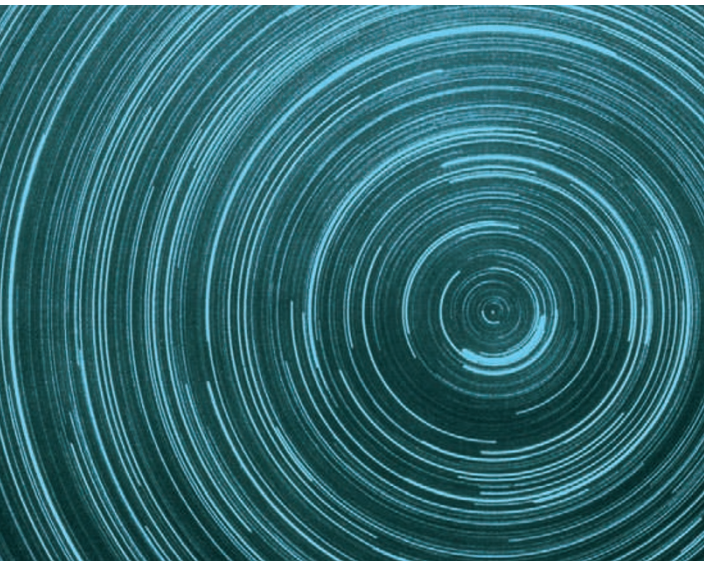
MICRORNA

Micro-loops in NF-κB signalling

> miR-301A downregulates NKRF expression, which in turn relieves the suppression of NF-κB activity



Nuclear factor-κB (NF-κB) signalling is constitutively activated in pancreatic adenocarcinoma, but genetic activation of members of the NF-κB pathway does not appear to be the cause. So, Yong Li and colleagues investigated what might activate this pathway in pancreatic adenocarcinoma.

MicroRNAs (miRNAs) have been shown to be effectors that function downstream of NF-κB activation, so Lu, Li and colleagues screened a library of miRNAs for those that modulate NF-κB activation. The most potent activator of NF-κB signalling was miR-301A, which upregulated NF-κB activity by fivefold. miR-301A was not predicted to bind any transcripts encoding subunits of the NF-κB transcription factor complex, but NF-κB repressing factor (*NKRF*), which encodes an inhibitor of NF-κB, was shown to be a target of miR-301A. NKRF protein and mRNA expression was reduced by ~threefold when miR-301A was exogenously overexpressed in 293T cells, and this upregulated NF-κB activity by ~2.5-fold. Knock down of miR-301A by antisense oligonucleotides in pancreatic cancer cell lines upregulated NKRF expression and downregulated the expression of five NF-κB target genes. One of these five genes, spindle and kinetochore associated complex subunit 2 (*SKA2*), encodes *miR-301A* in its first intron. The authors identified a functional NF-κB (specifically NFKB1–RELA) binding site upstream of *SKA2*, suggesting that *miR-301A* is also a target of activated NF-κB. Indeed, knock down of RELA and NFKB1 downregulated miR-301A expression. These data identify a feedforward loop in which miR-301A downregulates NKRF expression, which in turn relieves the suppression of NF-κB activity and activates target genes, which includes *miR-301A*. Moreover, they found that NKRF expression was reduced in pancreatic adenocarcinoma tissues compared with normal pancreas and tumour-adjacent tissue, and this correlated with miR-301A overexpression and NF-κB activation.

So, does miR-301A expression affect tumour growth? Stable knock down of miR-301A in PANC-1 cells significantly reduced RELA expression levels, tumour cell proliferation and the volume of the xenograft, and increased NKRF expression. There was also evidence of reduced blood vessel formation, suggesting that downregulation of the NF-κB target gene vascular endothelial growth factor C could be important for reduced xenograft growth. Finally, xenografts of PANC-1 cells in which *NKRF* transcripts could not be bound by miR-301A were significantly smaller than xenografts of tumour cells expressing wild-type *NKRF*. Similarly, knock down of *NKRF* in PANC-1 cells in which miR-301A was stably suppressed reversed the reduction in tumour growth. This suggests that the loss of miR-301A-mediated suppression of NKRF causes the reduced tumour growth observed.

Although it is likely that miR-301A has additional targets, Lu, Li and colleagues have identified an important feedforward loop that causes and maintains the activation of NF-κB in pancreatic cancer and could therefore be an effective therapeutic target.

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ORIGINAL RESEARCH PAPER Lu, Z. *et al.* miR-301a as an NF-κB activator in pancreatic cancer cells. *EMBO J.* 26 Nov 2010 (doi:10.1038/emboj.2010.296)