Swissliver Annual Board Meeting Report – Selicean Sonia-Emilia, PhD candidate

During this past year I have further studied the transcription factor ETS-related gene (ERG) and contributed to other projects in our group, as well as external collaborations.

Chronic liver disease (CLD) is characterised by profound liver sinusoidal endothelial cells (LSECs) dysfunction. Among the many stimuli participating to LSECs alteration, mechanical forces and inflammation play a leading role. ERG is an endothelial-specific transcription factor, involved in maintaining endothelial cell quiescence and homeostasis in adult vasculature and may be involved in the development of LSECs dysfunction. Little is known about ERG modulation and function during the development of CLD. In the past, we and others have demonstrated that the transcription factor ERG is downregulated in human cirrhotic tissue in a small cohort of patients from the Inselspital. Moreover, using an RNA-Seq based experiment, we showed that increased matrix stiffness enhances pathways related to inflammation in LSEC. In this context, we wanted to understand if the drivers of CLD progression, stiffness, and inflammation, could be responsible for ERG downregulation in CLD.

For these purposes, we used a cirrhotic rat models developed in male Sprague-Dawley rats by intraperitoneal thioacetamide injections (TAA) for 12 weeks, followed by a week of rest before further use. LSECs were freshly isolated by gradient centrifugation. Human umbilical vein endothelial cells (HUVECs) were used as human endothelial cell model. Cells were exposed to high (30 kPa) or low (0.5 kPa) stiffness polyacrylamide (PAA) substrates. Interleukin 1β (IL1β) was used as in vitro inflammatory stimulus. Immunofluorescence (IF) was performed on healthy and cirrhotic human and rat livers, and IF, Western Blot and qRT-PCR were performed on LSECs and HUVECs. ERG knockdown RNA sequencing data was obtained from the Gene Expression Omnibus database. Functional enrichment analysis was performed using the Panther software.

Human LSECs transcriptomic analysis from a database previously published by our group disclosed that ERG was among the top downregulated genes and the most downregulated transcription factor (FC=-102, $p<0.05$) in cirrhosis. A further IF performed on a new cohort of cirrhotic versus healthy human livers confirmed the downregulation of ERG in cirrhosis. In vitro, HUVECs showed ERG downregulation in response to 96h of exposure to increased stiffness. Moreover, IL1β treatment for 24 h also induced ERG downregulation under healthy stiffness conditions (0.5 kPa), but no further downregulation on the stiff substrate (30 kPa). In contrast, in the cirrhotic rat models (TAA and CCl4), ERG was less significantly downregulated, and freshly isolated rat LSECs displayed only mild, but significant downregulation of ERG in response to high substrate stiffness. When analysing our human cirrhotic LSECs transcriptomics dataset in conjunction with the publicly available ERG KD datasets, overlapping differentially expressed genes were involved in pathways related to angiogenesis, cell migration and cell-substrate interaction.

Given the less important response of ERG in rat tissue and isolated cells, future experiments will be planned on human samples only. We have established a collaboration with a hospital in Cluj-Napoca, Romania, and obtained samples from a larger cohort of well characterised CLD patients, whose liver ERG expression will be assessed and correlated with disease severity, portal pressure and liver stiffness, to understand the relationship between the degree of ERG downregulation and disease stage. These results will be submitted for publication during this year.

Besides work on the ERG transcription factor, I have contributed to the other project of the group concerning the small mechanoresponsive molecule, CIB1, by performing several experiments: immunofluorescence on freshly isolated rat LSECs in order to assess CIB1 behaviour in response to high stiffness, isolation of LSECs from healthy or cirrhotic rats, which were used in knockdown and RNA sequencing experiments and data analysis, such as Gene Set Enrichment Analysis. The results of this project will also be submitted for publication during this year.

Moreover, I have performed experiments and contributed to other projects of the group as well as to external collaborations, which resulted in a co-authored paper, currently submitted for publication, also listed below.

Publications:

Felli E, Nulan Y, **Selicean S**, Wang C, Gracia-Sancho J, Bosch J. (2023). Emerging Therapeutic Targets for Portal Hypertension. Current Hepatology Reports 2023, 1, 1–1

Selicean S, Wang C, Guixé-Muntet S, Stefanescu H, Kawada N, Gracia-Sancho J. Regression of portal hypertension: underlying mechanisms and therapeutic strategies. Vol. 15, Hepatology International. Springer; 2021. p. 36–50.

Guixé-Muntet S, Ortega-Ribera M, Wang C, **Selicean S**, Andreu I, Kechagia JZ, et al. Nuclear deformation mediates liver cell mechanosensing in cirrhosis. JHEP Reports. 2020 Oct;2(5):100145

Skorup I, Valentino G, Aleandri S, Gelli R, Ganguin AA, Felli E, **Selicean SE**, Marxer RA, Gracia-Sancho J, Berzigotti A, Ridi F, Luciani P. Polyenylphosphatidylcholines as bioactive excipient in tablets for the treatment of liver fibrosis. - Submitted to International Journal of Pharmaceutics

Felli E*, **Selicean S***, Guixé-Muntet S, Wang C, Bosch J, Berzigotti A, Gracia-Sancho J. Mechanobiology of portal hypertension. – Publication in progress in JHEP Reports

Wang C*, Felli E*, **Selicean S***, Nulan Y, Lozano JJ, Bosch J, Berzigotti A, Gracia-Sancho J. Novel insights on liver endothelial mechanobiology in cirrhosis: role of calcium integrin-binding protein 1. – in submission

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