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Commentary

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Transcriptional regulation of autophagy in RAS-driven cancers

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RAS-driven cancers exhibit variable dependency on autophagy for survival; however, it is not fully understood how. In this issue of the *JCI*, Cheong and colleagues demonstrate that RAS-dependent elevation of casein kinase 1 α (CK1 α) negatively regulates autophagy at the level of autophagy gene transcription. Moreover, combined inhibition of both CK1 α and autophagy reduced proliferation of RAS-driven tumors. The results of this study provide insight into the connection between mutant RAS and autophagy, and suggest targeting CK1 α as a potential therapeutic strategy to modulate autophagy in RAS-driven cancers.

The challenge of directly targeting RAS for cancer therapy

RAS is a canonical oncogenic driver, with RAS-activating mutations identified in 20%–30% of cancers. Constitutive RAS activation turns on many signaling pathways, including those that promote cell growth and survival. Despite the prevalence of RAS mutations in many forms of cancer, the development of drugs that directly target RAS has remained elusive. Small molecule RAS inhibitors have recently been discovered and shown to impair function in vitro (1); however, these will need to be further tested before clinical translation. Another focus for therapeutically targeting RAS-driven cancers has been the development of potent small molecule inhibitors against pathways downstream of mutant RAS, including MAPK and the PI3K/AKT/mTOR pathway (Figure 1). For instance, clinical trials of single agents, such as a MEK inhibitor (ClinicalTrials.gov, NCT01320085), and combination strategies that simultaneously target components of parallel pathways such as MEK and PI3K (NCT01363232) — or target the same pathway at two nodes, such

as MEK and CDK4 (NCT01781572) — are being conducted to evaluate these strategies for use against melanoma. Unfortunately, these combinations can produce substantial toxicities; therefore, efficacy of targeted combination therapies has not been proven to be superior to single-agent therapy or standard-of-care chemotherapy in melanoma or any other RAS mutant cancer.

Autophagy inhibition has potential

A number of studies have shown that autophagy is elevated in the setting of RAS transformation (2, 3), thereby providing another pathway — in addition to MAPK and PI3K — as a potential target for RAS mutant tumors. The intimacy between canonical growth factor kinase signaling pathways that are downstream of RAS and autophagy is underscored by the fact the MAPK signaling occurs on the surface of autophagic vesicles (4) and mTOR is physically attached to lysosomes (5). Autophagy as a therapeutic target is controversial, as autophagy can play different roles in early and late tumorigenesis (6). However, in the setting of advanced cancer, it is more and

more appreciated that increased autophagy is due to oncogenic and metabolic stress, and is further increased in response to anticancer therapies (7). Moreover, drug-induced autophagy is cytoprotective in most animal models of cancer therapy. Because autophagy is a complex molecular pathway, numerous efforts are underway to develop small molecule inhibitors of canonical autophagy proteins.

While specific autophagy inhibitors have yet to be clinically evaluated, the chloroquine (CQ) derivatives, which inhibit autophagy by impairing lysosomal function (but may also inhibit cancer cells in other ways), have begun to be tested in clinical trials. For example, six recent publications report on different clinical trials that examined use of hydroxychloroquine (HCQ) combined with various anticancer agents (8–13). These trials demonstrated that HCQ does modulate autophagy in human tissues; however, the magnitude of this modulation was modest at best, even in those given the highest FDA-approved doses. No catastrophic toxicity was observed in HCQ combination regimens that involved temsirolimus, bortezomib, or vorinostat, though toxicity was observed when HCQ was administered with a specific temozolomide schedule. Taken together, these preliminary results suggest that more potent lysosomal autophagy inhibitors, combined with more effective chemotherapy or other targeted therapies, may yield better results. More potent CQ derivatives such as Lys05 (14) are now being evaluated for potential clinical development, and a second generation of HCQ clinical trials that pair HCQ with more potent cancer therapeutics are currently underway. However, a missing element in these efforts is a predictive biomarker that would identify patients likely to respond to autophagy inhibitor therapies.

Initially, studies suggested RAS mutation as a potential biomarker for patient selection. In animal models of mutant RAS-driven cancer, genetic inhibition of autophagy dramatically impaired tumor

► Related Article: p. 1401

Conflict of interest: Ravi K. Amaravadi is a coinventor on a patent regarding Lys05 that was licensed to a biotechnology company by the University of Pennsylvania.

