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Review

Chromosome instability and carcinogenesis: Insights from murine models of human pancreatic cancer associated with *BRCA2* inactivation

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ABSTRACT

Chromosomal instability is a hallmark of human cancer cells, but its role in carcinogenesis remains poorly resolved. Insights into this role have emerged from studies on the tumour suppressor BRCA2, whose inactivation in human cancers causes chromosomal instability through the loss of essential functions of the BRCA2 protein in the normal mechanisms responsible for the replication, repair and segregation of DNA during cell division. Humans who carry heterozygous germline *BRCA2* mutations in the *BRCA2* gene are highly predisposed to cancers of the breast, ovary, pancreas, prostate and other tissues. Here, we review recent studies that describe genetically engineered mouse models (GEMMs) for pancreatic cancer associated with *BRCA2* mutations. These studies not only surprisingly show that *BRCA2* does not follow the classical Knudson "two hit" paradigm for tumour suppression, but also highlight features of the interplay between *TP53* inactivation and carcinogenesis in the context of *BRCA2* deficiency. Thus, the models reveal novel aspects of cancer evolution in carriers of germline *BRCA2* mutations, provide new insights into the tumour suppressive role of BRCA2, and establish valuable new preclinical settings for testing approaches to pancreatic cancer therapy; together, these features emphasize the value of GEMMs in cancer research.

Aberrations in the number and structure of chromosomes are a hallmark of cells derived from solid tumours. These aberrations not only include structural anomalies such as gross chromosomal rearrangements (including translocations, large deletions or inversions) and gene copy number variations, which have been connected to defective DNA replication or repair, but also anomalous chromosome number triggered by defective segregation between daughter cells during mitosis. Whereas much recent work has extensively documented the mechanisms underlying normal DNA replication,

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repair and segregation, what happens when these mechanisms are perturbed by the genetic alterations associated with cancer remains far less studied. Here, we will review this question from the perspective of recent work using transgenic models designed to recapitulate a specific human cancer involving mutations affecting the tumour suppressor gene *BRCA2*, associated with hereditary predisposition to breast, ovarian, pancreatic and other cancers. There is by now clear evidence to implicate the large, 3418 residue BRCA2 protein (3328 residues in the mouse) in the maintenance of chromosome integrity during cell division (Venkitaraman, 2009). BRCA2-deficient cells accumulate structural chromosome aberrations as they divide, and also become aneuploid through losses or gains in whole chromosomes (Gretarsdottir et al., 1998; Patel et al., 1998; Tutt et al., 1999; Yu et al., 2000). A large body of evidence connects the chromosomal instability observed in BRCA2-deficient cells to essential biological functions of the BRCA2 protein in DNA repair by homologous recombination (Connor et al., 1997; Patel et al., 1998; Moynahan et al., 2001), in progression through the S and G2/M phases of the cell cycle (Lomonosov et al., 2003; Ayoub et al., 2009; Menzel et al., 2011; Schlacher et al., 2011) and in mitotic cell division by cytokinesis (Daniels et al., 2004; Mondal et al., 2012). Thus, murine models that recapitulate the effect of cancer-associated mutations in human *BRCA2* on tissue-specific carcinogenesis provide an important opportunity to dissect the complex roles played by chromosomal instability during human carcinogenesis.

1. Modelling human pancreatic cancer associated with *BRCA2* inactivation

Pancreatic ductal adenocarcinoma (PDAC) represents the fourth leading cause of cancer mortality worldwide, with an incidence of approximately 217,000 new cases each year nearly matched by 213,000 deaths (Parkin et al., 2001). Several of the most frequent genetic events underlying the initiation and progression of human pancreatic cancer have been identified (Hezel et al., 2006; Maitra and Hruban, 2008). These include activating mutations in the *KRAS* proto-oncogene, which occur in >90% of PDAC (Caldas and Kern, 1995) and are considered as a key driver for pancreatic carcinogenesis, and mutations inactivating the *TP53* gene, which occur in 50–75% of patients (Redston et al., 1994).

