AWARD NUMBER: W81XWH-16-1-0700

TITLE: Furanyl Fatty Acid Inhibition of FABP5 as a Mechanism for Treatment and Prevention of Cancer

PRINCIPAL INVESTIGATOR: Liraz Levi

CONTRACTING ORGANIZATION: Case Western Reserve University
Cleveland, OH 44106

REPORT DATE: October 2018

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2018	Annual	30 Sep 2017-29 Sep 2018

4. TITLE AND SUBTITLE

Furanyl Fatty Acid Inhibition of FABP5 as a Mechanism for Treatment and Prevention of Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0700

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Liraz Levi, Gregory Tochtrop

E-Mail:lxl301@case.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

CASE WESTERN RESERVE UNIVERSITY
10900 EUCLID AVE
CLEVELAND OH 44106-17121

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We propose that inhibition of FABP5 represents a novel approach to diverting endogenous RA from pro—proliferative (PPARδ) to anti-proliferative (RAR) receptors, and further propose the use of furan—containing fatty acids as agents to target RA to RAR. We hypothesize that this pharmacologic inhibition will prevent the oncogenic effects of FABP5 overexpression in highly relevant breast cancer models that display a high ratio of FABP5/CRABP2 expression.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Introduction:
Retinoic acid (RA) is a potent anticarcinogenic agent that functions by regulating the expression of multiple genes through its ability to activate two nuclear receptors: RA receptors (RAR) and the peroxisome proliferator–activated receptor δ (PPARδ). However, RA's utility as a therapeutic agent is limited by RA resistance that is acquired in some tumors, and the paradoxical observation that in some tumors RA actually potentiates tumor growth. Activation of RAR results in inhibition of cancer cell growth, while activation of PPARδ leads to enhanced growth and survival. The key to regulating the partitioning of RA between these two opposing pathways lies in the two proteins that deliver RA to their respective transcription factors: cellular Retinoic acid binding protein 2 (CRABP2), which targets the hormone to RAR, and fatty acid binding protein 5 (FABP5), which transports it to PPARδ. Hence, cells that express a high level of FABP5 become resistant to RA-induced growth inhibition, and instead, display enhanced proliferation in response to RA being targeted to PPARδ. Recently, our laboratories have identified a class of furan-containing fatty acids (FAs) as a novel class of naturally occurring, dietarily available, high-affinity inhibitors of FABP5. Based on our RA signaling model we predict that by blocking FABP5, furanyl-FAs will specifically divert RA to RAR and consequently will overcome RA-resistance and suppress the growth of FABP5-overexpressing tumors. The goal of this work is to further investigate this partitioning between RAR and PPARδ, investigate the metabolic fate of furanyl-FAs, and determine if these molecules can serve as effective chemopreventive agents.

Major Goals:

Specific Aim 1. Define the ability of furanyl-FAs to perturb the CRABP2/FABP5 signaling balance.

1.1. Define a structure-activity relationship for naturally occurring furanyl-FAs.
1.2. Examine the ability of high affinity FABP5-binding furanyl-FAs to target RA to the CRABP2/RAR path.
1.3. Determine the ability of furanyl-FAs to inhibit the growth of cultured carcinoma cells.

Specific Aim 2. Define the metabolic fate(s) of furanyl-fatty acids.

2.1. Develop a comprehensive understanding of the metabolic and catabolic fate(s) of furanyl-FAs using a mass isotopomer approach in perfused organ systems.
2.2. Examine the rate(s) of metabolism/catabolism in normal tissues, and cancer cell lines.

Specific Aim 3. Assess the effects of furanyl-FAs on mammary tumor development *in vivo*.

3.1. Assess the efficacy of furanyl-FAs in inhibiting tumor development in xenograft mouse models of breast cancer.
3.2. Test the ability of furanyl-FAs to prevent tumor formation in the transgenic MMTV-Neu/Erb-B2 model of mammary carcinogenesis.

This report detailed the activities achieved in both Levi and Tochtrop lab.

Specific Aim 1. Define the ability of furanyl-FAs to perturb the CRABP2/FABP5 signaling balance.

1.1 Define a structure-activity relationship for naturally occurring furanyl-FAs.

These activities have been completed at the Tochtrop lab as was detailed in the previous report.

1.2 *Examine the ability of high affinity FABP5-binding furanyl-FAs to target RA to the CRABP2/RAR path.*

Studies were done in the Levi lab.

Several furanyl-FAs and other FA-like compounds (Fig. 1) were tested so far. Data established with the compound N-arachidonoylaminophenol is presented in this report as an example for analyses done with compounds that serve the two FABP5-inhibitor criteria. Transcriptional activation assays used to test the effect of compounds on activation of PPARδ (Fig. 2A), were performed for all compounds showing high binding affinity toward FABP5. Compounds were further tested for their ability to channel endogenous RA towards CRABP2/RAR path by measuring expression levels of RAR and PPARδ target genes (Fig. 2B, 2C). The triple-negative breast cancer line MDA-MB-231 and the mammary carcinoma cell line NaF were utilized for these assays. In addition to the target genes suggested in the proposal other target genes were tested including RAR target genes ADRRC3, CCND1 and CAS9 and PPARδ targets CD47, MMP9 and MMP2.

