MOLECULAR ONCOLOGY 7 (2013) 232–247

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Review

Genetically engineered mouse models of pancreatic adenocarcinoma

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ARTICLE INFO

Article history:
Received 5 February 2013
Accepted 14 February 2013
Available online 11 February 2013

Keywords:
Cancer models
Pancreatic ductal adenocarcinoma
Tumor microenvironment
Inflammation
Target validation
Therapeutic strategies

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal types of human cancer for which there are no effective therapies. Deep sequencing of PDAC tumors has revealed the presence of a high number of mutations (>50) that affect at least a dozen key signaling pathways. This scenario highlights the urgent need to develop experimental models that faithfully reproduce the natural history of these human tumors in order to understand their biology and to design therapeutic approaches that might effectively interfere with their multiple mutated pathways. Over the last decade, several models, primarily based on the genetic activation of resident KRas oncogenes knocked-in within the endogenous *KRas* locus have been generated. These models faithfully reproduce the histological lesions that characterize human pancreatic tumors. Decoration of these models with additional mutations, primarily involving tumor suppressor loci known to be also mutated in human PDAC tumors, results in accelerated tumor progression and in the induction of invasive and metastatic malignancies. Mouse PDACs also display a desmoplastic stroma and inflammatory responses that closely resemble those observed in human patients. Interestingly, adult mice appear to be resistant to PDAC development unless the animals undergo pancreatic damage, mainly in the form of acute, chronic or even temporary pancreatitis. In this review, we describe the most representative models available to date and how their detailed characterization is allowing us to understand their cellular origin as well as the events involved in tumor progression. Moreover, their molecular dissection is starting to unveil novel therapeutic strategies that could be translated to the clinic in the very near future.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal types of cancer, with an overall 5-year survival below 4%. This is mostly due to the advance state of the disease at the time of diagnosis in most patients, which makes current therapeutics rather ineffective (Hidalgo, 2010; Vincent et al., 2011). Histological, genetic, and clinical studies have identified three different ductal preneoplastic lesions as potential precursors of PDAC. They include pancreatic intraepithelial

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http://dx.doi.org/10.1016/j.molonc.2013.02.002

neoplasias (PanINs) that originate within intralobular ducts, intraductal papillary mucinous neoplasms (IPMNs) that arise in the main pancreatic duct or its major branches, and mucinous cystic neoplasms (MCN) that are mucin-producing epithelial neoplasms with a characteristic ovarian-type stroma. MCNs are classified according to their degree of epithelial dysplasia including mucinous cystadenoma, borderline mucinous cystic neoplasms and mucinous cystic neoplasms with in situ carcinoma (Wilentz et al., 2000). All of these preneoplastic lesions are likely to represent progressive stages of the disease (Hruban et al., 2000; Maitra et al., 2005).

PanINs are the best characterized preneoplastic lesions at the anatomopathological and molecular levels (Hruban et al., 2005). PanINs can be classified in four grades, PanIN-1A, PanIN-1B, PanIN-2, and PanIN-3 or carcinoma-in-situ, based on the degree of dysplasia (Figure 1A). PanIN lesions are frequently associated with lobulocentric atrophy and acinar ductal metaplasia (ADM) structures that have been proposed to be precursors of PanIN lesions (Brune et al., 2006; Detlefsen et al., 2005). These histological alterations are genetically characterized by the cumulative acquisition of mutations in both oncogenes, mainly KRAS, and tumor suppressor genes. The latter are also frequently inactivated by epigenetic mechanisms. In addition, telomere shortening is considered one of the early events in pancreatic tumorigenesis and is thought to be responsible for inducing genetic instability (van Heek et al., 2002). The best characterized mutational event during early PDAC development is the activation of the KRAS oncogene, mainly via single point mutations in codon 12. Indeed as many as 74% of low-grade PanIN lesions and 63% of ADM foci associated with PanINs in human patients contain such oncogenic mutations (Shi et al., 2009). This percentage increases with progression to invasive carcinoma up to more than 90% of the cases. The tumor suppressor most commonly mutated in PDAC is *CDKN2A*. This gene is found inactivated, deleted, or epigenetically silenced in 30–70% of PanIN lesions, a percentage that becomes as high as 95% in full-blown tumors (Wilentz et al., 1998). Mutations in two additional tumor suppressor genes, TP53 and SMAD4, are often associated (50–75% of the cases) with progression of PanIN-3 lesions to invasive PDAC tumors. Mutations in the RI and RII receptors for the transforming growth factor (TGF)-β have been identified with low frequency (<5%). Other alterations frequently found in advanced PanINs and PDAC tumors include over expression of other growth factors and their receptors as well as activation of signaling pathways driven by NFkB, STAT3 and SRC (Goggins et al., 1998; Hahn et al., 1996; Huang et al., 2012; Korc, 1998; Redston et al., 1994; Sclabas et al., 2003; Shields et al., 2011). More recently, analysis of exomic sequences from PDAC patients has revealed that these tumors carry as many as 50 mutations involving at least 12 different core signaling pathways (Jones et al., 2008).

