Romanian Academy

"Nicolae Simionescu" Institute of Cellular Biology and Pathology

PhD THESIS

STUDY OF THE PLASTICITY OF FETAL STEM CELLS WITH THERAPEUTIC POTENTIAL

Scientific supervisor:

Acad. Maya Simionescu

PhD student:

Andrei Constantinescu

Bucharest, 2013

CONTENTS

KEYWORDS

Fetal stem cells

Mesenchymal stem cells

Wharton’s jelly

Amniotic fluid

Human fetal vascular tissue

Cellular differentiation

Endothelial progenitor cells

Vascular recellularization

Flow cytometry

Cell sorting

ABSTRACT

This thesis **aims** at studying the plasticity of mesenchymal stem cells from fetal sources and evaluating the therapeutic potential of fetal stem cells and their implications in regenerative medicine. Thus, we wanted to investigate the differentiation ability of mesenchymal cells isolated from Wharton's jelly by endothelial progenitor cells and to evaluate the cardiovascular tissue regeneration potential thereof. To this end we intend to establish an *in vitro* model based on human fetal vascular tissue from umbilical cord arteries, in order to investigate the recellularisation potential of endothelial progenitor cells differentiated from fetal mesenchymal stem cells.

Also, we wanted to investigate the plasticity of mesenchymal stem cells from amniotic fluid by means of a thorough analysis of their ability to differentiate into endothelial progenitor cells, so that the therapeutic potential is assessed. To this end we wanted to investigate both immunophenotypic and transcriptomic profile of amniotic fluid mesenchymal cells but also the ultrastructural details of endothelial cells differentiated from mesenchymal cells in the amniotic fluid.

The thesis is divided in two parts. The first part, **Current state of knowledge**, is organized in three chapters describing aspects of stem cells (Chapter 1 of the *Current study of knowledge*) and the hierarchy of stem cells and their role (Chapter 1.1 of the *Current study of knowledge*) and their ability to differentiate (Chapter 1.2 of the *Current study of knowledge*). A description follows, concerning the types of cells depending on the source they are derived from and an overview of the isolation methods (Chapter 2 of the *Current study of knowledge*), and the last chapter is detailed using stem cells both in fundamental research and in pharmacological research and regenerative medicine (Chapter 3 of the *Current study of knowledge*).

In the second part of the thesis, **Original contribution**, the studies were divided in two chapters. In order to evaluate the potential for vascular recellularisation fetal stem cells isolated from Wharton's jelly (Chapter 1 of the *Original contribution*) I watched the isolation, characterization and differentiation of mesenchymal cells from Wharton’s jelly towards an endothelial line and the establishment of an *in vitro* model based on human fetal vascular tissue starting from umbilical cord arteries. Thus, we wanted to investigate the recellularisation potential of endothelial progenitor cells at both histological and immunophenotypic level.

The **results** obtained show the ability of mesenchymal stem cells in Wharton's jelly to differentiate towards endothelial progenitor cells with a real therapeutic potential in vascular regeneration. These cells could have a major impact in regenerative medicine due to their low immunogenicity and therapeutic potential.

In the study regarding the evaluation of the therapeutic potential of amniotic fluid cells (Chapter 2 of the *Original Contribution*), we aimed to achieve a complete characterization of these cells at an immunophenotypic, ultrastructural and molecular level. To this end we used flow cytometry to investigate surface markers, electron microscopy and real-time PCR technique (Section 2.3.1 of the *Original Contribution*). At the same time, we wanted to evaluate the potential of amniotic fluid cell differentiation by inducing the differentiation towards the endothelial cells (Chapter 2.3.2 of the *Original Contribution*). Also, I watched the determination of phenotype CD117 stem cells from amniotic fluid by positive sorting and immunophenotypic analysis of derived positive CD117 cell cultures (Chapter 2.3.3 of the *Original Contribution*).

The **results** show the ability of amniotic fluid cells to differentiate into mature endothelial cells in the presence of surface markers specific to these cells, but also in the presence of specific intracellular structures such as plasmalemmal vesicles and Weibel-Palade bodies. Moreover, we observed the immunophenotypical differences of CD117 positive cells in the heterogeneous cell population in the liquid, in order to establish relationships between positive CD117 and negative CD117 cells within the heterogeneous population.

To sum up, as regards the regenerative potential of cells isolated from Wharton's jelly, our results showed that decellularized human umbilical cord arteries can be used as an acellular matrix and they are a good experimental model *in vitro*. We have shown that EPCs differentiated from the Wharton's jelly MSCs can repopulate damaged/ decellularised vascular tissue outlined by endothelial phenotype expression of specific markers CD31+ / CD34+ / CD105+ / CD133+ at the level of recellularized arteries. The histology and flow cytometry data we obtained suggests that EPCs differentiated from MSCs of fetal origin have a high therapeutic potential through their ability of repopulating the damaged/decellularized blood vessels.

As regards cells isolated from amniotic fluid, we can conclude that these cells show an immunophenotype specific to mesenchymal stem cells, be means of expressing markers CD29 , CD90 and CD73 . Immunophenotype CD29+ / CD90+ / CD105+ / CD133+ / CD146+ of these cells suggests the presence of specific markers and other cell types, such as CD105 specific to endothelial cells and CD133 on the surface of hematopoietic stem cells and

endothelial progenitor cells. Despite the very high capacity of proliferating the cells in the amniotic fluid, their immunophenotype changes after 10 days in culture, thus becoming CD29+ /CD90-/CD105-/CD133-/CD146. At a molecular level, AFC sites show both mesenchymal stem cell markers such as ACAN, BGLAP, COL2A1 and PPARG, and embryonic stem cell-specific transcriptomic markers such as OCT4, REX1 or SOX2. One should note the presence of specific markers such as hematopoietic stem cells CD3D, CD4, CD8A or MME and neural stem cell-specific markers such as NCAM1, SIGMAR1 and S100B. These results lead us to the assumption that there is a limited number of specific markers at the level of stem cells. This phenomenon could be correlated with the differentiation potential, in response to external stimuli by the existence of “primer” molecules that lead the differentiation depending on the applied stimuli.

Also, AFCs show the capacity of differentiation towards the endothelial line. These cells can differentiate into cells with immunophenotype specific to mature endothelial cells, as shown by the flow cytometry technique, by the presence of endothelial markers CD31, CD105 and CD144. An important novelty is the ultrastructural analysis of AFC differentiated sites that showed the presence of intracellular structures specific to endothelial cells, such as plasmalemmal vesicles and the Weibel-Palade bodies. Thus, not only do these cells have the ability to be guided by endothelial line, but they may also become mature endothelial cells with the structures required for a possible fulfillment of their functions, such as, for example the plasmalemmal vesicles that may be involved in the processes of transcytosis.

As future **perspectives** we want to investigate the ability of amniotic fluid cells to repopulate damaged /decellularized blood vessels. In addition we aim a using the positive CD117 stem cells in a co-culture with endothelial progenitor cells isolated from cord blood, in order to investigate their regeneration effect, without the use of growth factors and the *in vitro* differentiation, in order to support a possible therapeutic strategy with clinical applicability.