THERAPEUTICS

Dependent on KRAS

Although activating *KRAS* mutations occur frequently in cancer, targeting this oncogene has proved difficult. Three groups have now identified pathways that have not been previously linked to *KRAS* — all of which may provide pharmacologically tractable anticancer targets — and that are crucial in cancer cells dependent on activated *KRAS*.

Steve Elledge and colleagues conducted a genome-wide RNA interference (RNAi) screen in an isogenic pair of DLD-1 colorectal cancer cell lines: one that carried an endogenous activated *KRAS* allele (*KRAS*^{WT/G13D} cells) and one in which this allele was disrupted (*KRAS*^{WT/-} cells). By finding short hairpin RNAs (shRNAs) that were selectively depleted over time in *KRAS*^{WT/G13D} cells compared with *KRAS*^{WT/-} cells, and refining their initial list using a second isogenic pair of HCT116 colorectal cancer cells, they identified a functionally diverse list of 50 genes that might have synthetic lethal interactions with activated *KRAS*. This list included many genes involved in mitotic regulation, such as Polo-like kinase 1 (*PLK1*) and genes that encode anaphase-promoting complex/cyclosome (APC/C) subunits. Cells expressing *KRAS*^{G13D} were sensitive to *PLK1* inhibition *in vitro* and in mouse xenograft models. The cells were also sensitive to inhibition of the ubiquitin ligase APC/C *in vitro*. As APC/C ultimately requires proteasome activity, the authors showed that the proteasome inhibitors MG132 and bortezomib were synthetic lethal with activated *KRAS*. Furthermore, non-small-cell lung cancer (NSCLC) cell lines were more sensitive to APC/C subunit knockdown if they harboured *Ras* mutations. Is this pathway relevant *in vivo*? Analysis of *Ras*-associated gene

signatures in human lung tumour samples indicated that those tumours with a positive *Ras* signature that also had a signature suggestive of decreased APC/C activity correlated with increased patient survival.

Gary Gilliland, Bill Hahn and colleagues performed a similar RNAi screen across eight cancer cell lines (four that expressed a *KRAS* mutant and four that were *KRAS* wild type) and found that shRNAs targeting the kinase *STK33* impaired cell viability and proliferation only in cells that were dependent on mutant *KRAS*. This was confirmed in several other cell types (25 in total), including both haematopoietic and epithelial cancer cell lines, and *STK33* shRNA reduced tumour formation by 4 different *KRAS*-dependent epithelial cancer cell lines in immunocompromised mice. Interestingly, DLD-1 cells, as used in the studies above, were also sensitive to *STK33* knockdown. Forcing *KRAS* dependence by expressing *KRAS*^{G13D} in two *KRAS*-independent acute myeloid leukaemia cell lines also led to sensitivity to *STK33*. What does *STK33* do? The authors examined the activation of several signalling pathways in cells with *STK33* suppression. Loss of *STK33* decreased phosphorylation and activity of the S6K1 kinase and downstream phosphorylation of the proapoptotic BH3-only protein *BAD*, leading to apoptosis.

Jeff Settleman and colleagues examined the effect of *KRAS* depletion in several lung and pancreatic cancer cell lines. By comparing *KRAS*-dependent cells (in which *KRAS* knockdown induced apoptosis) with *KRAS*-independent cells, they noted that *KRAS*-dependent cells tended to have an epithelial morphology, whereas *KRAS*-independent cells appeared to be

more mesenchymal. Induction of an epithelial–mesenchymal transition (EMT) in *KRAS*-dependent H358 NSCLC cells allowed these cells to become *KRAS* independent, and conversely, reversal of EMT in *KRAS*-independent cells rendered these cells *KRAS* dependent. The authors then derived a gene expression signature of *KRAS* dependency, which was also associated with well-differentiated primary lung tumours. From this signature, they found three pharmacologically tractable targets that were upregulated in *KRAS*-dependent cells: the kinase *SYK*, the RON receptor tyrosine kinase (encoded by *MST1R*) and $\beta 6$ integrin (encoded by *ITGB6*). shRNAs against *SYK* and *ITGB6* led to EMT and apoptosis in two *KRAS*-dependent cell lines, but not two *KRAS*-independent cell lines, whereas *MST1R* knockdown was context specific, inducing apoptosis in only one *KRAS*-dependent cell line. *KRAS*-dependent cells were also more sensitive to a pharmacological *SYK* inhibitor.

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ORIGINAL RESEARCH PAPERS Luo, J. et al. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the *Ras* oncogene. *Cell* **137**, 835–848 (2009) | Scholl, C. et al. Synthetic lethal interaction between oncogenic *KRAS* dependency and *STK33* suppression in human cancer cells. *Cell* **137**, 821–833 (2009) | Singh, A. et al. A gene expression signature associated with “K-Ras addiction” reveals regulators of EMT and tumour cell survival. *Cancer Cell* **15**, 489–500 (2009)