2024 NEOMED Summer Research Fellowship Program, Cytochrome P450 mediated eicosanoids mediate liver cirrhosis. Submit your application to Dr. James P. Hardwick

1. **Title:** Cytochrome P450 production of 20-HETE leads to liver cirrhosis and hepatocellular carcinoma.

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2. **Abstract of Project:**

Cirrhosis is the outcome of chronic liver disease due to progressive liver injury and fibrosis. Cirrhosis leads to portal hypertension and liver dysfunction, progressing to complications such as ascites, variceal bleeding, hepatic encephalopathy, hepatorenal syndrome, hepatopulmonary syndrome, cirrhotic cardiomyopathy, sarcopenia, hepatocellular carcinoma, and coagulation disorders. The cause of increased portal hypertension and ascites is believed to be vasoconstriction of the portal vein and vasodilation of the splanchnic arterial system. We have recently found the increased cytochrome P450 4a11 mediated increase in the vasoconstrictive 20-HETE eicosanoid in the progression of non-alcoholic fatty liver disease (NAFLD) in human patients. It is believed that 20-HETE mediates its vasoconstrictive effect by activating the GPR75 receptor in endothelial cells and hepatocytes, leading to vasoconstriction and hepatocyte proliferation respectively. This study aims to determine levels of 20-HETE and 12-HETE in human livers from patients with cirrhosis and hepatocellular cell lines, and whether blocking CYP4A11 production of 20-HETE or 20-HETE activation of the GPR75 receptor inhibits hepatocyte cell proliferation.

3. **Significance of overall research:**

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Hepatocellular carcinoma occurs most often in people with chronic liver diseases, such as cirrhosis caused by hepatitis B, hepatitis C infection, or non-alcoholic fatty liver disease. Systemic chemotherapy for HCC is usually not well tolerated by patients with significant underlying hepatic dysfunction, and chemotherapy may also be less effective in patients with substantial cirrhosis. Although combination therapies such as the Tyrosine kinase inhibitor Sorafenib and the anti-vascular endothelial growth factor inhibitor bevacizumab prolonged survival, they failed to increase the remission rate. Therefore, it is imperative to design new therapies to treat advanced liver cirrhosis and HCC. We have shown that the fatty acid omega hydroxylase P4504A11 is increased in patients with liver cirrhosis and HCC, producing vasoconstrictive 20-arachidonic acid and 12-hydroxylic acid. These fatty acids are converted to dicarboxylic acids, metabolized by peroxisome β-oxidation to acetate that feeds lipogenesis. Therefore, inhibition of peroxisome acyl-CoA oxidase will decrease acetate production for lipogenesis and increase dicarboxylic acid that can un-couple mitochondria respiration leading to cell death. These studies will provide insight into the beneficial effects of fasting in increasing the efficacy of chemotherapeutic drugs and reducing their subsequent side effects.

4. **Goals and Objectives of the summer research project:**

This research aims to determine the level of 20-HETE and 12-HETE in human samples of patients with Non-alcoholic fatty liver disease (NAFLD) and hepatocellular cell lines by high-pressure liquid chromatography and GC-MS/MS.

Investigative Research Methods:

Seven different HCC cell lines with different cell proliferation and metastatic rates will be used throughout this study. These cell lines will be maintained in cell culture and treated with 20-HETE, and the cell death and proliferation rate will be measured.

The proposed method of data analysis:

The student will be responsible for the following:

- Maintaining seven hepatocellular cell lines in culture
- Determine eicosanoid levels in NAFLD patient liver samples by HPLC/mass spectrometry.

The techniques and procedures the student will learn:

- Isolate total protein and RNA from cell cultures
- Separation and of eicosanoid levels in human tissues.
- Determine absolute mRNA content by real-time PCR
- Determine the level of CYP450 protein by Western immunoblot
- Determine the rate of cell proliferation in cell culture
- Determine the rate of apoptosis cell death

The student will meet daily with the PI to discuss the objective of the day's experiment and will be taught by the PI and laboratory technician how to perform each experiment. The student will be taught how to record and interpret experimental results using prism statistical programs. Finally, the students will attend weekly meetings of the liver focus group and present results at least once to the group over the eight weeks.

5. Significance of anticipated findings:

These results may lead to novel, innovative treatments for liver cirrhosis by targeted inhibition of vascular vasoconstriction and hepatocyte proliferation through CYP4A11-mediated production of 20-HETE.