RESEARCH HIGHLIGHT

Targeting an alternative route: autophagy in RAS-driven cancers

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Activating RAS mutations have been linked to overactivation of autophagy but how this can be differentiated from physiological autophagy is poorly understood. Wang and colleagues reveal a RAS-induced non-canonical mechanism of autophagy and propose a promising new target against RAS-mutant cancers.

RAS proteins, HRAS, KRAS and NRAS, are monomeric GTPases which are active in the GTP-bound state. Endogenous RAS proteins are mainly in the inactive GDP-bound state and are transiently activated. RAS exists at the center of a complex network of signaling cascades with effectors involved in regulating cell proliferation, differentiation and survival.[1] Activated KRAS is an essential driver for tumor growth, but a lack of drug-binding pockets makes it difficult to target.[2,3]

Canonical autophagy uses a conserved machinery to generate autophagosomes for degradation of cargo. Alternative or non-canonical forms of autophagy that utilize some components of the canonical machinery but are independent of others have been described in various contexts. One example of this is ATG5/ATG7-independent autophagy in mouse embryonic fibroblasts where double-membrane structures dependent on unc-51-like autophagy-activating kinase 1 (ULK1) and Rab9 are formed during erythrocyte differentiation.[4] Another example is LC3-associated phagocytosis where LC3 lipidation to single membranes occurs independently of the phosphatidylinositol 3-kinases class III complex (PI3KCIII) and WD repeat domain phosphoinositide interacting 2b (WIPI2b).[5]

Overactivation of autophagy in activated RAS mutant tumor cells has been shown to support oxidative metabolism and tumorigenesis, making autophagy an attractive target in the clinic.[6] However, a key challenge is that autophagy has an enigmatic role in cancer with both tumorigenic and tumor-suppressive effects. In a recent study published in *Cell Research* by Wang et al.,[7] oncogenic RAS-induced autophagy models are used to unpick the mechanism of aberrant autophagy in cancer. They reveal a novel, non-canonical autophagy pathway termed RAS-induced non-canonical autophagy via ATG8ylation (RINCAA), with distinct molecular features from canonical autophagy. During RINCAA induced by constitutively active KRAS(G12V), unique single-membrane structures form, RAS-induced multivesicular/multilaminar bodies positive of ATG8ylation (RIMMBA), which drive tumor growth.

By knocking down various autophagy factors and inducing RINCAA by expression of KRAS(G12V), the authors show that RINCAA differs from starvation-induced autophagy, utilizing some components of the canonical autophagy machinery (e.g., ULK1, WIPI2), but being independent of others (e.g., PI3KCIII, ATG2, ATG9). During canonical autophagy, ULK1 activation and inhibition is regulated by AMPK and mTOR, respectively. ULK1 subsequently phosphorylates PI3KCIII leading to PI3P generation and recruitment of WIPI2 to the autophagy initiation site. During RINCAA, RAS-activated P38 signaling activates ULK1 through phosphorylation at S556. ULK1 then regulates PI4KB by phosphorylation at S256 and S263 and subsequently PI4P generation. Notably, these phosphorylation sites are not required for PI4KB activity under normal conditions. WIPI2 puncta formation depends on PI4KB activity, suggesting a differential lipid binding to PI4P which is in contrast to PI3P binding during canonical autophagy. Overall, these findings reveal a P38-ULK1-PI4KB-WIPI2 signaling cascade, and the identification of a novel interaction between PI4KB and WIPI2. Co-localization of WIPI2 and LC3B on RIMMBA upon oncogenic RAS expression suggests recruitment of the ATG16L1 E3-ligase complex by WIPI2 in a similar manner described for canonical autophagy (Fig. 1a, b).

The distinct role of ULK1-mediated PI4KB phosphorylation during RINCAA makes it a promising target for therapeutic intervention in KRAS-mutated cancers (Fig. 1c). Expression of either a phosphorylation-deficient PI4KB mutant or treatment with a PI4KB peptide spanning S256 and S263 (Peptide 1) led to decreased RINCAA, reduced cell proliferation and tumor growth as well as increased life span in cancer cell lines, xenografts and pancreatic cancer mouse models, respectively. Combined with the MEK inhibitor trametinib, Peptide 1 showed improved anti-tumor efficacy compared to chloroquine and trametinib treatment. In contrast to chloroquine treatment and PI4KB depletion, which inhibit micropinocytosis and autophagy in RAS-mutated cancer cell lines, Peptide 1 did not affect micropinocytosis, suggesting that inhibiting autophagy-associated processes beyond nutrient scavenging are critical for treatment of RAS-driven cancers.

