BAP1 suppresses tumor growth via Interferon Beta and ISGF3 in ccRCC

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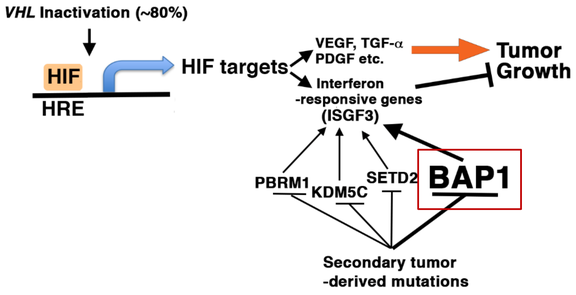
Abstract

BRCA1-associated protein 1 (BAP1) is a deubiquitinase mutated in 10-15% of clear cell renal cell carcinoma (ccRCC). Despite the association between BAP1 mutations and poor clinical outcome, the specific molecular function(s) of BAP1 in ccRCC remains unclear, and no specific therapies exist for BAP1-deficient ccRCC patients. Previously, we found that BAP1 activates Interferon Stimulated Gene Factor 3 (ISGF3), a transcription factor involved in type I interferon signaling, and ISGF3 is tumor suppressive in a ccRCC xenograft model. In this study, we found that Interferon Beta (IFN-β) is required for ISGF3 activation in ccRCC cells, and is tumor suppressive as well. BAP1 upregulates the expression of IFN-β and Stimulator of Interferon Genes (STING), both of which are required for BAP1's effect on ISGF3 activity. A STING agonist increases ISGF3 activity *in vitro* and suppresses the growth of BAP1-deficient tumors *in vivo*. Together, our results indicate that BAP1 loss reduces type I interferon signaling in ccRCC, and reactivation of this pathway may benefit ccRCC patients harboring BAP1 mutations.

Background and preliminary findings

Primary and secondary mutated tumor suppressors converge on ISGF3 to regulate tumor growth in ccRCC

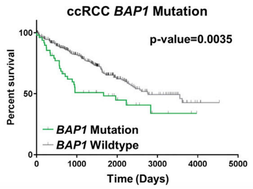


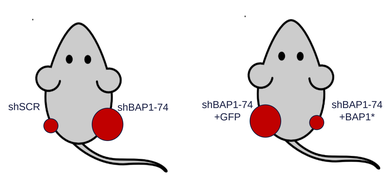
In ccRCC, VHL inactivation leads to constitutive HIF2α activity, which drives a pro-tumorigenic expression signature. HIF2α also activates ISGF3, the central transcription factor in the Type I Interferon pathway, which suppresses tumor growth. PBRM1, KDM5C, SETD2 and **BAP1** all enhance ISGF3 activity. Loss of any of these tumor suppressors reduces ISGF3 function and promotes tumor growth[1].

Our lab discovered that after VHL loss, HIF2α, the primary oncogene in ccRCC, increases the mRNA expression of IFN-β, an upstream activator of ISGF3

(unpublished)

We also discovered that BAP1 is a ccRCC tumor suppressor in a xenograft model





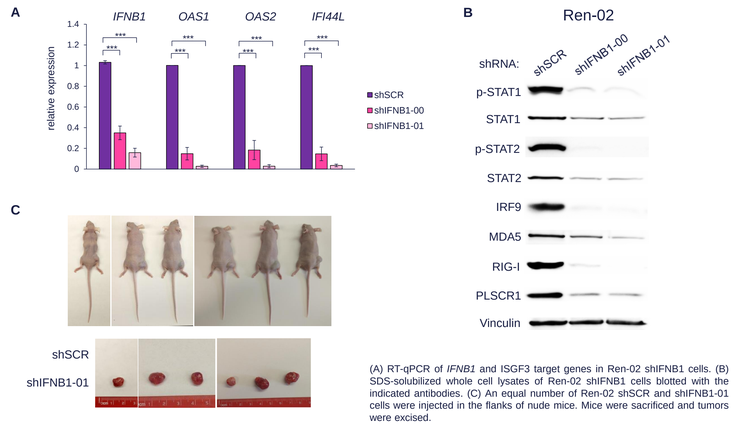
BAP1 is mutated in 10-15% of ccRCC, and its loss is associated with worse patient prognosis[2]. In the lab, we have established a model of BAP1's tumor suppressor function using Ren-02 ccRCC cells in nude mice xenograft experiments (unpublished, data not shown). BAP1-74: BAP1 shRNA; BAP1*: BAP1 cDNA harboring silent mutations that abolish targeting of shRNA.

