Commentary

Loss of Heterozygosity or Intragenic Mutation, Which Comes First?

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In the current issue of the *American Journal of Pathology*, Lüttges and colleagues¹ describe careful molecular and immunohistochemical analyses of the presumed precursors to invasive adenocarcinomas of the pancreas, “pancreatic intraepithelial neoplasias” (PanINs).¹ More than 80 lesions representing various grades of PanIN were microdissected and analyzed for allelic loss at chromosome arms 9p, 17p, and 18q. The authors coupled these molecular analyses with immunolabeling for the Dpc4 and p53 proteins, which are the products of established tumor-suppressor genes located on 18q and 17p, respectively.

The results of these studies were extremely interesting. No allelic losses were detected in the histologically low-grade PanINs (PanINs-1), moderate losses were detected in intermediate-grade PanINs (PanINs-2), and abundant losses were detected in high-grade PanINs (PanINs-3). Of interest, allelic loss at 17p and 18q was often seen in intermediate-grade PanINs with normal p53 and Dpc4 expression, suggesting that in these lesions allelic loss occurs as the first genetic hit at these loci.

Molecular Biology of Pancreatic Precursors

This work builds on a growing body of studies on the molecular genetics of precursor lesions in the pancreas² in several important ways.^{3,4} First, Lüttges and colleagues verify that PanINs are indeed neoplasms—that is, that they are clonal, proliferative lesions harboring genetic alterations in cancer-causing genes. The lesions are clonal because only clonal allelic losses could have been detected with the methods used. This study and others have shown that these clonal changes involve alterations in a variety of genes, including the *K-ras*, *p16*, *p53*, and *DPC4* genes.^{4–9}

Indeed, the evidence provided by this article and other studies lends support to the recent establishment of an international nomenclature to classify these intraductal proliferative lesions in the pancreas. Terms such as “hy-

perplasia,” “metaplasia,” and “dysplasia” were all replaced with the single, unified “pancreatic intraepithelial neoplasia (PanIN)” nomenclature.¹⁰ This unification of terminology and diagnostic criteria is a critical first step toward a better understanding of the precursors to invasive pancreatic cancer. With a single unified nomenclature, molecular analyses performed at one institution can be directly compared to analyses performed at another center. Unfortunately, although the establishment of uniform nomenclature and diagnostic criteria seems simple, it still has not been accomplished for several major tumor types, including breast neoplasia.

Second, molecular analysis of precursor lesions with varying degrees of histological atypia also helps establish the timing of these genetic events. Lesions with patterns of genetic alterations most closely approaching those seen in invasive cancers presumably are the latest or most advanced lesions, whereas lesions with fewer genetic alterations are presumably earlier ones. This approach has helped establish molecular histological progression models for a variety of neoplasms, including adenocarcinomas of the colorectum and pancreas.^{11–13}

Third, the molecular analysis of PanINs is also an important first step in the development of a new molecular taxonomy of precursor lesions (the subject of a recent National Cancer Institute-sponsored meeting organized by Donald Henson in Bethesda, MD). For example, Lüttges and colleagues¹ demonstrate two distinct genetic populations within a single histological grade of precursor lesions. Only a third of so-called “lower grade” PanINs-2 harbored allelic losses at 17p, whereas three quarters of “higher grade” PanINs-2 showed loss of heterozygosity (LOH) at 17p.¹ Molecular analyses may therefore help subcategorize lesions that were previously lumped together.

Such molecular taxonomies will have meaning only if they guide clinical decisions. Molecular taxonomies of precursor lesions that 1) delineate the subset of lesions

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that will progress to invasive cancer; 2) identify lesions that chemoprevention strategies can target; 3) advance our understanding of cancer etiology; and 4) single out targets for molecular screening, will all have real impact. By contrast, molecular taxonomies that subdivide lesions merely because they can be subdivided will only distract us from our ultimate goal, ie, finding a cancer cure.

LOH or Intragenic Mutation: Which Comes First?

In addition to helping refine the molecular taxonomy of precursor lesions of the pancreas, the article by Lüttges and colleagues also raises fundamental questions about the timing of genetic alterations, such as the inactivation of tumor-suppressor genes, in the development of an invasive neoplasm. Unlike the *K-ras* oncogene, which promotes carcinogenesis by activating only one of its two copies (alleles), tumor-suppressor genes such as *p53* and *DPC4* must inactivate both of their alleles to abolish the function of proteins involved in growth inhibition.

Inactivation of both alleles can occur by a variety of mechanisms. Most commonly, there is an intragenic mutation or deletion in one allele that is coupled with loss of the other allele (LOH).¹⁴ When both alleles are deleted, the term "homozygous deletion" is used. For example, the *DPC4* gene is inactivated by homozygous deletion in 35% of pancreatic cancers and by intragenic mutation coupled with LOH in 20%.^{15–17} In contrast, in pancreatic cancer the *p53* gene is almost exclusively inactivated by intragenic mutation in one allele coupled with LOH.¹⁸

This need for biallelic inactivation raises an important question about the timing of events in tumor-suppressor gene inactivation: which comes first—intragenic alteration (Figure 1A) or LOH (Figure 1B)? In this article Lüttges and colleagues present evidence that at first seems to resolve this issue. They contend that LOH is the first event in the two-hit model of tumor-suppressor gene inactivation.

