Commentary

Genetic Progression in the Pancreatic Ducts

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Precursors to Infiltrating Cancer

There are histologically well-defined precursors to inva-
sive carcinoma of the uterine cervix, colon, breast, and
other organs. This knowledge, coupled with effective
screening tests for these preclinical neoplasms, has
saved many lives that might otherwise have been lost to
cancer.[1–4] For example, simple annual screening of
asymptomatic individuals over the age of 50 for fecal
occult blood can lead to the early detection of adenomas
and cancers of the colorectum, and, remarkably, annual
screening can reduce mortality from colorectal cancer by
33%.[1]

By contrast, there is no effective screening test for
early pancreatic cancer. Most patients with pancreatic
cancer present late in the course of their disease after the
cancer has spread beyond the gland. Therefore, pancre-
atic cancer is almost universally fatal.[5] This year it is
estimated that ~28,000 Americans will be diagnosed
with and ~28,000 will die from pancreatic cancer.[5] One
cannot but believe that a sensitive and specific test for
early pancreatic cancer would reduce this extraordinarily
high mortality rate.

Early Pancreatic Cancer

The first step in developing a test for early pancreatic
cancer is recognizing and defining the precursors to
infiltrating pancreatic cancer. Although the recognition of
an early form of cancer may be relatively easy for organs,
such as the cervix and colon, which are accessible to
non-invasive biopsy, the study of early pancreatic cancer
is much more difficult because the organ can only be
sampled using invasive procedures. Therefore, sampling
generally does not occur until the patient is symptomatic
and the disease is advanced. The recognition and gen-
eral acceptance of early non-invasive precursor lesions
in the pancreas has, therefore, lagged far behind the
recognition of precursor lesions in other organs such as
the uterine cervix, colon, breast, and prostate.

Early Clues

The first hint that there may be histologically distinct
precursor lesions to infiltrating ductal adenocarcinoma of
the pancreas came from Sheldon Sommers.[6] He noted
pancreatic duct hyperplasias in 9% of people without
cancer and similar lesions in over one-third of those with
pancreatic cancer. The duct hyperplasias associated
with cancer generally had a more advanced papillary
architecture and cellular atypia. Cubilla and Fitzgerald
extended those observations in a seminal study of over
300 pancreata and again found that papillary proliferative
lesions in the ducts were more common in pancreata with
cancer than they were in pancreata without cancer and
that papillary proliferations with significant atypia were
seen only in the pancreata with cancer.[7] These observa-
tions have been confirmed by other groups.[8–10] In addi-
tion, Furakawa et al showed, using three-dimensional
mapping, that severely dysplastic areas often arise within
zones of milder atypia, a spatial pattern supportive of a
stepwise progression model.[8–10] Thus, morphological
observations provided the first critical step in the recog-
nition that intraductal proliferations in the pancreas are
the precursors to invasive ductal cancer.

These morphological observations, however, had sev-
eral significant limitations. First, although it was felt that
these lesions in the pancreatic ducts were the precursors
to infiltrating cancer, there was no direct evidence be-
yond morphological atypia that they actually progressed
to infiltrating cancer. As noted earlier, the relative diffi-
culty of biopsying the pancreas and the high operative
mortality rate associated with pancreatic surgery make
routine sampling the pancreas extremely treacherous. It
was therefore difficult to determine the natural history of
these duct lesions in the pancreas. Surgery on the pan-
creas has, however, become much safer,[11] and a grow-
ing number of patients who have had a portion of their
pancreas removed are alive today. Some of these pa-
tients have had pancreatic duct lesions, providing a
unique opportunity to study the natural history of these

lesions. As predicted, some duct lesions do indeed progress to infiltrating carcinomas.[12,13] For example, Brat et al reported three patients who developed an infiltrating adenocarcinoma of the pancreas 17 months, 9 years, and 10 years after partial pancreatectomy revealed atypical papillary duct lesions in their pancreata.[12] Brockie et al have reported two similar cases.[14] Although such anecdotal cases do not provide information on the frequency and speed at which duct lesions progress, they do establish that some duct lesions in the pancreas can progress to infiltrating carcinoma.

