AN IN VITRO MODEL FOR SCREENING THERAPEUTIC STRATEGIES TARGETING THE METASTATIC PROCESS: INTERCELLULAR CROSS-TALK, CHEMORESISTANCE AND MICRORNAS.

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Cancer cells manipulate their microenvironment to optimize conditions for growth and metastasis in multiple ways. Several evidences indicate that cancer cells communicate with each other and with non-neoplastic cells, via the release and delivery of miRNAs packed into exosomes, often inducing changes in targets on the recipient cells. We investigated whether exosomes are actively released by breast cancer MDA-MB-231 cells, the role of exosomal miR-210 in the cross-talk between primary cancer cells and metastatic cells and its contribution in regulating epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET). After 7 days of culture, a sub-population of breast cancer MDA-MB-231 cells spontaneously detaches the monolayer and starts to grow in suspension. The number of these cells progressively increases from the seventh to the fifteenth day in culture. These cells undergo epithelial-mesenchymal transition (EMT), display anoikis resistance and acquire a metastatic phenotype. Metastatic cells, in the presence of adherently growing MDA-MB-231, continue to grow in suspension whereas only if seeded in cell-free wells, are able to adhere again and to form E-cadherin positive and vimentin negative new colonies, suggesting the occurrence of mesenchymal-epithelial transition (MET). The chemosensitivity of MET cells to doxorubicin, paclitaxel and vincristin was diminished compared to adherent MDA-MB-231 cells. We also demonstrated that MDA-MB-231 secrete exosomes containing miR-210 and these exosome are taken up by metastatic cells where downregulate the expression of E-cadherin. When exosomes from adherently growing MDA-MB-231 culture media are administered to metastatic cells, the number of colonies formed by metastatic cells in cell-free wells, are significantly reduced. In conclusion, exosomes secreted by adherent MDA-MB-231 are taken up by metastatic cells where, through the delivering of miR-210, contribute to the downregulation of the adhesion molecule E-cadherin, inhibiting MET. Exosomal miR-210 modifies the adhesion dynamic of metastatic cells suggesting new molecular mechanisms involved in the dissemination of metastatic cells and chemo-resistance. This in vitro model is a useful tool for screening new therapeutic strategies targeting the metastatic process, for studying chemoresistance in EMT and MET cells and for exploring the potential of exosomal miRNAs as prognostic and predictive cancer biomarkers.