

REGULATION OF EMT BY THE MIR-200 FAMILY OF MICRORNAS AND ITS IMPLICATIONS FOR CANCER

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Epithelial to mesenchymal transition occurs during embryological development to allow tissue remodeling and is proposed to be a key step in the metastasis of epithelial-derived tumours. We used microRNA microarrays to identify microRNAs that change in level in epithelial-derived cells that have undergone EMT in response to overexpression of the protein tyrosine phosphatase Pez and found that all 5 members of the microRNA-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 were drastically down-regulated¹. These microRNAs were also downregulated in cells that have undergone EMT in response to Transforming Growth Factor (TGF)- β . Enforced expression of the miR-200 family alone was sufficient to prevent TGF β -induced EMT. We found these microRNAs cooperatively regulate expression of the E-cadherin transcriptional repressors ZEB1 (also known as δ EF1) and SIP1 (also known as ZEB2), factors previously implicated in EMT and tumour metastasis. Inhibition of the microRNAs was sufficient to induce EMT in a process requiring ZEB1 and or SIP1 upregulation. Conversely, ectopic expression of these microRNAs in mesenchymal cells initiated mesenchymal to epithelial transition (MET). Consistent with their regulation of EMT, expression of these microRNAs were found to be lost in invasive breast cancer-derived cell lines that have mesenchymal phenotype. We found that the level of miR-200 in invasive ductal breast cancers is correlated with the level of E-cadherin, while miR-200 expression is lost in regions of metaplastic breast cancer specimens that lack E-cadherin and display mesenchymal features. These data suggest that down-regulation of the microRNAs may be an important step in tumour progression. Examination of the genomic region encoding miR-200a, miR-200b and miR-429 indicated they are all encoded on a 7.5 kb polycistronic pri-miR transcript. We show the promoter for the pri-miR is located within a 300 bp segment located 4 kb upstream of miR-200b and this promoter region is sufficient to confer expression in epithelial cells and is repressed in mesenchymal cells by ZEB1 and SIP1 through their binding to a conserved pair of ZEB-type E box elements located proximal to the transcription start site. These findings establish a double-negative feedback loop controlling ZEB1/SIP1 and miR-200 family expression that regulates cellular phenotype and has direct relevance to the role of these factors in tumor progression.

¹Gregory,P.A. Bert,A.G.; Paterson,E.L.; Barry,S.C.; Tsykin,A.; Farshid,G.; Vadas,M.A.; Khew-Goodall,Y.; Goodall,G.J. The microRNA-200 family and miR-205 regulate epithelial-mesenchymal transition by targeting the E-cadherin repressors, ZEB1 and SIP1. *Nat. Cell Biol.* (2008) 10;593-601