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The review on doctoral thesis of Bianka Porubská.

The title (English): Comparison of the immunomodulatory properties of mesenchymal stem cells and Sertoli cells.

The title (Czech): Porovnanie imunomodulačných vlastností mezenchymálnych kmeňových buniek a Sertoliho buniek.

The main focus of the doctoral thesis of Mgr. Bianka Porubská was to address a new approach towards targeted cell therapies, particularly for specific pathologies to be utilised in human medicine. The goal of the study was to investigate an immunomodulatory potential of Sertoli cells (SCs) based on the hypothesis and recent scientific knowledge, that SCs possess immunomodulatory properties similar to mesenchymal stem cells (MSCs). SCs occupy testes, the specialised male reproductive organ, which is due to a blood testis barrier challenging for many standard medical interventions. For this reason, the SCs represent a hope towards treatment of inflammatory pathologies that stand behind several infertility issues and SCs therapy could result in an increase of sperm quality and rescue of male fertility.

The general outcome of the thesis delivered valuable findings showing ability of SCs to differentiate *in vitro* into mesenchymal cell lineages particularly to osteocyte, chondrocyte, and adipocyte, which was comparable with the ability of MSCs and provided evidence for SCs mesenchymal origin. Further, the impact of SCs as well as MSCs on activated immune cells *in vitro* resulted in immunosuppression and the details regarding the specific immune competent cells are described and discussed in the thesis and published literature.

Among other discoveries, there was shown SCs effect towards M2 macrophage induction, supported by the fact of strong up-regulation of CD206 molecule. Furthered, a unique ability of SCs facilitated a mitochondrial transfer to immune cells, which is in relevance to their MSCs ability towards the immune modulation.

The SCs therapeutic potential on testis inflammation treatment resulting in an increase of sperm quality was tested in elegant way, employing *in vivo* examination of SCs ability to suppress inflammation using mouse model of LPS-induced acute testicular inflammation, followed by SCs transplantation. In the results, SCs application brought the phenotype closer to wild-type healthy controls and sperm parameters, such as sperm count and sperm motility were preserved in animals treated with SCs supporting the original hypothesis that SCs possess a MSCs-like immunomodulatory properties, which are manifested mainly by suppressing a different type of inflammatory response. The results show that in LPS-induced inflammation, SCs transplantation had a positive effect on maintaining ongoing spermatogenesis and resulted in an increased sperm motility, as well as in decrease of specific leucocytes and immunoregulatory molecules.

The overall quality of the presented text is of high level and the individual chapters of the thesis are well balanced. I would like to compliment of the quality of English, as that is of top standards. In more specific way, the Abstract summarises very well the scientific goal and presents the achieved deliverables. The Introduction and individual scientific chapters target the topic of the thesis and are well supported by selected figures. I would probably expect chapters 2, 3 and 4 to be subchapters of the Introduction, but it is probably just the matter of individual taste. Similarly, I would prefer the chapter Publications to be the last chapter of the thesis, as the publications reflect the published results and the chapters such as Rationale and Objectives, Experimental Design and Methods, Results and Discussion would logically follow the introductory text. This order does not decrease the quality of presented text, just from my point of view overcomplicates it. The conclusions bring the key findings together as a list of discoveries.

The findings of the presented work have been summarised in four scientific publications in high quality peer-reviewed journals with an impact factor, such as *Clinical Science* IF 6.876; *Stem Cell Reviews and Reports*, IF 6.692; *Xenotransplantation* IF 3.788, when Mgr. Bianca Porubská is the first author in one of them, published in *Stem Cell Reviews and Reports*, (2021). Due to the fact that all the publications have been reviewed by experts in the field, I do not feel necessary to discuss them further. Moreover, the Discussion chapter covers all parts of the topic and retains the logical order.

Before few scientific questions, I would like to compliment on work that has been done and the delivered findings, which are of high merits. In conclusion I would like to stress the convincing data presenting SCs beneficial effect during acute phase of inflammation of testis and their ability to promote or rescue male fertility particular in terms of ongoing spermatogenesis and sperm motility after bacterial infection. The SCs therefore represent potential therapeutic agent and their targeted transplantation could be considered as clinical applications in personalised medicine.

I recommend to the scientific board members, to consider the presented thesis of Mgr. Bianca Porubská substantial to obtain a doctoral degree.

Kateřina Komrsková

At the end I would like to ask following technical and scientific questions:

Q1: I would like to ask, why the Experimental Design and Methods were divided into a single subchapter chapter 6.1. and extended as a chapter 7? I would expect to be more appropriate to have a chapter 7 as chapter 6.2.?

Q2: I would like to ask, why the rest of publications were not included in the text? I would believe that they should be part of the thesis, particularly, that the defender is the co-author and states her contribution to the individual articles.

Q3: The SCs transplantation improved sperm motility. Could you please elaborate on the individual motility parameters? Could you please show additional data, and describe the importance of changes of motility patterns. How is this relevant towards the fertilising ability of sperm?

Q4: Mitochondria transport is important parameter in many physiological processes, including tumour growth and survival or wound healing. SCs are critical somatic cells maintaining the spermatogenesis including cytoplasmic communication and molecular regulation. Could you please specify the detected mitochondria transfer and molecular mechanism behind? If that is not known, could you hypothesise what kind of mitochondria transport would be involved in your studied model.

Q5: Translational science has many challenges. Have you considered human studies? Do you have any data in men? If not, could you compare the mouse and human system, with relevance to your topic and draw some future steps how to proceed?