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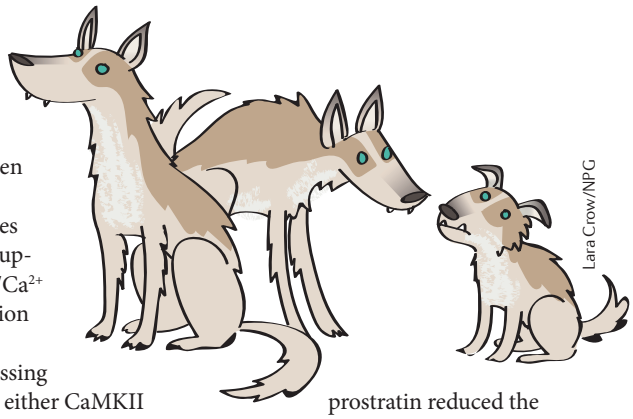
ONCOGENES

One of these things is not like the others



Lara Crow/NPG

The RAS isoforms — KRAS, HRAS and NRAS — are known to have similar biological functions; however there have been hints that KRAS might have a unique role in some tumour types. Wang *et al.* report that oncogenic KRAS, but not HRAS or NRAS, promotes self-renewal properties and tumour initiation by inhibiting non-canonical WNT/Ca^{2+} signalling.

Although HRAS-V12 and KRAS-V12 confer nearly identical phenotypes to NIH-3T3 cells, the authors noted that KRAS-V12-transformed cells had increased self-renewal and tumour-initiating potential, as assessed using sphere-forming assays and limited and serial transplantation into mice. In addition, knockdown of KRAS-V12 in human pancreatic cancer cell lines (PANC2.13 and PANC1) also reduced sphere formation and tumour initiation.

Profiling of stem cell-related gene expression in NIH-3T3 cells expressing KRAS-V12 or HRAS-V12 indicated that KRAS-V12 differentially downregulated Frizzled 8 (*Fzd8*), which usually functions to activate non-canonical WNT/Ca^{2+} signalling and repress β-catenin–TCF through activation of calmodulin-dependent kinase II (CaMKII). Consequently, β-catenin target genes were upregulated in cells expressing KRAS-V12 only. In mice, expression of wild-type KRAS or HRAS from the endogenous *Kras* locus followed by DMBA–TPA treatment to induce RAS mutations and skin tumours demonstrated that only mutant KRAS reduced FZD8 and CaMKII activation. Furthermore, knockdown of KRAS-V12 in PANC2.13 cells increased WNT/Ca^{2+} signalling.

The authors then established that stemness properties were induced by suppression of WNT/Ca^{2+} signalling. Inhibition of this pathway in HRAS-V12-expressing NIH-3T3 cells via either CaMKII inhibition or FZD8 knockdown enhanced sphere formation, and tumour initiation increased after subcutaneous injection of cells with FZD8 knockdown. Overexpression of FZD8 in KRAS-V12-expressing NIH-3T3 or PANC2.13 cells reactivated WNT/Ca^{2+} signalling and prevented the formation of xenograft tumours in mice. Consistent with a role for FZD8 as a tumour suppressor, the authors showed that its expression is frequently lost in human pancreatic adenocarcinoma relative to normal pancreatic tissue.

Interaction with calmodulin (CaM) is required for CaMKII activation. KRAS-V12, but not HRAS-V12 or NRAS-V12, was able to bind to CaM, thus reducing the amount of CaM available in the cell to activate CaMKII. Disruption of KRAS-V12–CaMKII by introducing a phosphomimetic mutation on S181 of KRAS-V12 in NIH-3T3 cells increased *Fzd8* and reduced β-catenin transcriptional activity; this effect was phenocopied in PANC2.13 and PANC1 cells by treatment with the atypical protein kinase C (PKC) activator prostratin. *In vivo*, prostratin reduced the initiation of PANC2.13 and PANC1 cell-derived subcutaneous and PANC2.13 orthotopic xenograft tumours. It also reduced the growth of established subcutaneous PANC2.13 and PANC1 cell-derived tumours, and lowered levels of human circulating cell-free DNA in mice bearing orthotopic PANC2.13 tumours, suggesting a reduction in tumour burden. Prostratin also prevented tumour initiation in non-immunocompromised mice with skin papillomas driven by oncogenic KRAS-D12 but not those driven by HRAS-V12.

Overall, these data indicate that the consequences of KRAS activation differ from those of HRAS and NRAS activation. This difference is crucial for tumour induction and maintenance by oncogenic KRAS, suggesting that KRAS-driven tumours might be specifically targeted by restoration of non-canonical WNT/Ca^{2+} signalling.

Sarah Seton-Rogers

> “the consequences of KRAS activation differ from those of HRAS and NRAS activation”

ORIGINAL ARTICLE Wang, M. T. *et al.* K-Ras promotes tumorigenicity through suppression of non-canonical Wnt signaling. *Cell* **163**, 1237–1251 (2015)