Award Number:  W81XWH-12-1-0442

TITLE:  Targeting Estrogen-Induced COX-2 Activity in Lymphangioleiomyomatosis (LAM)

PRINCIPAL INVESTIGATOR:  Jane Yu

CONTRACTING ORGANIZATION:
The Brigham and Women's Hospital Inc.
Boston, MA  02115-0110

REPORT DATE:  December 2014

TYPE OF REPORT:  Final Report

PREPARED FOR:  U.S. Army Medical Research and Materiel Command
   Fort Detrick, Maryland  21702-5012

DISTRIBUTION STATEMENT:  Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Targeting Estrogen-Induced COX-2 Activity in Lymphangioleiomyomatosis (LAM)

Estradiol increased the expression of cyclooxygenase-2 (COX-2), a rate-limiting enzyme in prostaglandin biosynthesis, which was also increased at baseline in TSC2-deficient cells, and was not affected by rapamycin treatment. However both Torin 1 treatment and Rictor knockdown, led to reduced COX-2 expression and phospho-Akt-S473. Prostaglandin production was also increased in TSC2-deficient cells. In preclinical models, both Celecoxib and aspirin reduced tumor development. LAM patients had significantly higher serum prostaglandin levels than healthy women. 15-epi-lipoxin-A4 was identified in exhaled breath condensate from LAM subjects and was increased by aspirin treatment, indicative of functional COX-2 expression in the LAM airway. In vitro, 15-epi-lipoxin-A4 reduced the proliferation of LAM patient-derived cells in a dose-dependent manner. Targeting COX-2 and prostaglandin pathways may have therapeutic value in LAM and TSC-related diseases, and possibly in other conditions associated with mTOR-hyperactivation.

15. SUBJECT TERMS: none listed

16. SECURITY CLASSIFICATION OF:

a. REPORT U
b. ABSTRACT U
c. THIS PAGE U

17. LIMITATION OF ABSTRACT

U

18. NUMBER OF PAGES

8

19a. NAME OF RESPONSIBLE PERSON

USAMRMC

19b. TELEPHONE NUMBER (include area code)
# Table of Contents

1. Introduction ................................................................. 4
2. Keywords ........................................................................ 4
3. Accomplishments ......................................................... 4
4. Impact ............................................................................ 5
5. Changes/Problems ......................................................... 6
6. Products .......................................................................... 6
7. Participants & Other Collaborating Organizations ........... 6
8. Special Reporting Requirements ..................................... 8
9. Appendices ...................................................................... 8
1. INTRODUCTION:
This proposal is focused on the molecular mechanisms underlying the pathogenesis of lymphangioleiomyomatosis (LAM), a devastating pulmonary disease affecting exclusively young women, often leading to end-stage lung disease. LAM is believed to affect approximately 30% of women with tuberous sclerosis complex (TSC). The only proven treatment for LAM is lung transplantation, which carries significant one-year mortality and after which LAM can recur in the transplanted lungs. The pathogenesis of LAM is very unusual: LAM cells are histological benign smooth muscle cells carrying TSC1 or TSC2 mutations that are believed to metastasize to the lungs where they cause lung degeneration. Cells lacking TSC1 or TSC2 exhibit hyperactivation of the mammalian target of rapamycin complex 1 (mTORC1), a master regulator of cell growth, protein translation, and metabolism. In LAM patients the mTORC1 inhibitor Rapamycin stabilizes lung function and improves symptoms. Our central hypothesis is that E2 induces COX-2 activity and production of prostaglandins (PGE2, PGD2, and 6-K-PGF1α), thereby promoting the survival and lung metastasis of TSC2-null cells. Furthermore, in preclinical models of LAM, molecular and pharmacologic suppression of COX-2 will block the E2-promoted lung metastasis and induce a regression of established lung lesions.

2. KEYWORDS:
Lymphangioleiomyomatosis (LAM), prostaglandin biosynthesis, cyclooxygenase-2 (COX-2), COX-2 inhibitors, xenograft tumors, bioluminescent imaging, cell proliferation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. To examine the cellular impact of COX-2 in TSC2-null LAM patient-derived cells in vitro. We proposed to test whether COX-2 is a key mediator for E2-enhanced prostaglandin production. We have successfully established two independent clones of COX-2 shRNA in human lung epithelial cells (Figure 1). We will use the same set of shRNA-COX-2 to develop COX-2 knock-down LAM patient-derived cells. We have also developed the ELISA assay for measuring PGE2 and 6-K-PGF1α in conditioned media.

Aim 2. To determine whether the molecular depletion of COX-2 suppresses estrogen-promoted lung metastasis of LAM patient-derived cells in vivo. We have successfully established two independent clones of COX-2 shRNA in TSC2-null LAM patient-derived cells. We will perform the in vivo experiment in the second funding year. To measure COX-2 activity in tumors of TSC2-null cells, we used noninvasive live imaging-XenoLight RediJect COX-2 probe. However, we did not detect tumor specific COX-2 signals in mice bearing xenograft tumors. We will continue optimizing the reagent and monitor tumor COX-2 activity.

Aim 3. To determine whether the COX-2 inhibitor Celecoxib can reduce the burden of established tumors or block E2-promoted lung metastases in preclinical models of LAM

What was accomplished under these goals?

1) COX-2 is a key mediator for E2-enhanced prostaglandin production. To inhibit COX-2 pharmacologically, we treated TSC2-deficient cells with aspirin or NS398, and found that both agents reduced COX-2 protein levels and production of PGE2 and 6-K-PGF1α. This result has been published (Figure 4j-k, Li et al., J Expt Med 2014, please see the Appendix 1).

