Loss of BAP1 Leads to More YAPing in Pancreatic Cancer

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Pancreatic cancer is increasing in incidence and is expected to be the second leading cause of cancer-related mortality by the year 2030. Understanding molecular pathways that contribute to pancreatic cancer initiation and progression provides the opportunity to uncover potential molecular vulnerabilities that can be exploited therapeutically. In this issue of *Cancer Research*, Lee and colleagues provide compelling evidence that BRCA1-associated protein (BAP1) functions as a tumor suppressor in pancreatic cancer by promoting the activity of the Hippo tumor suppressor pathway, highlighting YAP and TAZ, Hippo effectors, as attractive therapeutic targets in pancreatic ductal adenocarcinoma, especially in BAP1-deficient or low tumors.

See related article by Lee et al., p. 1656

Somatic mutations in *BAP1* are common in uveal melanoma and mesothelioma and have been noted in breast, lung, and renal cancers (1), but have not previously been appreciated as a significant somatic alteration in pancreatic ductal adenocarcinoma (PDAC). BAP1 was initially identified through a yeast two hybrid screen as an interactor with BRCA1 and it was the first nuclear localized ubiquitin hydrolase recognized (2). The human *BAP1* locus maps to 3p21.3, a hotspot for deletions, especially in lung cancer. Not surprisingly, multiple non–small cell lung cancer cell lines showed homozygous deletion of the *BAP1* locus (2). These early studies suggested that BAP1 might function as a tumor suppressor. Consistent with this, germline *BAP1* mutations result in a predisposition for cancer development (3).

BAP1 is a member of the ubiquitin C-terminal hydrolase (UCH) family of deubiquitinating enzymes (DUB). DUBs can remove ubiquitin from proteins, resulting in protection from degradation and release of free ubiquitin for recycling. Alternatively DUB activity can enhance protein degradation in some cases (4). UCHs have a complex and incompletely understood function in tumor development or control. For example, UCHL1, UCHL3, and UCH37 can function as tumor promoters, while BAP1 has tumor suppressor activity. The tumor suppressor activity of BAP1 was thought to be mediated through its interaction with BRCA1, yet BAP1 does not influence ubiquitin status of BRCA1 and BRCA1 is not a substrate for BAP1. Although BAP1 can restrain the E3 ligase activity of the BRCA1–BARD1 complex and BAP1 can deubiquitinate BARD1 (4).

Loss of *Bap1* in adult mice results in myeloid dysplasia, preventing significant investigation of contribution of Bap1 to homeostasis in other organs or tissues. Lee and colleagues (5) exploited an interesting strategy of crossing $Bap1^{fl/fl}$ mice with a tamoxifen-regulated *Cre deleter* strain and then reconstituted the bone marrow of the *Bap1*-deficient mice with bone marrow from wild-type (WT) mice. These *Bap1*-deficient animals were examined at multiple time points for tumor incidence and tissue/organ dysfunction. Five of 28 *Bap1*-deficient mice developed tumors of diverse origin compared with 1 of 27 control animals. However, virtually all of the *Bap1*-deficient animals developed exocrine pancreatic atrophy within a short time frame (∼4 weeks), leading the authors to investigate the consequences of Bap1 loss in the pancreas. Use of a pancreas selective $Pdx1^{Cre}$ transgene recapitulated the results from the nonhematopoietic deletion of *Bap1*, suggesting that Bap1 is important for pancreatic development and/or homeostasis. To extend these results and to determine the tumor suppressor function of Bap1 in PDAC, $Kras^{LSL\text{-}G12D}$; $Bap1^{fl/fl}$; $Pdx1^{Cre}$ animals were compared with $Kras^{LSL\text{-}G12D}$; $Pdx1^{Cre}$ (KC) animals. As anticipated, by 12 weeks of age all KC mice developed pancreatic intraepithelial neoplasia lesions (grade 1–3). In contrast in the same time frame, the absence of Bap1 cooperated with the Kras mutation such that 75% of the mice lacking *Bap1* developed PDAC (5), strongly supporting that Bap1 restrains PDAC development.

To investigate the clinical significance and implications of these results, a modest set of human PDAC samples ($n = 47$) were screened for BAP1 protein expression by IHC. In addition, copy number and mRNA expression of patients with PDAC in The Cancer Genome Atlas ($n = 149$) were analyzed. These data support that there are likely subsets of PDAC tumor cells that lose or have lower expression of BAP1, supporting that BAP1 contributes to human PDAC development.