Cancerization versus *Invasive Neoplasia*

The second limitation to the morphological observations described earlier, as Klöppel and colleagues have emphasized, is the fact that on careful morphological examination many of the atypical papillary lesions in pancreatic ducts can be shown to be intraductal growth of an invasive cancer.[15] This phenomenon, known as cancerization of the ducts, may account for a significant fraction of the markedly atypical papillary intraductal proliferations adjacent to infiltrating adenocarcinomas of the pancreas. Although cancerization of the ducts cannot account for the atypical papillary duct lesions seen in pancreata without cancer,[16] recognition of this phenomenon is essential for those studying the genetic alterations in these lesions.

Genetic Alterations in Duct Lesions

Though morphological observations and anecdotal case reports of progression established a foundation for the development of a progression model for pancreatic neoplasia, it was not until modern molecular genetic techniques were applied to the study of intraductal proliferations in the pancreas that such a model could be rigorously tested. The pathological term "hyperplasia" refers to an increase in cell numbers in the absence of neoplasia. If these lesions in the pancreatic ducts were simply hyperplastic, then they should not be clonal and they should not harbor functionally significant mutations in cancer-associated genes. If the lesions are merely intraductal growths of an invasive cancer, then they should be clonal and they should harbor the same mutations in cancer-associated genes as their associated infiltrating cancer. If, however, as the morphological observations would suggest, the duct lesions are indeed the precursors to invasive cancer, then the lesions should be clonal; they should harbor some, but not all, of the mutations found in their associated infiltrating cancer; and the prevalence of these genetic alterations should increase with increasing severity of dysplasia in the duct lesion. Pancreatic duct lesions have now been examined for loss of heterozygosity at a number of loci and for alterations in a number of genes and proteins including K-*ras*, HER-2/*neu*, *p16*, *p53*, *DPC4*, and *BRCA2*. In all instances the results of these analyses suggest that pancreatic duct lesions are the precursors to infiltrating ductal carcinoma.[16–23]

K-ras

Activating point mutations in codon 12 of the K-*ras* oncogene are among the most common genetic alterations identified to date in infiltrating ductal carcinomas of the pancreas.[24] These mutations can be found in 80 to 95% of these cancers. Mutations of codon 13 and 61 occur only occasionally.[25] A number of investigators have microdissected the duct lesions of the pancreas and analyzed them for K-*ras* mutations.[20,26,27] The first genetic evidence of a progression model for these duct lesions was provided by Caldas et al.[26,27] They noted that activating point mutations in codon 12 of the K-*ras* gene occur in about half of the nonpapillary duct lesions, but were present in the vast majority of papillary, more advanced lesions. It is this progression in K-*ras* mutational frequency that suggests that K-*ras* is not necessarily the first change, or gatekeeper, required for pancreatic ductal neoplasia.[28]

HER-2/neu

HER-2/*neu* is a member of the epidermal growth factor receptor family. Approximately 70% of infiltrating ductal carcinomas of the pancreas overexpress HER-2/*neu*.[18,29,30] Day et al examined the expression of HER-2/*neu* in a series of duct lesions using immunohistochemical labeling.[18] They found that although HER-2/*neu* was essentially not expressed in normal pancreatic duct epithelium, it was expressed in 82% of flat duct lesions, in 86% of papillary duct lesions without atypia, and in 92% of atypical papillary duct lesions.[18] The means of overexpression is unknown and does not seem to involve gene amplification.

p16

The *p16* tumor-suppressor gene on chromosome 9p encodes for a regulator of the cell cycle. The *p16* gene has been shown to be genetically mutated or its expression abrogated in duct lesions.[20,21] Although loss of the *p16* gene appears to occur slightly later than K-*ras* activation, the prevalence of *p16* gene loss also appears to increase with increasing degrees of atypia in duct lesions. For example, Wilentz et al, using immunohistochemical labeling for the *p16* gene product, showed that 30% of flat duct lesions without significant atypia, 55% of papillary duct lesions without significant atypia, and 71% of papillary duct lesions with significant atypia had loss of expression of the *p16* gene product.[20] In addition, the labeling pattern in the papillary duct lesions with atypia often differed from the patient's infiltrating carcinoma, establishing that these lesions were not simply an artifact of cancerization of the ducts.

