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Autophagy in intra-hepatic cholangiocarcinoma

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Intra-hepatic cholangiocarcinoma (IHCC) is a primary cancer of the liver that shares histological features with the hepatic bile ducts from which it is thought to arise. The incidence of this disease is increasing, possibly related to increased inflammatory liver diseases such as viral hepatitis and steatohepatitis (commonly called "fatty liver"), induced by obesity, diabetes, and other metabolic derangements. Its prognosis is generally poor with early metastasis and presently there are limited effective treatments. A basic understanding of the disease has long been hampered by limited tumor cell lines and good model systems. Similarly, such limitations have obstructed new therapeutic inroads.

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To address this need we created a genetically engineered mouse model of IHCC with somatic activation of $KRAS^{G12D}$ and deletion of TP53, two of the common mutations found in this cancer, targeted to the liver. Compound mutant animals rapidly develop primary IHCC that metastasizes to the lymph nodes and lungs. This engineered model not only recapitulates the histopathological characteristics of human IHCC, but also suggests that the tumors originate from a collection of precursor lesions called Von Meyenburg Complexes or biliary hamartomas and intraductal papillary biliary neoplasms (IPBN) which have long been observed in human, but have not been fully linked to advanced cancer. Tumors in our mouse model have an IHC profile consistent with a biliary origin, demonstrate activation of MAPK and PtdIns3K pathways, and incur spontaneous loss of the CDKN2A/p16Ink4a tumor suppressor, all features of the human disease.

Cell lines from these tumors offer an experimental system to test the importance of pathways and processes thought to be relevant to IHCC. Cancers harboring activating mutations in the RAS oncogene, such as lung and pancreatic cancer, have recently been characterized as having high levels of autophagy. Furthermore, inhibition of autophagy in these RAS mutant cancer cell lines by knockout of autophagy essential genes and pharmacological agents such as chloroquine result in decreased tumor formation and cell survival, providing a possible new treatment approach. Therefore, we evaluated whether autophagy may also have a role in our IHCC model with constitutively activated KRAS. In nutrient-rich conditions, cell lines derived from KRAS-TP53 IHCCs have elevated microtubule-associated protein 1 light chain 3, LC3, a marker of autophagy, compared with normal and KRAS-TP53 mutant liver. Visualization of GFP-LC3 autophagic puncta and chloroquine inhibition of autophagic flux confirmed this biochemical observation. Furthermore, chloroquine treatment inhibits the growth of four separate cell lines from both well and poorly differentiated IHCC. As autophagy is actively engaged in the IHCC cell lines derived from our KRAS mutant model, and chloroquine treatment suppresses their growth, inhibition of this metabolic pathway may provide a new therapeutic option in the treatment of IHCC.

How autophagy contributes to RAS-driven tumor formation and growth, when autophagy becomes critical to tumor progression, and what signaling pathways lie between activated KRAS and the molecular activation remain key questions both in IHCC and in other RAS-driven cancers. Future experimentation assessing levels of

autophagy in different stages of precursor lesions should elucidate at which stage the reliance on autophagy may be altered and at what point in tumor development may inhibition of autophagy best suppress tumor growth. Our model of IHCC provides the tools for these types of studies.

The clinical relevance of these findings and the breadth of any potential use of autophagy inhibitors in patients depend on a few critical and as yet untested questions. First, is autophagy a feature of all IHCC, which are genetically diverse and harbor a range of oncogenic mutations, or just those with RAS mutations? Second, to what extent do other cooperating mutations—such as loss of the TP53 tumor suppressor—influence the tumor's dependence on autophagy? It is possible that the high level of autophagy we observe in IHCC cell lines could result from the combination of somatic deletion of *TP53* with KRAS activation. In colon cancer models, the loss of TP53 and mutation in RAS cause synergistic alterations in a large number of genes in downstream networks representing a host of cellular processes including many metabolic functions. The loss of TP53 by itself increases cytosolic chromatin-binding protein high mobility group box 1 (HMGB1), which leads to increased autophagy in some contexts. Therefore, we speculate that mechanisms controlled by RAS activation and TP53 mutation likely cooperate to culminate in the deregulation of autophagy, including IHCC. The identification of genetically defined subsets of tumors with high levels of autophagy would enable selections of patients most likely to benefit from anti-autophagy therapies for clinical testing. Clinically, inhibition of autophagy has been achieved through the use of chloroquine and hydroxychloroquine, which are used to treat malaria and autoimmune diseases such as lupus. These drugs are currently being tested in clinical trials across a range of tumor types with and without traditional chemotherapy.

Finally, it would be ideal to identify positive regulators of autophagy specific to cancers that could be targeted therapeutically as opposed to generally inhibiting the process. As autophagy is essential to many normal physiological functions ranging from immune response to neuronal health, the consequences of widespread inhibition of autophagy could be significant. Pinpointing the connections between the underlying genetics of IHCC and activation of autophagy could expose a much needed treatment opportunity.