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The microRNA-200 Family Regulates Epithelial to Mesenchymal Transition

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The majority of human cancers originate from epithelial cells[1], with their local invasion and metastasis accounting for 90% of cancer-related death[2]. The progression of metastasis is a complex event thought to incorporate the reversible developmental process of epithelial to mesenchymal transition (EMT). This involves cancerous epithelial cells transitioning into motile mesenchymal cells, which invade distant sites in the body where they lodge and undergo mesenchymal to epithelial transition (MET) before proliferating into secondary tumours[3]. Essential to the maintenance of the polarised epithelial monolayer is the adherens junction protein, E-cadherin, which has been described as a tumour suppressor because its down-regulation is associated with invasion and metastasis[4]. During EMT, the increase of transcription factors, such as ZEB1, SIP1, Snail, and Slug, strongly represses the transcription of the E-cadherin gene, facilitating loss of the strong cell-cell interactions characteristic of epithelial cells.

First discovered in 1993, microRNAs (miRNAs) are an abundant class of noncoding, 18–25 nt, single-stranded oligoribonucleotides that function post-transcriptionally to negatively regulate the translation of messenger RNA (mRNA). Target recognition is based on complementary binding to the 3' untranslated region (3'UTR) of the target mRNA. Expression of miRNAs can vary from ubiquitous to highly site and/or temporal specific. They are predicted to regulate up to 30% of genes in eukaryotes[5] and have regulatory roles in cellular processes, such as proliferation[6,7,8], differentiation[9], apoptosis[10,11], metabolism[12], embryogenesis and developmental timing[13,14,15,16]. Consistent with their roles in maintaining normal cell function, the aberrant expression of miRNAs has been linked to oncogenesis[17] and, more recently, metastasis[18,19,20,21,22]. Since EMT is an essential developmental process and is implicated in metastasis, we postulated that miRNAs may be involved in its regulation.

Evidence that EMT is required for cancer metastasis is increasing with investigations into the signalling mechanisms driving EMT. A potent inducer of EMT is the cytokine transforming growth factor-β (TGF-β), which has been implicated in regulating transcription factors including Snail, Slug, ZEB1, SIP1, and basic-helix-loop-helix (bHLH) factors, such as Twist[23,24]. However, knowledge of the role of miRNAs and their potential target genes in EMT is limited. Previous studies have shown that

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miR-200c and miR-200b post-transcriptionally repress ZEB1 and SIP1, respectively[25,26]. The differential expression of miRNAs in primary tumours and their metastases has been reported, but it remains to be elucidated as to whether miRNAs are involved in the initiation of invasion in the context of EMT.

We conducted microarray analysis to assess differential expression of miRNAs in an EMT system in Madin-Darby canine kidney (MDCK) cells, induced by the protein tyrosine phosphatase (PTP) Pez[27]. Overexpression of PTP-Pez in MDCK cells leads to induction of TGF-β signalling and EMT, as observed in cells losing cell-cell adhesion and adopting a mesenchymal phenotype[27]. Quantitative real-time PCR of miRNA expression in the MDCK-Pez model confirmed that all five members of the highly related miR-200 family (miR-200a, -200b, -200c, -141, and -429) and miR-205 were down-regulated over 100-fold following EMT[28]. In addition, a TGF-β–induced EMT model also showed similar differential expression of these miRNAs. Stable overexpression of the miR-200s prevented TGF-β–induced EMT, implicating the miR-200s as key regulators of EMT. Computational searches using TargetScan[29] revealed multiple potential binding sites for the miR-200s and miR-205 in the 3'UTRs of the mesenchymal markers, ZEB1 and SIP1. Transfection of luciferase reporters containing either the ZEB1 or SIP1 3'UTR into the Pez-induced EMT model showed high expression in mesenchymal cells, but reduced expression in epithelial cells, consistent with the differences in miR-200s and miR-205 levels in these cells. This was verified by cotransfection of the reporters with either antisense inhibitors or synthetic precursors to miR-200s and miR-205 in epithelial and mesenchymal cells, respectively. Inhibition of the miR-200s in MDCK cells over a 19-day time course was sufficient to induce EMT, as demonstrated by loss of E-cadherin, gain of ZEB1 and SIP1, and an increase in cell migration. ZEB1 and SIP1 were shown to be essential for EMT by siRNA-mediated knockdown of their levels in the presence of the miR-200 inhibitors, which prevented this transition. Conversely, ectopic expression of the miR-200s in mesenchymal cells induced MET. This led to the conclusion that the miR-200 family is able to regulate EMT by repressing the translation of ZEB1 and SIP1. We extended this study to include analysis of miRNA, E-cadherin, ZEB1, and SIP1 in a range of epithelial and mesenchymal breast cancer lines. Strikingly, the epithelial lines expressed miR-200s and E-cadherin, and lacked ZEB1 and SIP1, whereas the mesenchymal lines were deficient in miR-200s and E-cadherin, but had high levels of ZEB1 and SIP1. *In vivo* analysis of miR-200 expression was conducted via real-time PCR of primary human ductal and metaplastic breast cancers, which exhibit epithelial and mesenchymal cell morphology, respectively. The ductal tumours had high levels of E-cadherin and miR-200s, while the invasive mesenchymal metaplastic tumours lacked these markers. This indicates that loss of the miR-200s may result in a more aggressive cancer, leading to metastasis.

In summary, we demonstrated that the miR-200 family and miR-205 maintain the epithelial phenotype by repressing the translation of ZEB1 and SIP1. The importance of this finding is supported by a number of subsequent reports that also demonstrate this relationship of the miR-200 family with ZEB1 and SIP1[30,31,32]. Loss of the miR-200s in invasive metaplastic breast cancers implicates these miRNAs as potential therapeutic agents that may have the ability to confine carcinoma cells to the primary tumour site, where the mass can be effectively resected.

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