p53

The *p53* tumor-suppressor gene on chromosome 17p is inactivated in 50 to 75% of infiltrating pancreatic carcinomas.[31–33] Although an imperfect marker of gene sta-
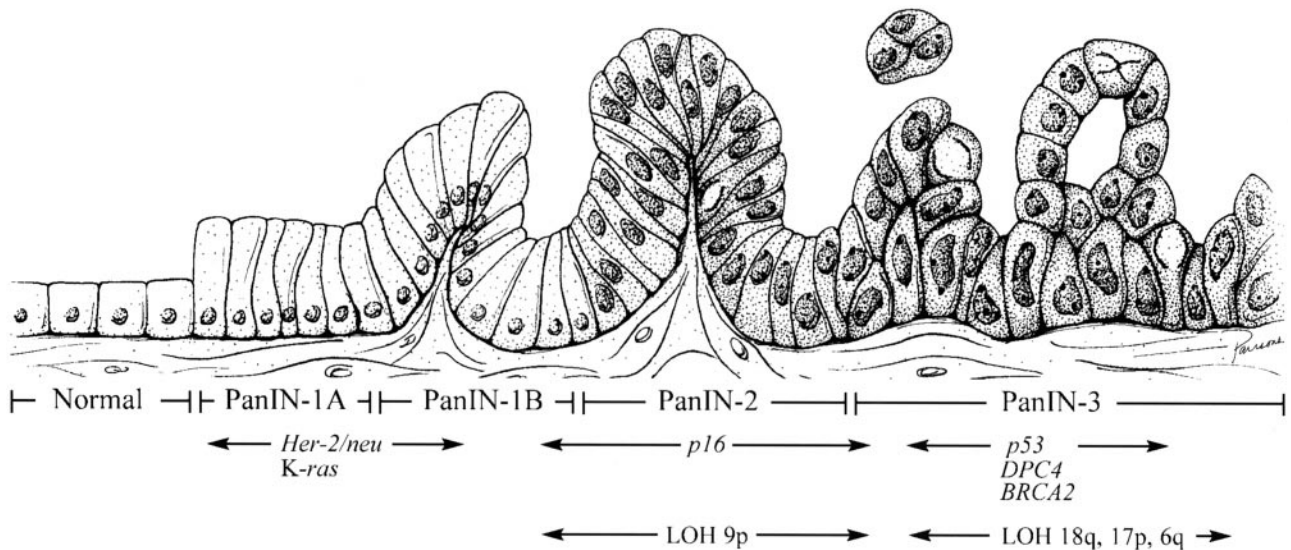
Figure 1. Progression model for pancreatic cancer. The progression from histologically normal epithelium to low-grade PanIN to high-grade PanIN (left to right) is associated with the accumulation of specific genetic alterations. (Artwork by Jennifer L. Parsons. Adapted from Reference 19 with permission.)

tus, immunohistochemical labeling for the *p53* gene product has been used to examine duct lesions for *p53* inactivation.[22] For example, DiGiuseppe et al found abnormalities in p53 labeling in 2 of 17 (12%) of histologically high-grade lesions (carcinomas *in situ*).[22] By contrast, all histologically lower-grade duct lesions labeled normally, suggesting that *p53* gene inactivation is a late event in genetic progression in pancreatic ducts.

