2024 NEOMED Summer Research Fellowship Program, Synthetic lethality of hepatocellular carcinoma mediated by fasting-induced *CYP4* P450. Submit your application to Dr. James P. Hardwick

1. **Title:** Cytochrome P450 production of dicarboxylic acid leads to synthetic lethality of hepatocellular carcinoma.

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2. **Abstract of Project:**

Cancer is the second leading cause of mortality worldwide and is suspected to be the foremost killer in the coming decades by the World Health Organization. Cancer treatments, including surgery, chemotherapy, and radiotherapy, have achieved considerable therapeutic efficacy, but damage to the normal tissue and the subsequent side effects are inevitable. Accordingly, besides the conventional therapy modalities, it is crucial to identify other assistant treatment methods to enhance the therapeutic efficacy further, reduce side effects, and improve prognosis. To improve chemotherapeutic effectiveness, multiple tumor pathways are targeted by drugs that show synthetic lethality (SL). Synthetic lethality is a novel strategy for anticancer therapies, whereby mutations of two genes will kill a cell, but mutation of a single gene will not. A growing number of recent studies in cancer treatments have suggested that factors in the categories of naturopathic medicine profoundly affect the initiation and treatment outcomes of cancer. Fasting therapy is a naturopathic treatment method used as a valid therapeutic modality for acute and chronic diseases in medicine worldwide. In cancer-bearing models, fasting therapy was reported to be a reproducible and efficient intervention in protecting mammals against tumors and prolonged overall survival. The chemotherapy-protection effects of fasting therapy in reducing chemotherapy side effects and related death were also shown in human clinical trials. There is little knowledge of how synthetic lethal chemotherapeutic drugs and fasting interplay improve drug efficacy and reduce systemic toxicity. We hypothesize that induction of omega fatty oxidation cytochrome P450 gene by fasting and inhibition of peroxisomal acyl-CoA oxidase (ACOX) will increase tumor dicarboxylic acids, causing synthetic lethality.

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 absolute expression levels of human ***CYP4*** genes in hepatocellular cell lines. This proposal seeks to determine if induction of CYP4 P450 and inhibition of ACOX1 can lead to synthetic lethality (SL) of hepatocellular cancer cell (HCC) lines through increased production of dicarboxylic acids.

Investigative Research methods:

Seven different HCC cell lines with different levels of cell proliferation and metastatic potential will be used throughout this study. These cell lines will be maintained in cell culture and treated with other chain-length dicarboxylic acids, and the rate of cell death and proliferation will be measured. The cell lines will be treated with ACOX1 inhibitors under fasting conditions, and the rate of cell and death and cell proliferation will be determined again. The absolute expression of CYP4 genes will be measured by real-time PCR and western immunoblot analysis.

The proposed method of data analysis:

The student will be responsible for the following:

- Maintaining seven hepatocellular cell lines in culture
- Isolate RNA from cell lines and produce cDNAs for determining expression levels of CYP4 genes and protein.
- Incubate HCC cell lines with different chain-length saturated dicarboxylic acids
- Determine the rate of cell proliferation in response to dicarboxylic acids
- Determine the rate of cell death in response to dicarboxylic acids
- Determine the effect of ACOX1 inhibitor on cell proliferation and death.

The techniques and procedures the student will learn:

- Isolate total protein and RNA from cell cultures
- Synthesis of cDNA
- 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 hepatocellular carcinoma by identification of synthetic lethality pathways of induction of fatty acid omega hydroxylase genes with the inhibition of peroxisomal acyl-CoA oxidase. The results may explain why intermittent fasting improves chemotherapeutic efficacy in patients and reduces the toxic side effects of chemotherapy. These studies may also provide evidence of a new synthetic lethality pathway in treating liver cirrhosis and HCC.