Review Article
Update on Pancreatic Intraepithelial Neoplasia

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Abstract: Pancreatic intraepithelial neoplasia (PanIN) is a histologically well-defined precursor to invasive ductal adenocarcinoma of the pancreas. PanINs are remarkably common lesions, particularly in the elderly population. Molecular studies have helped establish the progression of PanIN to invasive cancer, and recently genetically engineered mouse models have been generated that recapitulate the entire spectrum of lesions from precursor to invasive pancreatic cancer. Some PanIN lesions produce lobulocentric atrophy of the pancreatic parenchyma, and, when multifocal, this lobulocentric atrophy may be detectable using currently available imaging techniques such as endoscopic ultrasound. The association of acinar-ductal metaplasia with PanIN lesions has led some to hypothesize that PanINs develop from acinar cells that undergo acinar-ductal metaplasia.
Key Words: Pancreatic cancer, intraepithelial neoplasm, metaplasia, genetics, epigenetics

Introduction

Invasive adenocarcinoma of the pancreas is one of the, if not the, most lethal of all of the solid malignancies [1] It is usually not diagnosed until after the cancer has metastasized, and metastatic pancreatic cancer is a heterogeneous, complex and extremely challenging disease to treat. It will be difficult to make significant progress against pancreatic cancer if we focus our efforts solely on treating metastatic disease. Experience in the treatment of breast and colorectal neoplasms has demonstrated that the detection and treatment of early, non-invasive, disease have a major impact on cancer mortality [2-5]. We believe that the detection and treatment of early, non-invasive, pancreatic neoplasia will have a major impact on pancreatic cancer mortality.

Three morphologic forms of non-invasive pancreatic neoplasia have been defined. These are: 1) pancreatic intraepithelial neoplasia (PanIN), 2) mucinous cystic neoplasm (MCN), and 3) intraductal papillary mucinous neoplasm (IPMN). PanINs are by far the most common of these lesions. This review will focus on pancreatic intraepithelial neoplasia.

Clinical

A number of studies have examined the prevalence of PanINs in surgically resected pancreata and in autopsy material [6-8]. Like invasive pancreatic cancer, the prevalence of PanINs increases with age [9], and in most reports, PanINs are more common in the head of the gland than in the tail [6-8]. PanINs, and particularly higher grade PanINs (PanIN-3), are more common in pancreata with an invasive cancer than they are in pancreata with chronic pancreatitis, and PanINs are more common in pancreata with chronic pancreatitis than they are in controls. For example, Andea *et al* found a progressive increase in the number of PanINs and in the grade of PanINs when control pancreata were compared to pancreata with chronic pancreatitis and to pancreata with ductal adenocarcinoma [6]. PanINs were identified in 16% of the controls (none of which had PanIN-3), in 60% of the cases of chronic pancreatitis (4% had PanIN-3), and in 82% of pancreata with pancreatic cancer (40% harbored a PanIN-3) [9]. These clinical studies demonstrate that PanINs are very common lesions, and the clinical features of PanINs parallel those of invasive pancreatic cancer.
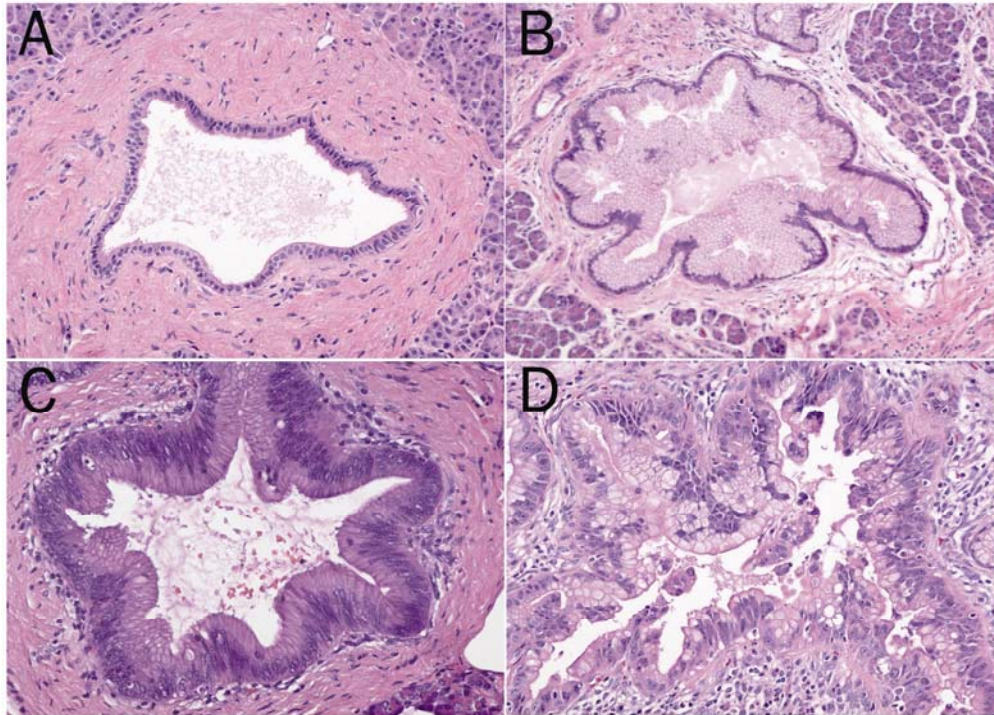
Figure 1 The progression from normal ducts (**A**) to pancreatic intraepithelial neoplasia (PanIN) (**B-D**) is associated with both architectural and cytological changes. In PanIN-1 the nuclei are uniform and basally oriented (**B**). The nuclei in PanIN-2 are slightly larger, more basophilic and there is some loss of nuclear polarity (**C**). The nuclear pleomorphism in PanIN-3 can be significant (**D**).