DPC4

The *DPC4* tumor-suppressor gene on chromosome 18q is inactivated in ~55% of infiltrating pancreatic carcinomas, and immunohistochemical labeling for the *DPC4* gene product has recently been shown to mirror *DPC4* gene status.[34,35] Wilentz et al studied a large series of pancreata using the anti-Dpc4 antibody. Dpc4 expression was intact in all of the low-grade duct lesions they examined, whereas 30% of the histologically high-grade lesions had a complete loss of Dpc4 expression.[19] Thus, just as is true for the *p53* gene, inactivation of the *DPC4* gene appears to be a late event in the genetic progression of pancreatic cancer.

BRCA2

The *BRCA2* tumor suppressor gene on chromosome 13q is inactivated in 7 to 10% of pancreatic carcinomas, and Goggins et al have recently demonstrated biallelic inactivation of *BRCA2* in a histologically high-grade duct lesion.[23] In this study, of patients that carried a germline *BRCA2* mutation, the remaining normal allele of *BRCA2* was intact in all of the histologically lower-grade duct lesions, suggesting that *BRCA2* inactivation, like *DPC4* and *p53* inactivation, occurs relatively late in neoplastic progression in the pancreas.

Loss of Heterozygosity

In this issue of *The American Journal of Pathology*, Yamano, Fujii, Takagati, Kadowaki, Watanabe, and Shirai report their studies of carefully microdissected duct lesions in which they detected loss of heterozygosity (LOH) using a panel of microsatellite markers.[17] The markers used included markers on chromosome arms 3p, 4q, 5q, 6q, 8p, 9p, 10q, 11q, 13q, 16q, 17p, and 18q. All histologically high-grade intraductal lesions exhibited LOH at more than one chromosomal locus, confirming the genetic complexity of this end of the progression model. The patterns of loss observed also confirm both the importance of the genetic targets and the sequence of gene inactivation in the genetic progression to pancreatic cancer. One allele of chromosome 9p (the location of the *p16* gene) was lost in ~13% of histologically low-grade duct lesions in dramatic contrast to the 90% of histologically high-grade duct lesions that had LOH of this chromosome arm. Chromosome arms 17p (the location of the *p53* gene) and 18q (the location of the *DPC4* gene) had LOH only in duct lesions with severe dysplasia. Their elegant technique also sets the stage for a cladistic understanding of neoplastic progression in the pancreas, genetically dissecting the heterogeneity that occurs after the initial invasive focus, as well as identifying the genetic combinations within the duct lesions that did not progress to invasive cancer.

Progression Model

Taken together, these morphological, clinical, and genetic observations all suggest a progression model for pancreatic neoplasia (Figure 1).[19,36] Just as there is a progression in the colorectum from adenoma to invasive cancer, so too is there a progression in the pancreas from

intraductal proliferation to invasive ductal carcinoma. This progression is associated with increasing degrees of cytological and architectural atypia and with the accumulation of genetic alterations in cancer-associated genes. K-*ras* activation and HER-*2/neu* expression appear to occur relatively early, *p16* inactivation is at an intermediate stage, and *p53*, *DPC4*, and *BRCA2* inactivation are late.

Implications of the Progression Model

This progression model for pancreatic carcinoma has a number of important implications. First, it suggests that the nomenclature used to designate proliferative duct lesions in the pancreas should reflect their neoplastic nature (most are clonal proliferations with genetic alterations in cancer-associated genes). The term "pancreatic intraepithelial neoplasia" (PanIN), as proposed by the National Cancer Institute-sponsored Pancreatic Think Tank held in Park City, Utah in September 1999, has been adopted. (For details on this nomenclature, see http://pathology.jhu.edu/pancreas/panin.) Second, this progression model suggests that, were the right tools to be developed, we might reasonably expect to be capable of detecting early, potentially curable pancreatic neoplasms. Third, this progression model suggests that, just as adenomas in the colon are a reasonable target for chemoprevention, so too are PanINs in the pancreas a potential target for chemoprevention trials.[37–39]

Summary

The genetic progression described by Yamano et al in this issue of *The American Journal of Pathology* helps establish a progression model for pancreatic ductal carcinoma. This progression model has a number of important implications for the classification of lesions in the pancreas and for the future early detection and chemoprevention of pancreatic carcinoma.

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