Final Report

Project 46PD/2018

Project Title: Molecular investigation of the exosomes-mediated interaction between stem and tumor cells (MIAMI)

The proposed objectives and their degree of achievement:

Stem cells derived from adipose tissue (hASCs) are stem cells of mesenchymal nature with beneficial properties for tissue engineering (TE) and wound healing, being currently considered a safe source for regenerative implants. However, there are a number of studies that have shown that hASCs (1) can respond to tumor signals by migrating from adipose tissue to a tumor site and that (2) they are susceptible to genomic instability and neoplastic transformation. Exosomes are nanovesicles that contain RNA and proteins and mediate intercellular communication.

In this context, the study hypothesis was that communication between hASCs and breast cancer-derived cells (BCCs) via exosomes, and this interaction could lead to a different response of hASCs during a healing / regenerative process by altering gene expression. and post-transcriptional mechanisms in hASCs.

The overall goal of the project was to investigate the exosome-mediated intercommunication between hASCs and BCCs, with the long-term perspective of testing the safety of using hASCs for stem cell-based therapies.

The general objectives were: (1) to investigate the exosomes secreted by hASCs and their miRNA content (phase 1/2018); (2) investigation of miRNA function derived from BCCs exosomes on gene expression and post-transcriptional mechanisms in hASC receptor cells (phase 2/2019) and (3) screening of non-coding RNA content in exosomes secreted by hASCs after interaction with BCCs (phase 3/2020).

Within the 46PD / MIAMI project, all the objectives and activities proposed according to the plan were achieved, both scientifically and at the level of the proposed result indicators. ***Regarding the scientific results obtained,*** a summary of each stage of the project is presented below.

The first stage of 46PD / MIAMI project was dedicated to the investigation of exosomes secreted by adipose tissue-derived stem cells (hASCs). These cells have the necessary properties and potential to be used successfully in medical practice for the purpose of tissue regeneration, being easy to isolate and having the ability to differentiate in all tissues of mesenchymal nature. In this constext, it is absolutely necessary to characterize the molecular mechanisms that take place in these cells, as well as the extracellular communication through exosomes. In this first stage we managed to optimize the microvessel isolation protocol from the hASCs culture. Subsequently, the microvesicles were characterized by electron microscopy and western blot to confirm their exosomal nature. The results indicated the obtaining of microvesicles with dimensions of 40-70 nm and expressing CD63 and CD81, molecules specific to exosomes. Next, the content of these exosomes was analyzed by isolating the miRNA fraction in order to identify miRNA species that condition the regenerative potential of hASCs. The isolated miRNAs were amplified by the miRNA PCR array, and the data were analyzed by specialized software to highlight miRNA species with a role in tissue regeneration. 10-12-fold increased expression in hASCs of miR-214-3p, miR-21-5p and let-7e-5p demonstrates the regenerative potential of hASC.

The second phase of 46PD / MIAMI project included the investigation of exosomal extracellular vesicles released by breast carcinoma tumor cells (from the MDA-MB-231 line) and the effects of the interaction of these exosomes with adipose tissue stem cells (hASCs). In the first phase, exosomes were isolated from tumor cells and validated by electron microscopy as extracellular vesicles measuring between 40 and 100 nm. The isolated exosomes were fluorescently labeled and then added to the stem cell culture system. The interaction of stem cells with exosomes from tumor cells was reflected at several levels: (i) at the level of gene expression, decreased p53 expression and increased telomerase expression were identified; (ii) at the posttranscriptional level - by activating miRNA species - miR-155-5p, miR-21-5p, let-7c, miR-96-5p, miR-210, miR-10b-5p and let-7b-5p .; (iii) at epigenetic level - by changes such as methylation and acetylation at histones H3 and H4. The obtained data were analyzed and integrated with specialized software and the obtained results were disseminated in 3 specialized conferences and an ISI article.

The third stage of 46PD / MIAMI project aimed to investigate extracellular vesicles of the type of exosomes secreted by adipose tissue stem cells (hASCs), after an interval of interaction with exosomes secreted by breast carcinoma tumor cells of the MDA-MB-231 line. After isolating the vesicles from the hASC culture medium, they were tested by electron microscopy and Nanoparticle Tracking Analysis (NTA) to validate their size (nm) and exosomal nature. The isolated vesicle fraction showed on average 150 nm, as well as specific exosomal markers. Subsequently, RNA was isolated from these exosomes and the miRNA and lncRNA fractions were analyzed to identify possible changes in the expression of non-coding RNA circulating in the exosomes, following the interaction with breast carcinoma cells. The interaction of stem cells with exosomes from tumor cells was reflected in the exosomal content by overexpression of miRNA species: miR-10a-5p, miR-93-5p, let-7i-5p, miR-206, miR-132-3p and miR -7-5p, as well as some lncRNA species: Malat1, Xist and Hotair. The obtained data were analyzed and interpreted with specialized software, and the obtained results were disseminated by publishing two ISI articles and a book chapter in the international publishing house.

Achieved results and their impact

The results proposed at the start of the project included the identification of non-coding RNA function in the hASCs-BCCs interaction and a conclusion on the safety of using hASCs for therapy, with a scientific basis at the molecular level.

