
### Timing Is Everything: Brca2 and p53 Mutations in Pancreatic Cancer

BRCA2 mutation is among the most common germline mutations responsible for familial pancreatic cancer and is also mutated in sporadic pancreatic cancer. In this issue of GASTROENTEROLOGY, Rowley et al use elegant genetically modified mouse models to show that p53 mutation is an obligate event before the loss of the remaining Brca2 wild-type allele. Indeed in the absence of p53 loss, Brca2 deletion slows tumor formation. Given the poor prognosis of pancreatic cancer patients and the recent development of poly adenosine diphosphate ribose polymerase (PARP) inhibitors for therapeutic targeting of Brca2-deficient cells, these findings have important implications for therapy.

The BRCA2 gene encodes a large protein that participates in DNA repair. Although familial mutations are associated most commonly with breast and ovarian cancer, mutation carriers also have a >5-fold increased risk of pancreatic cancer.1–4 Moreover, approximately 10% of sporadic pancreatic ductal adenocarcinomas (PDAC) carry mutations in BRCA2, although there is some debate whether these sporadic cases actually represent unidentifed germline BRCA2 carriers.5 Recent elegant studies have shown that cancer cell lines in vitro (such as the pancreatic cell line CAPAN1), and breast and ovarian cancers in vivo, which lack Brca2 (or Brca1), are sensitized to PARP inhibition.6–9 The reason for this is that inhibition of an additional DNA repair pathway in Brca1/2–deficient cells (which are deficient in homologous DNA double-strand break repair) causes synthetic lethality. Given the poor prognosis of pancreatic cancer and the frequency of BRCA2 mutation, this raises the potential efficacy of PARP inhibition-based therapy in a significant fraction of pancreatic cancer patients.

In this issue of GASTROENTEROLOGY, Rowley et al10 have assessed definitively the contribution of Brca2 loss to pancreatic cancer by using genetically modified mice to specifically delete Brca2 alone, or in combination with other major tumorigenic drivers of pancreatic cancer: either Kras-activating mutation or Trp53 loss-of-function mutation.11 Alone, Brca2 deletion (CB2) was not sufficient to drive PDAC. Remarkably, Rowley et al found that if Brca2 is co-deleted with Kras mutation (CKB2) in the absence of p53 loss, this suppresses PDAC formation, with those few tumors that arise carrying Trp53 mutations (or increased p53 levels, which is indicative of Trp53 mutation). These data are in contrast with a number of
recent studies showing that when other familial mutations such as Stk11/Lkb1, Cdkn2a, or Apc (β-catenin) are combined with activated Kras (which is mutated in up to 90% of PDAC), they cooperate to drive PDAC in mice. Examination of pancreata from Kras Brca2 (CKB2) animals showed increased apoptosis, indicating that double mutant cells were being deleted, thus preventing tumor initiation and progression. Therefore, these data strongly suggest that, for Brca2-deficient cells to be tolerated, other tumor suppressors have to be deleted.

In BRCA2 (and 1) breast cancers, p53 is often mutated, whereas mouse models of BRCA-related breast cancer also require additional p53 mutation. Thus, to examine this scenario in the pancreas, Rowley et al co-deleted p53 with Brca2 (CPB2) and found a strong cooperation with mice developing pancreatic cancer at much higher frequencies and reduced latencies. This requirement for Trp53 mutation in Brca2-deficient cells was confirmed in a recent study by Skoulidis et al, which showed that when Kras Brca2 (CKB2) mice carried an additional Trp53R270H mutation, mice now developed rapid PDAC. Tumorigenesis was also much faster than in the Kras Trp53R270H/+ mice alone, revealing that, once Trp53 is deleted, Brca2 deletion can cooperate with Kras mutation in the pancreas to drive PDAC. Therefore, this suggests a progression model of pancreatic cancer for BRCA2 mutation carriers (Figure 1). Here, after germline mutation of BRCA2, p53 must be lost before, or concomitantly with, the remaining copy of BRCA2. This also fits with the progression model of sporadic cancer, where BRCA2 mutation is a late event and presumably occurs within tumors that have already lost p53 and carry KRAS mutations.

Although KRAS mutation is common in human PDAC, it interesting to note that spontaneous Kras mutation occurred only in a small frequency of the Brca2 Trp53 (CPB2) pancreatic tumors (2/13). This suggests that BRCA2 carriers may develop tumors that have very different characteristics from sporadic cancers. To that end, it is interesting to note that the Brca2 Trp53 (CPB2) mice developed a wide spectrum of pancreatic cancers: conventional PDAC (40%), acinar carcinoma (15%), high-grade undifferentiated carcinoma (35%), and mucinous tumors. This could suggest that the increased genomic instability provoked by combined Brca2 and p53 deficiency might produce a very different spectrum of additional mutations compared with sporadic cancer, which could then affect subsequent tumor evolution. Alternatively, combined loss of Brca2 and p53 could allow a broader spectrum of mutations that would allow tumor initiation in a wider range of cell lineages across the pancreas. In support of this increased spectrum of pancreatic cancer in BRCA2 carriers, Skoulidis et al found that tumors arising in BRCA2999del5 carriers in Iceland that demonstrated loss of the wild-type allele of BRCA2 were predominantly acinar (3/4 tumors investigated). However, whether this is restricted to this specific mutation of BRCA2 is unclear because BRCA2 carriers normally develop PDAC rather than acinar cell carcinoma of the pancreas.

Figure 1. A mouse model for pancreatic cancer initiation in BRCA2 kindreds. In this model, 1 copy of Brca2 is inactivated from birth. LOH before the acquisition of further mutations is not sufficient to drive tumorigenesis, instead promoting chromosomal instability. Intriguingly, even in the presence of Kras activation, LOH at Brca2 inhibits tumor formation as long as wild-type p53 remains. When p53 is mutated, however, loss of the second copy of Brca2 accelerates pancreatic tumorigenesis in a Kras-independent manner.
The paper by Skoulidis found no evidence for loss of heterozygosity (LOH) of BRCA2 in 3 PDACs from these BRCA2994del carriers. Moreover, they discovered a slightly decreased tumor latency in Kras Trp533270H/+ (CKP) mice also heterozygous for a truncating Brca21492 mutation (median, Brca21492/+ = 143 days vs Brca2+/+ = 168 days) and no evidence of LOH in these tumors. This would also have important implications for treatment, because cell lines from these tumors were insensitive to PARP inhibition. These intriguing results would suggest that tumors from BRCA2 carriers should first be genotyped for LOH of the wild-type BRCA2 locus before PARP inhibition. Moreover, this would also suggest that patients where LOH has occurred should be sensitized to PARP inhibition. Some caution is needed in the interpretation of the effects on PARP inhibition. These intriguing results would suggest that PARP inhibition works in established murine tumors. Moreover, it will be important to characterize the stroma in BRCA2 carriers, because evidence from the murine model would suggest that stroma may not be so desmoplastic and drugs may penetrate these tumors more successfully.

In summary, Rowley et al have performed elegant proof-of-principle experiments in genetically engineered mouse models to elucidate the role of BRCA2 deletion in pancreatic cancer. Their work shows the kinetics of mutation are absolutely essential for tumorigenesis: Brca2 deletion in the absence of p53 can suppress pancreatic cancer, whereas combined Brca2 and p53 loss cooperate robustly to drive tumorigenesis. It will be exciting to see how PARP inhibition will work in vivo, in both preclinical models of PDAC, BRCA2 carrier patients and sporadic PDAC with BRCA2 mutations.

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Conflicts of interest
The authors disclose no conflicts.

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