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
Financing contract nr. PD44/2022
Period : 01.01.2023-31.12.2023
Budget : 250.000 LEI
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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 1.3 Evaluation of altered level transcripts in triple negative breast cancer cells and fibroblasts, using the microarray technique (months 7 – 8).

This step is based on the evaluation of transcripts with altered levels following co-culture between triple negative breast cancer cell lines and fibroblasts. To identify the presence of these transcripts, the microarray technique was used, which provides an overview of 60,000 oligonucleotides. Following the bioinformatic analysis, transcripts with altered levels, overexpressed and downregulated, were identified, which are involved in the modulation of various cellular and molecular processes. Thus, on each experimental condition, the following were identified:

1. for the Hs578T cell line in co-culture with fibroblasts: 183 down-expressed genes and 486 over-expressed genes.

2. for the Hs578T cell line resistant to doxorubicin therapy in co-culture with fibroblasts: 417 down-expressed genes and 1405 over-expressed genes.

3. for MDA-MB-231 cell line in co-culture with fibroblasts: 1778 overexpressed genes and 10588 overexpressed genes.

4. for the MDA-MB-231 cell line resistant to doxorubicin therapy in co-culture with fibroblasts: 1927 down-expressed genes and 13853 over-expressed genes.

An increased number of genes with altered levels are involved in the activation of therapy resistance processes. Resistance to therapy, in oncological research, represents a major impediment due to the decrease in the survival rate of patients diagnosed with various forms of cancer. Thus, an increased number of research are carried out on elucidating and countering these mechanisms. Special attention is directed towards the ABC transporter family, genes involved in therapy resistance. In the list of identified genes, the presence of an increased number of genes belonging to this family was observed, such as *ABCB3, ABCB1, ABCC6, ABCC13, ABCD3, ABCB5, ABCD2, ABCD3*, etc. The overexpression of these genes leads to an increased risk of resistance to therapy. By activating the genes *CXCL11, ZEB1, TGF-β1, IL6,* MALAT1, the activation of the mechanisms of resistance to therapy are also supported by the alteration of various biological processes, such as the epithelial-mesenchymal transition, inflammatory processes, tumor invasion, metastases, which conclude by decreasing the rate of survival of patients diagnosed with cancer.

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 2.2 Involvement of fibroblasts and therapy-resistant triple-negative breast cancer lines in the activation of the resistance mechanism in lines sensitive to doxorubicin therapy. Evaluation of the most significant microRNAs and genes by RT-qPCR technique (months 15-16).

After identifying the genes with altered expression level, the next step was to identify the microRNAs that are involved in the modulation of the aforementioned genes. Along with these microRNAs, the presence of the tumor microenvironment helps to support and modulate the processes of resistance to therapy and metastasis.

By using fibroblasts, a significant increase was observed in the *ABCB1, CXCL11, ZEB1, TGF-β1, IL6,* MALAT1 genes. In this case, the presence of fibroblasts, important components of the tumor microenvironment, supports the formation of the tumor site. Moreover, extracellular vesicles (exosomes) and growth factors (cytokines) are present in the composition of the tumor microenvironment. These entities ensure inter- and intracellular communication, modify the phenotype of the target cell, and modulate various biological processes through the transfer of genetic material (microRNA, DNA). Through bioinformatics analysis, the presence of some microRNAs targeting *ABCB1, CXCL11, ZEB1, TGF-β1, IL6,* MALAT1 genes was identified, namely miR-19b-3p, miR-21-5p, miR-125a-5p, miR-155-3p. MicroRNAs are direct targets of many genes, genes involved in different signaling pathways, biological processes, responsible for the development of tumor cells. The RT-qPCR technique validated the expression level of genes with an altered level, genes identified by the microarray technique, as well as the profile of the 4 microRNAs, miR-19b-3p, miR-21-5p, miR-125a-5p, miR-155-3p.

The next step is the validation of these microRNAs on biological samples (plasma/exosomes), samples collected from patients diagnosed with triple negative breast cancer.