RNA SPLICING

Layered regulation

Alternative splicing and epigenetic modifications, including the exchange of canonical histones for histone variants, are now widely accepted to contribute to the dysregulation of gene expression in cancer. Links between these layers of regulation are less clear, but a new study has uncovered a pathway in which alternative splicing of histone variant isoforms regulates the transcription of genes involved in tumour cell invasion.

Using the 4T1 mouse model of mammary tumour progression (a set of cell lines with a range of metastatic abilities when implanted in syngeneic mice), Dardenne *et al.* first observed overexpression of the DEAD box RNA helicase DDX17 in cells that are more likely to give rise to metastases (4T1 cells) compared with non-metastatic 67NR cells. DDX17 and its paralogue DDX5 often have redundant functions, and knocking down expression of either or both with small interfering RNAs (siRNAs) inhibited *in vitro* migration and invasion of 4T1 cells. DDX17 and DDX5 are known to regulate alternative splicing, and the authors found that DDX17 and DDX5 regulated the exon abundance of many genes that encode chromatin or DNA-binding factors. Of these, they focused on the gene encoding the histone variant macroH2A1 (also known as H2AFY), which has two splice isoforms

(*macroH2A1.1* and *macroH2A1.2*), because it has previously been linked to cancer. Depletion of DDX17 and DDX5 increased *macroH2A1.1* and decreased *macroH2A1.2* levels without affecting overall *macroH2A1* levels in both 4T1 cells and human HeLa cells. Furthermore, reversion to the endogenous splicing pattern was induced by re-expression of DDX17 and DDX5, and this was dependent on their helicase activity.

How might *macroH2A1* splice isoforms affect migration and invasion? Levels of *macroH2A1.2* were higher in invasive cells, and this was required for migration and invasion. Conversely, *macroH2A1.1* levels were higher in non-invasive cells, and loss of this isoform increased migration and invasion. Looking downstream, the authors found that several redox metabolism genes, including superoxide dismutase 3 (*SOD3*), were differentially regulated in the presence of either *macroH2A1* isoform, and by DDX17 and DDX5 depletion. *SOD3* converts extracellular superoxide radicals into hydrogen peroxide and inhibits cell invasion (possibly by reducing oxidative degradation of extracellular matrix components).

Consistently, the authors showed that *SOD3* depletion in 67NR cells increased migration and invasion, and that adding exogenous *SOD3* inhibited it in 4T1 cells. *SOD3* expression was higher in cells with increased *macroH2A1.1* expression or with lower DDX17 and DDX5 expression. Chromatin immunoprecipitation experiments indicated that *macroH2A1.2* was incorporated in the *SOD3* locus, leading to *SOD3* repression.

Similar data were obtained using invasive MDA-MB-231 human breast cancer cells. Importantly, a low ratio of *macroH2A1.1* to *macroH2A1.2* expression was associated with shorter metastasis-free survival, and high *SOD3* expression was associated with longer metastasis-free survival, in a cohort of 443 patients with breast cancer, indicating that this pathway may be relevant in human breast tumours.

Sarah Seton-Rogers

“ alternative splicing of histone variant isoforms regulates ... tumour cell invasion

ORIGINAL RESEARCH PAPER Dardenne, E. *et al.* Splicing switch of an epigenetic regulator by RNA helicases promotes tumor-cell invasiveness. *Nature Struct. Mol. Biol.* 30 Sep 2012 (doi:10.1038/nsmb.2390)