Targeting copper metabolism to defeat KRAS-driven colorectal cancer

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\textbf{ABSTRACT}

KRAS-driven cancers acquire profound metabolic dependencies that are intimately linked to tumor growth. Our work revealed that colorectal cancers that harbor KRAS mutations are addicted to copper metabolism. This adaptation renders tumor cells critically dependent on the copper transporter ATP7A, which reveals copper metabolism as a promising therapeutic target for KRAS-driven colorectal cancers.

Activating mutations in KRAS (Kirsten rat sarcoma 2 viral oncogene homolog) contribute to nearly half of all human colorectal cancers (CRC), and are commonly associated with resistance to first-line therapies. Consequently, almost four decades of intensive research have focused on the development of monotherapeutic approaches to directly or indirectly target oncogenic KRAS.\textsuperscript{1} Though early clinical studies have shown some promise, including the recent evaluation of KRAS\textsubscript{G12C} specific inhibitors, the emergence of resistance mechanisms has severely limited clinical outcomes. While several different combination therapies are currently being explored to counter drug resistance, the identification of novel vulnerabilities for KRAS-dependent cancers remains important for the design of tailored therapeutic approaches.\textsuperscript{2}

Metabolic adaptation in cancer is widely accepted as a critical aspect of tumor growth.\textsuperscript{3} In CRC, mutant KRAS actively rewires tumor cell metabolism to fuel the inexorable biosynthetic demand associated with aberrant cell growth and proliferation.\textsuperscript{4} Establishment of this new metabolic program is accompanied by a unique set of cellular features, including the ability to acquire and utilize essential nutrients required to maintain cancer cell fitness. Evidence suggest that cancer cell addiction to nutrient supply can be exploited for the design of efficient therapeutic approaches. In particular, KRAS-mutant cancer cells were shown to require different modes of nutrient uptake, such as macropinocytosis, which is increasingly recognized as a potential therapeutic target.\textsuperscript{5, 6} In recent years, tremendous efforts have helped shed light on the implication of mutant KRAS in nutrient uptake and metabolism, such as with glucose, lipid and amino acids. In contrast, much less is known regarding the role of mutant KRAS on the uptake and metabolism of micronutrients, such as trace minerals and vitamins.

Using a proteogenomic approach to study mutant KRAS-mediated transformation of intestinal epithelial cells, we have identified the copper-transporter ATP7A (Copper-transporting ATPase 1) as a potent synthetic lethal target for KRAS-mutant CRC.\textsuperscript{7} Combining cutting-edge cell-surface proteomics with CRISPR/Cas9 screens, we found that ATP7A is selectively elevated at the surface of KRAS\textsubscript{G12V}-transformed intestinal cells and plays essential roles in tumor growth in vitro and in vivo. Intriguingly, we found that ATP7A upregulation occurred in a transcription-independent manner that involved copper-dependent protein stabilization and intracellular transport, as described elsewhere.\textsuperscript{8} As these results suggested that KRAS-mutant cells have higher intracellular copper levels, we set out to measure basal copper levels in CRC cells and patient-derived colorectal tumor specimens. Using several methods, including the anti-correlated expression of the copper chaperone for superoxide dismutase (CCS), as well as inductively coupled plasma-mass spectrometry (ICP-MS), we found that KRAS mutation correlated with elevated intracellular copper levels. Using ratiometric fluorescent probes to monitor labile pools of copper,\textsuperscript{9} which contribute to metabolism, we found that KRAS mutation was linked to increased levels of bioavailable copper.\textsuperscript{7} Consistent with this, we found that ATP7A participates in the regulation of several copper-enzymes, including Cytochrome C Oxidase (CCO) and the pathway leading to ERK1/2 (extracellular signal-regulated kinases 1/2) activation, which helps explain its observed essentiality in KRAS-mutated cells.\textsuperscript{7}

Our results have shown that KRAS-mutated cells are more sensitive to copper chelators, such as ammonium tetrathiomolybdate (TTM), than wild-type counterparts, suggesting that copper metabolism may be a potential therapeutic target for KRAS-mutated CRC. Anti-Cu agents, such as TTM, choline tetrathiomolybdate (ATN-224), trientine, and D-penicillamine were previously shown to inhibit cancer cell growth in preclinical studies.\textsuperscript{10} A limited number of clinical trials have attempted to evaluate the effect of TTM on tumor growth, and found that the drug was generally well tolerated with manageable toxicity profiles. Notably, TTM treatment sensitized melanoma cells that are resistant to BRAF (Serine/threonine-protein kinase B-raf) inhibitors, suggesting that TTM could be repurposed to counter some forms of drug resistance.
As our data suggest that KRAS-mutant CRC are addicted to copper metabolism, an interesting possibility would be to determine the efficacy of anti-Cu agents in the context of KRAS-mutated cancers.

To determine the mechanism by which oncogenic KRAS increases intracellular copper levels, we investigated the involvement of the high-affinity copper-importer CTR1 (High affinity copper uptake protein 1). Our results have shown that KRAS-mutant cells express reduced levels of CTR1 compared to control cells, and that its expression is dispensable for KRAS-dependent tumor growth. Given that CTR1-independent routes for copper uptake are not well described in humans, we evaluated the contribution of macropinocytosis, which is upregulated in KRAS-mutated cells and previously shown to participate in nutrient uptake. Using the macropinocytosis inhibitor 5-(N-ethyl-N-isopropyl) amiloride (EIPA), we found that macropinocytosis regulates intracellular copper levels and is required for KRAS-mutated CRC growth. While macropinocytosis may not be the exclusive mode of copper uptake, our results indicate that it is required to fuel copper metabolism in KRAS-mutated CRC cells.

In summary, our findings suggest new ways to target KRAS-addicted CRC (see Figure 1). While copper metabolism could be targeted using anti-Cu drugs or ATP7A inhibitors, our results show that targeting copper entry via macropinocytosis could also be an efficient way to specifically affect KRAS-mutated cancer cells.

Disclosure of potential conflicts of interest
The authors declare no conflict of interest.

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