The role of the serum-circulating miR-662 in breast cancer bone metastasis.

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Breast cancer (BC) is the most common cancer in women, which often relapses in bone (~80%). Treatments for BC bone metastasis are only palliative, and new therapeutic approaches are urgently required. MicroRNAs (miRNAs), small non-coding RNAs, are post-transcriptional gene regulators within cells, inducing the degradation/ translational repression of messenger RNAs. MiRNAs are often dysregulated in BC cells, and contribute to tumour progression, also by promoting metastatic cell capabilities (e.g., increased stemness, proliferation and migration). Thus, miRNAs and their targets could be new therapeutic targets for BC patients. Moreover, miRNAs can be released from cells (circulating miRNAs), being extremely stable in the bloodstream, and could therefore be also used as biomarkers to follow BC progression and predict metastasis risk for BC patients.

We analysed the expression of >700 circulating miRNAs from the serum of 48 early-stage BC patients. Based on 10-year clinical patients' information, we identified a few circulating miRNAs associated with BC bone recurrence. Specifically, one miRNA, miR-662, resulted associated with bone recurrence and BC stemness cell properties in patient-derived xenograft models of primary BC. Thus, miR-662 might be a good candidate as prognostic biomarker to predict the risk of metastasis, and bone metastasis, in BC patients, which requires to be confirmed in a larger cohort of BC patients. Moreover, these findings led us to hypothesise that miR-662 could mediate BC bone metastasis progression. Thus, we decided to investigate miR-662 at functional level, using BC human (MDA-MB-231) and murine (4T1) cell models in cell-based assays as well as animal models of bone metastasis. We found that miR-662 over-expression increased stemness, proliferation, and migration abilities of BC cells *in vitro*. By RNA sequencing, we confirmed that the over-expression of miR-662 affected the expression of genes mainly involved in cancer

progression, immune suppression, and stemness. Interestingly, at an earlier stage of metastasis progression, miR-662 over-expression in MDA-MB-231 cells inhibited tumour burden, especially in bone, due to an inhibitory effect of miR-662 on osteoclastogenesis. However, this inhibition was only temporary. In fact, at later stages of BC metastasis, miR-662 over-expression in BC cells promoted metastasis in animal models.

Overall, miR-662 over-expression in BC cells promotes metastasis, especially in bone, making this miRNA interesting as a future therapeutic target in BC cells to reduce the risk of metastasis in BC patients.

Keywords: Bone metastasis, MicroRNA, Metastatic breast cancer, Biomarkers

Figure 1: Graphical abstract. MiR-662 over-expression in BC cells promotes metastasis progression, especially in bone, by increasing stem-like, proliferative and migration properties of BC cells. High circulating levels of miR-662 can be detected in the serum of early-stage BC patients, being a possible biomarker for high risk of metastatic relapse. In bone, miR-662 has a secondary effect on bone remodelling, by inhibiting osteoclastogenesis.