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REVIEW ARTICLE

Genetics and pathology of pancreatic cancer

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States [1,2]. Approximately 32 000 individuals in the USA and over 200 000 individuals worldwide die from the disease each year [1–3]. The incidence approximates the mortality rate, which reflects the poor prognosis of pancreatic cancer. Although there have been many advances in pancreatic cancer research, the 5-year survival rate for affected patients remains under 5% [2].

The aggressiveness that characterizes pancreatic cancer arises from multiple heterogeneous genetic changes that occur before the onset of clinical symptoms. Studies performed over the past decade have shed some light on the molecular and histological events that are associated with pancreatic carcinogenesis. This chapter will focus on the genetics and pathology of pancreatic ductal adenocarcinoma. Future progress in this area will hopefully lead to improved diagnostic tests, early detection, and new treatments for patients who suffer from this devastating disease.

Genetics

The accumulation of multiple nonrandom genetic changes over time is a hallmark of pancreatic cancer. Genetic abnormalities include alterations in chromosome or gene copy number, microsatellite instability, epigenetic silencing, intragenic point mutations, and gene overexpression secondary to increased transcription (Table I) [4].

Chromosomal alterations

The most common techniques used to study chromosome losses and gains are karyotyping (G-banding), comparative genomic hybridization (CGH), and allelotyping [4]. Metaphase spread karyotyping (G-banding) is a cytogenetic technique, developed in the 1960s, where chromosomes are stained and their banding patterns are examined during metaphase. Karyotyping can reveal large deletions, insertions, translocations, inversions, and other rearrangements, but suffers from a lack of submicroscopic resolution. Conventional CGH has an improved resolution (5–10 mB) [5], but in the post-genomic era, even this technology has become arcane. Array-based CGH, a molecular cytogenetic technique developed in the early 1990s, is an application of CGH using microarray technology. In microarray analysis, thousands of molecules may be immobilized on an insoluble solid support. This strategy enables the simultaneous detection of a large number of analytes [6]. A second application of microarray technology, gene expression analysis, will be discussed in greater detail below. The general principles of microarray analysis [7], applicable to both CGH and global gene expression studies, are illustrated in Figure 1.

As demonstrated in Figure 1, fragments of tumor DNA and non-tumor DNA are differentially labeled with fluorescent dye and allowed to compete for hybridization sites in either a metaphase chromosome spread (conventional CGH) or in an array of genomic probes (array CGH). The color and intensity of the

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Table I. Overview of the genetic abnormalities described in this paper.

Abnormalities observed in pancreatic ductal adenocarcinoma	Example
Chromosome alterations	Losses: 9p, 17p, 18q, 3p, 8p, 6q Gains: 3q, 5p, 7p, 8q, 20q
Microsatellite instability	Medullary carcinoma with mutations in *hMLH1*
Epigenetic silencing	Hypermethylation of promoter CpG islands in *ppENK*
Mutations	*p16, p53, DPC4*
Gene overexpression	Mesothelin

fluorescence emitted by the hybridized fragments of labeled DNA reveal areas of the genome that may be deleted or amplified in the test sample relative to the control sample [5,8]. In allelotyping analysis, microsatellite markers (short tandem repeats of DNA that are interspersed throughout the genome) in normal and cancer DNA from a single patient are PCR amplified and sequenced. Since individuals are often heterozygous at microsatellite loci, genomic deletions can be detected by evaluating microsatellite markers in tumor DNA for losses of heterozygosity (LOH). Chromosome positions that have frequent LOH in pancreatic cancer often harbor important tumor suppressor genes [9–11].

