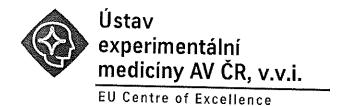# OPPONENT REVIEW

The thesis of M.Sc. Rishikaysh Pisal summarizes the results of his scientific work done at the Department of Histology and Embryology, Medical faculty Hradec Kralové. Mr Pisal has been working there for several years under the guidance of prof Mokry. The aims of the study was to derive and characterize iPSCs from natal DPSC and to differentiate them towards myogenic progenitors. He also optimized protocol for mycoplasma detection and prepared a vector optimized for microRNA expression. The study is based on 3 publications, where MSc Pisal is first author and 1 publication, which he co-authored. The topic of his study is important, since induced pluripotent stem cells plays an important role in regenerative medicine, not only as a tool for personalized medicine, but also for drug screening and toxicity testing mimicking early development stages or being differentiated into specialized cells (cardiomyocytes or neurons). Therefore it is important to search for suitable, cheap and accessible cell source as well as new efficient methods for cell reprogramming. As an interesting source for cell reprogramming was taken natal tooth and from that isolated DPSC. Obtained results support the hypothesis and objectives of the study. It was shown that natal DPSC derived iPS are pluripotent, express similar genes as hES and can differentiate into myogenic progenitors. This is the main outcome of the study, though new protocol for mycoplasma detection was optimized and suitable vector for miRNA expression was constructed.

The study is divided into Introduction, Methods, Results, Discussion and Conclusions. Introduction summarized the history of pluripotent stem cell research, derivation methods, the sources with its pros and cons. I would appreciate more figures or images in this part of the study. The weakest point of the whole study is the section Methods, where some of the paragraphs are very detailed, but on the other hand, some methods are lacking description at all (FASC, EB for immunohistochemistry were harvested only 21 DIV instead of 9 and 14, only three cell lines are mentioned instead of 6 i.e. none of the adult DPSC line is described and characterized, list and some classification of selected 83 genes is missing,). Also the list of abbreviations is very incomplete and many abbreviations are missing.

Chapter Results describes the major outcomes of the experiments. Unfortunately the overall quality of figures showing immunohistochemical staining could be better (more details with higher magnifications, showing also the cell morphology). Chapter Discussion discuss the expected and unexpected results, however, some comparison with similar studies in the field would be beneficial.

I have some comments and questions:

What is the difference between MSC isolated from dental pulp and DPSC and why did you choose iPS derived from 2 fibroblast lines for characterization and comparison and not iPS derived from adult DPSC. This would depict more the similarities and differences between adult and natal DPSC.

How did you assess your transfection? Was the viral vector marked with fluorescence tag? Since the WI38 line was less affected by transfection toxicity and the efficiency was the lowest, are you sure that the Sendai vector transfected the cells?

What is your explanation of the fact that gene expression pattern is similar in iPS-DPSC and WI38 and more different from iPS-HF?

Regarding the myogenic differentiation, what would be the values of positive control (gene expression in embryonic or adult myoblasts) and did you perform any electrophysiology measurements to see some of the physiological properties of the myoblasts?

What is your opinion on direct reprogramming in vitro and in vivo?

What is your opinion on Wharton Jelly MSCs as a source for cell re-programming.

Finally, I can conclude that M.Sc. Pisa is able to perform scientific work and I recommend his thesis for the defense.

Prague, 30th May 2018   Doc. RNDr. Pavla Jendelová, PhD.