Immunohistochemistry as a surrogate to molecular diagnosis in pancreatic tumors

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Abstract

Molecular and genomic characterization of the major neoplasms of the pancreas has produced a wealth of information and insight into the genetics involved in tumor initiation and progression. Many of these genetic discoveries can be interrogated with cost-effective immunohistochemical stains, which include: p53, Smad4, and mismatch repair proteins in pancreatic ductal adenocarcinomas; DAXX and ATRX in well-differentiated pancreatic neuroendocrine tumors; β-catenin and LEF1 in solid-pseudopapillary neoplasms; and, STAT6 in intrapancreatic solitary fibrous tumors. When combined with the gross and morphologic findings, the aforementioned immunohistochemical markers can enhance the diagnosis and prognostication of these neoplasms. The focus of this review is to explore the key molecular and genomic alterations that can be reliably assessed by immunohistochemistry in pancreatic neoplasms and discuss their clinical and pathologic applications.

Keywords ATRX; beta-catenin; DAXX; LEF1; p53; pancreatic ductal adenocarcinoma; pancreatic neuroendocrine tumor; Smad4; solid-pseudopapillary neoplasm; solitary fibrous tumor; STAT6

Introduction

The current classification system of pancreatic neoplasia is based on the gross configuration and predominant cell lineage or lineages within a neoplasm. The gross or radiographic appearance of the neoplasm can often be categorized as solid, cystic or intraductal. In addition, most pancreatic neoplasms recapitulate one or more components of the pancreas: ductal, endocrine, acinar and/or mesenchymal. However, despite the integration of macroscopic and microscopic findings, the pathologic diagnosis of the most common pancreatic neoplasms can be challenging. Furthermore, with the exception of rare histologic variants, a limited amount of information with regards to prognostication and treatment can be inferred from morphology alone.

Over the past few decades, DNA sequencing and gene expression technologies have rapidly evolved and reshaped our understanding of pancreatic neoplasms. Early studies using a candidate gene approach identified several genes that were frequently altered. Subsequent analyses of precursor lesions and distant metastases indicated the accumulation of these molecular and genomic alterations during tumorigenesis.[1] And, more recently, next generation sequencing technologies have uncovered the comprehensive genetic framework for the major pancreatic neoplasms.[2–5] Many of these genetic discoveries can be interrogated with cost-effective immunohistochemical stains and, when combined with morphologic observations, enhance the diagnosis and prognostication of these neoplasms. This review focuses on the key molecular and genomic alterations that can be reliably assessed by immunohistochemistry in pancreatic neoplasms with an emphasis on their clinical and pathologic applications.

Pancreatic ductal adenocarcinoma

Ductal adenocarcinoma constitutes the majority of pancreatic neoplasms and is the fourth leading cause of cancer deaths in the United States. In 2012, an estimated 43,920 individuals were diagnosed with pancreatic cancer in the United States, and approximately 37,390 died from this deadly disease. Although surgical resection offers the only possibility of cure, more than 85% of patients present with inoperable disease at the time of diagnosis. Therefore, chemotherapy and radiation are the mainstay of treatment in most patients. Despite aggressive combined modality treatment approaches, the 5-year survival rate of pancreatic cancer is 6% and has remained unchanged for the last 40 years.[6]

In 2008, a historic milestone was reached with the completion of the pancreatic cancer exome.[5] In a series of 24 pancreatic ductal adenocarcinomas, the coding regions of over 20,000 genes were sequenced and an average of 63 genomic alterations, the majority of which were point mutations, were found in each cancer. The most frequently mutated genes included the oncogene *KRAS* and tumor suppressor genes *CDKN2A*, *TP53* and *SMAD4*. These genes are well-recognized as contributing to pancreatic carcinogenesis and, thus, classifiable as "driver" genes for this tumor type. Among these four genes, abnormal nuclear labeling for p53 and loss of Smad4 expression by immunohistochemistry can be used as surrogate markers for their respective genetic alterations.

The *TP53* gene is mutated in up to 75% of pancreatic ductal adenocarcinomas and encodes for a stress-inducible transcription factor that exerts its tumor suppressive effect through the induction of either cell cycle arrest or apoptosis in damaged cells. Functional loss of the p53 protein enables cell survival and proliferation in the presence of DNA damage, which facilitates the accumulation of further genetic alterations. Inactivation of *TP53* typically occurs through intragenic missense mutations in one allele with loss of the second allele or loss of heterozygosity. Under normal conditions, wild-type p53 is a short-lived protein, but missense mutations prolong the half-life of p53 and result in nuclear accumulation that can be detected by immunohistochemistry.

Interpretation of p53 immunohistochemistry requires implementation of strict scoring criteria and can be separated into three staining patterns. In normal or reactive tissue, wild-type p53 has a mild-to-moderate nuclear intensity with a scattered

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staining distribution among proliferating cells. In contrast, *TP53* missense mutations result in a strong nuclear labeling for p53 protein in over 80% of tumor nuclei (Figure 1a and b). Lastly, in rare cases, a *TP53* deletion or truncating mutation is associated with complete absence of p53 expression within lesional cells. A potential pitfall is misinterpreting the absence of p53 immunolabeling as wild-type *TP53*. Several studies have shown that the assessment of p53 expression can aid in the diagnosis of malignancy on biopsies and cytologic specimens. van Heek et al. reported p53 immunohistochemistry had a 48% sensitivity and 97% specificity for malignancy.[7] However, in combination with cytologic assessment and additional molecular markers, the authors achieved a sensitivity and specificity of 86% and 94%, respectively. Hence, as part of an ancillary panel, p53 immunohistochemistry can be useful in distinguishing pancreatic ductal adenocarcinoma from reactive processes.

