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EPIGENETICS

Layer by layer

The view that cancer is purely a genetic disease has taken a battering over recent years, perhaps most extensively from the recent discovery that between transcription and translation sits a whole host of regulatory RNAs, chiefly in the guise of microRNAs (miRNAs). Now, we can add yet another layer of regulation: the evidence from three papers that protein-coding and non-coding RNAs influence the interaction of miRNAs with their target RNAs.

Pier Paolo Pandolfi and colleagues had previously suggested that the miRNA response element (MRE) in the 3′ untranslated region (UTR) of RNAs could be used to decipher a network of RNAs that are bound by a common set of miRNAs. RNAs within this network would function as competing endogenous RNAs (ceRNAs) that can regulate one another by competing for specific miRNAs. Using an integrated computer analysis and an experimental validation process that they termed mutually targeted MRE enrichment (MuTaME), Tay *et al.*, identified a set of *PTEN* ceRNAs in prostate cancer and glioblastoma samples. As predicted, some of these ceRNAs are regulated by the same set of miRNAs that regulate *PTEN* and have similar expression profiles to PTEN. For example, knockdown of the ceRNAs *VAPA* or *CNOT6L* using small interfering RNAs (siRNAs) resulted in reduced expression levels of *PTEN* and conversely, expression of the ceRNA 3′ UTRs to which the miRNAs bind resulted in an increase in expression of *PTEN* 3′ UTR–luciferase constructs. Importantly, the link between *PTEN*, *VAPA* and *CNOT6L* was lost in cells that had defective miRNA processing, indicating that miRNAs are crucial for these effects.

Pavel Sumazin, Xuerui Yang, Hua-Sheng Chiu, Andrea Califano and colleagues investigated the mRNA and miRNA network in glioblastoma cells using data from The Cancer Genome Atlas and a new multivariate analysis method called Hermes. They found a surprisingly large post-translational regulatory network, involving some 7,000 RNAs that can function as miRNA sponges and 148 genes that affect miRNA–RNA interactions through non-sponge effects. In tumours that have an intact or heterozygously deleted *PTEN* locus, expression levels of the protein vary substantially, indicating that other modulators of expression are at work. Analysis of 13 genes that are frequently deleted in patients with glioma and that encode miRNA sponges that compete with *PTEN* in the RNA network showed that a change in their mRNA expression had a significant effect on the level of *PTEN* mRNA. Specifically, siRNA-mediated silencing of ten of the 13 genes reduced PTEN levels and substantially increased proliferation of glioblastoma cells. Conversely, expression of the *PTEN* 3′ UTR increased the expression of these 13 miRNA sponges.

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Many of the genes that have previously been implicated in gliomagenesis, including *RB1*, *RUNX1*, *PDGFRA*, *STAT3* and *VEGFA*, form a dense subnetwork of mutually interacting RNAs. This might explain why high-grade gliomas often have deletion of either *PTEN* or *RB1*. These genes have 31 miRNAs in common, and loss of either *PTEN* or *RB1* profoundly affects and is affected by the expression of the remaining mRNAs in this subnetwork. These authors also confirmed non-sponge miRNA-mediated effects of several genes on *PTEN* and *RUNX1*.

Florian Karreth, Pier Paolo Pandolfi and colleagues validated the significance of ceRNA regulation in tumour development through the use of the sleeping beauty transposon system in a mouse model of melanoma. Of the 33 candidate *PTEN* ceRNAs in melanoma that were identified, the authors chose one of these, *ZEB2*, which is involved in regulating epithelial to mesenchymal transition, to evaluate further. The insertion of transposons near *ZEB2* reduced ZEB2 and PTEN expression, and transposon insertion near *PTEN* reduced its expression and that of ZEB2. This mutual regulation is lost in DICER-deficient cells, indicating that *ZEB2* functions as a ceRNA for *PTEN* and vice versa. In melanoma cells, miR-181, miR-200b, miR-25 and miR-92a interact with *PTEN* and *ZEB2* 3′ UTRs, and knockdown of either *PTEN* or *ZEB2* increased the availability of these miRNAs. Moreover, siRNA against *ZEB2* decreased PTEN expression and increased the growth of melanoma xenografts in nude mice. This interaction was also evident in human melanoma, colon carcinoma and glioblastoma gene expression data sets.

These results indicate that reduced expression of a specific set of mRNAs can affect the expression of other RNAs that form part of an miRNA–mRNA network. Moreover, they hint at the subtlety of changes that could be occurring during tumorigenesis, in which a small reduction in the expression level of a few mRNAs could have wide-ranging effects.

Nicola McCarthy

ORIGINAL RESEARCH PAPERS Tay, Y. *et al.* Coding-independent regulation of the tumour suppressor PTEN by competing endogenous mRNAs. *Cell* **147**, 344–357 (2011) | Sumazin, P. *et al.* An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. *Cell* **147**, 370–381 (2011) | Karreth, F. *et al.* *In vivo* identification of tumour-suppressive PTEN ceRNAs in an oncogenic BRAF-induced mouse model of melanoma. *Cell* **147**, 382–395 (2011)

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ERRATUM

Regulatory RNA: Layer by layer

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In the above article, the name of an author was misspelt in the text and should have been Pavel Sumazin. This has been corrected online.