2) Inhibition of COX-2 inhibits growth rate of TSC2-null cells. We found, aspirin or NS398, COX-2 inhibitors, suppressed the proliferation of TSC2-deficient cells. This result has been published (Figure 4i, Li et al., J Exp Med 2014, please see the Appendix 1).

3) Inhibition of COX-2 suppresses the growth of subcutaneous tumors. We have treated mice with the COX-2 inhibitor aspirin, and measured xenograft tumor burden in a subcutaneous tumor model of TSC2-null cells. We found that aspirin treatment for three weeks decreased the intensity of bioluminescence,
and decreased the tumor size. Tumors also had reduced expression of COX-2 and c-Myc, and increased levels of cleaved-caspase-3 and cleaved-PARP. This result has been published (Figure 5c-f, Li et al., J Exp Med 2014, please see the Appendix 1).

**What opportunities for training and professional development has the project provided?**

This project generated preliminary and published data that have been used for receiving two awards:
1. Chenggang Li, PhD, was awarded a three-year Postdoctoral Fellowship—the LAM Foundation (2014/1 – 2017/12)
2. Jane Yu, PhD, was awarded a three-year RO1-NIH/NIDDK

**How were the results disseminated to communities of interest?**


**What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to Report.

4. **IMPACT:**

**What was the impact on the development of the principal discipline(s) of the project?**

LAM is often a progressive disease which leads to respiratory failure and death in the absence of lung transplantation. The recent demonstration that rapamycin has clinical benefit in LAM is a major success. However, not all patients respond to rapamycin, and upon rapamycin withdrawal, lung function decline resumes. Hence lifelong treatment of LAM patients with rapamycin may be required to maintain benefit, with unknown long-term toxicities.

Our findings suggest that aspirin and/or other COX-1/COX-2 inhibitors may have significant benefit in slowing LAM progression. The well-known side-effect and toxicity profile of these drugs make them attractive candidates for long-term therapy in LAM patients.

**What was the impact on other disciplines?**

It is also possible that other neoplastic conditions associated with mTOR hyperactivation could be responsive to these agents. Further preclinical and clinical investigation is warranted to explore these possibilities.

**What was the impact on technology transfer?**

Nothing to Report.

**What was the impact on society beyond science and technology?**
We demonstrated that COX-1/COX-2 inhibition may have significant benefit in slowing LAM progression and make it promising for long-term therapy in LAM patients.

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

To measure COX-2 activity in tumors of TSC2-null cells, we used noninvasive live imaging-XenoLight RediJect COX-2 probe. However, we did not detect tumor specific COX-2 signals in mice bearing xenograft tumors. We will continue optimizing the reagent and monitor tumor COX-2 activity.

6. PRODUCTS:

- Publications, conference papers, and presentations

Journal publications.


Other publications, conference papers, and presentations.
1) Oral presentation at The International LAMposium 2014. April, 2014 Chicago IL. “Excessive prostaglandin biosynthesis exacerbate tumor development in LAM.”

- Inventions, patent applications, and/or licenses

Treatment of Lymphangioleiomyomatosis, January 1,2014. non-provisional, application number: 043214077221.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1) Name: John Blenis
   Project Role: Collaborator
   Researcher Identifier (e.g. ORCID ID): n.a.
   Nearest person month worked: less than 1
   Contribution to Project: Dr. Blenis has suggested and reviewed manuscript in the area of mTORC2 regulated COX-2 activity.
   Funding Support: National Institutes of Health Grant,GM51405

2) Name: Bruce D. Levy
   Project Role: Collaborator
   Researcher Identifier (e.g. ORCID ID): n.a.
Nearest person month worked: less than 1
Contribution to Project: Dr. Levy has provided, analysis, and reviewed area of 15-epi-LXA₄ profiles in LAM patients.
Funding Support: National Heart Lung and Blood Institute grants, HL68669

3) Name: Elizabeth P. Henske
Project Role: Collaborator
Researcher Identifier (e.g. ORCID ID): n.a.
Nearest person month worked: less than 1
Contribution to Project: Dr. Henske has provided, analysis, and reviewed area of metabolites profile signature of prostaglandins in ELT3 cells, xenograft tumors and in LAM patients.
Funding Support: The LAM Foundation, The Adler Foundation, The LAM Treatment Alliance, and National Heart Lung and Blood Institute grants HL118760

4) Name: David Kwiatkowski
Project Role: Collaborator
Researcher Identifier (e.g. ORCID ID): n.a.
Nearest person month worked: less than 1
Contribution to Project: Dr. Kwiatkowski has provided, analysis, and reviewed area of expression profile of prostaglandins in patient-derived cells, and in vivo study of effect of celecoxib on kidney cystadenoma in heterozygous mice.
Funding Support: National Cancer Institute grants, 1P01CA120964

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

1) Organization Name: Massachusetts General Hospital
Location of Organization: Boston
Partner’s contribution to the project (identify one or more)
• Collaboration (e.g., partner’s staff work with project staff on the project);
2) Organization Name: Peking Union Medical College

Location of Organization: Beijing, China

Partner’s contribution to the project (identify one or more)

- Collaboration (e.g., partner’s staff work with project staff on the project);

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) shall be updated and submitted with attachments.

9. APPENDICES:


SUPPORTING DATA:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PTGS2-shRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>p1, p2, p3</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Development of stable cells in which COX-2 is depleted. Human bronchial epithelial BEAS-2A cells were infected with lentiviral shRNA against PTGS2 (COX-2) and grown with puromycin 3 µg/ml for 1 week. After selection the cells were grown with puromycin 1 µg/ml. COX-2 levels were examined at passages p1-p3.