MicroRNAs — keeping cells in formation

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During epithelial–mesenchymal transition (EMT) cells loosen their intercellular contacts and leave the epithelial layer. Three microRNA (miRNA) families modulate EMT upstream of the key cell-adhesion protein E-cadherin, highlighting the potential importance of miRNAs in EMT-dependent processes, such as mesoderm development and tumour metastasis.

EMT is essential for animal development, for example during mesoderm development or neural plate formation in vertebrates[1]. In addition, EMT is thought to be important for wound healing and tumour metastasis. During EMT, downregulation of E-cadherin allows epithelial cells to undergo changes in cell morphology and motility so that they adopt mesenchymal characteristics. Expression of E-cadherin is under the control of several transcriptional repressors including Slug, Snail, Twist, E47, ZEB1 and ZEB2, which bind to E-boxes in their promoters[1,2].

Two recent reports, one by Gregory *et al.*[3] on page 593 of this issue and one by Park *et al.*[4] identify a new role for the miR-141, miR-200b and miR-205 families of miRNAs in EMT through the direct regulation of ZEB1 and ZEB2[3,4]. Belonging to a large class of short, non-coding RNAs found in many plants and animals, miRNAs often act post-transcriptionally to inhibit gene expression and are known to regulate several aspects of development and disease, including human cancer.

Gregory *et al.* set out to determine the miRNA expression profile of Madin Darby Canine Kidney (MDCK) epithelial cells before and after induction of EMT by either TGF-β treatment or ectopic expression of the protein tyrosine phosphatase Pez[3]. They found that either treatment downregulated all miR-141, miR-200b and miR-205 family members. Park *et al.* began their analysis by profiling miRNA expression of the NCI60 panel of human cell lines used for drug screening and observed that the miR-141 and miR-200b family of miRNAs were striking markers of the eleven epithelial cell lines in the collection[4].

Both groups identified the transcription factors ZEB1 and ZEB2 as direct targets of these miRNAs. Both ZEB1 and ZEB2 were attractive target candidates as they are known repressors of E-cadherin transcription and had been shown previously to be regulated by the miR-200b family of miRNAs[5,6]. However, Gregory *et al.* and Park *et al.* have gone beyond these earlier studies by demonstrating that the regulation of ZEB1 and ZEB2 by the miR-141, miR-200b and miR-205 family miRNAs is direct. They also show that knockdown of miR-141, miR-200b and miR-205 family miRNAs was sufficient to reduce E-cadherin expression, increase cell motility and thus induce EMT in MDCK[3] and HCT116 cells[4] (Fig. 1). Park *et al.* also noted that expression of the mesenchymal marker vimentin was inversely correlated with E-cadherin expression[4].

Conversely, ectopic expression of these miRNAs led to the re-expression of E-cadherin and epithelial phenotypes in MDCK cells that had undergone EMT[3] and in mesenchymal MDA-MB-231[4] cells. A particularly striking finding reported by both groups is that EMT induction by knockdown of these miRNA families was largely dependent on ZEB1 and ZEB2[3,4]. miRNAs can potentially act on hundreds of distinct target mRNAs and have also been proposed to have a modulatory rather than regulatory role. This appears not to be true for miR-141, miR-200b and miR-205 family miRNAs in EMT. Is ZEB1 and ZEB2 activity solely under the control of these miRNAs? This is unlikely as ZEB1 and ZEB2 mRNAs are substantially induced during EMT, suggesting a possible feedback-loop or additional regulation upstream of ZEB1 and ZEB2; it would be interesting to further explore the regulation of these two key transcription factors.

How do these findings relate to EMT during vertebrate development *in vivo*? In zebrafish, miR-141, miR-200b and miR-205 family miRNAs are highly expressed in epithelial cell types including the skin, digestive and respiratory systems and the olfactory epithelium[7,8]; in the chick, they are restricted to the endoderm and ectoderm[9]. In contrast, ZEB1 and ZEB2 are highly expressed in mesodermal tissues[10] in zebrafish and mice. Currently, no knockout analysis of miR-141, miR-200b or miR-205 family miRNAs has been reported. However, morpholino-mediated knockdown of the miR-141 and miR-200b miRNA families caused defects in the terminal differentiation of olfactory epithelia in zebrafish, phenocopying defects observed after inactivation of the miRNA biogenesis enzyme Dicer during olfactory epithelium development in mice[8]. Interestingly, the Zebrafish study identified a ZEB2 orthologue as a direct target of the miR-141 and miR-200b family miRNAs, suggesting that this regulatory pathway may be conserved in vertebrates[8].

