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Oncogenes calling on a lysosomal Ca^{2+} channel

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Oncogene-dependent cancers upregulate and use lysosomal biogenesis for nutrient supply. It is unclear which lysosomal proteins and functions are essential for cell proliferation and tumor growth. A study by Jung *et al* in this issue of *EMBO Reports* shows that increased levels of the lysosomal Ca^{2+} release channel TRPML1 and its activator $PI(3,5)P_2$ in $HRAS^{G12V}$-dependent tumors are essential for cell proliferation and tumor growth [1]. Notably, genetic and pharmacological inhibition of TRPML1 is equally effective in suppressing $HRAS^{G12V}$-dependent tumors.

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See also: **J Jung *et al***

The lysosome senses and responds to cell nutrients and the energetic status of the cell to determine cellular homeostasis. Lysosome proteases, lipases, and nucleases digest damaged intracellular organelles and proteins and endocytosed extracellular matrix to their building blocks, and export them to the cytoplasm using catabolite transporters [2]. The lysosome is also the site of several signaling pathways that control lysosomal function [3]. When sufficient nutrients are available, various kinases phosphorylate the master lysosomal biogenesis transcription factors TFEB and MiTF to retain them in the cytosol. Upon cell nutrient starvation and stress, TFEB and MiTF are dephosphorylated and translocate to the nucleus to activate the CLEAR (Coordinated Lysosomal Expression and Regulation) gene network, which induces lysosomal biogenesis [3].

Lysosomes have a crucial role in many cancers. Cancer cells are under severe nutrient stress and upregulate lysosomal biogenesis to increase metabolites and energy supply. Cancer cells also route a subpopulation of lysosomes to the cell periphery where they release lysosomal proteases that digest the extracellular matrix for additional nutrient supply and to aid in cell metastasis [4]. However, little is known about how the many lysosomal functions are regulated in cancer cells. In this issue of *EMBO Reports*, Jung *et al* [1] reported the upregulation and essential role of the lysosomal Ca^{2+} release channel TRPML1 in oncogenic HRAS ($HRAS^{G12V}$)-expressing tumor cells, as well as the correlation of elevated *TRPML1* with reduced survival in three types of squamous cell carcinoma.

Lysosomes are acidified by a coupled function of a vacuolar-type H^+ pump and a CLC7 $H^+/2Cl^-$ exchanger, and take up Ca^{2+} by an unidentified Ca^{2+}/H^+ exchange mechanism. Ca^{2+} release from the lysosomes is mediated by the TRP channel family TRPML1 and requires the NAADP-activated Ca^{2+} and Na^+ permeable two-pore channel TPC2 [5] (Fig 1). The only known and essential physiological activator of TRPML and TPC channels is the phosphoinositide lipid $PI(3,5)P_2$ [2]. The prominent role of TRPML1 in lysosomal and cellular function is evident from the human lysosomal storage disease mucolipidosis type 4, caused by mutations in *TRPML1*. TRPML1 has critical and permissive roles in lysosomal exocytosis, tissue repair, lysosomal positioning, and autophagy [6].

To identify key lysosomal proteins that regulate cell proliferation in tumor cells, Jung *et al* [1] screened $HRAS^{G12V}$-dependent tumors, revealing increased levels of *MiTF/TFEB* in $HRAS^{G12V}$-associated tumors compared with matched normal tissues. Notably, prominent within the cassette of differentially expressed cancer genes was *TRPML1*. Tumor cells differentially expressed *VAC14*, which is required for synthesis of the TRPML1 activator $PI(3,5)P_2$; tumors differentially downregulated *MTM1*, which codes for the phosphatase that reduces $PI(3,5)P_2$ levels. Consistent with these findings, TRPML1-mediated Ca^{2+} release from the lysosomes of $HRAS^{G12V}$-expressing cells was double that of control cells. To reveal the importance of increased lysosomal Ca^{2+} release for tumorigenesis, Jung *et al* showed that knockdown of *TRPML1* in several $HRAS^{G12V}$-expressing cancer cell lines inhibited cell proliferation by inhibiting the activated MAP kinase pathway. Furthermore, knockdown of *TRPML1* inhibited growth of $HRAS^{G12V}$-expressing cells in xenografts, and deletion of *trpml1* in drosophila expressing the activated fly Ras homologue $dRAS^{G12V}$ inhibited MAP kinase activation, cell proliferation, and tissue growth.

