Editorial Commentary

A mechanism for the tissue specificity in BAP1 cancer syndrome

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Cancer predisposition syndromes are caused by inherited mutations in tumor suppressor genes. Remarkably, despite the presence of the faulty tumor suppressor gene in virtually every cell in the body, individuals with a cancer predisposition syndrome develop tumors in specific organs but not others. The underlying mechanisms of the tissue specificity varies depending on the mutated tumor suppressor gene. A recent study by He *et al.* (1) sheds new light on the tissue specificity of the BAP1 cancer syndrome, which is caused by inheritance of a germline mutation in the tumor suppressor gene *BAP1*. This familial cancer predisposition syndrome exhibits an increased incidence of specific types of cancers, including mesothelioma, melanocytic tumors, uveal melanoma, renal cell carcinoma and others (2-9). He *et al.* demonstrate that conditional Bap1 inactivation in mice resulted in cell death in many cell types, but not in mesothelium and melanocytes, the same cell types from which tumors arise in *BAP1* mutation carriers in humans. Through experiments using CRISPR-based genetic screens, RNA sequencing (RNA-seq), and chromatin immunoprecipitation (ChIP), the authors provide new insight into the mechanism by which BAP1 loss causes apoptosis in certain tissues but not others.

BAP1 is a classic tumor suppressor gene that follows Knudson's two hit hypothesis, in which carriers of a heterozygous mutation undergo loss of heterozygosity (LOH) to lose the functional allele in tumors. Paradoxically, despite the fact that BAP1 loss promotes tumorigenesis, BAP1 inactivation causes cell death in many cell types. He *et al.* showed that *Bap1* knockout in mouse embryonic stem (ES) cells, fibroblasts, and keratinocytes caused cell death through the intrinsic apoptosis pathway (1), which is mediated by Bax/Bak oligomerization. Induced Bap1 inactivation in adult mice also caused Bax/Bak-dependent apoptosis in the liver and pancreas, leading to liver damage and pancreatic atrophy. Interestingly, Bap1 inactivation did not cause apoptosis in mesothelium and melanocytes. Because surviving BAP1 inactivation is prerequisite for development of BAP1-deficient tumors, the lack of apoptosis after Bap1 inactivation would provide an explanation for why tumors develop in mesothelium and melanocytes but not other cell types.

The *BAP1* gene encodes a deubiquitinase, an enzyme that reverses post-translational modification of proteins with ubiquitin. BAP1 inactivation can occur as a consequence of either missense mutations in the ubiquitin C-terminal hydrolase (UCH) domain in the N-terminal region, or frameshift or nonsense mutations that cause truncation of the BAP1 protein. Because the C-terminal region encodes nuclear localization signals and a regulatory domain necessary for activation of the UCH domain, the truncated proteins often mislocalize in the cytoplasm or lack deubiquitinase activity. Among the substrates that have been reported to be deubiquitinated by BAP1, deubiquitination of histone H2A is best characterized. Histone H2A is monoubiquitinated at Lys119 by the ubiquitin ligase polycomb repressive complex 1 (PRC1) and deubiquitinated by BAP1 (10). Because H2AK119ub is generally a repressive mark for transcription, BAP1 inactivation is expected to cause PRC1-dependent gene repression.

To gain more insight into the apoptosis that follows Bap1 inactivation, He *et al.* performed a genome-wide CRISPR-Cas9 screen and identified *Rnf2* as a gene that promotes the death of *Bap1* knockout MEFs (1). Rnf2 is a

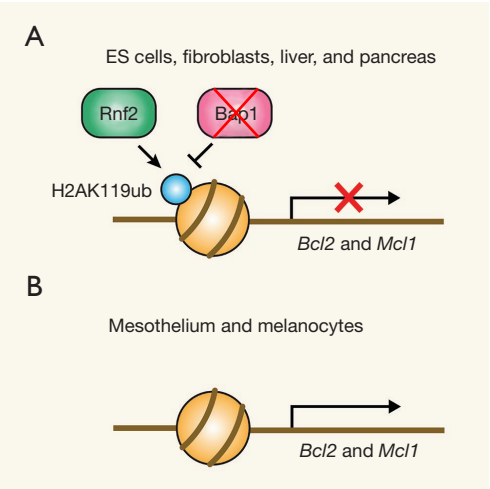

Figure 1 Tissue-specific effects of Bap1 inactivation on pro-survival gene expression. (A) In many tissues, *Bcl2* and *Mcl1* are regulated by histone H2A monoubiquitination (H2AK119ub), which is maintained by the equilibrium between ubiquitination by Rnf2 and deubiquitination by Bap1. Bap1 inactivation results in elevated H2AK119ub levels at the promoter and causes repression of the pro-survival genes; (B) in mesothelium and melanocytes, Rnf2 and Bap1 are not engaged in the regulation of *Bcl2* and *Mcl1* expression; therefore, Bap1 inactivation has minimum effect on the pro-survival gene expression.

