Uncapping NF- κ B activity in pancreatic cancer

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Living cells must be capable of rapidly responding to intrinsic and environmental cues to ensure normal development and homeostasis. The NF- κ B transcription factor mediates a series of such rapid-response programs, serving as a master regulator of gene transcription in cells subjected to inflammation, irradiation, or other stress states (Karin, 2009). While cells must be able to rapidly respond to NF- κ B stimuli, it is similarly important to restrain sustained high NF- κ B levels that can trigger chronic inflammation or cellular transformation. Results published by Lu *et al* (2011) in this issue of *The EMBO Journal* identify a novel mechanism that amplifies NF- κ B activity; NF- κ B upregulates mir301a, a microRNA (miRNA) that itself blocks the expression of an NF- κ B inhibitor. The resulting positive feedback mechanism potentiates NF- κ B target gene expression. While this pathway may be important for normal cellular stress responses, the authors show that its sustained induction in pancreatic cancer cells leads to constitutive NF- κ B activity that drives tumour progression.

NF- κ B is largely regulated at the level of protein stability via a well-characterized kinase cascade that controls the

targeting of this transcription factor to the proteasome (Karin, 2009). However, acute adaption to cellular stresses requires additional inputs that ensure rapid and potent NF- κ B target gene regulation. Conversely, tight controls are needed to restrain the dangerous pro-inflammatory and transforming functions of elevated NF- κ B signalling. Positive and negative feedback signalling networks are recurring themes in biology to achieve such regulation. Recent studies identified a major role of miRNAs as components of feedback signalling networks, as emphasized for NF- κ B by Lu *et al* (2011).

The authors sought to identify miRNAs that target the NF- κ B pathway, with a particular focus on identifying candidates that activate NF- κ B-mediated transcription. miRNAs (or miRs) are an evolutionarily conserved class of non-coding double-stranded RNA molecules that suppress expression of their target genes by imperfect binding to the 3'UTR of target mRNA, leading to either blocked translation or degradation of the message (Garzon *et al*, 2010). Using an miRNA library screen based on a luciferase reporter, they identify miR301a as a striking activator of NF- κ B. *In silico* approaches predict numerous putative targets of miR301a, including the known NF- κ B-regulator Nkrf. Nkrf represses NF- κ B transcriptional

Figure 1 Schematic representing the NF- κ B-miR301a signalling circuit. NF- κ B coordinately upregulates the FAM33a gene as well as miR301a, which is located within the first intron of FAM33a. miR301a targets the Nkrf mRNA transcript, which encodes a repressor of NF- κ B transcriptional activity. Hence, NF- κ B-mediated induction of miR301a creates a positive feedback loop driving increasing levels of NF- κ B transcriptional regulation.

activity through multiple mechanisms, including blocking its access to DNA and modulating its stability (Feng *et al*, 2002). In an elegant series of experiments including inhibition of miR301a function and mutation of the 3'UTR miR301a binding site, the authors show that Nkrf is a *bona fide* target miR301a.

miRNAs are often located within the introns or UTRs of other genes (Garzon *et al*, 2010). In these cases, the miRNA expression follows cues that govern expression of the parent gene. Notably, miR301a is located within intron 1 of the Fam33A gene, which is an established NF- κ B target. Correspondingly, the authors demonstrate that miR301a is itself an NF- κ B target gene. Hence, activation of NF- κ B signalling creates a positive feedback loop through induction of miR301a and consequent inactivation of the Nkrf repressor (Figure 1).

miR301a was shown in an earlier study to be highly elevated in pancreatic cancer, a tumour type in which NF- κ B is constitutively active and appears to be required for tumour maintenance (Wong and Lemoine, 2009). The authors extend these findings to show that miR301 overexpression is associated with loss of Nkrf in pancreatic cell lines. Conversely, inhibition of miR301a function results in increased expression of Nkrf and decrease in NF- κ B target genes. Most importantly, miR301a inactivation impairs growth of pancreatic cancer xenografts, indicating that miR301 has a key oncogenic role.

This study adds to the emerging literature regarding important functions of miRNAs in cancer pathogenesis. A number of miRNAs localize at 'hotspots' of chromosomal deletions in cancer; moreover, expression profiling studies have consistently shown that miRNAs are broadly down-regulated in cancer tissues (Garzon *et al*, 2010). Mouse knockout studies have validated specific miRNA, such as miR17-92 and miR106b-25, as tumour suppressors (Ventura *et al*, 2008). More broadly, disruption of miRNA processing by deletion of the pre-miRNA processing nuclease dicer leads to enhanced tumour formation, suggesting that miRNAs comprise a general tumour suppressor mechanism (Kumar *et al*, 2009). On the other hand, specific miRNAs have been shown to be upregulated in cancers and to contribute to oncogenic transformation. For example, data from cell lines

and mouse models have established miR21 as a potent regulator of the initiation and maintenance of lung cancer (Medina *et al*, 2010). There is considerable ongoing investigation aimed at the systematic characterization of miRNA expression in cancer and the use of miRNA signatures to predict patient prognosis. In light of this emerging clinical and genetic data, a key current challenge is to elucidate the mechanisms by which miRNA expression is deregulated and to identify the relevant target pathways.

NF- κ B deregulation can have devastating consequences in promoting cancer and other disease states (Karin, 2009). The current study by Lu *et al* (2011) provides insight into how NF- κ B signalling can go awry through an miR301a-mediated circuit, while also raising important questions about the normal physiological role of this circuit, and about how it relates to other modes of NF- κ B regulation. In this regard, NF- κ B pathway is governed by negative feedback loops to keep the pathway in check. One of the primary targets of NF- κ B transcriptional activity is its repressor I κ B; I κ B binds and prevents nuclear translocation of the p65 subunit of NF- κ B, thereby serving to restrain NF- κ B activity. So the question arises as to how this intrinsic negative feedback mechanism is affected by the miR301a circuit. In addition, it remains to be determined whether upregulation of miR301a is important for the normal functions of NF- κ B, such as in the regulation of inflammation or innate immunity. NF- κ B is a pro-survival pathway activated in a number of cancers and is a specific effector of activated Kras in lung cancers (Barbie *et al*, 2009; Meylan *et al*, 2009). Does miR301a have a general function in Kras mutant tumours in addition to pancreatic cancer, such as lung and colorectal cancers? Finally, recent work from Iliopoulos and Struhl implicate NF- κ B-mediated induction of miRNAs as a key early event during transformation of multiple cell types (Iliopoulos *et al*, 2009); hence it will be interesting to determine whether miR301a signalling acts in an even broader manner to promote tumourigenesis.

Conflict of interest

The authors declare that they have no conflict of interest.

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