Lüttges and colleagues make this argument by studying both LOH and gene expression in the same set of PanINs. They detect LOH with traditional genetic analysis and expression of the *p53* and *DPC4* genes with immunohistochemical techniques. Immunohistochemical overexpression of the p53 protein is a good marker of a *p53* mutation because mutated p53 protein is longer lived than its wild-type counterpart.¹⁹ In contrast, immunohistochemical loss of the Dpc4 protein is a good marker of *DPC4* inactivation because most intragenic alterations in the *DPC4* gene are either deletions or nonsense mutations.²⁰

The authors show that LOH at 17p and 18q (the locations of the *p53* and *DPC4* genes, respectively) occurs in lesions with normal p53 and Dpc4 expression. For example, they detect LOH at 17p and 18q in 77 and 58% of moderately dysplastic PanIN-2 lesions. In contrast, only 11 and 13% of the same lesions show abnormal immunohistochemical expression of the p53 and Dpc4 proteins. This means that only 11 and 13% of moderately dysplastic lesions have inactivation of both alleles of these genes, seemingly implying that inactivation of the second allele by intragenic alteration is a later event than

Figure 1. Inactivation of tumor-suppressor genes. Inactivation of a tumor-suppressor gene requires inactivation of both of its alleles. This commonly occurs by intragenic mutation (IM) in one allele coupled with loss of the other allele (LOH). This diagram presents three different scenarios for the timing of these events. The **circle** represents a previously characterized gene allele. The **square** represents a yet undiscovered gene allele. An **X** represents an IM in one of these two genes. **A:** IM occurs before LOH of a single gene. **B:** LOH is the first hit. **C:** IM occurs in a yet undiscovered gene (**square**) that is located on the same chromosomal arm as a previously characterized gene under study. LOH then takes place on this chromosomal arm. Lastly, the gene under study (**circle**) becomes mutated, and it too is biallelically inactivated. (Artwork by Jennifer L. Parsons, M. A.)

LOH at the *p53* and *DPC4* gene loci. The fact that later PanIN-3 lesions show an increased rate of immunohistochemical expression abnormalities seems, at first blush, to fit with the authors' contention that LOH is the first hit in tumor-suppressor gene inactivation.

An Alternative Explanation

Thus, Lüttges and colleagues do show that LOH at 17p and 18q occurs before the inactivation of the *p53* and *DPC4* genes. This, however, does not mean necessarily that LOH is the first hit of the two needed to inactivate a tumor-suppressor gene. This LOH may actually represent the inactivation of other tumor-suppressor genes at 17p and 18q that have not yet been discovered (Figure 1C). The LOH at 17p and 18q could represent the second hit occurring after intragenic inactivation of first alleles of yet unidentified genes. The first hit (an intragenic mutation) is not recognized because the gene is not known.

Three lines of evidence support this alternative explanation for the authors' data. First, by studying familial cancers, we know that there are inherited cancer syndromes in which intragenic alteration comes first. For example, Goggins and colleagues⁸ have shown that a subset of familial pancreatic cancers is the result of inherited germline mutations in the *BRCA2* gene, with loss of the other allele occurring later, at the PanIN-3 stage. Of course, it is possible that sporadic cancers behave differently from inherited ones, but inherited cancers do

provide some evidence that intragenic mutations can occur before LOH.

Second, there already is good evidence that there are additional, yet undiscovered tumor-suppressor genes on 17p and 18q. This evidence is seen within the present study and others as well. For example, Lüttges and colleagues report that 60 and 88% of PanIN-3 lesions show LOH at 17p and 18q, respectively, even though only 41 and 41% of PanIN-3 lesions show inactivation of the *p53* and *DPC4* genes, respectively. Similarly, 91 and 82% of invasive adenocarcinomas show LOH, whereas 67 and 55% show inactivation of the *p53* and *DPC4* genes by immunohistochemistry.^{7,20} The large differences between the percentage of tumors with LOH on these chromosome arms and inactivated genes believed to be targeted by these losses suggest other genes are involved. Indeed, original allelotyping of pancreatic cancer shows similar results.²¹

Third, from a purely theoretical vantage, an intragenic mutation as the first hit may provide a selective growth advantage to neoplastic cells. When intragenic mutation is the first hit, the protein product of this allele may interfere with the function of the normal protein produced by the remaining intact allele. This situation would provide a growth advantage to those cells with a mutated allele, even if the other allele has not yet been lost. In contrast, LOH without accompanying mutation would be unlikely to provide a selective growth advantage to neoplastic cells, as one normal allele is present.¹² Of course, this line of reasoning assumes that there is no dose effect, that is, that having only one normal allele of a gene does not result in protein concentration changes that have an effect on cell growth.

Importance of This Study

Whichever explanation is correct, the paper by Lüttges and colleagues remains important and thought provoking. If LOH is the first hit, a paradigm shift is needed to explain the mechanisms for this LOH and the selective advantage afforded a cell by isolated LOH. If intragenic mutation is the first hit, the authors' data suggest that further studies refining the boundaries of LOH on 18q and 17p in precursor lesions may lead to the discovery of new tumor-suppressor genes.

Therefore, this article solidifies the belief that PanINs are indeed neoplastic and also supports the previously proposed model of genetic changes in pancreatic neoplastic progression.¹¹ In addition, the article raises the issue of the timing of intragenic mutation and LOH in the inactivation of tumor-suppressor genes involved in this progression model. This article helps us to consolidate what we already know about pancreatic cancer progression, and it also encourages us to explore the fundamental nature of the changes in pancreatic cancer progression.

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