Progress in cancer genetics: Lessons from pancreatic cancer

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Summary

Background: In the near future advances in the molecular basis of cancer are expected to facilitate cancer diagnosis, to rationalize treatment, to facilitate screening, and to identify individuals requiring cancer prevention strategies.

Methods: The literature was reviewed concerning the genetic alterations that contribute to pancreatic cancer development.

Results: Virtually all pancreatic cancers have inactivation of the p16 pathway, and the majority inactivate the TGF beta/DPC4 and p53 tumor-suppressive pathways. Pancreatic cancers with mismatch repair deficiency have a characteristic histology and may have an improved prognosis. The recently discovered tumor suppressor genes, ALK-5, MKK4, and STK11 (the gene responsible for Peutz-Jeghers syndrome) are all targeted for mutation in a small proportion of sporadic pancreatic cancers. Germline mutations of the BRCA2 gene are present in 5-10% of patients with pancreatic cancer. Typically such patients do not have a family history of pancreatic cancer and are mistaken as patients with sporadic disease. Five to 10% of patients with pancreatic cancer have first-degree relatives that will develop pancreatic cancer. Some such families also have a family history of melanoma and harbor germline p16 mutations. However, the gene(s) responsible for much of the inherited predisposition to pancreatic cancer remain to be identified.

Conclusion: Further advances in pancreatic cancer molecular genetics are needed to facilitate the development of molecular screening tests, to identify additional familial susceptibility genes, and to identify targets for rational therapeutic targeting.

Key Words: ALK-5, BRCA2, DPC4, mismatch repair, pancreas cancer, p16, tumor suppressor genes.

Introduction

Pancreatic cancer is a genetic disease. Mutations to cancer-associated genes give cells a selective advantage which drives the evolution of sequentially more autonomous clones until a cancer has evolved. Many classes of cancer-causing genes are involved in the formation of cancer. Defects in growth regulatory genes alone may not be sufficient to cause cancer. Increasingly cancers are characterized by defects in genome maintenance. Hence, the quest for the pancreas cancer genotype has widened considerably to encompass the identification of the many potential tumor suppressor genes and oncogenes, to identifying the genetic causes of chromosomal instability, and to identifying the genes that give rise to DNA methylation-induced gene silencing.

Cancer-causing genes: Gatekeepers, Caretakers and Guardsmen

Several eponyms are now used to describe cancer-causing genes. A "gatekeeper" is a gene whose sole function is to prevent neoplasia, and whose alteration is sufficient to cause neoplastic transformation [1]. A gatekeeper gene is so vital to the prevention of neoplasia in a given tissue that germline disruption of a gatekeeper typically leads to a recognizable inherited cancer syndrome. Examples of gatekeepers include the Rb gene responsible for retinoblastoma, and the APC gene mutated in the majority of colorectal cancers [2]. Vogelstein and Kinzler have coined the term "caretaker" for a gene involved in genome maintenance whose inactivation facilitates the development of additional DNA damage thereby accelerating the evolution to cancer. The BRCA2 and DNA mismatch repair genes are examples of caretakers. The classification of cancer-related genes is extended by the "guardsmen" concept [3]. Guardsman is used to describe the function of most tumor suppressor genes not directly involved in genome maintenance that function to suppress neoplasia, but whose sole inactivation is not sufficient to cause neoplasia.

Tumor suppressor genes and their pathways

Genetically targeted tumor suppressor genes have been demonstrated to suppress oncogenesis by multiple mechanisms. These functions include maintaining cell cycle control, transcription regulation, apoptosis control, cytoskeletal function, and growth suppression mediated by several signal transduction pathways.

The TGF/β/DPC4 pathway

TGF beta normally suppresses oncogenesis by binding to TGFBR2 enabling the latter to heterodimerize with ALK-5. ALK-5 signals through SMAD’s 2, 3, and 4 [4], driving DPC4 to the nucleus where it binds DNA and transactivates multiple downstream genes [5]. The introduction of normal DPC4 into cancer cells that lack DPC4 results in their growth arrest [6]. ALK-5 activates other SMADs besides DPC4, suggesting the existence of some non-DPC4-mediated TGF beta signaling pathways [4]. Resistance to TGF beta-mediated growth suppression has been
demonstrated in vitro in pancreatic cancer and other tumor types in up to 80% of the cancer cell lines studied [7]. DPC4 (SMAD4) [8, 9], TGFBR1 (ALK-5) [10], TGFBR2 [11, 12], and SMAD2 [13, 14] have been identified as targets of inactivation by mutation, homozygous deletion, or loss of expression (in the case of TGFBR2 and ALK-5) in several cancers.

