In Pilsen, 13th February 2024

The review of doctoral thesis *"Mechanotransduction of Hepatic Cancer Cells cultured in a 3D Collagen Scaffold" written by Ph.D. applicant Adam Frtús, M.Sc.*

TOPIC AND SIGNIFICANCE OF THE WORK

The interplay between cells and the surrounding extracellular matrix (ECM) is crucial for both physiological and pathological performance of each tissue. In tumors, the changes in intercellular contacts and cell-ECM connections reflect the inner ongoing processes. To mimic the 3D environment of tissues in *in vitro* research belongs to highly growing scientific area mining from wide interdisciplinarity. This doctoral thesis combines the material engineering, cellular biology and advanced microscopic techniques together to contribute the understanding, how liver tumor cells could react on the impulses coming from their extracellular environment. The doctoral thesis explores the role of mechanical signals coming from *in vitro* culture substrates in the behavior of cells cultivated within these substrates. Particularly, two commonly used human cell lines of hepatic carcinoma (HepG2 and Alexander) were cultured in 3D collagen scaffolds (CS) and on 2D standard glass-bottom dishes (MC) for 7 days, and the selected cellular responses on mechanical impacts originating either from collagen 3D matrix or from rigid glass surface were evaluated. The proliferation and cell plasticity and related signaling pathways, organization of cytoskelet concerning the cytoskeletal proteins and adhesion molecules or mitochondrial functions represent the cellular processes analyzed and compared in respect to stiff 2D and soft 3D matrix.

METHODOLOGICAL APPROACHES

The doctoral thesis utilizes *in vitro* models of human hepatocellular carcinoma cells cultured on both stiff 2D glass bottom dishes and soft 3D collagen scaffold. It combines the engineering of scaffold, structural and mechanical analysis of scaffold, photobiomodulation of cells by low power laser, immunoblot of cellular extracts, qPCR (for human liver samples), fluorometric analysis of pyruvate and lactate content, and advanced confocal microscopy including quantitative analysis based on ImageJ macros. The statistical analysis of experiments includes the sample size determination utilizing previous literature recommendations.

FORMAL EVALUATION

This doctoral thesis has all obligatory parts and is very synoptic and well-arranged. The experimental part is based on two first-author publications of the applicant. The introduction starts with basic biology of the cell, introduces principle of mechanotransduction and YAP signaling pathway, continues with liver morphology, function and pathology and goes over collagen connective tissue in liver, and finally focuses on the summary of 3D culture systems for hepatocytes. The aims of the work are clearly stated. The detailed description of all used experimental methods and results follows. The results are expressed by thematically orientated and very well elaborated figures with clear and detailed legends. The discussion goes systematically through all reached results and compares them with relevant literature sources. The thesis is finalized by concise conclusion. The lists of abbreviation, bibliography, figures, tables and publications is included. The appendix of this thesis contains two topic related first-author publications of the applicant and the applicant contributions to the work are declared. The schemes and some figures are created by BioRender.com. and look professionally.

PUBLICATION ACTIVITY

The publication activity of the Ph.D. applicant is excellent. The applicant is the first-author of 4 publications and the co-author of 5 publications. The total number of citations was 147 (November 2023) and H-index of the applicant is 6 (acc. Scopus).

FINAL EVALUATION AND DECISION

The doctoral thesis covers the actual topic of the role of mechanical signals in hepatic tumor cell behavior and the applicant utilizes engineered 3D collagen scaffolds and especially advanced microscopic techniques to explore the consequences between mechanics of culture substrates (soft 3D vs. stiff 2D) and possible tumorigenic phenotype of *in vitro* cultured hepatocytes. The doctoral thesis is very well organized, with concise content and illustrative both theoretical and experimental figures. The applicant exhibits already advanced publication activity (H-index 6) and is the first author of 4 publications and the co-author of 5 publications. **The applicant reaches all obligations and requirements needed for Ph.D. degree, and I recommend to acknowledge the Ph.D. degree to Adam Frtús, M.Sc.**

QUESTIONS TO THE APPLICANT

1) HepG2 and Alexandra cells are tumor cell lines with slightly different morphological and functional features compared with primary human hepatocytes (PHH). Did you consider to perform your experiments also with PHH (isolated by your team or commercially provided)?
2) The cell lines were seeded on the top of the scaffolds. From attached videos is evident, that cells populated not only the surface, but also the inner pores. Which depth did the cells reach? Did you consider to encapsulate cells into 3D scaffold during its preparation procedure to get cells into total volume of scaffold?
3) You observed and interpreted the different morphology of cell lines when attached closely to collagen fibers and when located in the center of pores of 3D collagen scaffold (Figure 13). Did you consider to analyze also the intercellular junctions and cell polarization typical for hepatocytes including the formation of biliary capillaries at apical pole of cells? It makes sense to expect that the cells in centers of pores will be stimulated to keep their epithelial phenotype and, on the other hand, the cells attached to collagen fibers will be stimulated to lost this phenotype and to switch to some like mesenchymal phenotype.
4) Did you evaluate the sufficient distribution of nutrition (glucose/O2) to deeper located cells in 3D scaffolds? The decrease of proliferation, mitochondrial activity and expression of Bcl-2 could be also affected by the lack of nutrition in deeper located cells in the zones called “necrotic zone” or “necrotic core” (e.g. https://doi.org/10.3390/mi9030094).
5) Additional, optional question: What would you do in different way if you would have the chance to repeat your experiments performed within your doctoral studies?

Thank you for your responses to my questions and good luck in your further scientific career.

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