IPMN and MCN are cystic lesions less well characterized at the molecular level. Recent studies have identified an average of 27 mutations in IPMNs and 16 in MCN lesions by exome sequencing (Wu et al., 2011a, 2011b). IPMN mutations included those in KRAS as well as in GNAS (a gene encoding for the guanine nucleotide binding, alpha stimulating protein) and RNF43 (RNF43 codes for a protein with intrinsic E3 ubiquitin ligase activity) genes. MCN mutations included, in addition of KRAS and RNF43 genes, inactivation of the TP53 tumor suppressor (Wu et al., 2011a, 2011b). Although KRAS appear in early lesions, their incidence increases with progressing dysplasia. In contrast, TP53 mutations appear late and are observed only in carcinomas, in combination with mutant KRAS genes (Jimenez et al., 1999).

In the case of IPMNs, KRAS and/or GNAS mutations are present in more than 95% of these lesions. Interestingly, most of the invasive adenocarcinomas in close association with IPMNs display the GNAS mutations present in these preneoplastic lesions (Wu et al., 2011b), indicating that IPMNs might be precursors of PDAC. An independent study using exome sequencing also revealed a high proportion of GNAS mutation (>40%) in IPMN lesions (Furukawa et al., 2011). The results indicate that GNAS mutations are selective for IPMNs suggesting that activation of G-protein signaling may play a key role in the development of these lesions. Interestingly, IPMNs can be detected by computed tomography, allowing their surgical removal before they progress into malignant lesions.

2. Genetically engineered mouse (GEM) models of PDAC: prenatal models

Early attempts to express KRas oncogenes in ductal cells failed to induce PDAC (or any other type of tumor) in mice. The first model to faithfully reproduce the natural history of human PDAC in mice involved expression of a resident (knocked-in) KRasG12D oncogene in all pancreatic lineages during early embryonic development (Hingorani et al., 2003) (Table 1). Briefly, a mouse strain carrying a conditional KRasG12D knocked-in allele silenced by a floxed STOP transcriptional cassette (LSL-KRasG12D) was crossed to transgenic strains that expressed the bacterial Cre recombinase under the control of either the Pdx1 or the Ptf1a/P48 promoters (Hingorani et al., 2003). The resulting strains, designated as Pdx1-Cre; LSL-KRasG12D (from now on KC, according to the nomenclature given in the original publication) and Ptf1a-Cre; LSL-KRasG12D, express the KRasG12D oncogene in all pancreatic lineages from early embryonic development (E8.5 in KC mice and E9.5 in Ptf1a-Cre; LSL-KRasG12D animals). These compound strains develop, with complete penetrance, the full spectrum of PanIN lesions histologically indistinguishable from those present in human patients. Indeed, these mouse PanINs also express mucins, cytokeratin-19, and components of signaling pathways that include Cyclooxygenase-2 (Cox-2), EGFR, MMP-7 and Hes1 (Hingorani et al., 2003). In addition, these mice display ADM, a benign lesion that precedes the appearance and possibly be a precursor of PanIN lesions (Zhu et al., 2007). PanIN lesions progress with long latencies to PDAC in a percentage of mice (Table 1). Addition of mutations in loci encoding tumor suppressors known to be mutated or inactivated in human PDAC such as INK4A, TP53, LKB1 or SMAD4 accelerate the progression of these PanIN lesions leading to the generation of invasive PDAC with complete penetrance (Table 2). A percentage of these compound mice also develop metastatic tumors. Some of the most important GEM PDAC models are summarized in recent reviews (Mazur and Siveke, 2012; Perez-Mancera et al., 2012; Qiu and Su, 2012; Westphalen and Olive, 2012) and in Table 2.