Abstract

The Habilitation Thesis ***"Stem cell therapies for ischemic muscle regeneration"*** reflects the activity of the author, performed between 2006 and 2016, together with the perspective for the evolution and development of the professional, scientific and academic career, the research pathways, targets, and practical applications.

The first section of the thesis details original contributions obtained so far in the field of ***stem cell biology and regenerative cell therapies***.

Alexandrina Burlacu performed studies in three main field-related topics:

1. Effects of DNA demethylation agents on the stem cell multipotency;

2. Characterization of various populations of stem cells and their potential for tissue regeneration by cell therapy;

3. Regenerative therapies of ischemic muscle using adult stem cells.

The first chapter describes the studies performed with the aim of inducing differentiation of adult stem cells into cardiomyocytes based on the ability of the de-methylation agent 5-azacytidine to remove the inactivation of some determination loci that are transcriptionally inactivated, allowing induction of differentiation of stem cells into multiple phenotypes. These results have been published into 4 articles (Arch Physiol Biochem. 2006; Eur J Cell Biol. 2008; Ann Rom Soc Cell Biol. 2010; Stem Cells Dev. 2011) and in principal they show that: (i) mesenchymal stem cells (MSC) retained multipotency after one pulse with 5-azacytidine; (ii) additional pulses with 5-azacytidine resulted in a restricted differentiation potential of MSC, with a concomitant increase in their ability to undergo chondrogenic differentiation; (iii) 5-azacytidine promoted the expression of muscle-specific proteins and genes in MSC, however this was not sufficient for MSC differentiation to CMCs; (iv) 5-azacytidine fosters initial myogenic differentiation of MSC over a relatively short period of time.

The second chapter describes studies focused on embryonic, foetal and adult stem cells and provides insights into some practical aspects of their derivation, purification methods and differentiation potential. The results were published into 4 manuscripts (Biotechnol Appl Biochem. 2010; Tissue Cell 2013; Annals Rom Soc Cell Biol. 2015; J Tissue Eng Regen Med. 2016). In summary, data show that: (i) MSC with multipotent abilities represent a valuable source of stem cells for regenerative medicine and can be successfully derived from mouse bone marrow in culture without losing their potential; (ii) 3D aggregation of MSC induces changes which impact the resistance and behaviour of cells post-transplantation but do not affect their multipotency; (iii) a progenitor cell population can be obtained from bone marrow by simply exploiting the physical properties of these cells; (iv) endothelial progenitor cells (EPC) secrete VEGF-A and SDF-1, which have partially-overlapping functions i.e., the absence of either one from EPC-conditioned medium could be rescued by the presence of the other one, so that the overall angiogenic effects remain unchanged.

The third chapter focuses on the mechanisms of CMC death after myocardial infarction, mechanisms of MSC engraftment into the ischemia-damaged tissues after transplantation, as

well as on the paracrine effect of MSC on ischemic muscle regeneration. The results have been published into 7 manuscripts (Comp Med 2010; Ann Rom Soc Cell Biol. 2010; Stem Cells Dev. 2013; Cell Biol Int 2012; Stem Cells. 2014; Biochem Biophys Res Commun. 2015; Biomaterials. 2015; J Tissue Eng Regen Med 2016), one editorial (Curr Stem Cell Res Ther. 2013) and one patent (A/00845-2013). Briefly, the data show that: (i) Exogenous oxidants at the time of reperfusion have a major role in completing the apoptotic process associated to myocardial ischemia-reperfusion; (ii) Stimulation of MSC with FGF-2 prior to transplantation may facilitate their access among the myocardial cells and increase the functional coupling between transplanted and host cells; (iii) MSC and EPC exert several distinct paracrine effects on vascular endothelial cells (EC) and neither one by itself may simultaneously support cell adhesion and proliferation. Since their effects are complementary, the combination of these two progenitor cell populations, or the factors they secrete, has a profound positive effect on EC behaviour in vitro and may successfully stimulate the angiogenesis in vivo, after transplantation; (iv) concurrent transplantation of endothelial colony forming cells (ECFC) and soluble factors released by circulating angiogenic cells (CAC) increase in situ retention of transplanted cells into the ischemic tissues and promote vascular maturation of newly-formed vessels; (v) Subcutaneous transplantation of MSC induces remote cardioprotection against ischemia. Transplanted cells proliferate at the site of injection with no detectable migration and secrete molecules that are possibly involved in an endocrine/paracrine fashion. These results unravel new facets regarding the behaviour of MSC after in vivo transplantation and may contribute significantly to the development of cell remote transplantation as a viable option for ischemia-reperfusion injury and other clinical applications.

The second section of the thesis presents current international collaborations of the author and her future research directions. Thus, for the next couple of years, the author's aim is to exploit the versatility and potential of MSC to reduce inflammation for consolidating a clinically-relevant cell therapeutic approach to cure diabetic autoimmunity by targeted delivery of apoptotic signals using MSC as vehicles. To this aim, a systematic approach will be undertaken to consolidate a pragmatic pre-clinical therapeutic strategy that integrate three mechanistic steps based on unique principle: use of killer MSC (genetically modified so that to transiently express TNF death ligands on their surface) in conjunction with maximal immunomodulation and minimal lymphoreduction.