



Research Project Proposal
Academic year 2019-2020

Project Nº 09	
Title: <i>Project title Cell competition as a strategy to increase interspecies chimerism</i>	
Department/ Laboratory <i>Programa de Medicina Regenerativa, lab. 1.01, CIMA</i>	
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<p>Summary Blastocyst complementation is an extraordinarily promising approach to generate humanized organs in pigs, that could be used for therapeutic xenotransplantation, therefore solving the global problem organs' shortage for transplantation and as an exceptional model for drug toxicity test. Nevertheless, to be able to generate such organs we need to improve the interspecies chimerism upon PSCs microinjection into pre-implantational embryos.</p> <p>Cell competition is a natural process that takes place in the early embryo development for the selection of the epiblast cell pool. This process is mediated by relative cMyc levels: cells expressing higher levels of cMyc have higher fitness and proliferate, while cells presenting lower levels of cMyc enter in apoptosis and die. The aim of this TFM project is the use of this system to improve rat-mouse inter-species chimerism. For that purpose, we will conduct microinjection experiments with WT and Myc overexpressing rat PSCs in preimplantational mouse embryos derived from Myc^{+/-} crossings. Rat cells will be modified with the CRISPR-Cas9 system to introduce a single copy of Myc in the Rosa 26 locus, therefore inducing a constitutive basal expression of Myc in rat PSCs. Microinjected embryos will be then transferred to the uterus of foster mothers to complete their development until E9.5 and then the level of interspecies chimerism will be analyzed by FACS analysis and histology, as rat cells are genetically marked with a GFP reporter. Additionally, if the chimeric potential will be increased with this system and if the time will allow, Myc overexpressing cells will be microinjected in vascular- and cardiac- deficient mice models to achieve interspecies blastocyst complementation. In this way, we aim to create rat heart and vascular system inside a mouse. This project will be the base for future application of this system in human-pig model.</p> <p>The master thesis student will have the opportunity to learn several techniques: stem cell culture, FACS analysis, histological analysis, genotyping, embryo handling and others. Therefore, we are looking for a very motivated student</p>	
yes	<input checked="" type="checkbox"/>
no	<input type="checkbox"/>
Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?	