To examine potential mechanisms by which increased lysosomal Ca^{2+} release increases cell proliferation in $HRAS^{G12V}$-expressing cells, Jung *et al* [1] analyzed plasma membrane (PM) cholesterol and $HRAS^{G12V}$ nanoclustering. To affect cell proliferation, all activated RAS oncogenic proteins are organized into nanoclusters in defined non-overlapping PM lipid domains [7]. Increased lysosomal Ca^{2+} release was essential for intracellular cholesterol trafficking, which was in turn required for $HRAS^{G12V}$ nanoclustering. The sequence of events proposed by Jung *et al* is illustrated in Fig 1. In cells expressing $HRAS^{G12V}$, elevated *MiTF* and *TFEB* induce the

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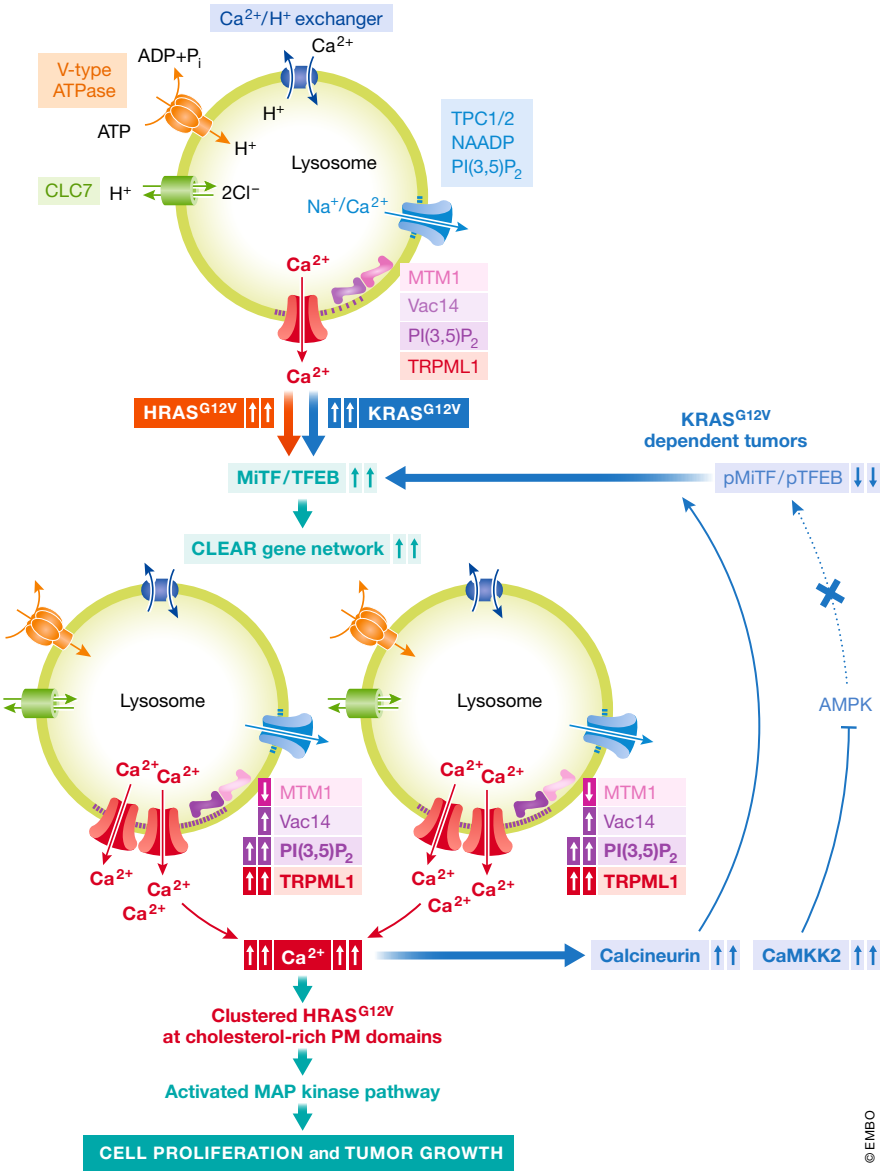

