Project title: Exosomal miRNA profiling to depict mechanisms of drug resistance in triple negative breast cancer.
Project acronym: SUNRISE
Competition: P1 – National system development of CD – Postdoctoral research project (PD)
Code : PN-III-P1-1.1-PD-2021-0471
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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
Project manager : CSIII Dr Jurj Maria-Ancuța
Mentor: Prof. Univ. Dr. Ciuleanu Tudor Eliade

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.

WP3. Evaluating the altered non-coding transcrips (miRNAs) in plasma-derived exosomes isolated from TNBC patients as a marker of therapy resistance prediction (months 1-24)

Activity 3.1 – Patient's selection criteria & Ethical committee approval (months 1 - 12). This activity involved obtaining written informed consent from all participants. Patients aged 18 years and older were eligible for inclusion in the study.

The subsequent stages, **Activity 3.2. Human specimen collection (blood samples) (months 4 - 18)** and **Activity 3.3. Preparation of blood samples (months 4 - 20)** involves the collection of blood samples from both breast cancer patients and healthy subjects. Biological samples were collected using EDTA tubes, and plasma was extracted for exosome isolation. The process began with centrifugation at 4°C for 10 minutes at 4000 x g to separate plasma from other blood components. The obtained plasma underwent ultracentrifugation for 2 hours at 100,000 x g to isolate exosomes from microvesicles and apoptotic bodies. Subsequently, the isolated exosomes underwent physical characterization.

The final two activities, **Activity 3.4. Physical and molecular characterization of plasma-derived exosomes (months 20-22)** and **Activity 3.5. Validation of the most significant miRNAs in TNBC-derived exosomes using RT-qPCR (months 22 - 24),** focus on the physical and molecular characterization of exosomes. Physical characterization utilized NanoSight and

Transmission Electron Microscopy (TEM) to determine exosome size and morphology, including the characteristic 'cup-shape.' Following confirmation of exosome presence, RT-qPCR was employed to assess the expression levels of miR-19b, miR-21, and miR-125a. These miRNAs have been implicated in critical biological processes such as therapy resistance, reduced survival rates, metastasis, and TNM staging in breast cancer patients. Thus, they hold potential as prognostic and diagnostic biomarkers for breast cancer.