Project title: Exosomal miRNA profiling to depict mechanisms of drug resistance in triple negative breast cancer.
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Coordinator : Research Center for Functional Genomics, Biomedicine and Translational Medicine from „Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
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Specific objectives of the project:

The project has the following objectives:

1. MiRNA analysis in exosomes isolated from resistant and sensitive TNBC cell lines and CAFs (cancer associated fibroblasts), focusing on the miRNAs altered pattern in relationship with drug resistance.
2. Determination of the effect that CAFs exhibit on sensitive TNBC cells biology, showing the modulation of doxorubicin resistance through the transfer of CAF-derived exosomes and soluble factors.

WP1. Analysis of microRNAs in exosomes isolated from doxorubicin-resistant and sensitive triple-negative breast cancer cell lines and fibroblasts with emphasis on therapy resistance.

Activity 1.1 involves isolating exosomes from both doxorubicin-resistant and sensitive triple-negative breast cancer cell lines, as well as fibroblasts, using differential centrifugation (months 1 - 5).

Activity 1.2, the focus shifts to the physical and molecular characterization of exosomes obtained from doxorubicin-resistant and sensitive triple-negative breast cancer cells (months 5-6).

To perform these activities, culture medium collected from the specified cell lines and fibroblasts underwent ultracentrifugation. This process involved the successive removal of microvesicles through centrifugations and filtrations, resulting in the isolation of exosomes smaller than 200nm. Following ultracentrifugation, NanoSight and Transmission Electron Microscopy (TEM) were employed for physical characterization, revealing an exosome diameter of approximately 100 nm, consistent with existing literature, and showcasing a distinctive "cup-shape" morphology in microscopic images. For molecular characterization, the Western Blot

technique was utilized, confirming the presence of specific proteins, namely CD9, CD63, and CD81, within these exosomes.

WP2. Evaluation of the effect of fibroblasts on therapy-sensitive triple-negative breast cancer cells, showing modulation of doxorubicin resistance by transfer of exosomes and soluble factors secreted by fibroblasts (months 11-18).

Activity 2.1 entails the co-culturing of exosomes, secreted by doxorubicin-resistant triple-negative breast cancer cell lines and fibroblasts, with doxorubicin-sensitive triple-negative breast cancer cell lines (months 11 - 14).

Activity 2.2, a statistical analysis will be conducted on the data obtained from the co-cultures performed on triple-negative breast cancer cell lines (months 11 - 14).

Exosomes, characterized by their biocompatibility and biodegradability, play a crucial role in inter and intracellular communication. Their involvement in genetic material transfer from donor cells to target cells modulates the phenotype of the latter. Following isolation, physical and molecular characterization, the co-cultivation of exosomes with breast cancer cell lines allowed for the observation of internalization at the target cell level. Within 2 hours, a significant presence of exosomes in the cytoplasm of the target cells was noted, underscoring their facile internalization. Additionally, when administered to cells not treated with doxorubicin, exosomes induced changes in cell morphology, affirming their role in modulating the epithelial-mesenchymal transition process. Moreover, exosomes were found to be instrumental in influencing therapy resistance mechanisms, contributing to increased resistance in initially sensitive cells. As integral components of the tumor microenvironment, exosomes exhibit the ability to target cells within their vicinity or from distant sites.

Utilizing bioinformatics analysis, we identified genes implicated in therapy resistance by comparing microarray data from Hs578T/Dox and MDA-MB-231/Dox with their non-Dox counterparts, Hs578T and MDA-MB-231. To assess the clinical significance of these genes, we conducted an analysis on Doxorubicin-treated patients from TCGA BRCA 2018 (n = 314). Our investigation revealed that certain genes overexpressed (*CFB, CXCL1, CXCL2, CXCL3, DUSP1, EGR1, IL8, RRAD, TMEM88*) are associated with a poor prognosis in terms of disease-free range, while genes downregulated (*SHISA3*) are linked to a positive prognosis in terms of disease-free interval.

Considering the use of exosomes as a liquid biopsy modality, it's noteworthy that the expression profile of microRNAs within these entities is more stable and easier to assess than that of mRNAs. Employing miRTargetLink 2.0, we observed that three microRNAs—miR-1-3p, miR-335-5p, and miR-98-5p—target the most genes in this network (four genes). Interestingly, all three microRNAs target both CXCL2 and CXCL8. These findings suggest that these microRNAs could be potential candidates for evaluation from the plasma of breast cancer patients to predict the acquisition of resistance to doxorubicin therapy.