BIOCHEMICAL SOCIETY

Manchester Metropolitan University

Biochemical Society Summer Studentship Report

Role of PDZ and LIM (PDLIM) Proteins in Liver Fibrosis.

Introduction

Fibrosis refers to the thickening and scarring of tissues in any body organ. In the UK, there are over 10,000 deaths in a year related to liver disease (Peng et al., 2019). The end stage of chronic fibrosis is liver cirrhosis which leads to liver cancer. Fibrosis in the liver occurs when increased amounts of extracellular matrix (ECM) proteins, such as collagen accumulation, are present in most. The primary collagen-producing cells are activated hepatic stellate cells (HSCs), portal fibroblasts and myofibroblasts of bone marrow (Friedman et al., 1985). HSCs are activated by different fibrogenic cytokines, including TGFb1, angiotensin II and leptin (Bataller and Brenner, 2005). The mechanical properties of the ECM (Extracellular Matrix) are altered by fibrosis. The altered mechanical environment drives and maintains HSC activation (Wallace et al., 2008).

One of the effectors of mechano-signalling is the Yes-associated protein (YAP-1), expressed by activated HSCs. Though we know that YAP-1 drives HSC activation, it is not well understood how YAP1 is activated. The PDLM family proteins, also known as Enigma proteins, are significantly identified as a potential driver of YAP-1 mechano-activation (Martin et al., 2016). My project investigated PDLIM5 expression in HSCs. Inhibition of YAP-1-related pathways could be a novel strategy for anti-fibrotic treatments.

Aim and Hypothesis-

Aim: Investigate PDLM protein expression in a human HSC cell line (LX-2) using qPCR and Western blot techniques.

Hypothesis: PDLM family members are expressed by Hepatic Stellate Cells. (LX-2).

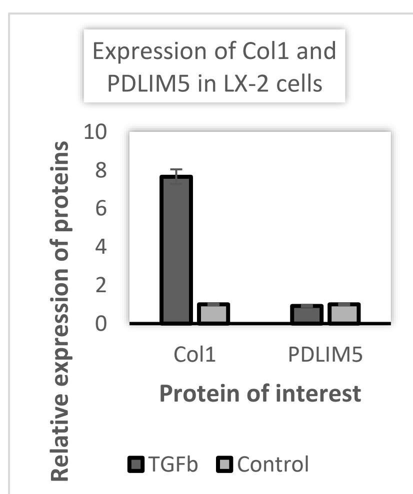
Description and Methods Used-

The LX-2 cell line was used from Hepatic Stellate cells. These cells are immortalised human cell lines that have characteristics similar to HSCs. The lining was brought from Mount Sinai School of Medicine, New York. The isolated HSCs were cultured in M199 containing 1% of DMEM (Xu, 2005).

LX-2 cells were maintained in DMEM with 1% serum at 5% CO_2 and 37°C. The cells were split when over 70% confluent. To stimulate HSC activation LX-2s were treated at 0 and 24hours with 5ng/ul TGFb, and lysed at 48 hours.

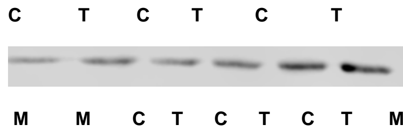
Protein was collected by lysing cells in RIPA buffer, and samples were mixed with 5x Laemmle buffer for loading in the gel. For PDLIM5, N=9 and for Col1 N=3. The SDS gel (10%) was prepared, and chameleon protein ladders from Licor were used as markers for the blot. 2 primary antibodies were used: PDLIM5 and Col1. The blots were then imaged in Li-Cor Odyssey to express the proteins.

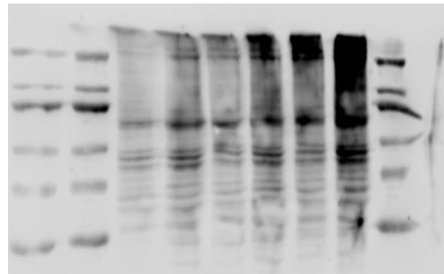
Results and Outcome-



The quantification of the western blot showed an expression of Col1 in LX-2 cells. There was no significant difference between control and the expression of PDLIM5 in 38-hour treated TGFb cells. But there was an expression of PDLIM5. There can be different reasons behind the low expression of PDLIM5 in LX-2 cells. The time limitation of the TGFb treatment can be a reason for low expression. Moreover, there were only three repeats done for PDLIM5. Several repeats could decrease the standard deviation and lead to more significant data. Moreover, qPCR and immunocytochemistry could not be performed due to limited time. These experiments could validate and enrich the data analysis, and more reliability of the hypothesis could be concluded. Moreover, fluorescence microscopy and flow cytometry could be used to investigate the expression of proteins. The data proves the expression of Col1 in the activation of fibrotic HSCs.

Figure 1. Expression of PDLIM5 and Col1 in TGFb treated and untreated LX-2 cells.





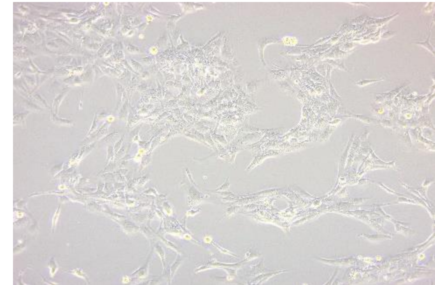


Figure 2. PDLIM5 protein expression and total protein stain.

Figure 3. Immortalised LX-2 cells

Future Direction In which the project can be taken-

The immunostaining of the treated cells could be done to have a better understanding of the expressions. More trials can be done with different primary antibodies to compare the expression of PDLIM5. The cells can also be treated with other fibrogenic cytokines such as angiotensin II and leptin1 so that there is a diverse way of understanding the mechano-transduction in the whole pathway.

Value of the studentship to the student and the lab-

From the studentship, I have received both lab and transferrable skills. I was trained to work under laboratory conditions and completed risk assessments. I learnt the process of western blot and densitometry. I have learnt the standard process of mammalian cell culture. In addition, I have learnt communication skills in a lab, planning before a task, time management, and analytical and problem-solving skills throughout the weeks. These skills will help me progressively build my career. The research group valued the studentship as my data showed whether there was an expression of PDLIM5 in HSCc. It adds to the interest of the ongoing research to understand the mechano-signalling pathways of the activation of HSCs and the role of PDLIM5.

Figure 4. Mohammad in the molecular biology lab with Dr. James Pritchett and Mohammad in the cell culture lab performing LX-2 cell culture.

References-

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