The Hippo signaling pathway is a major signaling pathway commonly altered in human cancer (6). Consistent with this, YAP and TAZ, the two major effectors of the Hippo pathway, have been shown to be significantly elevated in human PDAC (7). Lee and colleagues (5) found that loss of Bap1 resulted in elevation of Yap and Taz and canonical target genes *Cyr61* (*Ccn1*) and *Ctgf* (*Ccn2*) in tumor tissue and tumor cells derived from the Kras-driven mouse tumors. Importantly, they also found that loss of Bap1 resulted in sensitivity of PDAC cells to knockdown of Yap/Taz. To explore the link between Bap1 and Hippo signaling, the components of the Hippo pathway were examined in the context of Bap1 loss in PDAC cells. Loss of Bap1 resulted in reduced levels of Lats1, Lats2, and Mob1, but expression of upstream regulators (e.g., Mst1, Mst2, and Nf2) was not altered. Further studies demonstrated that Bap1 interacts with Lats1 and Lats2 and that Lats2 but not Lats1 is a substrate for Bap1. In addition, Bap1 increased pYap1 in a Lats2- but not Lats1-dependent manner. These data directly link Bap1 tumor suppressor activity to the Hippo pathway in PDAC cells.

YAP has recently been implicated as a major driver of a squamous subtype of PDAC (7). Squamous PDAC is associated with a quasi-mesenchymal tumor cell phenotype and poor outcome. Interestingly, Artegiani and colleagues (8) showed that loss of BAP1 in human liver

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organoids coincided with a loss of epithelial features, consistent with epithelial plasticity. Epithelial plasticity is associated with therapy resistance, a hallmark of PDAC. Consistent with this, BAP1-proficient mesothelioma cells were sensitive to gemcitabine, while BAP1-deficient cells were resistant. Thus it is tempting to speculate that BAP1 loss or lower expression is more frequent in squamous PDAC and is selected for by standard chemotherapy regimens, which have been shown to induce tumor cell phenotypic change, potentially through the induction of YAP activity. Targeting YAP activity or YAP effectors in PDAC and in squamous PDAC in particular is attractive and has been validated in animal models of PDAC (9). An alternative approach being considered in mesotheliomas is targeting EZH2. Global methylation was found to be elevated in *BAP1*-deficient mesothelioma cells. Further *BAP1*-deficient mesothelioma cell lines and tumors were sensitive to EZH2 inhibition (10).

In summary, Lee and colleagues (5) highlight the rationale for targeting YAP in PDAC and provide a mechanistic insight into how BAP1 activity is critical to the tumor suppressor activity of the Hippo signaling pathway in PDAC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Okonska A, Felley-Bosco E. BAP1 missense mutations in cancer: friend or foe? Trends Cancer 2019;5:659–62.
2. Jensen DE, Proctor M, Marquis ST, Gardner HP, Ha SI, Chodosh LA, et al. BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. Oncogene 1998;16: 1097–112.
3. Walpole S, Pritchard AL, Cebulla CM, Pilarski R, Stautberg M, Davidorf FH, et al. Comprehensive study of the clinical phenotype of germline BAP1 variant-carrying families worldwide. J Natl Cancer Inst 2018;110:1328–41.
4. Fang Y, Shen X. Ubiquitin carboxyl-terminal hydrolases: involvement in cancer progression and clinical implications. Cancer Metastasis Rev 2017;36:669–82.
5. Lee HJ, Pham T, Chang MT, Barnes D, Cai AG, Noubade R, et al. The tumor suppressor BAP1 regulates the Hippo pathway in pancreatic ductal adenocarcinoma. Cancer Res 2020;80:1656–68.
6. Zheng Y, Pan D. The Hippo signaling pathway in development and disease. Dev Cell 2019;50:264–82.
7. Tu B, Yao J, Ferri-Borgogno S, Zhao J, Chen S, Wang Q, et al. YAP1 oncogene is a context-specific driver for pancreatic ductal adenocarcinoma. JCI Insight 2019;4: pii: 130811.
8. Artegiani B, van Voorthuijsen L, Lindeboom RGH, Seinstra D, Heo I, Tapia P, et al. Probing the tumor suppressor function of BAP1 in CRISPR-engineered human liver organoids. Cell Stem Cell 2019;24:927–43.
9. Murakami S, Nemazanyy I, White SM, Chen H, Nguyen CDK, Graham GT, et al. A Yap-Myc-Sox2-p53 regulatory network dictates metabolic homeostasis and differentiation in Kras-driven pancreatic ductal adenocarcinomas. Dev Cell 2019;51:113–28.
10. Yap TA, Aerts JG, Popat S, Fennell DA. Novel insights into mesothelioma biology and implications for therapy. Nat Rev Cancer 2017;17:475–88.