



AUTHOR'S VIEWS



BAP1 suppresses tumor development by inducing ferroptosis upon SLC7A11 repression

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ABSTRACT

The tumor suppressor BRCA1-associated protein 1 (BAP1) is a deubiquitinase that removes histone 2A ubiquitination. How BAP1 suppresses tumor development remains elusive. Our recent study identified the cystine transporter solute carrier family 7 member 11 (SLC7A11) as a critical BAP1 target, and showed that BAP1 promotes ferroptosis (a non-apoptotic cell death) through repressing SLC7A11 expression, resulting in tumor suppression.

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BRCA1-associated protein 1 (BAP1) encodes a deubiquitinase (DUB) which predominantly localizes in the nucleus. Although BAP1 was originally identified as a BRCA1-binding protein through a yeast two-hybrid screen, subsequent proteomic analyses from many studies have not identified BRCA1 as a major binding protein of BAP1. Instead, these studies showed that BAP1 mainly interacts with several transcription factors and chromatin-associated proteins, including ASXL1, ASXL2, FOXK1, FOXK2, HCFC1, KDM1B, MBD5, MBD6, and OGT, indicating a role of BAP1 in regulating gene transcription.¹ A seminal study from *Drosophila* revealed that BAP1 and its associated proteins form the so called polycomb repressive deubiquitinase (PR-DUB) complex, which mainly functions to remove monoubiquitination of H2A (H2Aub) at lysine 118 in *Drosophila* and lysine 119 in vertebrates.² Subsequent studies confirmed this finding in mammalian cell lines. These data thus suggest a model that the BAP1-containing PR-DUB complex regulates gene transcription through epigenetic mechanisms.

Research interest in BAP1 has substantially increased in recent years. A survey of PubMed revealed less than 15 BAP1-related publications per year before 2012, increasing to 40–60 per year between 2012 and 2014, and more than 100 per year since 2015. Arguably, this increase largely results from recent cancer genomic studies which identified prominent mutations of *BAP1* in several human cancers, including 36–65% mesotheliomas, 32–47% uveal melanomas, 20–30% cholangiocarcinomas, and 10–15% clear cell renal cell carcinomas.¹ More than 40% *BAP1* mutations are inactivating mutations (such as truncating mutations), and *BAP1* is located at the 3p21.1 locus which is frequently deleted in human cancers, indicating *BAP1* is a tumor suppressor. Functional analyses showed that *BAP1* restoration in *BAP1*-deficient cancer cells inhibited xenograft tumor development, while *Bap1* deletion in mouse promoted tumor development in various contexts,¹ further supporting BAP1's tumor suppression function. It is

conceivable that, similar to other tumor suppressors that regulate gene transcription such as TP53 (best known as p53), BAP1 exerts its tumor suppression function at least partly via its downstream transcriptional targets. However, the identity of such transcriptional targets and their associated biological processes in BAP1-mediated tumor suppression still remain largely unexplored.

To identify potential targets that underlie BAP1-mediated tumor suppression, we conducted both RNA sequencing (RNA-seq) and H2Aub chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) analyses in *BAP1*-deficient cancer cells that stably express an empty vector or BAP1.^{3,4} Integration of both RNA-seq and H2Aub ChIP-seq data sets identified 541 potential target genes (including more than 300 BAP1-upregulated and around 200 BAP1-downregulated genes) with BAP1-dependent H2Aub reduction, suggesting that BAP1-mediated deubiquitination of H2Aub is associated with both transcriptional activation and repression of target genes. Gene ontology (GO) analysis revealed a striking enrichment of metabolism-related biological processes in BAP1 target genes. Cancer genomic analysis identified *solute carrier family 7 member 11 (SLC7A11)* as a top BAP1 target gene in human cancers, and *SLC7A11* is also among “oxidative stress response”, a top ranking biological process from GO analysis. We subsequently confirmed that BAP1 decreases H2Aub occupancy on the *SLC7A11* promoter and potently represses *SLC7A11* expression in cancer cells. We further showed that other proteins in the PR-DUB complex, such as ASXL1, strongly bind on the *SLC7A11* promoter, suggesting that these other proteins in the PR-DUB complex might function to recruit BAP1 to the *SLC7A11* promoter to regulate H2Aub levels.

SLC7A11 is an amino acid transporter that imports extracellular cystine.⁵ Studies in recent years have linked this nutrient transporter to a new form of regulated cell death termed ferroptosis, which is caused by over-accumulation of

