Nature Reviews Genetics | AOP, published online 29 April 2014; doi:10.1038/nrg3741

CANCER GENETICS

Exosomally derived miR-105 destroys tight junctions

Several studies have implicated gene regulation by microRNAs (miRNAs) in tumorigenesis, but the role of exosomal miRNAs is less clear. A new study by Zhou *et al.* showed that exosomal miR-105 targets tight junction protein ZO-1 (also known as zona occludens protein 1) and thus facilitates migration of cancer cells to distant locations.

Exosomes are small secretory vesicles that are released into the extracellular environment and can also be taken up by recipient cells. To study the effect of cancer-secreted exosomes on the endothelium, Zhou *et al.* used human microvascular endothelial cells (HMVECs) and incubated them with purified fluorescently labelled exosomes that were derived from either MDA-MB-231 metastatic breast cancer cells or the non-cancerous mammary epithelial cell line MCF-10A. Although HMVECs took up both cancer-derived and non-cancer-derived exosomes with high efficiency (>90%), HMVECs incubated with the cancer-derived exosomes showed significantly higher migration efficiency in a transwell migration assay. This effect was recapitulated when HMVECs were incubated with small RNAs or total RNA from cancer-derived exosomes but not from non-cancer-derived exosomes.

In order to determine the specific small RNA that induced migration in cancer-derived exosomes, small RNAs from exosomes derived from MDA-MB-231 and MCF-10A cells were sequenced and profiled. The secretion of the miRNA miR-105 was found to be specific to MDA-MB-231 cells, and its expression level was significantly higher in exosomes derived from MDA-MB-231 cells than in exosomes derived from MCF-10A cells. Moreover, when the authors examined a panel of breast cancer lines, the expression and secretion of miR-105 were specific to highly metastatic lines.

miR-105 was predicted by several algorithms to target the tight junction protein ZO-1. The authors then used several *in vitro* assays to confirm that expression of ectopic miR-105 or treatment with exosomes from MDA-MB-231 cells led to downregulation of ZO-1 at both mRNA and protein levels in HMVECs, and that this could be rescued by addition of a miR-105 hairpin inhibitor. These effects were recapitulated using exosomes secreted by MCF-10A cells that were stably transfected with miR-105, which suggests that additional exosomal components from MDA-MB-231 cells are not required.

The functional effects of targeting ZO-1 were examined using *in vitro* permeability and sprouting assays, which showed that targeting endothelial ZO-1 by miR-105-containing exosomes resulted in increased endothelial permeability and the destruction of vascular sprouts. These effects *in vitro* suggest a role for miR-105 in promoting metastasis *in vivo*. Accordingly, mouse xenografts derived from cells containing high levels of miR-105 were treated with an anti-miR-105 compound, and this treatment reduced both distant metastasis and the volume of the primary tumour relative to controls.

As miR-105 is secreted by breast cancer cells, the authors asked whether secreted miR-105 is found in the serum of patients with breast cancer and whether it is indicative of cancer stage. In 38 patients examined with stage II and stage III breast cancer, miR-105 was detected in serum-derived exosomes and found to be at a significantly higher level in patients who later developed distant metastasis than in patients who did not develop metastasis. In addition, there was a significant correlation between high miR-105 and low ZO-1 levels in tumours.

This study provides insights into gene expression regulation by exosomally derived miRNAs and suggests that miR-105 has clinical applications as both a prognostic biomarker and a therapeutic target for breast cancer.

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ORIGINAL RESEARCH PAPER Zhou, W. *et al.* Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis. *Cancer Cell* **25**, 501–515 (2014)