Chromosome losses are more common than chromosome gains in pancreatic cancer [4]. According to allelotyping data in pancreatic cancer xenografts, DNA loss in xenografts ranges from 1.6% to 32% of the genome [10]. Despite this variability, certain reproducible patterns have been observed. The most common regions of genomic loss in primary pancreatic cancers and pancreatic cancer xenografts include chromosome arms 9p, 17p, 18q, 3p, 8p, and 6q [10,12,13]. Some of these loci contain known tumor suppressor genes such as *CDKN2A/P16/MTS1* (9p21), *p53* (17p13), and *MADH4/SMAD4/DPC4* (18q). Further investigations may reveal additional tumor suppressor genes in the other LOH 'hot spots' [10].

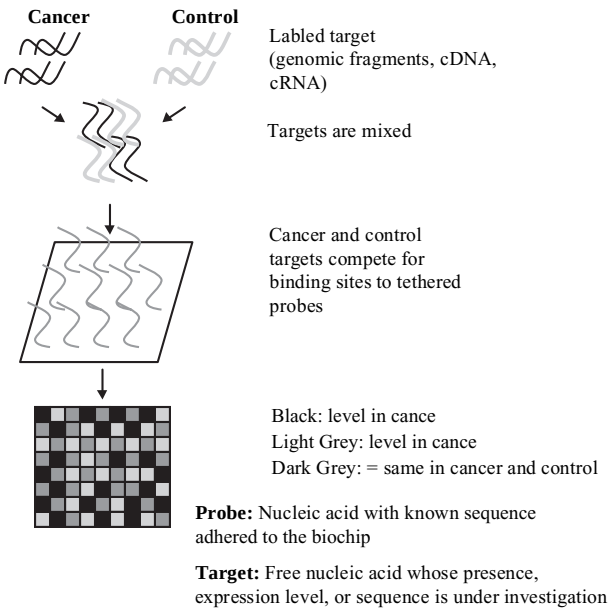

Figure 1. Microarray technology is based on high throughput competitive hybridization of fluorescently labeled sample DNA and control DNA (target sequences) with an organized array of sequences deposited on a biochip (probe sequences). Molecular cytogenetics (e.g. CGH) and gene expression analysis (e.g. cDNA microarray) are two widely used applications of microarray technology. The cartoon is based on a schematic from Coe and Antler [7].

Frequent gains of DNA, secondary to unbalanced chromosome rearrangements, have been observed in primary pancreatic cancers on chromosome arms 3q, 5p, 7p, 8q, and 20q [12,14]. Fluorescent *in situ* hybridization (FISH) performed on pancreatic cancer cell lines demonstrates amplified regions of DNA at 19q, 12p, 12q, 17q, and 20q. These amplified sites correspond to the locations of the following oncogenes: *AKT2, KRAS2, MDM2, ERBB2*, and *AIB1*, respectively [13,15].

Microsatellite instability

Medullary carcinoma, a subtype of pancreatic adenocarcinoma seen in approximately 5% of operative cases, often contains a defective DNA mismatch repair (MMR) mechanism [16]. Altered MMR gives rise to microsatellite instability (MSI), characterized by hypermutability at so-called minisatellite repeat sequences. Unlike most ductal adenocarcinomas of the pancreas, MSI tumors show minimal LOH and aneuploidy [17]. They also frequently lack mutations in KRAS2 and P53. Histologically, medullary cancers are poorly differentiated. They grow in a syncytial pattern, exhibit pushing borders, and demonstrate extensive necrosis. Interestingly, these tumors appear to have a better prognosis than the more common ductal adenocarcinoma [16].

Epigenetic silencing

Epigenetic phenomena are heritable DNA modifications that do not involve alterations in DNA sequence [18]. Examples include structural changes to chromatin, such as post-translation histone modifications and nucleosome rearrangements, and deregulation of cytosine methylation at promoter CpG islands. The expression of many tumor suppressor genes and oncogenes appears to be influenced through this mechanism. Genes that are silenced in pancreatic cancer by epigenetic changes include *ppENK* (90%), *RARB* (20%), *CDKN2A/P16* (18%), *CACNA1G* (16%), *TIMP3* (11%), *CDH1* (7%), *THBBS1* (7%), and *hMLH1* (4%) [19,20]. Approximately