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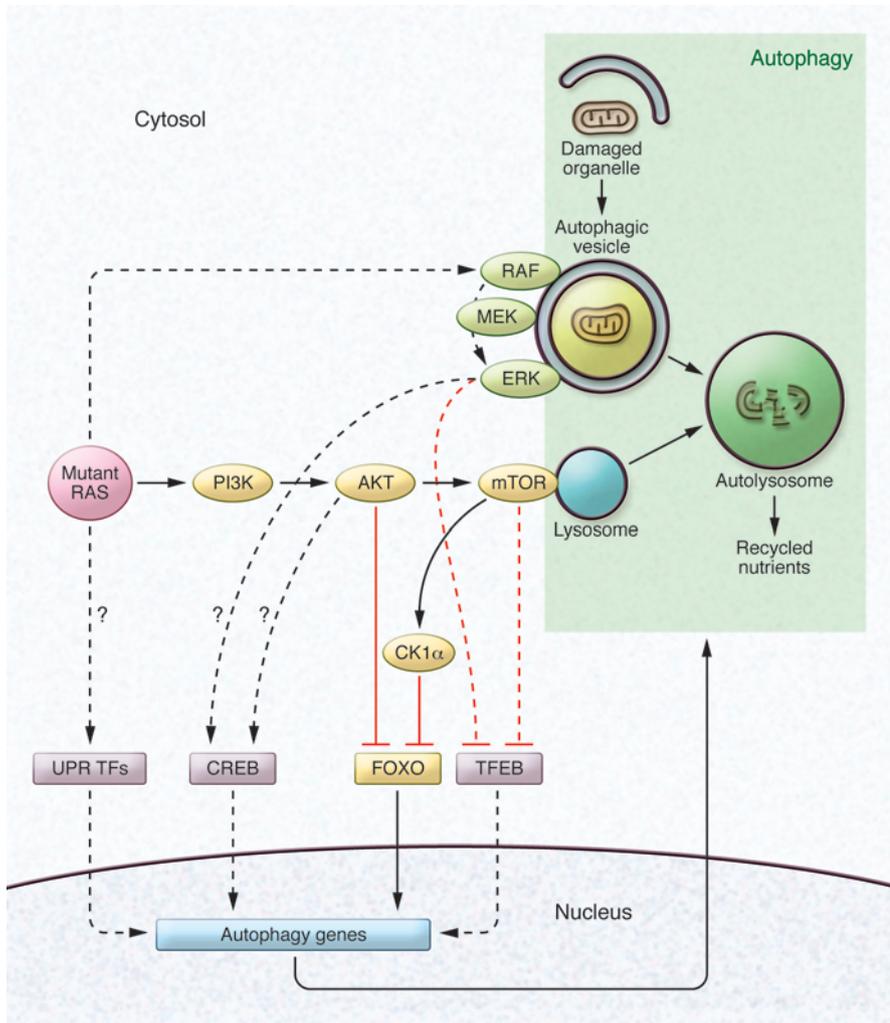


Figure 1. Transcriptional regulation of autophagy in RAS-driven cancers. Mutant RAS activates several canonical growth factor signaling pathways, including the MAPK pathway (RAF/MEK/ERK) and the PI3K pathway (PI3K/AKT/mTOR). MAPK and PI3K signaling events take place in part on the surface of autophagic vesicles and lysosomes, respectively. Autophagy consists of the sequestration of damaged organelles within autophagic vesicles followed by fusion with the lysosome. A subset of known transcriptional regulators of autophagy genes are depicted, along with their regulation by growth factor kinase signaling pathways under the control of RAS. In this issue, Cheong et al. demonstrate that RAS-driven PI3K signaling increases levels of CK1 α , which in turn phosphorylates and inhibits nuclear localization of FOXO3A, a transcription factor that positively regulates the expression of key autophagy genes (this pathway is denoted in yellow). Dashed lines indicate pathways described in other reports. Arrows indicate activation; lines ending in T indicate inhibition. UPR, unfolded protein response; TF, transcription factor.

progression, leading to the notion that RAS mutant tumors are “addicted” to autophagy. For instance, a genetically engineered mouse model of KRAS-driven cancer and xenografts derived from patients with pancreatic cancer, which often harbor KRAS mutations, were strikingly susceptible to CQ derivatives (15); however, as the efficacy of autophagy inhibitors has been tested in more cell lines, it has been shown that RAS mutation alone does not adequately predict susceptibility to autophagy inhibition. There is clear evidence that some RAS-driven can-

cers are autophagy dependent, while some are autophagy independent (16).