DPC4 is genetically inactivated in 50% of all pancreatic cancers [8], somewhat less often in colorectal and biliary cancers [15, 16], and occasionally targeted in other cancers such as breast and head and neck cancer [6]. The TGFBR2 gene is inactivated in 3-15% of cancers, most commonly occurring in colorectal cancers with microsatellite instability [7-9]. Mutations and deletions outside the mononucleotide repeat have also been described in pancreatic, gastric and head and neck cancer [7, 16]. We have also identified homozygous deletions of the ALK-5 (TGFBR1) gene in pancreatic and biliary cancer demonstrating that ALK-5 is also a tumor suppressor gene [7]. Loss of TGFB beta type I and the type II receptor expression has been described with moderate frequency in many types of cancers [17].

The p16/Rb pathway
p16 is a cyclin-dependent kinase inhibitor that prevents the cooperative action of the cyclin-dependent kinase CDK4 and cyclin D1 from phosphorylating Rb, an activity closely linked to the moving of the cell cycle out of late G1 phase [18, 19]. Several cancer types, such as small cell and non-small cell lung cancer, melanoma, and pancreatic cancer have inactivation of this pathway in virtually 100% of tumors studied [20-23]. For small cell lung cancer, Rb inactivation is almost universal, while for pancreatic cancer and non-small cell lung cancer, p16 inactivation by mutation, deletion or methylation are the mechanisms by which this important pathway is inactivated. Patients with germline p16 mutations are at increased risk of melanoma [21] and pancreatic cancer [22].

The p53 pathway
The p53 tumor suppressor gene is one of the most frequently altered genes in cancer [24]. In pancreatic cancer, p53 is mutated in approximately 75% of tumors [25, 26]. Studies continue to refine our understanding of its function, to simplify the detection of its mutant forms, to identify new upstream and downstream genes involved in p53 responses, and to characterize p53 analogues. p53 is a transcription factor induced in response to DNA damage (reviewed in [27]). Proteins known to induce p53 include p14ARF, a gene that resides in the p16 locus [28-31]. The viral oncoproteins SV40, adenovirus E1B, and HPV E6 all target p53 [24]. Hypoxia induces p53 and this induction can be mediated by HIF-1α (hypoxia inducible factor 1 alpha) [32-35], suggesting that the hypoxia present in a cancer nodule selects for cells that have lost p53-mediated hypoxia-induced apoptosis. p53 causes G1 cell cycle arrest through p21 induction and apoptosis mediated by bax and several PIG genes [27, 36]. p53 also contributes to radiation-induced G2 arrest [37,38] which requires the cooperation of the ATM gene product [39]. Experimentally wild type p53 suppresses angiogenesis through inhibition of VEGF [39]. Additional genes transcriptionally activated by p53 include mdm-2, GADD45, IGFBP3 [27], and p33ING1 [40]. A detailed analysis of genes that are induced by p53 was carried out by Polyak et al, using the powerful molecular tool, SAGE [36]. The authors identified a large number of p53-induced redox-related genes (PIG genes) leading them to hypothesize that these genes induce apoptosis by stimulation of oxygen radicals culminating in mitochondrial destruction. The recent identification of mitochondrial DNA mutations in colorectal cancer raises the possibility that cancers may evade apoptosis and cell death by selecting mitochondria more resistant to apoptosis [41].

The BRCA2 gene
The BRCA2 gene product is thought to function as a mitosis maintenance gene. Working with the recombination protein Rad51, BRCA2 is thought to prevent DNA strand breaks that can occur during normal cell division [42, 43]. A potentially significant therapeutic consequence of BRCA2 function is the associated increased radio-sensitivity of BRCA2 deficient cells that has been observed in vitro and in animal models [42, 43]. Investigations are currently underway to determine whether or not cancers from patients with germline BRCA2 mutations are more sensitive to radiotherapy. Mammography could pose additional risks for BRCA2 mutation carriers for the same reason. Patients with germline BRCA2 mutations have an increased lifetime risk of breast, pancreatic, ovarian, and possibly prostate cancer (25-50% for breast cancer, and probably 5% risk for pancreatic and ovarian cancer) [44-47]. The age of onset of cancer in BRCA2 mutation carriers appears to be relatively late for a familial cancer gene. Although most attention is given to the breast cancer risk posed by carrying a germline BRCA2 mutation, pancreatic cancer is almost uniformly fatal, early stage disease can not be reliably detected by screening techniques, and currently there are no effective preventative strategies. Current evidence suggests that 5-10% of patients with clinically sporadic pancreatic cancer harbor germline mutations of the BRCA2 gene [45, 46]. If BRCA2 mutation carriers are recognized as having a familial predisposition to cancer, they are likely to be recognized as a member of a breast, rather than a pancreas cancer family. Epidemiological studies defining familial pancreatic cancer as families with two first-degree relatives with the disease underestimate the inherited influences on pancreas cancer development. The incomplete penetrance of cancer in such carriers and their families complicates clinical decision making it difficult to identify gene carriers by clinical features alone.

