RAI2 as novel suppressor of cancer cell dissemination to the bone marrow

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Background & objectives

Disseminated tumor cells (DTCs), the seeds of metastases detected in the bone marrow (BM), can be found years after the primary diagnosis and its persistence in the BM is related to minimal residual disease. We have shown that the presence of DTCs in the BM at both the time of diagnosis and after chemotherapy is an independent predictor of poor prognosis in non-metastatic breast cancer. We identified retinoic acid–induced 2 (RAI2) as a novel metastasis suppressor gene significantly associated with both positive DTC status and poor prognosis, especially among hormone-receptor positive breast cancer patients.

The goal of this project is to better understand the precise role of RAI2 on the tumor cell dissemination to the bone in breast cancer.

Methods

Xenograft models were established by injecting orthotopically RAI2 KO (CRISPR/Cas9 mediated RAI2 depletion) and parental KPL1 cells in immune deficient SCID/J mice. The *in vitro* effect of RAI2 on the BM cells was studied with osteoblast and osteoclast differentiation assays either with conditioned media from parental and RAI2 KO (MCF7 and KPL1) cells or with extracellular vesicles (EVs) isolated by ultracentrifugation from the supernatant fractions. miRNA content of the EVs was assessed by NGS and validated in plasma from a cohort of bone and brain metastatic breast cancer patients, non-metastatic breast cancer patients and in healthy donors.

Results

Xenograft experiments in SCID/J mice showed an increased early tumor dissemination (numbers of CTCs and DTCs) when RAI2 KO cells were injected orthotopically versus the parental cell line. Both conditioned media as well as the EV fraction from RAI2 KO cells significantly increase osteoclast differentiation. Five miRNA candidates were identified to be involved in this RAI2-mediated crosstalk between tumor and osteclasts. Validation assays identified miR-135a-5p to be lower expressed on EVs from bone metastatic patients compared to heathy donors and non-metastatic patients.

Conclusion

RAI2 is a novel inhibitor of breast cancer tumor cells dissemination in the BM, attenuating the vicious cycle of tumor crosstalk with the bone microenvironment. Results suggest that miR-135a-5p in EVs may suppress the development of bone metastasis, which needs to be further confirmed by future functional analyses.