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Tumor suppressor functions of BNIP3 and mitophagy

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There is a growing realization that tumor cells rely on healthy mitochondria to promote their growth under changing microenvironmental stresses and do so by dynamically modulating both their mitochondrial mass and state of mitochondrial fusion. Our recent work adds to this appreciation by showing that the mitophagy receptor BNIP3 functions as a tumor suppressor in mammary tumorigenesis and also as a prognostic indicator of progression to metastasis in certain sub-types of human breast cancer.

Keywords: BNIP3, cancer, hypoxia, HIF-1, mitophagy, mitochondrial dysfunction, ROS, Warburg effect

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BNIP3 is a HIF1A target gene that is induced by hypoxia but is also transcriptionally regulated by RB1-E2F1, TP53, FOXO3, NFKB/NF-κB, and other tumor-relevant transcription factors. BNIP3 at the outer mitochondrial membrane interacts with processed LC3 at phagophore membranes to promote sequestration of mitochondria within the autophagosome for degradation. We showed that BNIP3-dependent mitophagy is required to limit mitochondrial mass and reactive oxygen species (ROS) levels in growing tumors and to prevent stabilization of HIF1A, the upstream regulator of BNIP3, revealing a novel negative feedback loop between HIF1A and one of its target genes. The tumor suppressor properties of BNIP3 could not be attributed to increased cell death in the MMTV-PyMT tumor model either in vitro or in vivo. Loss of BNIP3-dependent mitophagy leads to HIF1A-dependent increases in tumor growth and increased progression to metastasis (**Fig. 1**).

The role of HIF1A in promoting glycolysis and angiogenesis is well recognized and both of these tumor-promoting processes are increased in tumors forming in BNIP3 null mice, consistent with the tumor suppressor effects of BNIP3 being mediated through repression of HIF1A. Indeed, restoring BNIP3 prevents HIF1A accumulation in tumor cells, and HIF1A inhibition reduces tumor cell growth. Elevated HIF1A levels have been linked to mutation of IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) and IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) that leads to increased production of 2-hydroxyglutarate (2HG) and reduced prolyl hydroxylase activity. However, metabolomic analyses failed to detect any significant differences in levels of 2HG (or α-ketoglutarate) in BNIP3 null tumors nor were we able to detect IDH1/2 mutations, indicating that this was not the cause of increased HIF1A in our system. Rather, our results show increased production of ROS in BNIP3 null tumor cells and quenching ROS in vivo reduces HIF1A levels and attenuates tumor growth and metastasis. We attribute increased ROS in BNIP3 null tumor cells to increased overall mitochondrial mass because we do not detect mutations in mtDNA encoding respiratory chain subunits. However, our work does not rule out a direct role for BNIP3 in regulating complex I or complex III activity, the major sources of ROS at the mitochondria.

Our analyses identified triple negative breast cancer (TNBC) as the subtype of human breast cancer where copy number loss of BNIP3 at chr.10q26.3 is most common. Intriguingly, elevated HIF1A levels are also most commonly associated with TNBC than any other human breast cancer subtype suggesting a selective advantage to tumors that inactivate BNIP3 in the context of high HIF1A expression, and indeed our results show that loss of BNIP3 predicts poorer metastasis-free survival in HIF1A high-expressing tumors.