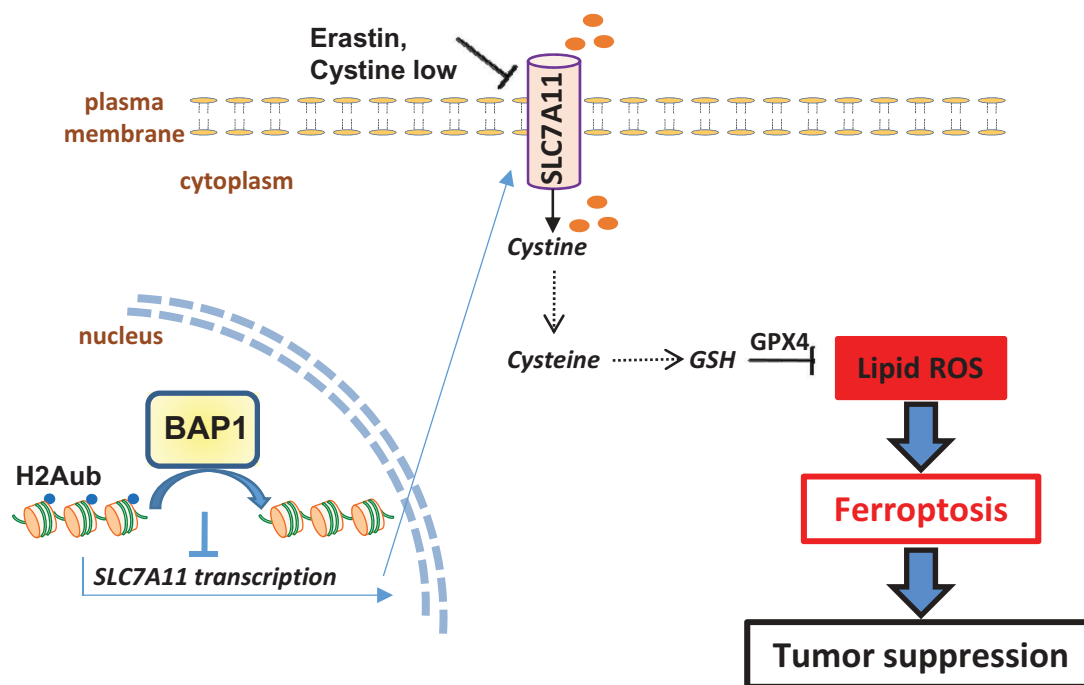


Figure 1. BAP1 suppresses tumor development through repressing *SLC7A11* and inducing ferroptosis. Solute carrier family 7 member 11 (*SLC7A11*) imports extracellular cystine, which is subsequently converted to cysteine in cells. Cysteine is a rate-limiting precursor for glutathione (GSH) biosynthesis. GSH is used as a cofactor by glutathione peroxidase 4 (GPX4) to reduce lipid reactive oxygen species (ROS), particularly lipid hydroperoxides, to lipid alcohols. Overproduction of lipid ROS in cells results in ferroptosis. Similar to apoptosis, ferroptosis might serve as a tumor suppression mechanism. Cystine depletion or inhibition of *SLC7A11*-mediated cystine transport by erastin promotes lipid ROS and induces ferroptosis. BRCA1-associated protein 1 (BAP1) represses *SLC7A11* transcription likely through deubiquitination of histone 2A monoubiquitination (H2Aub) at lysine 119 on the *SLC7A11* gene, resulting in decreased cystine import, reduced GSH synthesis, enhanced sensitivity to ferroptosis, and tumor suppression.

intracellular lipid hydroperoxides in an iron-dependent manner.⁶ Glutathione peroxidase 4 (GPX4) uses glutathione as a cofactor to convert lipid hydroperoxides to lipid alcohols, therefore protecting cells from undergoing ferroptotic cell death. Since cystine is a critical precursor for glutathione biosynthesis, ferroptosis can be induced by cystine depletion or blockage of *SLC7A11*-mediated cystine transport by drugs such as erastin (Figure 1). Consistent with this, we showed that, through repressing *SLC7A11* expression, BAP1 inhibits cystine uptake and glutathione synthesis, enhances lipid peroxidation, and sensitizes cancer cells to ferroptosis.

Cell death resistance is one hallmark of cancer, and the role of apoptosis in tumor suppression is well established.⁷ However, whether ferroptosis plays any role in tumor suppression is less clear. Our studies provided several lines of evidence to support the model that ferroptosis indeed is critical for BAP1-mediated tumor suppression. First, electron microscopy analysis revealed that many tumor cells from *BAP1*-expressing xenograft tumors exhibit morphologic features of ferroptosis, and immunohistochemistry (IHC) analysis showed increased staining of 4-hydroxynonenal (a lipid peroxidation product) in *BAP1*-expressing tumors, confirming that BAP1 promotes lipid peroxidation and ferroptosis in vivo. In addition, restoration of *SLC7A11* or treatment of a ferroptosis inhibitor partially restored tumor development in *BAP1*-expressing xenograft tumors, suggesting that BAP1 inhibits tumor development at least partly through repressing *SLC7A11* and promoting ferroptosis. Finally, we showed that

most cancer-associated *BAP1* mutations we have analyzed are defective in inhibiting *SLC7A11* and inducing ferroptosis.

In summary, our results show that BAP1 executes its tumor suppression function at least partly through its regulation of ferroptosis, and identify a hitherto unrecognized regulatory mechanism to link ferroptosis to tumor suppression (Figure 1). Our study also raises several important questions. Since H2Aub is generally associated with gene repression,⁸ it remains unclear how BAP1, a H2Aub DUB, represses the expression of its target genes including *SLC7A11* through decreasing H2Aub levels. Our study, together with other recent studies,⁹ supports the emerging role of ferroptosis in tumor suppression; however, it remains to be determined how common this new tumor suppression mechanism occurs in human cancers. Finally, a convenient assay similar to cleaved caspase-3 IHC in apoptosis studies is needed to routinely characterize ferroptosis in tumor specimens. We envision that novel mechanistic insights will be revealed and important research tools be established in this emerging field in the upcoming years.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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