In 46PD / MIAMI project, the following scientific results were obtained, having potential impact in research and medicine:

(1) Identification and validation of efficient methods for isolating extracellular vesicles from the culture medium of some cell cultures

(2) Characterization and validation of the exosomal nature of isolated vesicles by methods such as electron microscopy, western blot for specific exosomal markers or Nanoparticle Tracking Analysis (NTA)

(3) Development of an in vitro interaction model between adipose tissue isolated stem cells (hASC) and breast carcinoma cells from the MDA-MB-231 line, which would allow intercellular communication through exosome exchange

(4) Characterization of the molecular profile of non-coding miRNA-type RNA at the level of hASC with potential in tissue regeneration, as well as at the level of hASC after interaction with tumor cells, in order to identify differences

(5) Characterization of the molecular profile of non-coding RNA of long non-coding RNA (lncRNA) at the level of hASC with potential in tissue regeneration, as well as at the level of hASC after interaction with tumor cells, in order to identify differences

(6) Identification of epigenetic changes in hASC stem cells after interaction with tumor cells

(7) The evidence of changes that occur at the molecular level (transcriptional, post-transcriptional and epigenetic) in hASC stem cells and in the content of exosomes secreted by them after interaction with breast cancer carcinoma cells leads to the hypothesis of a susceptibility to transformation of these cells to transcriptional and post-transcriptional level, and implicitly at risks related to their use in regenerative medicine.

These results have an impact on the scientific and medical fields by improving knowledge in cancer research, tissue engineering based on mesenchymal stem cells, exosome-mediated intercellular communication, etc. A particular impact is the identification of changes that stem cells acquire at the transcriptional, post-transcriptional and epigenetic level after communication with tumor cells through exosomes, because it addresses the ethical aspects of using hASCs in medicine in stem cell-based therapies. The results of the project draw an alarm signal on the risks of using these mesenchymal stem cells in implants intended for tissue regeneration, without an advanced investigation of the changes that may occur at the molecular level.

Fulfillment of result indicators:

The result indicators ***proposed*** at contracting stage included: (1) the development of an experimental model of interaction between stem cells and tumor cells; (2) preparation of 3 stage research reports and a final activity report; (3) presentation of a project website; (4) dissemination of data at 2 conferences on the subject of the project and (5) publication of an ISI article.

Result indicators ***achieved*** in 46PD / MIAMI project:

(1) The experimental model of interaction between stem cells and tumor cells was developed, as described in the above scientific report.

(2) the 3 research reports for each stage were made and uploaded, as well as the present final activity report

(3) a website dedicated to the project was developed and updated at https://pd2016.wixsite.com/46pdmiami

(4) the data obtained in the project were disseminated at 3 national / international conferences on the subject of the project, as follows:

- ✓ **Dinescu S.,** Ignat SR., Balahura R., Selaru A., Costache M., Human adipose-derived stem cells display altered exosomal miRNAs profile after interaction with breast cancer cells, FSEV Congress, 14-15.10.2019, Nantes, Franta.
- ✓ **Dinescu S**., Zarnescu O., Costache M., Human adipose-derived stem cells produce exosomes that carry pro-regenerative signals, Al 11-lea Congres National de Biologie Celulara cu participare internationala si a 37-a Sesiune Stiintifica Anuala a Societatii Romane de Biologie Celulara, 20-23.06.2019, Constanta, Romania.
- ✓ **Dinescu S**., Chitoiu L., Fertig TE., Zarnescu O., Costache M., Isolation and characterization of the exosomes released by the triple negative breast cancer cell line MDA-MB-231, Conferinta Internationala a SRBBM 2019, 26-27.09.2019, Iasi, Romania.

(5) ***3 articles were published in ISI journals and a book chapter in the international publishing house:***

- ✓ **Dinescu S**., Ignat S., Lazar A., Constantin C., Neagu M., Costache M., Epitranscriptomic signatures in lncRNAs and their possible roles in cancer, *Genes* (MDPI ISSN: 2073-4425) 2019, 10(1):52. [ISI 3,331].
- ✓ Chitoiu, L.; Dobranici, A.; Gherghiceanu, M.; **Dinescu, S**.*; Costache, M. Multi-Omics Data Integration in Extracellular Vesicle Biology- Utopia or Future Reality? *International Journal of Molecular Sciences* 2020, 21, 8550. DOI: 10.3390/ijms21228550 [ISI 4,556].
- ✓ Dobre, E.G.; **Dinescu, S**.*; Costache, M. Connecting the Missing Dots: ncRNAs as Critical Regulators of Therapeutic Susceptibility in Breast Cancer. *Cancers* 2020, 12(9), 2698. DOI: 10.3390/cancers12092698 [ISI 6,126]

1 book chapter in an international publishing house:

- ✓ **Dinescu S**., Dobranici A., Tecucianu R., Selaru A., Balahura R., Ignat S., Costache M. (2020) Exosomes as Part of the Human Adipose-Derived Stem Cells Secretome- Opening New Perspectives for Cell-Free Regenerative Applications. In: Advances in Experimental Medicine and Biology. Springer, New York, NY; DOI: 10.1007/5584_2020_588

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