancreatic cancer vs. normal pancreas and found aberrant methylation of hundreds of promoters and CpG islands in pancreatic cancer cells [48]. Recently, we developed a novel method called "microarray coupled with methyl-CpG targeted transcriptional activation" (MeTA-array) [49], and Shimizu et al. applied this method to searching for methylation-mediated transcriptionally silenced genes in pancreatic cancer [50]; 16 methylated genes that have never been previously detected by 5Aza-dC were identified. These include *TRH*, *CYP26A1*, *TMEM204*, *GAD1*, *CSMD2*, *FRG2*, *ARC*, *SLC32A1*, *FOXJ1*, *TBX21*, *HOXA7*, *ANKRD35*, *HBA2*, *SP5*, *TNXB*, and *GRASP*. Among these, 90% (19/21) of *CSMD2*, 100% (21/21) of *SLC32A1*, 95% (20/21) of *TMEM204*, and 100% (21/21) of *TRH* were methylated in pancreatic cancer cell lines. Furthermore, *CSMD2, SLC32A1*, and *TRH* were also hypermethylated in primary pancreatic cancers in a tumor-specific manner [50]. Many of these genes are aberrantly methylated in a high proportion of pancreatic cancers and can be detected with MSP analysis, making them attractive candidates for early detection of pancreatic cancer. Table 1 provides a selected list of genes identified as aberrantly hypermethylated in pancreatic cancer.