1. How does BAP1 promote ISGF3 activity?

2. Can a drug that activates ISGF3 slow the growth of BAP1-deficient tumors?

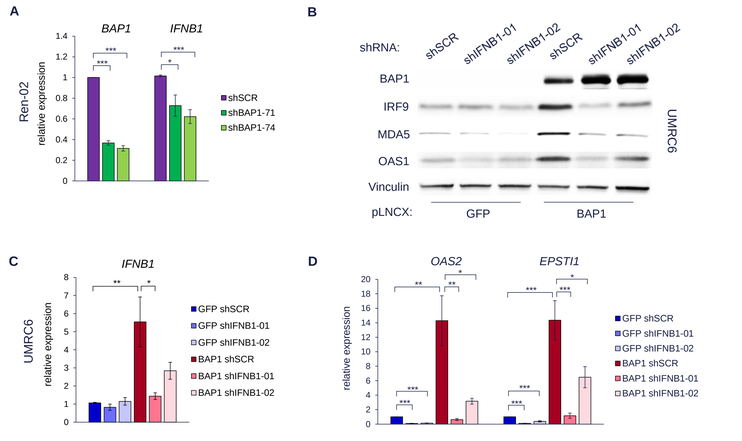
Results

IFN-β activates ISGF3 and functions as a ccRCC tumor suppressor



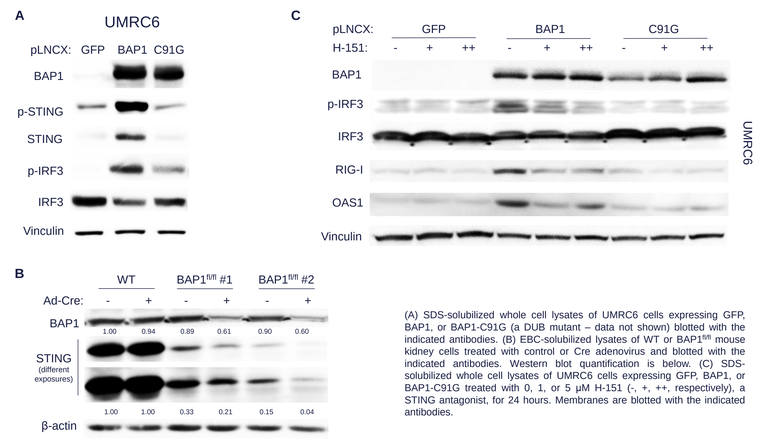
(A) RT-qPCR of *IFNB1* and ISGF3 target genes in Ren-02 shIFNB1 cells. (B) SDS-solubilized whole cell lysates of Ren-02 shIFNB1 cells blotted with the indicated antibodies. (C) An equal number of Ren-02 shSCR and shIFNB1-01 cells were injected in the flanks of nude mice. Mice were sacrificed and tumors were excised.