There have only been a handful of isolated case reports of patients with PanINs who later developed an invasive pancreatic cancer [10, 11]. The frequency and speed at which PanINs progress to invasive pancreatic cancer remain two important unanswered clinical questions. The only estimate of the progression of PanINs that we are aware of is a "back of the envelope" calculation reported by Terhune *et al* in a footnote to a paper on *KRAS2* gene mutations [12]. Based on estimates of the prevalence of PanINs and the known prevalence of invasive pancreatic cancer, they estimated a 1% probability of a single PanIN lesion progressing to invasive cancer [12].

Morphology

Lesions which we now call PanINs have been recognized for over a century, but their significance was not recognized until recently [13-15]. As a result, these lesions were called by a wide variety of names including duct hyperplasia, hypertrophy, metaplasia, dysplasia, and, in German, zwishenformen [13-15]. PanINs arise in the smaller pancreatic ducts, they measure less than 0.5 cm, and

they are classified morphologically into three grades. PanIN-1 lesions are composed of columnar epithelial cells with basally oriented uniform and round nuclei (**Figure 1**) [13, 14]. PanIN-1 lesions can be flat (PanIN-1A) or papillary (PanIN-1B) [13, 14]. PanIN-2 lesions are architecturally slightly more complex than PanIN-1 lesions, and they have more nuclear changes including loss of nuclear polarity, nuclear crowding, variation in nuclear size (pleomorphism), nuclear hyperchromasia, and nuclear pseudostratification [13, 14]. PanIN-3 lesions show the greatest degree of dysplasia. These lesions are architecturally complex, forming papillae and cribriform structures, and in some instances clusters of cells bud off of the epithelium into the lumen of the duct [13, 14]. Cytologically, the nuclei in PanIN-3 lesions are enlarged, pleomorphic, and poorly oriented [13, 14]. Nucleoli are often prominent and mitotic figures, and even abnormal mitoses, can be seen [13, 14].

PanINs need to be distinguished from the larger intraductal papillary mucinous neoplasms (IPMNs) [16]. IPMNs are larger than PanINs, and IPMNs tend to have longer

and more mucinous papillae than PanINs [14, 16]. Most PanINs cannot be appreciated grossly and most are smaller than 0.5 cm [14, 16]. Most IPMNs are larger than 1 cm, and most are easily seen grossly and radiographically [14, 16]. The expression of the mucin MUC2 also favors the diagnosis of an IPMN [17]. The two lesions are easy to distinguish at the extremes, but it is becoming increasingly clear that a number of precursor lesions fall in between the two. These in between lesions not only pose diagnostic challenges, but they also suggest that in some instances small lesions which we currently classify as PanINs may progress to larger lesions which we would classify as an IPMN. Additional studies are needed to establish this possible continuum.

