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> manipulation of energy availability by cancer cells … can fuel metastatic colonization

Understanding the formation of pre-metastatic niches is crucial for devising effective strategies to prevent or treat metastasis. Two studies have examined how manipulation of energy availability by cancer cells, mediated by changes in microRNAs (miRNAs), can fuel metastatic colonization.

Loo *et al.* used *in vivo* selection of a colon cancer cell line (LS-174T) to derive two independent lines (LS-LvM3a and LS-LvM3b) that had an increased ability to colonize the liver — a major site of colon cancer metastasis. They also screened 661 miRNAs in two different colon cancer cell lines to identify metastasis-suppressive miRNAs. Two of these, miR-483-5p and miR-551a, were silenced in the LS-LvM3a and LS-LvM3b cells relative to LS-174T cells.

Creatine kinase, brain type (CKB) is a target of both miR-483-5p and miR-551a; indeed, overexpression of CKB enhanced metastasis of poorly metastatic cells, and CKB knockdown prevented liver colonization. CKB catalyses the formation of phosphocreatine, which is stored in cells and used to generate ATP when levels are low, as might occur when colon cancer cells enter the hypoxic liver microenvironment. RNA interference-mediated depletion of CKB reduced survival of LS-LvM3b cells under hypoxia *in vitro* and decreased liver colonization *in vivo*; both effects were reversed by pre-incubating the cells with phosphocreatine. However, cancer cells arriving in the liver are likely to have low ATP levels, and using ATP to create phosphocreatine intracellularly would create a futile cycle. The authors hypothesized that CKB might be secreted by cancer cells to catalyse extracellular phosphocreatine generation using extracellular ATP. Indeed, LS-LvM3b cells secreted CKB, and cells depleted of miR-483-5p and miR-551a had lower levels of extracellular ATP. Delivery of exogenous phosphocreatine increased metastasis of CKB-depleted cells, and cells that only produced a secreted form of CKB were also able to metastasize.

To investigate the therapeutic potential of this pathway, the authors used adeno-associated virus to deliver miR-483-5p and miR-551a. A single dose significantly reduced colonization of the liver by LS-LvM3b or SW480 cells following intrasplenic injection. A small-molecule inhibitor of CKB also reduced metastasis of LS-LvM3b cells. In patients, miR-483-5p and miR-551a levels were shown to be lower in liver metastases than in primary colon tumours, and CKB levels were higher, suggesting that this pathway operates in human tumours.

Fong *et al.* previously identified that high levels of circulating miR-122 are predictive of metastasis in patients with breast cancer. They found that several breast cancer cell lines had high levels of secreted miR-122 in extracellular vesicles (EVs; including exosomes), and that miR-122 could suppress glucose metabolism by reducing pyruvate kinase (PKM) expression. To determine the function of extracellular miR-122, the authors treated lung fibroblasts and brain astrocytes with miR-122-containing EVs isolated from breast cancer cell lines. EV-derived miR-122 reduced expression of the PKM isoform PKM2 and decreased glucose uptake in the fibroblasts and astrocytes. Similar effects were observed using EVs that were derived from the sera of a patient with breast cancer.

Intravenous injection of miR-122-containing EVs suppressed PKM expression and reduced glucose uptake in the brain and lungs of mice, indicating that this pathway also occurs *in vivo*. Furthermore, pre-treatment of mice with miR-122-containing EVs promoted metastasis of a breast cancer cell line to the brain and lungs following intracardiac injection. Overexpression of miR-122 in tumorigenic MCFDCIS cells reduced primary tumour size in orthotopic xenografts but increased metastasis to the brain and lungs. Mice bearing xenograft tumours of miR-122-expressing cells had reduced glucose uptake in the brain and lungs at the pre-metastatic stage, which was reduced further as the tumours metastasized. Anti-miR-122 treatment of these mice prevented metastasis and promoted glucose uptake in the brain. Overall, these data suggest that miR-122-containing EVs derived from cancer cells can prevent glucose utilization in pre-metastatic niches, thus increasing the amount of glucose available to metastasizing cancer cells.

Therapeutic manipulation of the pathways identified in these studies could potentially reduce or prevent metastasis.

Sarah Seton-Rogers

ORIGINAL RESEARCH PAPERS Loo, J. M. *et al.* Extracellular metabolic energetics can promote cancer progression. *Cell* http://dx.doi.org/10.1016/j.cell.2014.12.018 (2015) | Fong, M. Y. *et al.* Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. *Nature Cell Biol.* http://dx.doi.org/10.1038/ncb3094 (2015)