DESCRIPTION FOR THE GENERAL PUBLIC

Fibrosis is a pathological condition of the unbalanced extracellular matrix remodelling, estimated to contribute up to 45% of all deaths in the developed countries. Diseases of the exocrine pancreas represent some of the most dramatic examples of tissue fibrosis with deadly implications. In pancreatic cancer, fibrotic stroma severely limits the delivery of drugs to the tumour, directly protecting it against chemotherapy. In chronic pancreatitis, a necrotising disease of the pancreas, living components of the tissue become destroyed and replaced by a fibrotic scar. Although the vast majority of the exocrine pancreas consists of enzyme storing pancreatic acinar cells (PACs), pancreatic fibrosis is attributed to pancreatic stellate cells (PSCs), which constitute as little as 4-7% of all cells in the organ.

Normally quiescent and seemingly redundant, pancreatic stellate cells become activated upon tissue damage or in response to inflammatory mediators, such as TGF-β. Although these star-shaped cells are clearly required for tissue repair, in pancreatic disorders activated PSCs become "the villains" engaged in the overproduction of extracellular matrix components that replace live pancreatic tissue leading to dysfunction of the organ.

Excessive alcohol consumption, one of the most serious global problems of our modern society, is the major cause of chronic pancreatitis and pancreatic fibrosis. The products of alcohol metabolism, reactive oxygen species (ROS) and fatty acid ethyl esters (FAEEs), were shown to induce pathological Ca^{2+} signals in PACs, triggering premature activation of digestive enzymes in these cells, followed by autodigestion of the tissue, necrosis and inflammation. Our preliminary data show that alcohol metabolites also induce pathological Ca^{2+} signals in PSCs and that there is an astonishing reduction of these signals upon PSC activation with TGF-β. This resistance to alcohol metabolites is at least partially due to the shifts in the components of intracellular Ca^{2+} homeostasis upon PSC activation. The main goal of this project is to investigate the alterations in physiology of quiescent *vs* activated PSCs and to understand what causes those differences.

To achieve this, we plan to conduct a series of real-time intracellular Ca^{2+} measurements to compare Ca^{2+} signals induced by alcohol metabolites in quiescent and activated PSCs. This will be followed by analysis of shifts in Ca^{2+} homeostasis, i.e. comparing the intracellular Ca^{2+} store content and the mechanisms of Ca^{2+} fluxes across the plasma membrane in activated PSCs.

Since Ca^{2+} homeostasis is tightly associated with the cellular redox balance, mitochondria and cell death, we will conduct a series of experiments to compare mitochondrial functions, ROS signals and the extent of cell death caused by ethanol metabolites in quiescent *vs* activated PSCs.

In order to understand why activated PSCs are so different from quiescent PSCs, we plan to compare the transcriptome and proteome of these cells. The focus will be placed on Ca^{2+} channels and proteins controlling programmed cell death.

Further, we will use confocal Raman spectroscopy to investigate characteristic biochemical shifts present in activated PSCs, which could be used as markers of PSC activation in future studies and diagnostics.

Finally, we will test whether the alterations in gene / protein expression found in activated PSCs *in vitro*, also occur *in vivo* in a mouse model of alcohol-induced chronic pancreatitis

Although this is a typical basic research project expected to deliver novel insights into the pathophysiology of PSCs, the new knowledge obtained in this study might contribute to the development of new therapeutic procedures or medical policies for alcohol-induced pancreatitis or pancreatic cancer. Transcriptome analysis of PSCs will provide us with a large amount of data and will be made available for future research; whereas biochemical characteristics of activated PSCs revealed by spectroscopic methods might be used as templates for machine learning algorithms in automated histopathological assessment of collected tissues / biopsies.