Three new observations on the morphology of PanIN lesions deserve special note. First, Brune *et al* and Detlefsen *et al* have reported that distinctive lobulocentric atrophy is associated with many PanIN lesions, even low-grade PanIN lesions [18, 19]. This lobulocentric atrophy raises the possibility that PanIN lesions hamper the flow of acinar secretions producing a localized form of chronic pancreatitis [18, 19]. This may initiate a vicious cycle in which an early PanIN lesion causes localized duct obstruction, inflammation, and epithelial injury and regeneration which conspire to promote the progression of the PanIN lesion [18]. In addition, the foci of lobulocentric atrophy are larger than the PanIN lesions themselves, and, as will be discussed in greater detail later, these larger lesions may be clinically detectable [18, 20].

Second, PanINs, and particularly PanINs in individuals with a strong family history of pancreatic cancer, can be multifocal [18, 20, 21]. Brune *et al* reported eight patients with a strong family history of pancreatic cancer who underwent partial pancreatectomy because a screening endoscopic ultrasound revealed an early pancreatic lesion, and, remarkably, 11% of the duct profiles in these eight pancreata harbored a PanIN lesion (range 1.0% to 27.3%) [18]. This multifocality of disease has profound implications for patient management [20, 22]. If a patient with a strong family history of pancreatic cancer undergoes partial pancreatectomy and is found to have multi-focal PanINs, they are likely to have additional PanIN lesions in the unresected portion of

their gland. On the plus side, the combination of lobulocentric atrophy and multifocality may produce a distinctive change in the pancreas that is detectable using currently available imaging technologies. For example, Canto *et al* screened seventy-eight high-risk patients (72 from familial pancreatic cancer kindreds, 6 individuals with the Peutz-Jeghers syndrome) and found that multifocal lobulocentric atrophy caused by multifocal PanIN lesions produced endoscopic ultrasound changes similar to those seen with chronic pancreatitis [18, 20]. This major advance establishes that early pancreatic neoplasia, in this case multifocal PanIN lesions, can be detected in asymptomatic individuals before an invasive cancer develops.

Third, a growing number of observations have highlighted acinar to ductal metaplasia in association with PanIN lesions [18, 23]. The acini in the lobules of lobulocentric atrophy described earlier are often characterized by prominent acinar-ductal metaplasia. As will be discussed in greater detail later, the association of acinar-ductal metaplasia with PanIN lesions has led investigators working with genetically engineered mouse models of pancreatic cancer to suggest that PanINs develop from acinar cells that undergo acinar-ductal metaplasia [23, 24].

Genetics

The genetic changes in PanIN lesions have been determined using a variety of techniques. Activating point mutations in the *KRAS2* gene have been described, as has bi-allelic inactivation of the *MAD4/DPC4*, *TP53* and *p16/CDKN2A* genes [12, 25-35]. Recently, telomere shortening has been shown to be an early event in PanIN development [36]. As one would expect for a precursor lesion, the genes mutated in PanINs are many of the same genes mutated in invasive pancreatic cancer.

Two important observations have been made in the genetic analyses of different grades of PanIN. First, although the mutations do not occur in a specific well-regulated order, some mutations usually occur before others. *KRAS2* gene mutations and telomere shortening tend to appear first (in PanIN-1 lesions), followed by the inactivation of *p16/CDKN2A* (usually in PanIN-2), and finally the inactivation of *TP53* and *MAD4/DPC4* (typically in PanIN-3 lesions) (**Figure 2**) [25-35, 37]. Second, the prevalence
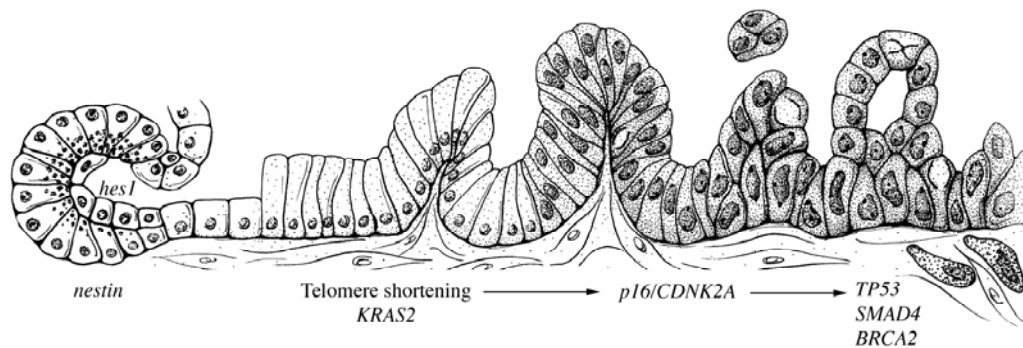
Figure 2 Model for the histological and genetic progression from normal cells (far left) through PanIN lesions (center), to invasive pancreatic cancer (far right). (Reprinted, with permission, from Annual Review of Pathology: Mechanisms of Disease. 2008;3:157-188 © by Annual Reviews. www.annualreviews.org).

