Regulation of autophagy by two products of one gene: TRPM3 and miR-204

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In clear cell renal cell carcinoma (ccRCC), oncogenic autophagy dependent on microtubule-associated protein 1 light chain 3 α and β (LC3A and LC3B) is stimulated by activity of the transient receptor potential melastatin 3 (TRPM3) channel through multiple complementary mechanisms. The Von Hippel-Lindau (VHL) tumor suppressor represses this oncogenic autophagy in a coordinated manner through the activity of miR-204, which is expressed from intron 6 of the gene encoding TRPM3. TRPM3 represents an actionable target for ccRCC treatment.

Control of autophagy by VHL provides an example of VHL functioning as a master tumor suppressor in ccRCC beyond its role in regulating hypoxia-inducible factors. We have previously reported that loss of VHL inhibits expression of a tumor suppressing microRNA, miR-204, and that the loss of miR-204 in turn leads to augmented activity of oncogenic autophagy dependent on microtubule-associated protein 1 light chain 3 β (MAP1LC3B, best known as LC3B), as LC3B is a direct target of miR-204.1 In our current publication, we expand these studies by showing that loss of VHL in ccRCC leads to disinhibition of an entire regulatory network that stimulates oncogenic autophagy at multiple levels (Fig. 1).2 In particular, loss of VHL and miR-204 leads to augmented expression of the transient receptor potential melastatin 3 (TRPM3) channel, a direct target of miR-204. We have demonstrated that TRPM3 stimulates oncogenic autophagy dependent on microtubule-associated protein 1 light chain 3 α (MAP1LC3A, best known as LC3A) and LC3B and promotes the growth of ccRCC. A clinically important aspect of this investigation is the potential to therapeutically target TRPM3. Indeed, we observed that inhibition of TRPM3 by a specific inhibitor, mefenamic acid (MFA),3 inhibited ccRCC tumor growth in xenograft assays. Our interest in TRPM3 initially arose because miR-204 is expressed from intron 6 of the gene encoding TRPM3.4 TRPM3 expression was found to be elevated in human ccRCC tumor samples with inactivated or mutated VHL compared to matched kidneys, as well as in human ccRCC cell lines with loss of VHL compared to cell lines with intact VHL. Whereas cells with TRPM3 knockdown failed to form tumors, reconstituting TRPM3 in these cells with shRNA-resistant TRPM3 allowed the formation of tumors that were comparable in size and incidence to those formed by unadulterated cells expressing endogenous TRPM3. MFA, a non-steroidal anti-inflammatory (NSAID) that is approved by the Food and Drug Administration for the treatment of pain, is known to be a specific inhibitor of TRPM3. MFA treatment of VHL-mutated ccRCC cell lines resulted in the reduction of TRPM3 expression at protein and mRNA levels. MFA treatment of mice bearing ccRCC xenograft tumors led to a significant reduction of tumor growth and, in some cases, tumor regression.

Next, we demonstrated that TRPM3 expression is controlled by miR-204 directly by inhibition of TRPM3 translation and indirectly by inhibition of translation of caveolin 1 (CAV1), which is necessary for TRPM3 expression. miR-204 directly inhibits the translation of TRPM3 by binding to a miR-204 site in the 3′ untranslated region (3′UTR). Similarly, miR-204 downregulates the expression of CAV1 through a miR-204 site in the 3′ UTR of CAV1.

Intriguingly, TRPM3 regulates autophagy in a manner opposite to miR-204. Whereas miR-204 inhibits LC3B-dependent autophagy, TRPM3 stimulates LC3A and LC3B autophagy through Ca2+ influx and calmodulin-regulated...
First, it stimulates autophagosome formation through Ca\textsuperscript{2+}-dependent activation of calcium/calmodulin-dependent protein kinase 2, β (CAMKK2) and AMP-activated protein kinase (AMPK, also known as PRKA) and the resulting phosphorylation of unc-51 like autophagy activating kinase 1 (ULK1). Second, we established that TRPM3 activity inhibits expression of miR-214, which directly targets LC3A and LC3B. The VHL tumor suppressor inhibits expression of TRPM3 directly and indirectly through the effect of miR-214 on CAV1. In addition, miR-214 directly targets LC3B. AMPK, AMP-activated protein kinase; CAMKK2, calcium/calmodulin-dependent protein kinase kinase 2; VHL, Von Hippel-Lindau.

Figure 1. Robust control of the autophagic network by microRNAs and calcium- and zinc-activated pathways. Calcium and zinc entering the cell through the TRPM3 channel stimulate oncogenic autophagy mediated by LC3A and LC3B through a dual mechanism. Calcium stimulates phagophore initiation through Ca\textsuperscript{2+}-dependent activation of CAMKK2 and AMPK, and the resulting phosphorylation of ULK1. Calcium and zinc also inhibit miR-214, which directly targets LC3A and LC3B. The VHL tumor suppressor inhibits expression of TRPM3 directly and indirectly through the effect of miR-214 on CAV1. In addition, miR-214 directly targets LC3B. AMPK, AMP-activated protein kinase; CAMKK2, calcium/calmodulin-dependent protein kinase kinase 2; CAV1, caveolin 1; LC3A, microtubule-associated protein 1 light chain 3\textsuperscript{a}; LC3B, microtubule-associated protein 1 light chain 3\textsuperscript{b}; TRPM3, transient receptor potential melastatin 3; ULK1, unc-51 like autophagy activating kinase 1; VHL, Von Hippel-Lindau.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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