The Peutz-Jeghers (STK11) gene
Giardiello et al found that patients with Peutz-Jeghers syndrome have an increased incidence of pancreatic cancer [48]. We have identified germline mutations in the STK11 gene in patients with Peutz-Jeghers syndrome who have developed pancreatic cancer, and have also found that this gene is somatically mutated in a small proportion of pancreatic and biliary cancers [49].

Other tumor suppressor genes
Other genes responsible for familial pancreatic cancer include the cationic trypsinogen gene responsible for familial pancreatitis. Carriers have a lifetime risk of pancreas cancer approaching 50% [50,51]. M KK4 is a target of mutation in
pancreatic cancer [52]. Currently, the selective advantage of MKK4 loss in cancers is poorly studied. Several recently identified tumor suppressor genes are not targeted in pancreatic cancer. These include beta-catenin and the APC genes [53], and the PTEN gene responsible for Cowden’s disease [54]. As the field of molecular genetics continues to grow, more genes will inevitably be studied for their potential in cancer development. In response to the growth in the field, an on-line journal (NOGO, http://www.NOGO.org; The Journal of Negative Observations in Genetic Oncology) publishes negative studies in cancer genetics.

The K-ras oncogene

K-ras is mutated in over 90% pancreatic cancers [55, 56]. No other oncoproteins are commonly mutated in pancreatic cancer although several oncoproteins are overexpressed in pancreatic cancer including Her-2/neu [57]. The few pancreas cancers that harbor wild-type K-ras are often RER+ [58]. Although both chronic pancreatitis and neoplastic precursor lesions in the pancreas occasionally harbor mutated K-ras [59, 60], several investigators have investigated K-ras as a diagnostic and screening test [61-63]. Varying rates of mutant K-ras detection have been demonstrated in the blood of pancreatic cancer patients probably reflecting differences in the stage of the disease and the sensitivity of tests used for K-ras detection between different studies. Mutant K-ras is more often found in the blood of patients with unresectable lesions [61]. On its own, mutant K-ras detection will probably not suffice as a pancreatic cancer screening tool, but could be of value as a member of a panel of specific pancreatic cancer markers.

The function of the K-ras pathway remains incompletely understood. Mutant K-ras can induce transformation of cells in culture under certain conditions, and such studies suggest that several downstream signals are induced by mutant K-ras. Implicated pathways include the MAP kinase pathway, protein kinase C activation, COX-2 and the TGF beta pathway. The overexpression studies of K-ras in vitro that form the basis of our understanding of the K-ras pathway have been criticized as they do not mimic the more modest level of activation of the K-ras pathway found with mutant K-ras in vivo [64].

The K-ras protein requires farnesylation and methylation for binding the cell membrane and function. The ability of farnesyl transferase inhibitors to inhibit K-ras signal transduction has led to clinical trials of their use in cancer [65].

Mismatch repair genes

The establishment of well-defined criteria (the “Amsterdam criteria”) for the identification of kindreds with the hereditary non-polyposis colon cancer syndrome (HNPPC) [66, 67], was instrumental in the discovery that tumors from patients with HNPPC typically have microsatellite instability (RER*) suggestive of a mismatch repair defect [68]. Six human DNA mismatch repair genes have been identified to date and they include hMSH2, hMLH1, hPMS1, hPMS2, hMSH6/GTBP and hMSH3. Microsatellite instability has been demonstrated in a wide variety of cancers including colorectal, pancreatic, endometrial, stomach, urothelial, and lung cancer. 15-20% of colorectal carcinomas have microsatellite instability (predominantly due to bi-allelic inactivation of hMSH2 and/or hMLH1) [68], while a lower rate is typical of most other types of cancer [reviewed in ref 58]. Once both copies of a mismatch repair gene are inactivated, de novo mutations are not repaired appropriately and carcinomas evolve relatively rapidly. This altered pathway of tumorigenesis may account for the clinicopathological differences between RER* and RER+ cancers [58, 69, 70]. Approximately 4% of pancreatic cancers show microsatellite instability and have a similar clinicopathological pattern to RER+ colorectal carcinomas with a medullary pathology, a suggestion of an improved prognosis, and a distinct mutational and karyotypic spectrum [58, 69, 70, 71].