of mutations increases in parallel with the degree of dysplasia. For example, Lohr *et al* conducted a meta-analysis of the studies published between 1988 and 2003 that provided information on *KRAS2* gene mutations in PanIN lesions from pancreata of patients with pancreatic cancer [37]. As has been reported for *p16/CDKN2A*, they found a stepwise increase in *KRAS2* gene mutations with the grade of dysplasia of the PanIN lesion [32, 37]. *KRAS2* gene mutations were found in 36%, 44%, and 87% of PanIN-1A, 1B, and 2-3 lesions, respectively (trend statistic P <0.001) [37]. Thus, in PanIN lesions, we see a progression from apparently harmless precursors to deadly invasive cancers.

These findings have a number of important implications. First, the finding that the same genes are targeted in PanINs and in invasive pancreatic cancer lends strong support to the hypothesis that PanINs are a precursor to invasive pancreatic cancer. Second, the finding that the prevalence of mutations increases with the degree of dysplasia validates the histological grading system that has been developed for PanIN lesions [13]. Third, knowledge of the relative order in which genes are mutated in PanINs has helped guide the creation of genetically engineered mouse (GEM) models of pancreatic cancer [38-40]. As discussed in greater detail later in this review, the major breakthrough in the creation of GEM models that mimic human disease came when Hingorani *et al* introduced endogenous *KRAS* gene mutations into the embryonic mouse pancreas [39]. Finally, an understanding of the relative order at which genetic alterations

occur in PanINs forms an essential foundation for the development of early detection tests based on genetic changes. For example, *KRAS2* gene mutations are a natural target for gene-based screening tests because these mutations occur relatively early in the development of pancreatic neoplasia [41, 42].

Epigenetic Alterations

Gene expression can be controlled by promoter methylation, and a number of genes have been shown to be abnormally methylated in pancreatic cancer [43-45]. For example, the *p16/CDKN2A* gene is genetically inactivated in ~80% of invasive pancreatic cancers, and in another 15-20% it is inactivated by promoter hypermethylation [46]. As is true for genetic mutations, the prevalence of these abnormally methylated genes increases with the grade of the PanIN lesion [47, 48]. For example, Fukushima *et al* reported that the *p16/CDKN2A* gene is hypermethylated in 7% of PanIN-1 lesions and in 21% of PanIN-3 lesions [47]. A number of tests have been developed to detect abnormally methylated genes in a variety sample types, and this abnormal methylation can therefore potentially form the basis of a detection test for early, non-invasive, disease [49, 50].

Protein Expression

As one would expect for a precursor lesion, many of the same proteins expressed in invasive pancreatic cancer are also expressed in PanIN lesions [51-55]. Global analyses of gene expression have shown that a number of

markers of gastric epithelial differentiation, such as pepsinogen C, MUC6, KLF4 and TFF1, are expressed in PanINs [55]. In addition, several cell adhesion molecules, such as claudin 18, and proteins that play a role in calcium regulation, such as the S-100 proteins, are expressed in both PanINs and in invasive pancreatic cancers [51-55]. Some of these proteins are expressed in early lesions (PanIN-1 lesions), while other proteins aren't expressed until late, in PanIN-3 lesions [51, 52]. For example, Bhanot *et al* reported an almost exponential increase in survivin transcript levels from low-grade PanINs (PanIN-1) to high-grade PanINs (PanINs-2 and 3) to invasive cancer [51]. The patterns of protein expression in PanIN lesions are important because the proteins expressed in low-grade PanINs may be reasonable chemoprevention targets, while those expressed late (in PanIN-3 lesions) are potential markers for the early detection of pancreatic neoplasia. For example, Maitra *et al* have shown that cyclooxygenase-2 (COX-2) is expressed in human PanINs and Funahashi *et al* have shown that COX-2 inhibitors delay the progression of PanINs to invasive cancer in a mouse model of pancreatic neoplasia [52, 56]. S100A11 is a protein expressed in PanINs that may serve as a good marker for the early detection of pancreatic neoplasia [53].

