A splicing mastermind for EMT

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Alternative splicing of mRNA precursors is a prevalent mode of gene regulation in multicellular organisms. Important open questions include whether most alternative splicing variants display distinct physiological functions and whether cell type-restricted regulatory factors orchestrate cell type-specific splicing decisions. In this issue of *The EMBO Journal*, Warzecha *et al.* (2010) uncover a network of alternative splicing changes contributing to epithelial–mesenchymal transition (EMT), which is controlled by downregulation of the Epithelial Splicing Regulatory Proteins 1 and 2 (ESRP1 and ESRP2).

The conversion of epithelial to mesenchymal cells, known as EMT, has important functions in cell differentiation and organogenesis. It also contributes to tissue repair and to disease, with alterations in EMT associated with tissue fibrosis, immunosuppression and carcinogenesis (Thiery *et al.*, 2009). EMT involves striking cellular changes, including loss of cell–cell adhesion and polarity and gain of migratory and even invasive properties. Numerous overlapping signaling pathways (e.g. Wnt and TGF- β) initiate, amplify and modulate EMT, inducing gene expression changes that bring

about the cell's phenotypic transitions (Thiery and Sleeman, 2006; Thiery *et al.*, 2009). Thus, activation of transcriptional regulators similar to Snail and transcriptional changes such as down-regulation of E-cadherin or up-regulation of Vimentin are classical landmarks of EMT. The picture emerging from the work of Warzecha *et al.* (2010) in this issue of *The EMBO Journal* and a previous report from the Carstens' group (Warzecha *et al.*, 2009) is that alternative splicing provides an additional layer of gene regulation that contributes to shape the EMT process.

Classical genetic work in *Drosophila* established the existence of sex-specific splicing regulators, with the female-specific Sex-lethal protein acting as a master regulator (Penalva and Sánchez, 2003). This triggered an interest in the identification of tissue-specific regulatory factors that could explain tissue-specific alternative splicing decisions. In contrast to transcriptional regulation, however, only a handful of tissue-restricted factors were found, leading to the idea that alternative splicing in higher eukaryotes is largely controlled by the relative activity of rather ubiquitous factors (Chen and Manley, 2009). Recent genetic screens in

Figure 1 ESRP proteins regulate epithelial-specific splicing. The scheme on the left represents splicing patterns influenced by ESRP proteins present specifically in epithelial cells. ESRP binding to GGU/UGG motifs 3' of regulated exons enhances exon inclusion, whereas ESRP proteins enhance exon skipping when its binding sites are located 5' of and/or within the regulated exon. The absence of ESRP proteins in mesenchymal cells allows the alternative patterns of splicing to occur, leading to gene expression changes that can contribute to EMT.

Caenorhabditis elegans and mammalian cells, however, suggest that the list of developmentally and tissue-restricted splicing factors is larger than previously thought (Ohno *et al.*, 2008; Warzecha *et al.*, 2009). Thus, ectopic expression of two related, epithelial cell-specific factors (ESRP1 and ESRP2) was found to induce the expression of epithelial-specific isoforms of fibroblast growth factor receptor 2 as well as of other genes (Warzecha *et al.*, 2009).

Could ESRP1 and ESRP2 act as master regulators of epithelial cell-specific splicing, as Sex lethal does for sex-specific splicing in *Drosophila*? To address this question, Warzecha *et al.* analysed splicing profiles derived from ectopic expression of ESRP1 in mesenchymal cells, as well as from knockdown of ESRP1/ESRP2 in epithelial cells using splicing-sensitive microarrays covering the majority of human alternative splicing events supported by mRNA and EST evidence, and validated microarray predictions by high-throughput RT-PCR analyses. About 100 alternative splicing events showed reciprocal changes in the two experimental situations and are, therefore, candidate targets of ESRP regulation during EMT. These are likely to be direct targets of ESRP proteins because UGG/GUU-rich sequence motifs enriched in the alternatively spliced regions are similar to known ESRP-responsive motifs and can be bound directly by ESRP proteins *in vitro*. Further sequence and experimental analyses generated a predictive 'RNA Map' in which binding of ESRP proteins within or 5' of an alternative exon leads to exon skipping, whereas binding to the downstream intron leads to exon inclusion (Figure 1), a result remarkably similar to RNA Maps generated for various other regulatory factors (Licatalosi *et al.*, 2008; Yeo *et al.*, 2009; Llorian *et al.*, 2010).

A second important question is whether ESRP1/2 coordinate a biologically coherent set of alternative splicing decisions contributing to important physiological processes such as EMT? Knockdown of ESRPs results in loss of characteristic morphological features of epithelial cells, concomitant

with increased motility and expression of invasive markers, suggestive of acquisition of mesenchymal phenotypes. Some expression changes characteristic of EMT (e.g. up-regulation of Vimentin) were also observed, whereas others (e.g. down-regulation of E-cadherin) were not. Collectively, these observations suggest that ESRP down-regulation contributes to loss of epithelial properties, although EMT requires additional changes in gene expression. Future experiments (e.g. conditional knockout in mice) could delineate the extent to which ESRPs contribute to EMT and its widespread physiological and pathological effects. Although it is not straightforward to predict how the alternative splicing events regulated by ESRPs contribute to physiological changes, ESRP targets include numerous genes representing functional categories relevant to EMT such as cell polarity and motility or cell-cell and cell-matrix adhesion. One interesting example includes components and regulators of the vesicular transport system, which strongly influence cell polarity and adhesion properties.

A significant contribution of the work of Warzecha *et al.* is a splicing signature of 10 exons characteristic of epithelial cells, as confirmed in a number of epithelial and mesenchymal cell lines. Such signature can be a valuable marker of EMT, and one of potential clinical use, as cancer cells undergoing EMT are known to display more aggressive tumour phenotypes (Thiery *et al.*, 2009). Evaluation of this splicing signature in a diverse set of carcinomas is, therefore, an important line for future studies, opening also the possibility that ESRPs can themselves be considered therapeutic targets. More generally, these considerations further argue that full understanding of physiological and pathological gene expression programmes requires a detailed analysis of alternative splicing.

Conflict of interest

The authors declare that they have no conflict of interest.

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