Transcriptional regulation of autophagy in RAS-driven cancers

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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; 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

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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 CK1α, 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.
cific inhibitors of other CK1 isoforms (20), no specific inhibitors of CK1α have been reported. This study by Cheong and colleagues, along with other reports that support a role for CK1α 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 CK1α and autophagy depend on CK1α-dependent regulation of FOXO3A? Alternatively, could the benefit of combined therapy be due to one or more of the other pathways that CK1α regulates, such as β-catenin/WNT, circadian rhythms, or p53 signaling? Second, how does PI3K signaling alter levels of CK1α? Finally, can CK1α 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 CK1α-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-119 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 cancers that would be susceptible to thera-

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