BAP1 increases ISGF3 activity by enhancing IFN-β expression



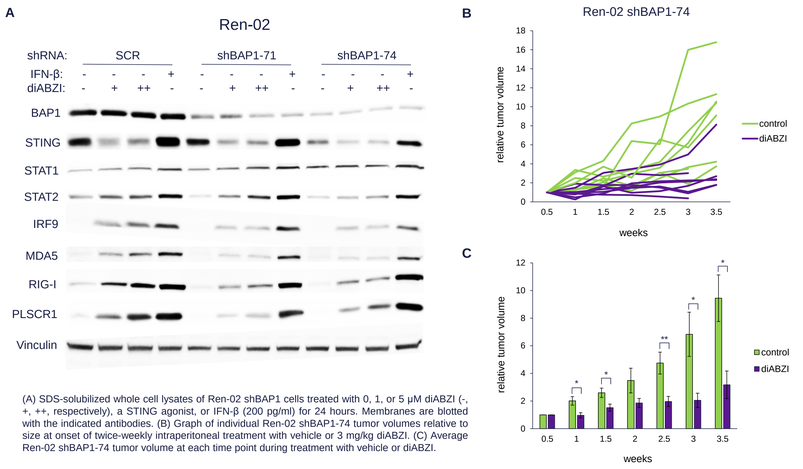
(A) RT-qPCR of the indicated transcripts in Ren-02 shBAP1 cells. (B) SDS-solubilized whole cell lysates of UMRC6 cells expressing GFP or BAP1 and shIFNB1 blotted with the indicated antibodies. (C) RT-qPCR of *IFNB1* in UMRC6 GFP or BAP1-expressing cells with shIFNB1. (D) RT-qPCR of ISGF3 target genes in UMRC6 GFP or BAP1-expressing cells with shIFNB1.

BAP1 activates STING, which is upstream of IFN-β, to regulate ISGF3



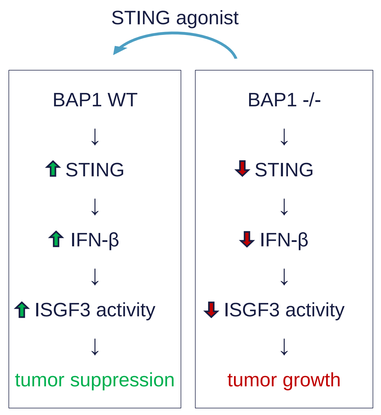
(A) SDS-solubilized whole cell lysates of UMRC6 cells expressing GFP, BAP1, or BAP1-C91G (a DUB mutant – data not shown) blotted with the indicated antibodies. (B) EBC-solubilized lysates of WT or BAP1$^{fl/fl}$ mouse kidney cells treated with control or Cre adenovirus and blotted with the indicated antibodies. Western blot quantification is below. (C) SDS-solubilized whole cell lysates of UMRC6 cells expressing GFP, BAP1, or BAP1-C91G treated with 0, 1, or 5 μM H-151 (-, +, ++, respectively), a STING antagonist, for 24 hours. Membranes are blotted with the indicated antibodies.

STING agonist treatment promotes ISGF3 activity and slows the growth of BAP1-deficient ccRCC tumors



(A) SDS-solubilized whole cell lysates of Ren-02 shBAP1 cells treated with 0, 1, or 5 μM diABZI (-, +, ++, respectively), a STING agonist, or IFN-β (200 pg/ml) for 24 hours. Membranes are blotted with the indicated antibodies. (B) Graph of individual Ren-02 shBAP1-74 tumor volumes relative to size at onset of twice-weekly intraperitoneal treatment with vehicle or 3 mg/kg diABZI. (C) Average Ren-02 shBAP1-74 tumor volume at each time point during treatment with vehicle or diABZI.

Conclusions



In ccRCC cells, IFN-β drives ISGF3 activation and suppresses tumor growth.

BAP1 promotes IFN-β expression which, in turn, activates ISGF3.

BAP1 upregulates STING-IRF3 signaling in a deubiquitinase-dependent manner, which is required for ISGF3 activation.

Loss of BAP1 reduces STING function and IFN-β production, which results in low ISGF3 activity.

Activation of STING using a small molecule agonist enhances ISGF3 activity and suppresses the growth of BAP1-deficient tumors.

Future Directions

- Investigate the cause of IFN-β production in ccRCC cells
- Decipher the mechanism by which BAP1 regulates STING expression and activity
- Assess the importance of STING function in BAP1-mediated tumor suppression in ccRCC and other cancers
- Examine the role of the immune system in mediating STING- and ISGF3-dependent ccRCC tumor suppression

Acknowledgements

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References

1. Liao, L. et al. Multiple tumor suppressors regulate a HIF-dependent negative feedback loop via ISGF3 in human clear cell renal cancer. *eLife* 7:e37925 (2018).
2. Ricketts, C.J. et al. The Cancer Genome Atlas comprehensive molecular characterization of renal cell carcinoma. *Cell Reports* 23:313-326 (2018).