ubiquitin E3 ligase component of PRC1, responsible for the deposition of the H2AK119ub mark. This finding suggests that apoptosis after Bap1 inactivation requires H2AK119 ubiquitination by Rnf2, an observation consistent with the antagonistic relationship between a ubiquitin ligase and a deubiquitinase for the H2AK119ub mark. Subsequent RNA-seq experiments revealed that *Bap1* knockout in ES cells causes Rnf2-dependent down-regulation of the pro-survival genes *Bcl2* and *Mcl1*. Ectopic expression of Bcl2 and Mcl1 prevented apoptotic cell death of *Bap1* knockout ES cells, suggesting that the down-regulation of these pro-survival factors is the key event triggering cell death after Bap1 inactivation. This is consistent with the fact that the apoptosis after Bap1 inactivation is Bax/Bak-dependent, given that Bcl2 and Mcl1 promote cell survival by restraining the activity of cell death mediators Bax and Bak, respectively. Subsequently, He *et al.* employed ChIP-seq to demonstrate that the Rnf2 and Bap1 proteins localize to the *Bcl2* promoter in ES cells, and *Bap1* knockout leads to an Rnf2-dependent increase in the H2AK119ub mark at the *Bcl2* promoter. Therefore, in many cell types, it seems that the basal expression levels of *Bcl2* and *Mcl1* are maintained through an equilibrium of Rnf2-dependent H2AK119 ubiquitination and Bap1-dependent deubiquitination at their promoters (*Figure 1A*). When this equilibrium is disturbed by Bap1 inactivation, it results in down-regulation of the pro-survival factors and apoptotic cell death.

In stark contrast to ES cells, however, primary melanocytes and mesothelial cells maintained normal levels of Bcl2 and Mcl1 proteins after Bap1 inactivation and did not undergo apoptosis (1). He *et al.* found that melanocytes and mesothelial cells had minimal Rnf2 binding to the *Bcl2* promoter regardless of the *Bap1* gene status. Therefore, in melanocytes and mesothelial cells, it appears that Rnf2 does not engage in the regulation of *Bcl2* and *Mcl1* expression, and as a result, neither does Bap1 (*Figure 1B*). The authors propose that this difference in the engagement of Rnf2 and Bap1 in the regulation of the pro-survival gene expression determines the cellular fate after Bap1 inactivation. When a cell spontaneously loses functional BAP1 through LOH in *BAP1* mutation carriers, that precancerous cell vanishes due to apoptotic cell death in the majority of tissues. In mesothelial and melanocytic tissues, however, such a precancerous cell with inactive BAP1 would persist because apoptosis is not triggered in those cell types. Having survived BAP1 loss, those precancerous cells in melanocytic tissue and mesothelium are poised to respond to additional tumor-promoting effects of BAP1 inactivation.

In fact, He *et al.* also provided insight into the tumor-promoting effect of BAP1 inactivation in melanocytes (1). The authors showed that melanocyte-inducing transcription factor (*Mitf*), a melanoma oncogene, is upregulated after *Bap1* knockout or inactivation in melanocytes. The molecular mechanism for this upregulation is not provided in this study, but it will be worth investigating whether *Mitf* is directly regulated by Bap1 and whether the upregulation involves histone H2AK119ub or ubiquitination of other Bap1 substrates such as the transcriptional regulator HCF-1 (11,12) or O-linked *N*-acetylglucosaminyl transferase (OGT) (13), both of which could influence transcription. The function of BAP1 is also not limited to transcriptional regulation. BAP1 regulates Ca^{2+} release from endoplasmic reticulum and mitochondria by deubiquitinating and stabilizing inositol 1,4,5-trisphosphate receptor type 3 (IP3R3), promoting apoptosis after genotoxic stress (14). BAP1 is also involved in DNA repair (15,16) although the relevant substrate remains unclear. Given the diversity of the cellular processes that BAP1 regulates and the possibility that BAP1 influences expression of many target

genes, it is possible that the tumor-promoting effect of BAP1 inactivation is also tissue-specific. Therefore, it will be important to investigate the mechanism underlying the tumor-promoting effect of BAP1 inactivation in each cell type.

In summary, this study demonstrated that Bap1 loss promotes tumorigenesis only in the tissues in which Bap1 is not engaged in pro-survival gene expression, providing an explanation for the tissue specificity of the BAP1 cancer predisposition syndrome. Somatic mutations in *BAP1* have been reported in the sporadic form of mesothelioma, uveal melanoma, and renal cell carcinoma (2,4,17-19), suggesting that the reported mechanism for the tissue specificity applies to the sporadic form as well. However, somatic *BAP1* mutations have also been reported in other types of spontaneous tumors including cholangiocarcinoma (20,21), hepatocellular carcinoma (22), thymic epithelial tumors (23), uterine corpus endometrial carcinoma (24), and others. Therefore, a key question will be how spontaneous tumors in other tissues bypass cell death when BAP1 is inactivated. Given that resisting apoptosis is a hallmark of cancer (25), it will be important to understand the preceding oncogenic events that promote survival of BAP1-deficient cancer cells.

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