Linking RAS and autophagy provides an attractive target

In this issue, Cheong et al. identify a link between mutant RAS and autophagy, and demonstrate that CK1 α is a key negative regulator of autophagy in RAS-driven tumors (17). CK1 α is a constitutively active kinase that has been implicated in numerous signaling pathways (Figure 1), including a previous study that identified CK family mem-

bers as important regulators of autophagy in an siRNA screen performed in breast cancer cells (18). Cheong and colleagues investigated whether members of CK1 could provide a mechanistic link between oncogenic RAS and autophagy by evaluating the effects of knockdown of CK1 family members on autophagy in RAS-transformed cells. Knockdown of CK1 α , but not other CK1 isoforms, increased autophagic flux but only in the presence of oncogenic RAS. CK1 α knockdown perturbed the transcription of a number of canonical autophagy genes, all of which are regulated by FOXO3A. Using a series of FOXO3A mutants, Cheong et al. demonstrated that CK1 α phosphorylates FOXO3A on a specific serine residue that is distinct from the residue phosphorylated by AKT, and this CK1 α -dependent phosphorylation impaired FOXO3A-dependent transcription of multiple autophagy genes, such as LC3B. Moreover, CK1 α inhibition, via genetic means or with small molecules in RAS-transformed cells, resulted in nuclear localization of FOXO3A and induction of autophagy. Activation of PI3K, which is downstream of RAS, increased levels of CK1 α , though this increase was not the result of elevated CK1A transcription. Cheong and colleagues combined CK1 α inhibition with CQ to simultaneously induce autophagy and inhibit clearance of autophagosomes in RAS-mutant cancer cells. Compared with single-agent administration, this combination strategy impaired tumor cell proliferation in vitro and profoundly inhibited tumor growth in a xenograft model (17).

The study by Cheong et al. provides some important conceptual advances that link autophagy and cancer therapy, and identifies a feedback loop downstream of constitutively activated RAS that impacts the transcriptional regulation of autophagy genes. Importantly, the results of Cheong and colleagues provide a preclinical rationale for combining CK1 α and autophagy inhibitors in RAS mutant cells; however, clinical translation is limited at this time by a lack of potent and specific CK1 α inhibitors. CK1 family members have been difficult drug targets. Each isoform has been shown to perform different functions, depending on the cell context (19); therefore, the identification of selective inhibitors that are both potent and specific to a particular CK1 isoform has been challenging. While a number of groups have reported on spe-

cific inhibitors of other CK1 isoforms (20), no specific inhibitors of CK1 α have been reported. This study by Cheong and colleagues, along with other reports that support a role for CK1 α in promoting or limiting tumorigenesis in a subset of malignancies (19), provides a rationale for the focused development of such inhibitors.

Remaining questions and future directions

There are several questions that the work by Cheong and colleagues raises. First, does the augmented efficacy observed with combined inhibition of CK1 α and autophagy depend on CK1 α -dependent regulation of FOXO3A? Alternatively, could the benefit of combined therapy be due to one or more of the other pathways that CK1 α regulates, such as β catenin/WNT, circadian rhythms, or p53 signaling? Second, how does PI3K signaling alter levels of CK1 α ? Finally, can CK1 α levels be used to subclassify RAS-mutated tumors into autophagy dependent and autophagy independent categories to determine treatment options?

In a broader context (Figure 1), the CK1 α -dependent transcriptional regulation of autophagy genes identified by Cheong et al. can be added to a growing list of PI3K pathway-dependent mechanisms that suppress autophagy. AKT-dependent phosphorylation results in cytoplasmic retention of FOXO transcription factors, preventing autophagy gene transcription (21). mTOR activation downstream of mutant RAS inactivates unc-like kinase 1 (ULK1) (22) and traps the master regulator of autophagy genes transcription factor EB (TFEB) in the cytoplasm (23). TFEB is also phosphorylated and sequestered in the cytoplasm by ERK (24). These negative regulatory events do not explain the observation that autophagy is elevated and required in some RAS-driven tumors. Potential RAS-dependent mediators for positive regulation of autophagy gene transcription include unfolded protein response transcription factors (25) and the cyclic AMP response element binding protein (CREB), which was recently identified as a master regulator of autophagy (26). CREB is an attractive candidate because it is phosphorylated and activated by p90RSK and AKT, which are downstream of the MAPK and PI3K pathways, respectively. While the CREB dependency of mutant RAS-driven autophagy has not been exper-

imentally established, it is reasonable to speculate the presence of such a pathway. The identification of multiple positive and negative regulatory loops suggests that each aspect of autophagy may be quite dynamic in RAS-transformed cells, especially in the context of therapies that modulate PI3K and MAPK pathways. As highlighted by Cheong and colleagues, a better understanding of factors that regulate transcription of autophagy genes may point the way forward toward the ability to identify subsets of RAS-driven cancers that would be susceptible to therapeutic strategies that modulate autophagy.

Acknowledgments

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