Moreover, several lines of evidence implicate mutations inactivating the *BRCA2* tumour suppressor in an estimated 5–20% of familial PDAC (Hahn et al., 2003; Couch et al., 2007). Germline carriers of deleterious *BRCA2* mutations that commonly truncate the encoded protein exhibit an increased lifetime risk of developing PDAC, in addition to their well-known predisposition to cancers of the breast and ovary (Breast Cancer Linkage Consortium, 1999). Within high-risk pancreatic cancer kindreds, inherited mutations in *BRCA2* represent the most frequently encountered germline genetic alteration (Hahn et al., 2003). The incidence of germline *BRCA2* mutations in apparently sporadic pancreatic cancers may be as high as in breast or ovarian cancer (Goggins et al., 1996). More recently, *PALB2*, which encodes a BRCA2-interacting protein also essential for homology-directed DNA repair, has emerged as a pancreatic cancer susceptibility allele (Jones et al., 2009).

Three new transgenic models for pancreatic adenocarcinoma associated with *BRCA2* inactivation have recently been described (Skoulidis et al., 2010; Feldmann et al., 2011; Rowley et al., 2011) [Figure 1]. One of these models does not incorporate activation of the *Kras* oncogene (Feldmann et al., 2011). In contrast, the other two models (Skoulidis et al., 2010; Rowley et al., 2011) use a conditional gene-targeted allele developed by Tuveson, Jacks and colleagues (Jackson et al., 2001; Johnson et al., 2001), in which tissue-specific activation of oncogenic *Kras*G12D is driven on a single allele by *loxP*-CRE mediated recombination, mimicking a genetic event that frequently triggers *Kras* activation in human cancers. CRE recombinase expression is controlled by the PDX1 promoter, which is expressed at E8.5 and required for organogenesis of the pancreas, whereby loss of the gene is associated with an absence of pancreatic formation (Jonsson et al., 1994; Offield et al., 1996). The expression of the PDX1-CRE transgene therefore occurs throughout the pancreatic cellular compartment, albeit in a stochastic manner, to trigger *Kras* activation (Hingorani et al., 2003).

Patients who carry germline mutations affecting *BRCA2* harbour the germline mutant allele in all somatic tissues, whereas the second *BRCA2* allele is wildtype (Wooster et al., 1994). It has been widely believed that loss of the second, wild-type *BRCA2* allele in nascent cancer cells (termed 'loss of heterozygosity' or LOH) is necessary for the emergence of tumours in germline mutation carriers. The pancreatic cancer model developed in our laboratory (Skoulidis et al., 2010) models this presumed sequence of events. It carries in all somatic tissues a truncated allele of murine *Brca2* ($Brca2^{Tr}$), which truncates the gene in an evolutionarily conserved and functionally critical region encoded by exon 11, resembling deleterious germline mutations found in human carriers (Friedman et al., 1998). The second *Brca2* allele, $Brca2^{F11}$ (Jonkers et al., 2001) can be conditionally disrupted to remove exon 11 by *loxP*-CRE recombination in the pancreas. This event is driven by PDX1-CRE, and therefore occurs in the same tissues which undergo *Kras* activation. There is evidence that both the $Brca2^{Tr}$ and $Brca2^{F11}$ alleles can express a truncated protein product (Patel et al., 1998; Choi et al., 2012), a point we will later return to. Notably, the models developed by Rowley et al. and Feldmann et al. both exclusively use a strain homozygous for the conditional $Brca2^{F11}$ allele; thus, germline heterozygosity for *BRCA2* is not modelled in their experiments.

All three of the new pancreatic cancer models incorporate conditional alleles that inactivate *Tp53* in the pancreas, to mimic the frequent loss of this tumour suppressor in human pancreatic cancers. Somewhat different *Tp53* alleles are used by each group, an important distinction given that the nature of *Tp53* mutations is thought to affect pancreatic cancer development (Olive et al., 2004). Two of the studies employ gain-of-function mutants affecting a single allele. These are either the structural mutant $Tp53^{R172H}$ (Feldmann et al., 2011) or the contact mutant $Tp53^{R270H}$ (Skoulidis et al., 2010). Both these *Tp53* mutants are associated with the development of carcinomas (Olive et al., 2004). In contrast, the third study uses a null allele of *Tp53*, wherein exons 2–10 are deleted by PDX1-CRE

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