Figure 1. Lysosomal Ca^{2+} release in oncogene-dependent tumor growth.
Lysosomal Ca^{2+} release by TRPML1 is supported by $PI(3,5)P_2$ and requires TPC channels. $HRAS^{G12V}$ expression leads to increased levels of the lysosomal biogenesis master transcription factors MiTF and TFEB and activation of the CLEAR gene network in which *TRPML1* and *VAC14* are upregulated and *MTM1* is downregulated. This results in increased lysosomal Ca^{2+} release, $HRAS^{G12V}$ nanoclusters at cholesterol-rich plasma membrane (PM) domains, MAP kinase pathway activation, cell proliferation, and tumor growth. In $KRAS^{G12V}$-dependent tumors, released lysosomal Ca^{2+} activates the phosphatase calcineurin and the kinase CaMKK2. Calcineurin dephosphorylates, whereas CaMKK2 phosphorylates and inhibits the AMP kinase, which phosphorylates MiTF and TFEB to retain them in the cytosol. Dephosphorylated MiTF and TFEB translocate to the nucleus to activate the CLEAR gene network.

CLEAR gene network of endolysosomal genes, including *TRPML1* and genes that upregulate the TRPML1 activator $PI(3,5)P_2$. This results in increased lysosomal Ca^{2+} release, nanoclustering of $HRAS^{G12V}$ in cholesterol-rich PM domains, MAP kinase pathway activation, and ultimately cell proliferation and tumor growth. It is of interest that TRPML1-mediated lysosomal Ca^{2+} release induced proliferation in $HRAS^{G12V}$-expressing cells but not control cells. Moreover, partial deletion of *TRPML1* was sufficient to inhibit cell proliferation and tumor growth, and pharmacological inhibition was as effective as *TRPML1* knockdown in $HRAS^{G12V}$-expressing cells. This suggests that TRPML1 inhibition will be a feasible therapeutic strategy for $HRAS^{G12V}$-dependent tumors.

These findings raise several questions and may have implications for other forms of oncogene-driven cancers. One key question is how an increase in lysosomal Ca^{2+} release can support tumor growth. One potential mechanism is suggested by the role of lysosomes in $KRAS^{G12V}$-dependent tumors. As illustrated in Fig 1, lysosomal Ca^{2+} activates the Ca^{2+}-regulated phosphatase calcineurin and the Ca^{2+}/calmodulin-dependent kinase CaMKK2. Calcineurin dephosphorylates MiTF and TFEB [8], and CaMKK2 phosphorylates and inhibits AMP kinase (AMPK), which phosphorylates MiTF and TFEB [9]. This results in dephosphorylation of MiTF and TFEB and their translocation to the nucleus and increased lysosomal biogenesis.

Other open questions are how exactly TRPML1 affects PM cholesterol level, and how TPC channels may affect oncogene-driven cancers. PM cholesterol is determined by lipid transfer proteins such as oxysterol binding protein (OSBP)-related protein 2 (ORP2), which translocates endolysosomal cholesterol to the PM [10]. It should be of interest to determine expression of ORP2 and other OSBPs that transport cholesterol in oncogene-dependent tumors and their regulation by lysosomal Ca^{2+} release. Finally, the TPC channels that are required for lysosomal Ca^{2+} release are implicated in cancer development [5]. Considering the findings of Jung *et al*, it should be of interest to determine their status and activity in oncogene-dependent tumors.

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