A new regulatory loop in cancer-cell invasion

The epithelial-to-mesenchymal transition (EMT) converts epithelial cells into mesenchymal cells that are able to invade and migrate. In the context of cancer pathogenesis, the EMT contributes to metastatic progression (Thiery, 2002; Acloque *et al*, 2008). One of the characteristics of EMT is the functional loss of E-cadherin, which is crucial for the progression to invasive carcinoma (Perl *et al*, 1998). Transcriptional repression has emerged as a fundamental mechanism for E-cadherin loss during EMT and several repressors have been characterized. These include zinc-finger E-box-binding homeobox 1 (ZEB1), ZEB2/SIP1, Snail1, Snail2 and Twist, which strongly repress *E-cadherin* transcription through their direct binding to the E-box motifs within the *E-cadherin* promoter (Barrallo-Gimeno & Nieto, 2005; Peinado *et al*, 2007). During the past decade, it has emerged that microRNAs (miRNAs) are a fundamental mechanism of gene-expression regulation, and several recent reports have shown that these small molecules are involved in the control of *E-cadherin* expression in cancer cells and tumours (Hurteau *et al*, 2007; Christoffersen *et al*, 2007; Gregory *et al*, 2008; Park *et al*, 2008). Furthermore, in this issue of *EMBO reports*, Burk and colleagues have provided a beautiful example of gene regulation in which miRNAs and transcription factors are linked to one another in a gene-regulatory network to control E-cadherin expression and the invasive phenotype in cancer cells (Burk *et al*, 2008).

MiRNAs regulate gene expression through binding to mRNA target sequences typically in their 3' untranslated regulatory regions (UTRs), which results in the degradation of target mRNAs and/or the inhibition of the translation process (Filipowicz *et al*, 2008). Several miRNAs are deregulated or mutated in many human cancers, including lung, breast, brain, liver and colon cancers, making them attractive targets in biomedical research and anti-tumour therapies (Esquela-Kerscher & Slack, 2006; Zhang *et al*, 2007; Couzin, 2008).

MiRNAs can function as tumour promoters or as tumour suppressors. For example, miR-10b, miR-373 and miR-520c are highly expressed in metastatic breast cancer cells in which they positively regulate cell migration, invasion and metastatic properties (Ma *et al*, 2007; Huang *et al*, 2008). Similarly, miR-21 is increased in breast and colon carcinomas and is upregulated during the transforming growth factor- β (TGF β)-induced EMT in cancer-derived cell lines (Zavadil *et al*, 2007). Conversely, Tavazoie and colleagues showed that miR-126, miR-206 and miR-335 suppress breast cancer metastasis (Tavazoie *et al*, 2007), and they were able to confirm clinical relevance for miR-126 and miR-335. Similarly, let-7 is a

marker for differentiated cancers and seems to impair tumour progression by preventing the re-expression of early embryonic genes during the initial steps of neoplastic transformation. However, let-7 is unlikely to be an EMT regulator as its expression cannot be associated with changes in the expression of EMT markers (Shell *et al*, 2007; Park *et al*, 2007).

Given the relevance of both EMT and miRNAs in tumour progression, the question became whether other miRNAs had a role in the induction of EMT. Indeed, two recent reports indicate that the EMT inducers ZEB1 and ZEB2/SIP1 are direct targets of miR-200 family members, suggesting that miRNAs can indirectly regulate E-cadherin expression in tumour cell lines (Christoffersen *et al*, 2007; Hurteau *et al*, 2007). Two additional comprehensive studies have now confirmed that members of the miR-200 family directly repress the ZEB1 and ZEB2/SIP1 factors in a cooperative manner, and that there is a strong correlation between the expression of miR-200 and that of E-cadherin in several cancer cell lines and human tumours (Gregory *et al*, 2008; Park *et al*, 2008). Conversely, the expression of the miR-200 family is lost in mesenchymal and invasive cells, as it is in poorly differentiated and aggressive metastatic breast cancers. Interestingly, the silencing of miR-200 family members is sufficient to induce EMT and invasion in epithelial cell lines, whereas their overexpression can trigger the reverse process—the mesenchymal-to-epithelial transition (MET; Gregory *et al*, 2008; Park *et al*, 2008). These data suggest that the loss of the miR-200 family might be crucial for the induction of EMT during tumour progression.

Once it had been established that the miR-200 family represses *Zeb* gene transcription, and given that several family members are lost in invasive carcinomas, Burk and colleagues took one step further and asked whether the forced downregulation of ZEB1 modifies miRNA expression in cancer cells (Fig 1A; Burk *et al*, 2008). They found that the miR-200 family members miRNA-200c and miRNA-141 are upregulated, and that their putative regulatory elements contain several binding sequences for ZEB1, some of which can also be recognized by other EMT inducers. Indeed, ZEB1 directly suppresses the transcription of miR-200c and miR-141, giving rise to a negative-feedback loop of transcriptional coregulation of miRNAs and their targets as previously modelled by Tsang and colleagues (Fig 1B; Tsang *et al*, 2007). Interestingly, miR-141 also represses the EMT inducer TGF β 2 (Burk *et al*, 2008), perhaps reinforcing the epithelial state. Indeed, the normal epithelial condition is characterized by high levels of miR-200 that maintain high E-cadherin levels and tissue homeostasis. In cancer cells, the increase in the signals that activate *Zeb* genes and other EMT inducers might overcome this control and favour the transition to the mesenchymal state.

The past few months have witnessed the emergence of a plethora of studies linking miRNAs with tumour invasion and metastasis. A tight connection between the miR-200 family and EMT in tumour progression has unveiled how transcription factors and miRNAs are linked to one another in gene-regulatory networks. This network might function to maintain epithelial homeostasis or to trigger a ZEB1

Fig 1 | Schematic representation of the regulatory loop between the miR-200 family and ZEB factors. **(A)** Various cellular states are characterized by the relative activity of *Zeb* genes and members of the miR-200 family. The epithelial cell expresses high levels of miR-200 and E-cadherin, whereas the mesenchymal cell expresses high levels of ZEB and vimentin. Active genes are shown in black and inactive genes in grey (adapted from Gregory *et al*, 2008; Park *et al*, 2008 and Burk *et al*, 2008). **(B)** The two cellular states can be two outcomes of a circuit in which a signal (in this case $S = \text{TGF}\beta$) induces the expression of transcription factors (in this case $\text{TF} = \text{ZEB factors}$). These transcription factors are the targets of the microRNAs (miRNAs) that they repress (in this case the miR-200 family), generating a negative-feedback loop (see Tsang *et al*, 2007 for coordinated transcriptional and miRNA-mediated regulatory models). The miRNA negatively regulates not only the expression of the TFs but also the expression of the signal ($\text{TGF}\beta$). EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition; SIP1, Smad interacting protein 1; $\text{TGF}\beta$, transforming growth factor- β ; ZEB, zinc-finger E-box-binding homeobox transcription factors.

self-enhancing loop of epithelial repression and invasion in cancer cells. It will be interesting to determine whether other EMT inducers are regulated by the miRNA-200 family and whether miRNAs are able to repress cancer progression *in vivo*. Indeed, the reversibility of the EMT process induced by miR-200 overexpression provides some hope for the use of miRNAs as therapeutic anti-invasive drugs.

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