IN VITRO CHARACTERIZATION OF NOVEL 'PEPTIDE B'

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Background

The portal vein is an essential compartment of the gut-liver axis. Mediators entering the portal vein and transported into the liver have far-reaching consequences on liver function and hepatic diseases. 'Gut-mediated effects' on the liver are modulated by the integrity of the gut barrier, which is affected by portal venous pressure among other factors. To study gut-liver crosstalk, portal vein and peripheral blood of patients with severe portal hypertension and liver pathology undergoing transjugular intrahepatic portosystemic stent (TIPS) were profiled by mass spectrometry. Two peptides were enriched in portal compared to peripheral blood. MS sequencing identified these features as peptides derived from the parent proteins 'protein kinase c-beta type' (from here on referred to as: 'peptide β') and 'protocadherin fat' [1].

Hypothesis:

These novel peptides might be gut-derived mediators with functional consequences on the liver.

Aim:

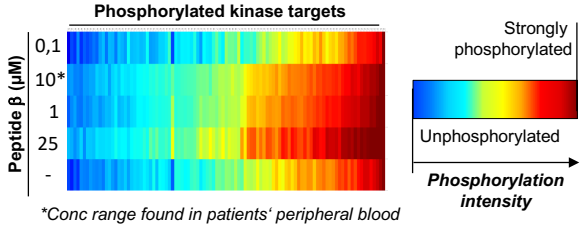
The effect of the novel peptides on liver inflammation and fibrosis, two crucial processes underlying chronic liver disease, will be examined *in vitro* using hepatocytes, hepatic stellate cells and macrophages.

Methods

Kinase activity profiles with increasing concentrations of peptide β (0-10µM) were analysed after 15 minutes of incubation using a commercial "kinase activity assay". Cell viability (MTT) and proliferation assay (CyQUANT, Invitrogen) was performed according to manufacturer's instructions. For gene expression, cells were processed for RNA isolation, cDNA synthesis and quantitative real-time PCR according to standard protocols, analyzing markers for myofibroblast activation and fibrosis (α-SMA, Col1α1 and TGF-β1, MMP-2, TIMP-1, Vimentin).

Results

Kinase activity profiling in hepatocytes (HepG2) revealed an impact of peptide β on cellular signaling at clinically relevant concentrations **(Figure 1)**.



**Conc range found in patients' peripheral blood*

Figure 1: Heat map of the kinase activity assay from dose response with peptide β. HepG2s were lysed 15min after stimulation. The heat map displays intensities (AU) of phosphorylation levels of a range of kinase targets.

In hepatocytes, peptide β does not affect cellular viability/metabolic activity **(Figure 2A-B)**. In contrast, peptide β is pro-proliferative in hepatic stellate cells (HSCs) **(Figure 2C)**

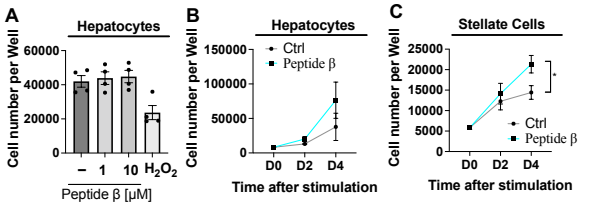


Figure 2: Peptide β is pro-proliferative in hepatic stellate cells. Cells were treated with 1µM peptide β. (A) Metabolic activity in HepG2s after 1 day (n=4). (B) Proliferation in HepG2s (n=5). (C) Proliferation in TWNT-4s (n=5). Mean with SEM.

Peptide β significantly reduced gene expression levels of α-SMA, Col1α1 and TGF-β1 in human hepatic stellate cells (**data not shown**), indicating a less activated and ECM-producing myofibroblast-like phenotype. Gene expression of vimentin, MMP-2 and TIMP-1 were unchanged (**data not shown).**

Discussion

We revealed a novel peptide that is increased in the portal vein of patients with portal hypertension ('peptide β'). Peptide β impacts cellular signaling and function at concentrations found in patients' blood. Peptide β reduces the activation into a myofibroblast-like phenotype and decreases collagen production, while it increases the pro-proliferative capacity of hepatic stellate cells. Both effects might be mutually interrelated. Transdifferentiated, highly ECM-producing myofibroblasts are less proliferative than activated proto-myofibroblasts [2]. Although peptide β limits the myofibroblast-like phenotype, we suggest a profibrotic nature of the novel peptide β because of its pro-proliferative effects on stellate cells. Direct effects of peptide β on inflammation in liver cells remain to be elucidated. Furthermore, it is uncertain whether the peptide derived from the 'protocadherin fat' parent protein exhibits functional consequences on any liver cell.

References

1. Bagarolo et al, Submitted, 2024
2. Gibb et al, Circ Res, 127(3):427-447, 2020.