Hepatic Stellate Cells and Hepatocytes – Raman-Based Imaging and Differentiation

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Hepatocytes and hepatic stellate cells (HSCs) are two cell types in the liver which play an important role in the course of liver fibrosis. The cell-cell interactions which lead to the morphological and functional changes are only incompletely understood. Elucidation of those interactions requires a method suited for investigations in a multi-cell environment.

Raman spectroscopy holds the potential to observe physiological changes in single cells in complex surrounding. However, reliable Raman-based identification of a cell type requires deep knowledge of the vibrational signature of the cells of interest. HSCs need special attention since they undergo dramatic phenotype alterations once they change from their quiescent to the activated state. Activation of the HSC is observable in the course of fibrosis but also during prolonged cell culture. Accompanying changes are expected to be reflected in the Raman spectra.

Against this background, Raman spectroscopy was utilized to characterize primary HSCs and hepatocytes obtained from mouse liver. Raman maps of formalin-fixed cells were acquired at different time points ranging from 0 to 14 days after isolation to study activation-related changes in HSC spectra. HSC activation state was confirmed by immunofluorescence labeling after Raman measurements. First experiments were carried out to identify HSCs in fresh mm-slices from mouse liver.

An upright micro-Raman setup (CRM 300, Witec) was used for spectra acquisition. A 532 nm Nd:YAG laser served to probe fixed cells, a 785 nm laser diode for excitation within living samples. Raw spectra received a pre-treatment containing spike removal, noise reduction, baseline subtraction and normalization. Vertex component analysis followed to derive compartment-connected information from fixed single cells. Lipid droplets, nucleus and cytoplasm could be visualized. Differing Raman spectroscopic characteristics of HSCs and hepatocytes were found. Especially, spectra obtained from quiescent HSCs contain a retinol signature which is not present in hepatocytes. Upon activation, the retinol content in HSCs decreases until no retinol could be detected in remaining lipid droplets any more. Cultured HSCs and hepatocytes could be successfully distinguished based on the Raman spectra belonging to nucleus or lipid droplets by principal component analysis. In fresh tissue slices HSCs could be delineated against their surrounding by their spectral retinol signature.

Future work will be focused on stimulation-triggered overall biochemical changes in the course of HSC hepatocyte interaction.

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