Funded Application:
Example #3

Advice from the student:

Make sure you explore several options before finding a topic you’re really interested in, and make plenty of visits to mentor’s laboratory to discuss about research. Also be familiarized with equipments in the lab, so that you know what you would be doing specifically. Plan it ahead and communicate with mentor as much as possible. Doing background research always helps, and build up strong knowledge and ideas to impress the readers.

Tai Ho Shin
Tai_Ho_Shin@brown.edu
Please complete ALL of the following sections:

### BIOGRAPHICAL INFORMATION

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Email Address</th>
<th>Project Title</th>
<th>Mentor Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shin</td>
<td>Tai Ho</td>
<td><a href="mailto:Tai_Ho_Shin@brown.edu">Tai_Ho_Shin@brown.edu</a></td>
<td>Effect of Resveratrol on AMPK Signaling Pathway in Swine Hypercholesterolemic Models</td>
<td>Dr. Frank Sellke</td>
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</tbody>
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### Summer Funding

Summer funding for which you are applying:

- [ ] Summer Assistantship
- [x] Basic and Translational Research Program
- [ ] Summer Stipend

### Faculty Mentor Information

<table>
<thead>
<tr>
<th>Name</th>
<th>FRANK SELLKE, BA, MD, MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Department/Institution</td>
<td>Karlson and Karlson Professor of Cardiothoracic Surgery Bio Med Medicine</td>
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</tr>
</tbody>
</table>

### Summer Project Overview

Please attach a narrative (maximum of 3 pages) that includes ALL of the following: see attachment
1. Project Title;

2. Beginning and ending dates of your summer work;

3. A description of the goals of your summer work;

4. A description of your proposed methodology (include information regarding your specific role in the project and how you will be spending your time);

5. A description of how the project will contribute to general knowledge of the topic and/or contribute to the community;

6. A description of any potential problems you anticipate;

7. A description of the ways in which this project fits into your personal development and educational goals (include information about any ways in which your project work will continue beyond the summer if applicable).

Other Funding (SA applicants only)
If you are seeking additional funding please indicate sources to which you have applied, or plan to apply: none
1.
2.
3.

Application Submission
All applications and application materials must be submitted electronically as email attachments to Emily_Green@brown.edu no later than February 24, 2010.
Effect of Resveratrol on AMPK Signaling Pathway in Swine Hypercholesterolemic Models

Overview

I would like to spend this summer of 2010 and year II-IV of the medical school for the molecular research of resveratrol, which has been the subject of the many exciting anti-aging and cardiovascular research in decades. My research this summer will last 10 weeks, from June 7th, and until August 13th, mainly involving laboratory works up to 40 hours per week.

Introduction

Resveratrol, a key component in grapes and red wine, has powerful antioxidant properties that can protect the body against cardiovascular disease and many other metabolic diseases. The study of the benefits of red wine, and specifically resveratrol began due to what is known as the “French Paradox”. That term came from a study that found that French were less likely to die of a heart disease than Americans, despite the fact that both share similar high fat diets. The study concluded that some components of the red wine may protect us from heart disease, suggesting further studies are needed to investigate the benefits of resveratrol.

Some studies show that resveratrol might increase life expectancy, as fish model increased their life span by 56% when given resveratrol supplement diet\(^1\). Resveratrol’s anti-cancer and anti-inflammatory in vitro action on mammalian cell cultures also suggests a possible positive effect on human\(^2\). Resveratrol may be beneficial in reducing deaths from obesity-related conditions. In a study carried out on mice, increased resveratrol intake meant that fat related deaths decreased by 31%. The mice that were fed with resveratrol also performed much better in agility and movement assessments than the mice that were not fed resveratrol\(^3\). To study the mechanism of resveratrol is obviously a particularly relevant question on various diseases, especially the ones of high co-
morbidity with cardiovascular disease, including type II diabetes, hypercholesterolemia and hypertension.

The most well-known contribution to be made to longevity by resveratrol is its on-target binding to SIRT1 gene\(^4\). This gene activates the silent information regulator two protein (sirtuin)\(^5\). Sirtuin is either histone deacetylase or mono-ribosyltransferase and regulates aged cells to repair itself and avoid apoptosis by stress resistance\(^3\). However, this is controversial because resveratrol also activates several off-target molecules including 5′-AMP activated kinase (AMPK), which also regulates insulin sensitivity and mitochondrial biogenesis.\(^6\)

Preliminary studies from our group have shown that AMPK level is increased by resveratrol-treated swine models of myocardial ischemia (unpublished). While using similar model, I plan to examine off-target pathways of resveratrol more closely and distinguish more relevant factors leading to its effect. My specific role in the project will be to study the possible changes in the AMPK pathway in the non-ischemic and chronic ischemic cardiac cells from left ventricle of our pig models with hypercholesterolemia and metabolic syndrome treated with resveratrol or placebo.