Wang et al. have revealed key mechanistic differences between RINCAA and physiological autophagy, providing a promising target for RAS-driven cancers. Previously, autophagy has been shown to be activated during HRAS-induced senescence (RIS). During RIS, a unique compartment forms, TOR-autophagy spatial coupling compartment (TASCC), which contains LC3-positive autolysosomes.[8] Further investigation into the relationship between RINCAA and RIS might reveal commonality in the machinery between these degradative pathways. At the mechanistic level, the discovery of RINCAA opens intriguing questions for future research. The fact that several autophagy factors are shared

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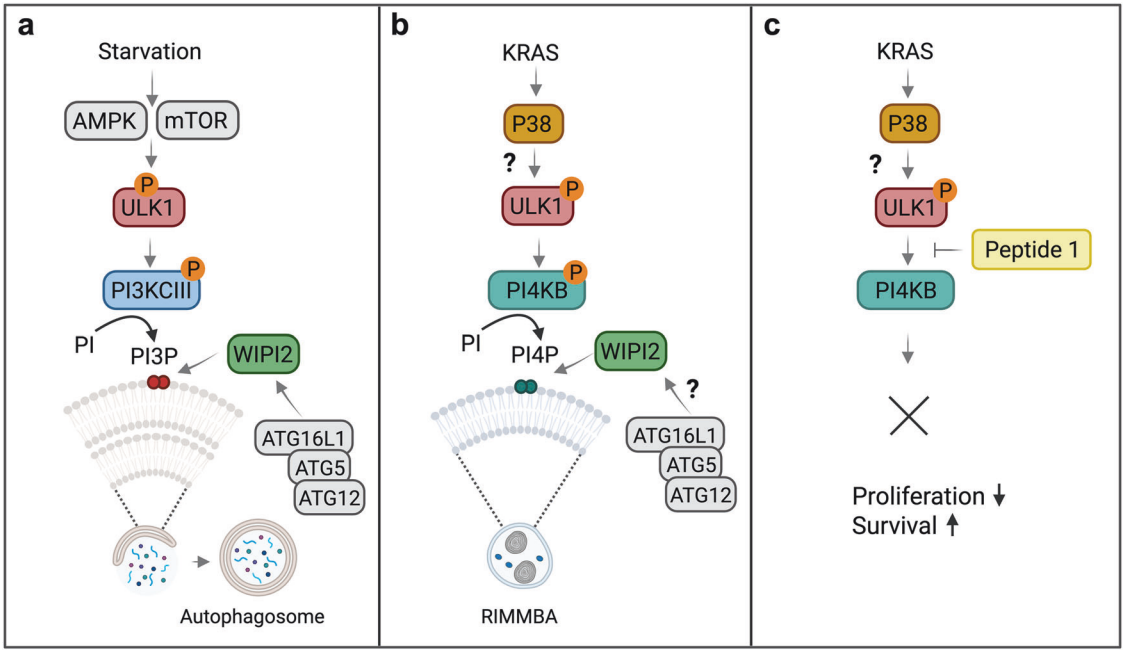

Fig. 1 Model of molecular mechanism of starvation-induced autophagy and RINCAA. This model is created in BioRender. Ueffing, A. (2025) https://BioRender.com/q1kc0si. **a**, **b** While starvation induced-autophagy relies on ULK1, PI3KCIII and WIPI2 axis (**a**), RINCAA operates under the P38-ULK1-PI4KB-WIPI2 axis (**b**). **c** Peptide 1 inhibits phosphorylation of PI4KB, leading to decreased tumor proliferation.

between canonical autophagy and RINCAA leaves the question as to how the two processes are coordinated and whether competition arises between shared factors. One of these is ULK1 and the authors show that ULK1 S556 phosphorylation is critical for RINCAA. However, recent reports of ULK1 inactivation through phosphorylation at S556 by AMPK[9] suggest that additional factors regulating differential ULK1 activity remain to be identified. Another central mechanistic question is how WIPI2 shifts from acting as a PI3P to PI4P effector and which function WIPI2 and subsequently recruited autophagy factors have during RINCAA. Are these factors involved in RIMMBA formation, analogous to their role in double-membrane autophagosome biogenesis or do they regulate downstream degradative processes? Finally, identifying the precise mechanism of action of Peptide 1 will reveal whether ULK1 directly phosphorylates PI4KB during RINCAA or whether additional intermediate kinases are involved. Accordingly, the effect of Peptide 1 or corresponding small molecules on physiological autophagy in non-tumor cells will have to be investigated to gain further mechanistic insight while validating its applicability as a therapeutic strategy.

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COMPETING INTERESTS

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ADDITIONAL INFORMATION

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