A number of mucins are also expressed in PanIN lesions [17, 57-60]. PanINs, particularly high-grade PanINs, express MUC1, MUC4, MUC5AC, and MUC6 [14, 17, 58-60]. These mucins can be used to distinguish PanINs from IPMNs, because PanINs, in contrast to IPMNs with intestinal differentiation, do not express MUC2 [17]. These mucins are also potentially detectable by imaging [61] and they may be useful for screening and as therapeutic targets for the treatment of precursor lesions [62, 63].

Mouse Models

An understanding of the genetics of human PanINs and invasive pancreatic cancer was critical to the development of genetically engineered mouse models of pancreatic cancer [64]. The first model to successfully recapitulate the human disease was developed by Hingorani *et al* [39]. They directed the endogenous expression of mutant *KRAS(G12D)* to progenitor cells of the mouse pancreas using the PDX1 and PTF1 promoters [39]. *KRAS* was chosen because, as discussed earlier, *KRAS2* gene mutations occur early in human PanIN lesions and are almost ubiquitous in invasive pancreatic cancer [39]. These genetically engineered mice first developed lesions morphologically very similar to human PanINs and months later they developed invasive ductal adenocarcinomas [39]. The fact that these animals developed PanIN lesions before they developed invasive cancer has helped to validate the hypothesis that PanINs can progress to invasive cancer.

Once a *KRAS* gene-based genetically engineered mouse model was developed, the next natural step was to add additional genetic alterations to define the role of these additional genetic changes in the development of pancreatic neoplasia [64]. The genetic alterations that have been combined with *KRAS* mutations, based on the genes targeted in human pancreatic cancer, include *p16(ink4a)* mutations, *TP53* mutations and *MAD4/DPC4* [40, 65-68]. Invasive pancreatic cancer developed faster with the addition of *p16/CDKN2A* and *TP53* mutations, and, of interest, cystic neoplasms developed with the addition of *MAD4/DPC4* mutations [40, 65-68]. These experiments are complex and sometimes produce varying results. Nonetheless, they do help define the role of these genes in the progression of pancreatic neoplasia.

Mouse models can also be used to examine the role of other medical conditions and environmental factors in the development of pancreatic cancer [38, 69]. For example, Guerra *et al* demonstrated that when *KRAS* mutations are created in adult mice these genetically engineered mice do not develop lesions, but when pancreatitis is induced these same mice develop PanINs and pancreatic cancer [38]. Studies such as this one, when integrated in with observations made in humans, can help define the contribution of genetic, medical and environmental factors in the development of pancreatic cancer [38, 70].

Mouse models are potentially useful tools for the discovery of markers of early pancreatic neoplasia. One of the limitations in studying human disease is that it is rare to identify patients with documented PanINs who do not also have an invasive neoplasm. By contrast, it is relatively easy to obtain biosamples from

genetically engineered mice with PanINs but no invasive cancer. Indeed, Bardeesy *et al* have recently reported that they were able to identify serum markers of human pancreatic cancer through proteomic analysis of biosamples from genetically engineered mouse models of PanIN and pancreatic cancer (presented at the 2007 SPORE Meeting, Baltimore, MD).

Finally, the issue of "cell of origin" has been examined in genetically engineered mouse models using different promoters to target the expression of mutant *KRAS* in different cell populations [24, 38, 71-73]. While the early models targeted pancreatic progenitor cells using the PDX1 and PTF1 promoters, the expression of mutant *KRAS* in acinar and centroacinar cells as directed by the nestin and even the elastase promoters produces PanIN and invasive pancreatic cancer [38, 71]. Surprisingly, the expression of mutant *KRAS* in mature ductal cells as driven by the cytokeratin 19 promoter did not induce PanINs nor did it produce invasive cancer [74]. Careful examination of the pathologic changes in these models suggests that acinar-ductal metaplasia and centroacinar cell expansion play a role in the formation of PanINs, at least in mice (**Figure 2**) [39, 72, 73].