Inactivation of *SMAD4*, either by homozygous deletion or intragenic mutation with loss of heterozygosity, occurs in approximately 55% of pancreatic ductal adenocarcinomas. Smad4 is a key mediator of the transforming growth factor beta (TGF-β) signal transduction pathway. Activation of the pathway begins with binding of a TGF-β ligand to type I and type II serine/threonine kinase cell surface receptors. This, in turn, triggers phosphorylation of Smad2/3 to form a complex with Smad4, and together they translocate into the nucleus where, in association with other cofactors, they regulate gene expression. Loss of *SMAD4* and hence canonical TGF-β signaling in pancreatic ductal adenocarcinoma results in abrogation of TGF-β-induced growth suppression and cell death.

By immunohistochemistry, Smad4 is ubiquitously expressed within all cell types of the pancreas and exhibits both nuclear and cytoplasmic staining. The complete absence of Smad4 expression within neoplastic cells, but preserved staining in surrounding stromal cells, accurately mirrors *SMAD4* somatic inactivation (Figure 1c and d). Thus, Smad4 immunohistochemistry can be used as a diagnostic tool in the separation of pancreatic ductal adenocarcinoma from its non-neoplastic mimics.[8] In addition, Smad4 status has prognostic significance. Tascilar et al. immunolabeled a large cohort of surgically resected pancreatic ductal adenocarcinomas for Smad4 and found the absence of protein expression correlated with poor overall survival.[9] Subsequent studies demonstrated the absence of Smad4 was often associated with widespread metastases.[10] Smad4 loss may therefore be predictive of metastatic disease and serve to stratify borderline resectable patients for systemic therapeutic regimens.

In addition to somatic mutations, a subset of patients with pancreatic ductal adenocarcinoma harbor predisposing germline genetic alterations. Nearly 10% of pancreatic ductal adenocarcinomas have a familial component. Among inheritable conditions, pancreatic ductal adenocarcinomas have been observed in patients with Lynch syndrome, an autosomal dominant condition caused by defects in the DNA mismatch repair (MMR) genes. These genes include *MLH1*, *PMS2*, *MSH2* and *MSH6*. While colorectal and endometrial cancers are the most common cancers in this condition, patients have an 8.6-fold increased risk of developing pancreatic ductal adenocarcinoma.[11] Similar to their colorectal counterparts, pancreatic tumors in Lynch syndrome patients often have a distinctive medullary appearance that is characterized by poor differentiation, syncytial growth, pushing borders and prominent intraepithelial lymphocytes.[12] Further, these adenocarcinomas frequently demonstrate microsatellite instability (MSI) due to inactivation of one of the MMR genes.

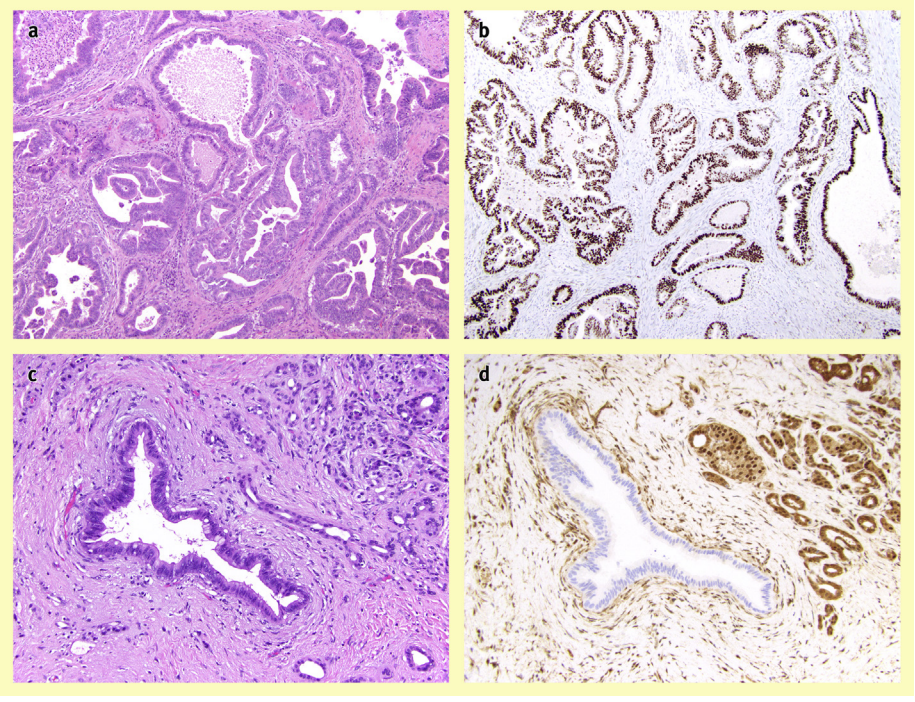

Figure 1 *TP53* and *SMAD4* are two of the most commonly mutated genes in pancreatic ductal adenocarcinoma (**a** and **c**). By immunohistochemistry, *TP53* missense mutations result in a strong nuclear labeling for p53 protein in the majority of tumor nuclei (**b**). In contrast, somatic mutations in *SMAD4* correlate with the absence of Smad4 expression (**d**).