EMT-like transitions are a hallmark of tumour invasion and metastasis. The work of Gregory *et al.* and Park *et al.* now suggests that miR-141, miR-200b and miR-205 family miRNAs may be important during these processes. In support of this hypothesis, high expression of miR-141 and miR-200b family miRNA is correlated with epithelial phenotypes of several human cancer cell lines[3,4]. Furthermore, miR-141 and miR-200b family miRNAs are downregulated in metaplastic

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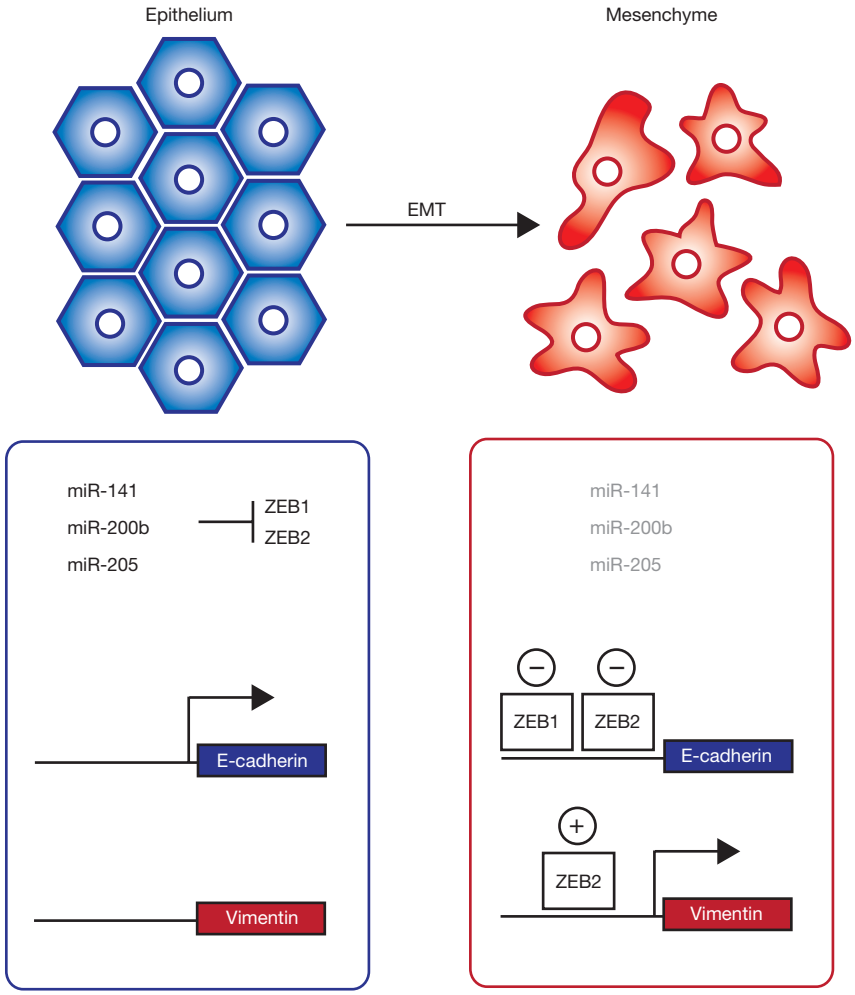

Figure 1 The miR-141, miR-200b and miR-205 families directly regulate repressors of E-cadherin transcription. In epithelial cells, miR-141, miR-200b and miR-205 family miRNAs inhibit the expression of the E-box-interacting transcription factors ZEB1/δEF1/TCF8 and ZEB2/SIP2/SMADIP1/ZFXH1B allowing E-cadherin transcription. During EMT these miRNAs are downregulated and ZEB1 and ZEB2 now repress E-cadherin transcription. ZEB2 also upregulates vimentin expression directly.

breast cancers that have a substantial number of mesenchymal cells, compared with invasive ductal carcinomas that are epithelial in nature[3]. Finally, expression of miR-200c, a miR-200b family miRNA, correlated with E-cadherin expression in primary serous papillary ovarian cancer tissues[4]. The case for a potential role of the miR-205 miRNA in metastasis is less convincing, as a correlation with epithelial phenotypes was observed in human breast cancer cell lines, but not in the NCI60 panel nor in the metaplastic breast cancer versus ductal carcinoma analysis[3,4]. It will be of great interest to explore the role of these miRNAs in tumour metastasis further, possibly by taking advantage of mouse models for EMT and metastasis[11-13].

Does loss of miRNA expression generally correlate with tumour metastasis? Fortunately, the answer is not that simple. Gregory *et al.* and Park *et al.* found that downregulation of miRNA expression during EMT is specific to miR-141, miR-200b and miR-205 family miRNAs[3,4]. In contrast, previous work demonstrated that a distinct miRNA, miR-10b is upregulated by Twist to drive breast cancer cell invasion and metastasis[14] and miR-373 may also promote metastasis[15]. Finally, miR-335 has been shown to inhibit breast cancer metastasis[16]. So, a complex interaction between miRNAs and tumour metastasis is emerging with future potential for cancer diagnosis and therapy.

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