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Understanding the role of miR-122-5p in colorectal cancer liver metastasis

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Metastasis is the leading cause of colorectal cancer (CRC) deaths and the liver is the most common metastasis site. Nearly 50% - 60% CRC patients are diagnosed with synchronous metastases, 80% of which have liver metastases. Metastasis itself is not a single process but rather, a constellation of multiple events that culminate with the colonization of distant sites by the primary tumor.

As preliminary work for the project of this thesis, the whole genomes of 12 patients with advanced colorectal cancer were sequenced with the Illumina next generation sequencing platform in the Allgayer department. Bioinformatics analysis and subsequent validation showed that the miR-122 gene locus was deleted in primary tumors and corresponding metastases of most patients. Interestingly, while the expression of miR-122 was suppressed in primary tumors, it was significantly increased in metastatic lesions. miR-122 itself is highly abundant and specific to the liver and this microRNA plays a critical role in liver homeostasis by regulating the expression of a large number of target mRNAs and also by suppressing non-hepatic genes.

We found that miR-122 was secreted in the conditioned media and exosomes of Huh7 liver cancer cells, which meant that miR-122, could be potentially transferred extracellularly through exosomes. Furthermore, in co-culture experiments, we observed an increase of miR-122 expression in RKO cells from the third day of co-culture with Huh7 cells. This expression was still significantly enhanced on the fourth and fifth days. On the other hand, miR-122 expression was significantly upregulated in RKO and DLD1 cells when we directly added the exosomes extracted from conditioned media of Huh7 cells into their normal medium, respectively. These two approaches both demonstrated that colorectal cancer cells could take up miR-122 secreted from Huh7 cells. This explains our previous paradoxical finding and elucidates where the overexpression of miR-122 in the colon cancer cells came from. To date, there are no reports about an exosomal interaction involving the delivery of miR-122 to colorectal cancer cells in a paracrine fashion.

We identified and validated two novel target genes of miR-122, RIMS1 and RABL6. RIMS1 is a member of the RAS gene superfamily and regulates synaptic vesicle exocytosis. It also regulates voltage-gated calcium channels during neurotransmitter and insulin release. This protein has not been reported yet to be exposed in the colon or liver. RABL6, also known as C9orf86 (chromosome 9 open reading frame 86), or RBEL1 (Rab-like protein 1), is a novel subfamily within the Ras superfamily. There are no reports implicating RABL6 in colorectal cancer. In our study, we found that P21 protein was upregulated in RABL6 knockdown CRC cells, or CRC cells treated with active miR-122. This suggested that the miR-122 induced suppression of RABL6 caused a upregulation of P21, although the mechanism by which RABL6 controls P21 is still unknown.

Our data also demonstrated that forced overexpression of miR-122 significantly suppressed cell proliferation and colony formation in at least 2 different CRC cell lines. No significant difference in cell invasion/migration was however observed, possibly due to the function of the main target gene, RABL6.

In general, our findings suggest that miR-122 is a tumor suppressor in colorectal cancer, and regulates tumor growth by multiple mechanisms including the silencing of RABL6 as well as enhancing P21 activation. This particular activity in the context of liver metastasis implicates miR-122 as a potential line of defence against the establishment of CRC liver metastasis, and also supports a potential use of miR-122 in the therapy of advanced CRC with liver metastasis.