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Role of MKP-2 in Hepatic Inflammatory Response to Fasting in Mice

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Project Title: Role of MKP-2 in Hepatic Inflammatory Response to Fasting in Mice

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Project Description

Obesity is a major problem globally and its incidence is increasing at an alarming rate. An assessment from the World Health Organization (WHO) shows that about 1 billion adults worldwide are overweight, and about 300million of these are clinically obese. Currently, obesity affects 78.6 million people (33%) in the United States and it has been estimated that together with increases in diabetes over the next two to three decades, the finances and resources of the US healthcare system would be significantly stressed. Obesity predisposes to the development of cardiovascular disease, type 2 diabetes, nonalcoholic fatty liver disease, atherosclerosis, and increased risk of insulin resistance and even some cancers. Mitogen activated protein kinases (MAPKs) are established physiological regulators of metabolic homeostasis and MAPK dysfunction promotes metabolic disease. The identity and functional contribution of the negative regulators of MAPKs in metabolism remains unclear.

The mitogen-activated protein kinase phosphatases (MKPs) are a family of dual-specificity phosphatases (DUSPs) which function to terminate the activity MAPKs. One MKP of renewed interest is the inducible nuclear phosphatase MKP-2, which dephosphorylates ERK and JNK *in vitro*. Unhealthy eating behaviors increase the risk of obesity and metabolic diseases, however the fundamental mechanisms are not fully understood. MKP-2 has been shown to be a key regulator of proinflammatory cytokines in macrophages. How MKP-2 inflammatory function contributes to the pathogenesis of obesity and metabolic diseases is not known. There is now need to investigate the mechanisms in the liver and hepatocytes to uncover the contribution of MKP-2 on hepatic metabolism and inflammation in obesity.

Aims of the study is (1) to determine the influence of high fat diet on MKP-2 expression in the livers of mice, and (2) to determine the impact of MKP-2 deletion on hepatic inflammation. **Hypothesis:** MKP-2 mediates liver inflammatory response elicited by unhealthy eating behavior.

Research Plan: In this project we will utilize a novel MKP-2 knockout mouse model to

investigate the impact of MKP-2 deletion on the hepatic inflammatory responses using the fasting/refeeding paradigm. MKP-2 wild-type and MKP-2 knockout (KO) mice will be fasted for 48 h and then refed either chow or high fat diet. Inflammatory genes expression in the liver will be assessed for 2, 6 and 12 h after refeeding. Hepatic MKP-2 protein expression and p38 MAPK, JNK, and ERK phosphorylation will be examined. RNA will be isolated from liver tissue derived from male MKP-2 wild-type and MKP-2 KO mice using an RNeasy kit. Liver tissue will be homogenized in RIPA buffer. Protein concentrations will be determined by Pierce BCA Protein Assay kit. Lysates will be resolved by SDS-PAGE and transferred to

nitrocellulose membranes, which will be incubated with phospho-specific antibodies followed by enhanced chemiluminescence detection.

Student Duties, Contributions and Outcomes: At the beginning of the project, background reading of peer-reviewed scientific articles related to the project and training in handling mice and isolation of tissue samples will be required. Subsequently, the student will be trained in RNA and protein extraction and RT-PCR and immunoblotting. The project will require the student to work full-time up to 40 hours per week for a period of 10-12 weeks. During this period the student will learn mouse *in vivo* studies and multiple molecular biology and biochemical techniques, including western blotting, RT-PCR etc. Specifically, the student will administer the fasting and refeeding-paradigm in mice and collect tissue samples from mice at the end of each experiment. It will require protein and RNA isolation from liver tissue, generation of cDNA and real time PCR analysis. Analysis of protein expression and phosphorylation using specific antibodies by immunoblotting. The student researcher will be will be required to send a weekly report of activities to Dr. A. Lawan and to present project progress at our regular lab meetings to share the data and get feedback and from members of the lab. In addition, the student will learn how to document the experimental work and keep laboratory notebook with the comprehensive description of all experiments performed. The student will be encouraged to prepare a poster for subsequent presentation at a scientific meeting. At the end, the student researcher will be required to write up a report with suggestions for future development of the project and analyses of the obtained results that can be used in a future publication that the student will be part of. In addition, this short term project will provide pilot data that will direct our future investigations as well as adding to the knowledge gained from our *in vivo* studies in the MKP-2 KO mouse model.

Student Selection Criteria: Students from juniors to seniors will be selected. **Project Mentorship:** The student must have completed some courses in Anatomy and Physiology,

Biochemistry and Molecular Biology at UAH. The student will be required to undergo recommended EHS training for the lab. The mentoring will involve day-to-day teaching and supervision of the student project by Dr. A. Lawan and graduate students in the Lab. Dr. Lawan will be having one-on-one meeting with the student to explain the project, and present some of the published work from the Lab to commence literature review of the project. Dr. Lawan will supervise and assist in data analysis generated from this project. Also, student researcher will be required to present progress report of the project during our regular lab meetings. Dr. Lawan will guide the stduent in writing up a report for the data generated during the course of the project and preparation for the final presentation.

Prior Awardees: Dr. A. Lawan is new to the UAH RCEU program.

Contingency Plan for Restricted Face-to-Face Interactions

An alternative strategy to complete the proposed project

- Substituting all face-to-face with virtual/on-line one-on-one meeting with student researcher
- Literature review of the role of MKP-2/MAPK in hepatic inflammation and development of obesity and metabolic diseases.
- Use video and diagrams to demonstrate some of the methods proposed in the proposal • Remote Data analysis using Excel and Graph-Pad prism 8
- Substituting all face-to-face with virtual/on-line presentation of project progress