Integrating Mouse Models and Human Disease

Although they are often performed in isolation, it is helpful to integrate the observations made with human disease with the results produced by genetically engineered mouse models. First, both the experience in humans and the observations in mice support the importance of genetic alterations, including mutations in the *KRAS2*, *p16/CDKN2A*, *TP53* and *MAD4/DPC4* genes, in the development of pancreatic neoplasia [39, 40, 46, 65-67, 68, 75, 76]. These mutations are common in human disease and can drive the formation and/or progression of disease in mouse models. Second, both the experience in humans and the observations in mice support the importance of non-genetic factors, such as chronic pancreatitis, contributing to disease [38, 69, 70]. Third, both the experience in humans and the observations in mice support the hypothesis that invasive pancreatic cancers can evolve from PanIN lesions [13]. Finally, observations made in the mouse models and then translated to observations in

humans, suggest that PanINs may not be the earliest precursor lesions; that centroacinar cells and acinar-ductal metaplasia may play a role in some instances [39, 73].

Early Detection

It is our belief that the early detection of precursor lesions, such as PanINs, offers the greatest hope of saving lives that would otherwise be lost to pancreatic cancer [2]. A number of potential targets for the early detection of PanIN lesions have been discussed, including mutant genes, aberrantly methylated genes, and over expressed proteins. While these are all exciting markers for the future, we need to do something now. This need is felt greatest in individuals with a strong family history of pancreatic cancer because they appear to be the most at-risk for developing pancreatic cancer [77]. Patients with three or more first-degree relatives with pancreatic cancer have a 14 to 32-fold increased risk of developing pancreatic cancer, and this risk is significant [77]. For example, as of September 1, 2007, 56 incident pancreatic cancers have developed in at-risk family members in the National Familial Pancreas Tumor Registry and almost all of these patients presented with metastatic disease [77].

Endoscopic ultrasound (EUS) is one of the best available technologies to image the pancreas and M. Canto *et al* and Brentnall *et al* have used EUS to screen asymptomatic, apparently healthy, members of families in which there have already been several pancreatic cancers [20, 22, 78]. Close to 10% of the individuals screened by Canto *et al* were found to have a significant precursor lesion in their pancreas [20, 78]. Most of the lesions detected were intraductal papillary mucinous neoplasms [78]. In addition, as discussed earlier in the section on the morphology of PanINs, it was noted that multifocal PanINs, because they produce multifocal lobulocentric atrophy, can be detected by EUS because they give the pancreas an appearance similar to that of chronic pancreatitis [18]. Based on these early successes, a multicenter study, called "CAPS 3", examining the efficacy of EUS and other imaging modalities in the early detection of pancreatic neoplasia is underway.

Unanswered Questions

While the last two decades have witnessed an explosive growth in our understanding of PanIN lesions and the critical role they play as precursors to invasive pancreatic cancer, there remain several important unanswered questions. First, long-standing pancreatitis, and familial pancreatitis in particular, has been shown to significantly increase the risk of pancreatic cancer in humans [70]. Similarly, the induction of pancreatitis in genetically engineered mouse models can promote pancreatic neoplasia [38]. Yet, most patients with pancreatic cancer don't have a clinical history of pancreatitis. This leads to the question: is subclinical pancreatitis common in the population? Second, if subclinical pancreatitis is common in the population what is the mechanism by which pancreatitis promotes the development of pancreatic cancer? Is it the result of inflammation, injury and repair, or does pancreatitis induce acinar to ductal metaplasia and thereby promote pancreatic neoplasia? The answer to this question may guide intervention strategies. Should efforts to prevent the progression of normal cells to PanINs to cancer focus on preventing acinar to ductal metaplasia or should they focus on reducing inflammation [53, 62, 79]? Third, what is the natural history of untreated PanIN lesions [10, 80]? What percentage of PanINs progress and how fast do the various PanIN lesions progress to invasive carcinoma? Finally, does a PanIN arising in an individual with a strong family history of pancreatic cancer behave the same as PanIN arising as a sporadic lesion [18]? This is a critical issue for screening efforts directed at the high-risk population with a family history of pancreatic cancer.

Conclusion

Pancreatic intraepithelial neoplasia is a precursor to one of the deadliest of all of the solid malignancies. Efforts to prevent, to detect, and to treat these lesions have the potential to save many lives.

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