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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.

Autophagy is an important homeostatic process by which cells generate nutrients from intracellular sources and perform quality control on their organelles. Autophagy also plays an important role in the survival of cancer cells. Our recent investigations have demonstrated a process by which the autophagy networks in clear cell renal cell carcinoma (ccRCC) are remodeled in a manner dependent on loss of the von Hippel-Lindau (VHL) tumor suppressor.

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 Ca^{2+} influx and calmodulin-regulated

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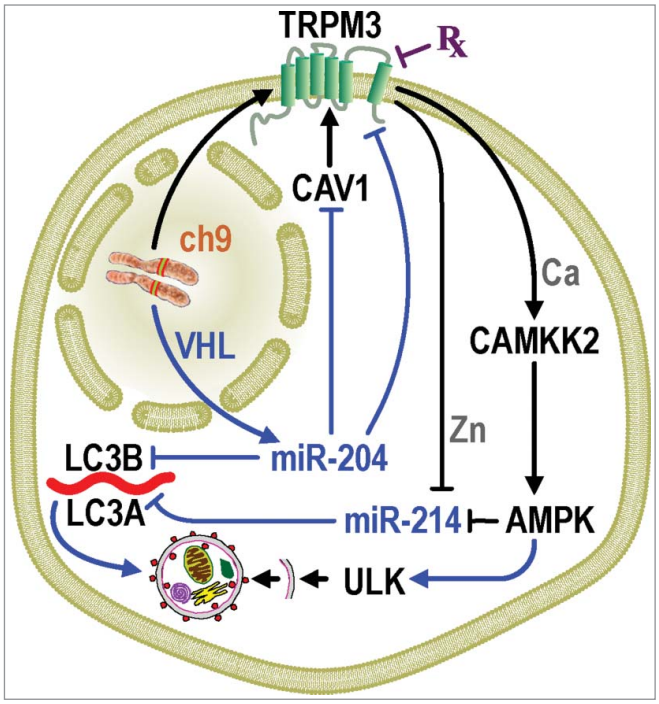

Figure 1. Robust control of the autophagic network by microRNAs and calcium- and zinc-activated pathways. Calcium and zinc entering the cell through the TRPM3 channel stimulate oncogenic autophagy mediated by LC3A and LC3B through a dual mechanism. Calcium stimulates phagophore initiation through Ca^{2+}-dependent 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-204 on CAV1. In addition, miR-204 directly targets LC3B. AMPK, AMP-activated protein kinase; CAMKK2, calcium/calmodulin-dependent protein kinase kinase 2, β; CAV1, caveolin 1; LC3A, microtubule-associated protein 1 light chain 3 α; LC3B, microtubule-associated protein 1 light chain 3 β; TRPM3, transient receptor potential melastatin 3; ULK1, unc-51 like autophagy activating kinase 1; VHL, Von Hippel-Lindau.

signaling pathways at multiple levels. First, it stimulates autophagosome formation through Ca^{2+}-dependent activation of calcium/calmodulin-dependent protein kinase 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, a microRNA with decreased expression in ccRCC compared to normal kidney.[5,6] We determined that miR-214 directly targets LC3A and LC3B, therefore TRPM3 increases expression of LC3A and LC3B via its control of miR-214. The inhibition of miR-214 by TRPM3 requires Ca^{2+}-stimulated activity of the CAMKK2-AMPK pathway and influx of Zn^{2+}.

Thus, it is our assertion that TRPM3 and its regulation are integral to the development and progression of ccRCC, in particular tumors with *VHL* loss. In the context of the current feverish interest in identifying "targetable" pathways and "actionable" cellular processes in order to develop novel therapeutics, we believe that inhibition of oncogenic autophagy through regulation of TRPM3 may hold great promise. Potentially, autophagic pathways allow cancer cells access to an additional intracellular source of energy and may provide an escape mechanism to avoid the "energy starvation" induced by current therapies against RCC that target angiogenic pathways. Additionally, metastatic tumors may utilize oncogenic autophagy to survive the stressful process of metastasis and specifically to provide the crucial energy needed during periods of limited supply.

In conclusion, the pathway linking TRPM3 with autophagy provides a novel, VHL-regulated, central mechanism for the regulation of oncogenic autophagy in ccRCC. Elucidation of the role of this pathway in ccRCC tumor growth provides a platform for targeted inhibition that has a clinical rationale. The enduring hope is that this will present an additional opportunity for a targeted assault on ccRCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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