Mitotic checkpoint genes

Genetic instability is an inherent feature of cancer, but investigators are only beginning to identify the molecular mechanisms underlying this instability. Lengauer et al have demonstrated that all colorectal cancers can be classified as having one of two forms of genetic instability, microsatellite instability (MIN) or chromosomal instability (CIN) [72]. Cahill et al found that CIN cancers have a defect in mitotic spindle checkpoint function [73]. In response to a mitotic spindle toxin, MIN cells with an intact checkpoint stop undergoing mitosis, but CIN cells do not. Two genes that regulate the mitotic checkpoint, BUB1 and BUBR1 were mutated in 5-10% of CIN cancers. The deregulation of mitotic checkpoint genes and other related genes probably contributes to the aneuploidy commonly seen in cancer. The role of these genes in pancreatic neoplasia has not been determined to date.

DNA Methylation

Abnormalities in DNA methylation include the global DNA hypomethylation in cancer [74, 75], and the more focal hypermethylation of promoters that often results in reduction, or loss of transcription of a small number of specific genes [23]. DNA hypomethylation may contribute to genome instability [76, 77]. The origin(s) of the deregulation of DNA methylation in neoplasia is not known. A large number of genes have demonstrated as targets of DNA hypermethylation including p16, p15, hMLH1, p27, estrogen receptor, N33, HIC1, GSTP1, and neurotensin [78]. A closely related form of epigenetic DNA regulation is that of genomic imprinting. Imprinting is a form of silencing of gene clusters within selected chromosomal regions that occurs early in embryonic development. DNA methylation contributes to the gene silencing of imprinted genes [79]. Imprinting appears to influence cancer development in two ways. First, the imprinting of one allele of a tumor suppressor gene can facilitate tumorigenesis if the wild type gene is inactivated. Colorectal cancers display a loss of imprinting (LOI) of the IGF2 gene, a finding frequently
associated with the presence of microsatellite instability in the cancer [80]. In pancreatic cancer p16 methylation occurs in the 20% of cancers that do not have p16 mutation or homozygous deletion [23].

Chromosomal alterations in pancreatic cancer

A comprehensive allelotype of loss of heterozygosity patterns in pancreatic patterns has helped to identify areas of the genome that may harbor tumor suppressor genes [81]. Karyotyping studies have helped to identify important chromosomal changes present in lymphoid cancers [82], but structural alterations such as amplifications and translocations have been detected less commonly in epithelial solid tumors such as pancreatic cancer [83-85].

Telomerase

Telomerase is an enzyme with reverse transcriptase activity, an RNA template, and several subunit proteins that function to prevent telomere shortening that would otherwise occur with each cell division. Telomerase activity is lost in most somatic cells after development, but it is retained in lymphocytes and in approximately 90% of cancers and less often in pre-cancerous lesions [86], and may contribute to the intraimmortalization of cancer cells. Telomerase detection has been extensively studied as a screening test for several cancers. Almost 90% of patients with pancreatic cancer have telomerase activity in their pancreatic juice [87]. Like K-ras detection, because of imperfect specificity, telomerase detection will not be sufficiently specific alone to diagnose pancreatic cancer.

Discussion

Many genetic alterations are required for cancer evolution. Bringing the knowledge of cancer genetics into clinical practice for screening, prognosis and therapeutic decision making has proved to be a more elusive goal than first realized. Most genes are inactivated by various mechanisms. Often such losses cannot be screened for in biological fluids due to the presence of contaminating normal DNA. Gene chip technology may soon facilitate the rapid and inexpensive identification of cancer-causing mutations.

The deadly nature of pancreatic cancer is focussing increasing efforts towards identifying individuals at-risk and to detect cancers arising in such individuals at an early stage disease. Early evidence suggests that patients with RER+ pancreatic cancers have an improved prognosis. These patients may benefit from an aggressive therapeutic approach.

The pancreatic cancer patients with germline BRCA2 mutations are noteworthy for the absence of a clear-cut family history suggestive of an inherited predisposition to cancer. This leads to the prediction that other inherited genes may commonly predispose to pancreas cancer in a low-penetrant fashion.

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