I will be addressing those changes of pathway using the immunohistochemistry, immunobloting and Real time PCR. Dr. Frank Sellke’s lab has all the techniques and equipments available that are standard in his lab. He has published several papers using these techniques as well the animal models. All samples will be available when I start my summer research project.

**Hypothesis**

My hypothesis is that AMPK-α activity is lower in hypercholesterolemic swine models as compared with swine with normal diet. In resveratrol treated-swine, AMPK activity will be increased to levels similar to normal diet pigs. AMPK activity is regulated by various activators and inhibitors, which are to be investigated extensively throughout my research. A few of questions to be answered include;
1) Which upstream activators and inhibitors of AMPK-α are responsible for the increased activation of AMPK-α?
2) Are AMPK-β and γ also regulated by resveratrol?
3) Which cells in the heart are the main targets for resveratrol (cardiomyocytes vs. endothelial cells)

Methods
Animal Model
Yorkshire miniswine (Parsons Research, Amherst, MA) were divided to groups of three and fed differently throughout the 11 weeks prior to the experiment. The first group was given 500 grams of a hypercholesterolemic diet daily (HCD, n= 7) composed of 4% cholesterol, 17.2% coconut oil, 2.3% corn oil, 1.5% sodium cholate, and 75% regular chow. A second group was fed the same hypercholesterolemic diet supplemented with 100 mg/kg/day of resveratrol (HCD-R, n=7). The third group of swine was fed regular chow (control, n= 7) and served as the control. Animals were observed during feeding to ensure complete consumption of food and supplement.

Seven weeks after ameroid placement, swine would again be anesthetized and the heart would be exposed through a median sternotomy with taking physiologic measurements, followed by euthanasia. The heart would be harvested and two 1-cm-thick transverse slices would be cut at the midventricular level and then sectioned into 8 segments identified clockwise starting from the anterior junction of the right and left ventricles. Samples would be divided and rapidly frozen in liquid nitrogen (molecular studies) or 10% formalin (immunohistochemistry studies). All experiments are approved by the hospital Institutional Animal Care and Use Committee (included in the application).

Molecular studies
Western blotting would be performed by first homogenizing myocardial samples with RIPA buffer. Each samples will be fractioned by gel electrophoresis and visualized with specific primary antibodies, including anti-phospho-AMPK, anti-AMPK-α, β and γ, followed by appropriate secondary antibodies. Bands will
be visualized with chemiluminescence detection system and quantified by densitometry of autoradiograph films.

Immunohistochemistry

Frozen sections of ventricular tissue will be used for immunohistochemistry. Tissue will be fixed in acetone and blocked with phosphate buffered saline and 1% bovine serum albumin. The slides would be applied with primary antibodies used in molecular studies and appropriate secondary antibodies, followed by viewing with confocal microscope to determine the cellular localization (vascular cells vs. myocytes) of AMPK (isoforms, active, and total forms). Photomicrographs will be taken and analyzed using repeated measures analysis of variance.

I will spend first two weeks of summer to familiarize with new techniques and lab environment, while setting up for the experiments. Then I would spend the next 7 weeks mainly for the data collection, while the last week would be for the data analysis and writing up the research summary. During the following school years, I plan to continue my participation mainly by data analysis and collecting data at least one session per week.

Discussion

Despite its importance and wide availability in the red wine, resveratrol has not been studied carefully until recently. Currently there is only small number of data on the impact of resveratrol in human and large animal models of metabolic syndrome. This research project will hopefully discover more details about its effect on AMPK pathway on swine models, and contribute to help patients with cardiovascular disease.

However, working in the laboratory does not always produce predictable results. I expect my work will be modified by several changes to optimize the procedures. I will have a regular, weekly meeting both during the summer and throughout Year II with Dr. Sellke, to present working process, receive feedback, and develop new ideas. I have also spoken with Dr. Cesario Bianchi, who would provide soliciting feedback and advice so that I might
refine and improve this project. He has agreed to meet with me and to offer guidance and support throughout this process with open door office, as I progress the research. The feedback and guidance from these individuals will help me to find answers to those potential problems.

This study of resveratrol during my first summer will provide me with the background and experience necessary to broaden my understanding of complex body metabolism and survival mechanism from basic cellular level. Working closely with Dr. Sellke and Dr. Bianchi, and fellow students in the laboratory, I would learn several techniques in research field and develop more skills. This will help me to pursue in becoming a cardiac surgeon, as well as spending time in research as much as possible in the future.
Reference


