PANCREATIC CANCER

Eliminating protective autophagy in *KRAS*-mutant cancers

95% of pancreatic ductal adenocarcinomas (PDACs), which are responsible for a large number of cancer-related deaths, are driven by oncogenic *KRAS*, an as-yet pharmacologically intractable oncogene. Two studies recently published in *Nature Medicine* show that in the presence of KRAS pathway inhibition, these tumours become dependent on autophagy for survival, and that removing this protective mechanism through the inhibition of MEK or ERK kinases combined with inhibitors of autophagy is likely to be therapeutically beneficial in patients with PDAC.

The autophagy inhibitor hydroxychloroquine is in clinical trials for PDAC, as basal levels of autophagy are upregulated in *KRAS*-mutant tumours, but has so far shown limited benefit as a monotherapy. In hopes of developing better therapeutic strategies, Bryant et al. examined the mechanisms of autophagy in a panel of *KRAS*-mutant human PDAC cells. Surprisingly, suppression of KRAS using short hairpin RNA or the KRAS-G12C inhibitor ARS-1620 further increased rather than decreased levels of autophagic flux. They observed similar results in cells derived from an inducible $Kras^{G12D}$-driven mouse model of PDAC. Autophagic signalling was increased in cells with suppressed oncogenic *KRAS* (but not in PDAC cells with suppressed wild-type *KRAS*),

> “inhibition of autophagy and either MEK or ERK inhibition [had] a synergistic effect in *KRAS*-mutant PDAC”

as was transcription of autophagy-associated genes. These results were phenocopied by treating cells with the ERK1 and ERK2 selective inhibitor SCH772984, indicating that the increased autophagic flux requires the RAF–MEK–ERK effector pathway downstream of KRAS.

Similar observations were reported by Kinsey et al. Autophagic flux increased in three PDAC cell lines expressing either oncogenic *KRAS* or *BRAF* following treatment with either SCH772984, a different KRAS-G12C inhibitor (ARS-853) or the MEK1 and MEK2 inhibitors cobimetinib or trametinib.

Bryant et al. further examined the metabolic effects of oncogenic KRAS suppression or ERK inhibition in KRAS-mutant PDAC cells. In addition to the upregulation of genes supporting autophagy, transcription of genes supporting glycolysis decreased. Cells with suppressed KRAS or ERK also had reduced glucose uptake and decreased levels of glycolytic intermediates. Because withdrawal of glucose from the media of *KRAS*-mutant PDAC cells increased autophagic flux, the authors proposed that cells with oncogenic KRAS signalling have increased glycolysis, and when this pathway is suppressed, cells respond by increasing autophagy.

The transcription of mitochondrial biogenesis genes was also reduced in response to oncogenic KRAS suppression or ERK inhibition in KRAS-mutant PDAC cells. In addition, mitochondrial fusion increased; although this increased mitochondrial potential as expected, it surprisingly did not increase oxygen consumption or ATP production, suggesting that increased mitochondrial potential in these cells is not used to produce energy. Further studies will need to decipher the nature of the relationship between mitochondrial activity and morphology in these cells.

Both studies presented data supporting the hypothesis that inhibition of autophagy and either MEK or ERK inhibition would have a synergistic effect in *KRAS*-mutant PDAC. Both groups found that treatment of *KRAS*-mutant PDAC cells in vitro with the non-specific autophagy inhibitor choloroquine plus either trametinib (Kinsey et al.), SCH772984, a distinct ERK inhibitor, or the MEK inhibitor binimetinib (Bryant et al.) significantly increased cell death. Specific inhibition of autophagy similarly synergized with MEK–ERK inhibition, suggesting that the synergistic effects of chloroquine are largely due to suppression of autophagy. Synergy and reduced tumour growth with combination treatment compared to MEK–ERK inhibition or chloroquine alone were also observed in several *KRAS*-mutant xenograft and patient-derived xenograft (PDX) mouse models of PDAC. In addition, Kinsey et al. found that treatment of two PDAC PDX models with chloroquine plus trametinib was superior to the PDAC standard of care chemotherapy regimen of gemcitabine plus nab-paclitaxel.

Kinsey et al. extended these findings to other tumour types, showing that PDX models of *NRAS*-driven melanoma and *BRAF*-mutant colorectal cancer were also sensitive to chloroquine plus trametinib treatment.

Finally, Kinsey et al. initiated off-label treatment of a patient with metastatic PDAC with trametinib and hydroxychloroquine (both of which are approved by the US FDA for other indications). They observed a partial response in this patient, noting a 50% reduction in tumour burden. Although this case study is intriguing, suitable clinical trials will need to be conducted to determine the benefits of this therapy for patients with PDAC as well as for patients with other activating mutations in the RAS–RAF–MEK–ERK pathway.

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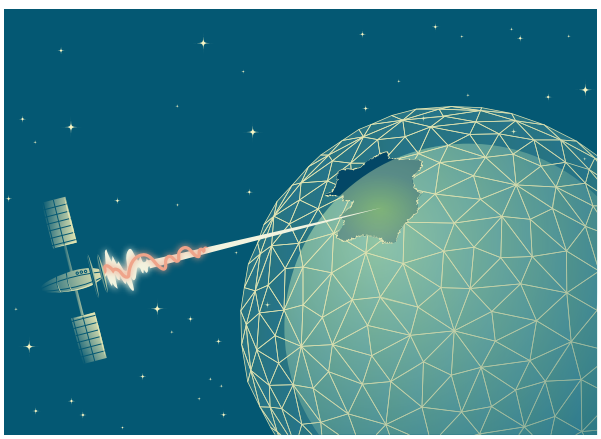

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ORIGINAL ARTICLES Bryant, K. L. et al. Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer. *Nat. Med.* https://doi.org/10.1038/s41591-019-0368-8 (2019) | Kinsey, C. G. et al. Protective autophagy elicited by RAF → MEK → ERK inhibition suggests a treatment strategy for RAS-driven cancers. *Nat. Med.* https://doi.org/10.1038/s41591-019-0367-9 (2019)