METASTASIS

Converging targets

> melanoma cell-derived APOE systemically suppresses metastasis

MicroRNAs (miRNAs) have been shown to regulate various processes of metastasis, so Tavazoie and colleagues investigated their role in melanoma metastasis.

Pencheva *et al.* compared small RNA profiles of lung metastatic human melanoma cell lines with their poorly metastatic parental cell lines (MeWo and A375 cells) and found that four miRNAs were significantly overexpressed in the derivative cell lines compared with the parental cell lines. Overexpression of three of these miRNAs, miR-199a-5p, miR-1908 and miR-199a-3p, individually in MeWo cells increased lung colonization when the cells were injected into the tail veins of nude mice. Importantly, primary tumour growth was unaffected when the miRNAs were overexpressed, indicating that they have a role in driving metastasis. Furthermore, miRNA profiling of 71 human primary melanoma samples revealed that the expression of miR-199a-5p, miR-1908 and miR-199a-3p was significantly increased in patients who subsequently developed metastasis.

So, how do these miRNAs drive metastasis? Overexpression of miR-199a-5p, miR-1908 or miR-199a-3p in MeWo cells increased their invasive capacity in Matrigel and their ability to recruit endothelial cells *in vitro*, whereas knockdown of these miRNAs in two metastatic derivative cell lines, MeWo-LM2 and A375-LM3, had the opposite effect. Furthermore, lung metastases that formed from tail vein injection of MeWo-LM2 and A375-LM3 cells in which each of the miRNAs was knocked down had reduced endothelial cell content and vascularization.

The authors reasoned that as altering the expression of each of the miRNAs conferred similar phenotypes, they may have overlapping target mRNAs. Indeed, they found that the expression of apolipoprotein E (APOE) was suppressed by miR-199a-5p and miR-1908, and the expression of DNAJA4 was suppressed by miR-199a-3p, miR-199a-5p and miR-1908. Moreover, APOE and DNAJA4 knockdown increased the ability of MeWo cells to invade Matrigel and recruit endothelial cells *in vitro*, indicating that these miRNAs drive melanoma metastasis by suppressing these targets.

Interestingly, the authors found that DNAJA4, a heat shock protein, regulated the expression of APOE such that DNAJA4 overexpression increased the levels of both *APOE* mRNA and secreted APOE protein. Furthermore, treating MeWo cells with recombinant APOE prevented the increased cell invasion and endothelial recruitment that resulted from DNAJA4 knockdown. Therefore, APOE seems to be the target of three metastasis-promoting miRNAs, and by using various tissue microarray data sets, the authors confirmed that APOE expression was reduced in human melanoma metastases.

How does APOE suppress melanoma metastasis? Consistent with APOE being a secreted protein, the authors found that injecting MeWo-LM2 cells that had been cultured with recombinant APOE significantly reduced metastatic colonization in wild-type and *Apoe*$^{-/-}$ mice, indicating that melanoma cell-derived APOE systemically suppresses metastasis. Next, the authors knocked down the four APOE receptors in MeWo-LM2 cells and found that knockdown of LRP1 increased Matrigel invasion and knockdown of LRP8 on endothelial cells further increased their recruitment to MeWo-LM2 cells. Finally, the authors showed that intravenous injection of antisense locked nucleic acids to the three miRNAs reduced lung colonization of tail vein-injected MeWo-LM2 cells in nude mice, indicating that this pathway may have therapeutic potential.

In summary, the authors have identified three miRNAs (miR-199a-5p, miR-1908 and miR-199a-3p) that, through downregulation of DNAJA4 and APOE, abrogate metastasis suppressor pathways that are mediated by APOE–LRP1 in melanoma cells and by APOE–LRP8 in endothelial cells.

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ORIGINAL RESEARCH PAPER Pencheva, N. *et al.* Convergent multi-miRNA targeting of ApoE drives LRP1/LRP8-dependent melanoma metastasis and angiogenesis. *Cell* 8 Nov 2012 (doi:10.1016/j.cell.2012.10.028)