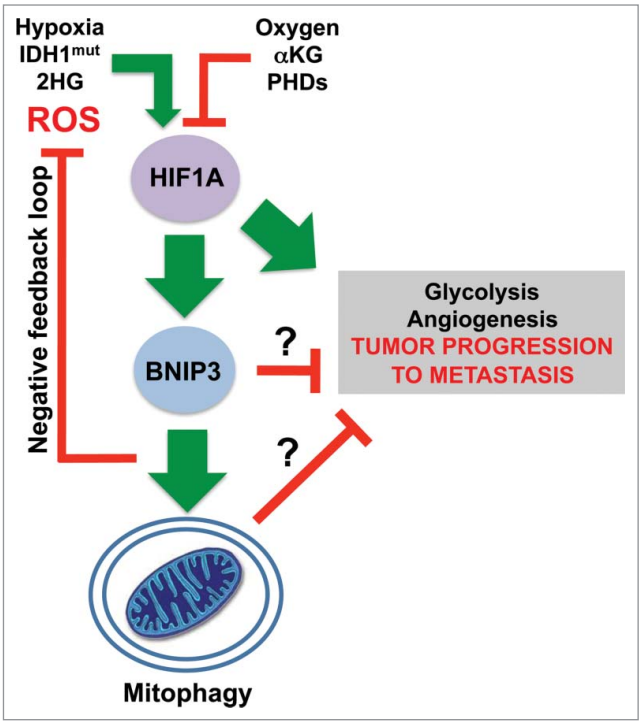

Figure 1. BNIP3-dependent mitophagy feeds back to inhibit HIF1A stabilization by reducing mitochondrial mass and promoting the integrity of the mitochondrial pool, thereby limiting generation of reactive oxygen species (ROS). HIF1A stabilization is influenced by a number of factors, including oxygen levels and the mutational state of IDH1/IDH2 that produces 2-hydroxyglutarate (2HG) but we show that in the mammary tumor model studied, the primary signal promoting HIF1A stabilization is excess ROS production at the mitochondria. Increased HIF1A leads to HIF1A-driven glycolysis and angiogenesis that promotes tumor progression to metastasis. Thus, we have defined a novel negative feedback loop from BNIP3 to its upstream regulator, HIF1A, that acts to mitigate against the tumor-promoting activity of HIF1A. These studies do not rule out additional functions of BNIP3 in tumorigenesis, and the broader role of mitophagy in cancer also remains to be fully elucidated. PHDs, prolyl hydroxylases.

Alongside elevated HIF1A and reduced BNIP3, TNBC shows the most Warburg-like phenotype of all breast cancer subtypes possibly due to elevated HIF1A activity driving glycolysis and inhibiting oxidative metabolism. Mitochondrial dysfunction could also be contributing, as we see in tumors developing in MMTV-PyMT mice when *Bnip3* is deleted, and more in-depth examination of mitochondrial mass and function in primary TNBCs is ongoing.

Our work examined defective mitophagy in a very specific setting—loss of BNIP3 in mammary tumorigenesis. Clearly, there are other regulators of mitophagy, such as BNIP3L/NIX, PARK2/Parkin, and FUNDC1, and it will take additional studies to determine the extent to which each of these molecules can compensate for each other. Chromosomal deletion of *PARK2* has been detected in human cancers, including breast and ovarian cancers, and *Park2* null mice are susceptible to spontaneous liver tumors and sensitized to irradiation-induced lymphomagenesis indicating that like BNIP3, PARK2 has tumor suppressor properties. BNIP3L levels are elevated in BNIP3 null tumors, but this is not sufficient to compensate for loss of BNIP3, and indeed elevated BNIP3L may simply reflect increased mitochondrial mass and hypoxia in *BNIP3* null tumors. Nevertheless, it seems likely that not all mitophagy is defective in *BNIP3* null tumors but rather only mitophagy driven by the signals that would normally induce BNIP3, such as hypoxia or nutrient deprivation.

Our studies thus far have been restricted to understanding the role of mitophagy in mammary tumorigenesis. Given the marked phenotypic differences between tumor models in which general autophagy is inhibited (failure to progress to malignancy) and those in which mitophagy is specifically inhibited (increased malignancy), we are actively assessing the consequences of selective mitophagy defects in tumor models in which general autophagy defects were previously examined (pancreas, lung, liver). Interestingly, BNIP3 is epigenetically silenced in human pancreatic ductal adenocarcinomas that are highly hypoxic due to their avascular nature and thus expected to have high HIF1A levels that, combined with BNIP3 silencing, could be playing a similar tumor-promoting role to what we observe in mammary tumor models.

Finally, a major question in the field remains how rapidly mitochondria become dysfunctional once mitophagy is inhibited. Clearly, the first outcome of defective mitophagy is increased mitochondrial mass, and initially these mitochondria are likely to be largely functional and only become dysfunctional over time. Initial increases in mitochondrial mass could confer a selective advantage to tumor cells by providing more ATP and key metabolic intermediates that may select in favor of (epi)-genetic changes that block mitophagy. Only later, as damaged mitochondria accumulate, would the tumor need to adapt to increased ROS, and decreased oxidative potential, perhaps by increasing mitochondrial biogenesis or fusion or by undergoing the switch to Warburg metabolism. Given their high growth rate and nutrient-deprived environment, it might also be predicted that tumor cells would accumulate dysfunctional mitochondria more rapidly than normal cells. Exactly how tumor cells adapt dynamically over time to loss of mitophagy needs to be examined further and in particular to assess how this is affected by tissue type, nutrient stresses or the driving oncogene.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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