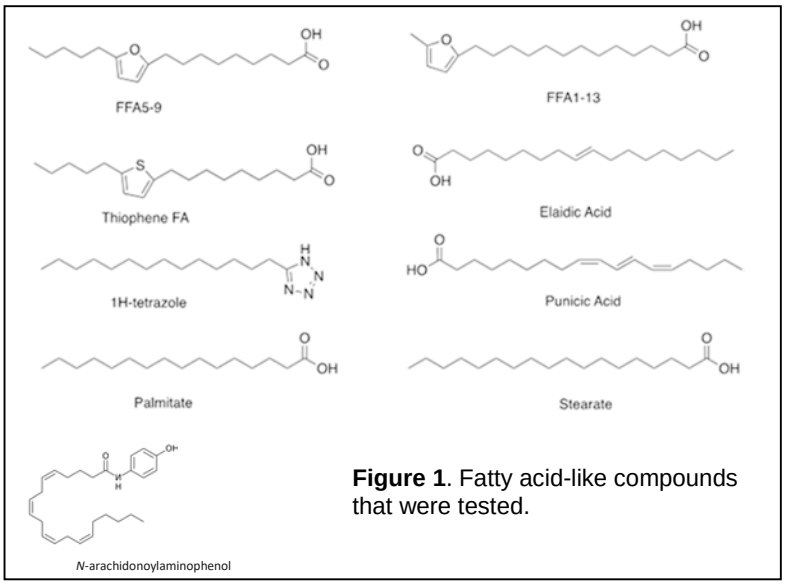

Figure 1. Fatty acid-like compounds that were tested.

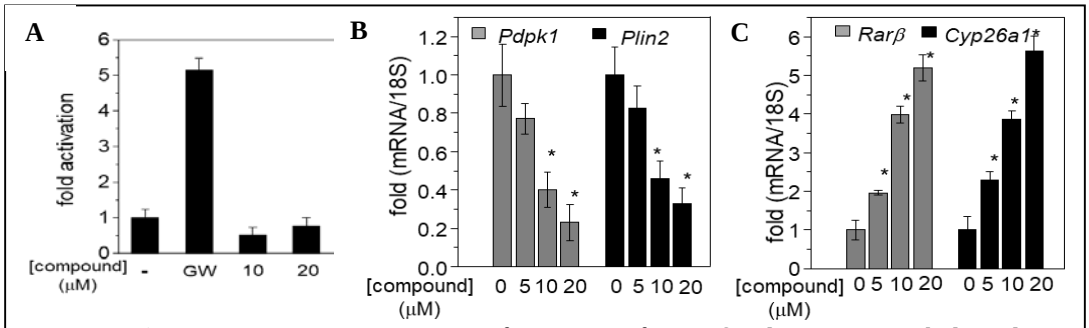

Figure 2. **A**) Transcription activation assays for activity of PPARδ indicating N-arachidonoyl-aminophenol treatment does not activate the recptor. PPARδ is activated by the PPARδ specific agonist GW0742 (GW). **B**) Expression levels of PPARδ targets are reduced after N-arachidonoylaminophenol treatment (10μM, 6 hrs). **C**) Expression levels of RAR targets are induced after N-arachidonoylaminophenol treatment (10μM, 6 hrs). Data in all panels are mean (n=3). *p<0.01

1.3 *Determine the ability of furanyl-FAs to inhibit the growth of cultured carcinoma cells.*

Studies were done in the Levi lab.

Several compounds that serve the criteria for FABP5 inhibitors were further utilized in proliferation assays. MDA-MB-231 and NaF cells were utilized as models for carcinoma cell that highly expresses FABP5 while the breast carcinoma line MCF-7 and the normal mammary epithelial cells HMEC was used as a model for cell in which FABP5 expression is low (Fig. 3A). In addition, three stable 231 lines were established that stably overexpress FABP5 shRNA or control shRNA (Fig. 3C), and two MCF-7 lines that stably overexpresses human FABP5 or empty vector (Fig. 4D). These lines are used to validate the effect of tested compound on cells is mediated by FABP5 (Fig. 3B).

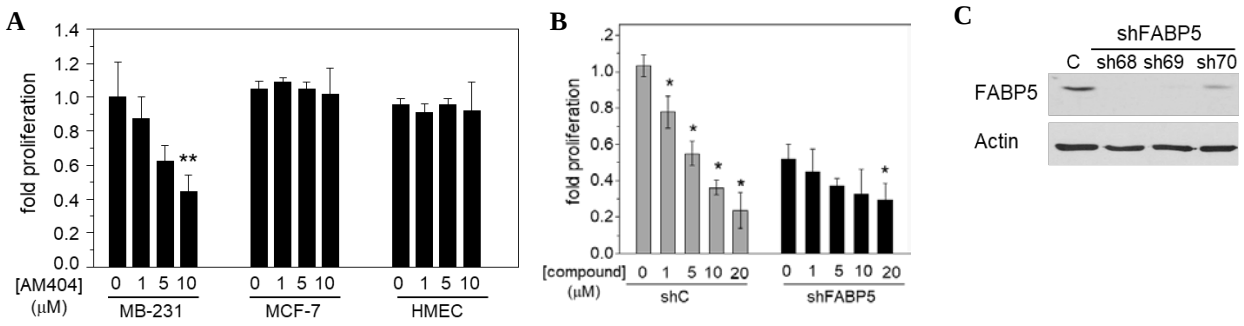

Figure 3. **A**) Cells were treated with N-arachidonoylaminophenol for 4 days and proliferation was measured by PrestoBlue. **B**) MDA-MB-231 lines stably expressing FABP5 shRNA or control shRNA were utilized in proliferation assays (4 days). N-arachidonoylaminophenol inhibited cell growth only in the line expressing FABP5. Data in all panels are mean (n=3). *p<0.01. **C**) Immunoblots indicating levels of FABP5 in stable MB-231 lines. **D**) Immunoblots indicating levels of FABP5 in MCF-7 lines stably overexpressing FABP5 or empty vector.

Specific Aim 2. Define the metabolic fate(s) of furanyl-fatty acids.

2.1. *Develop a comprehensive understanding of the metabolic and catabolic fate(s) of furanyl-FAs using a mass isotopomer approach in perfused organ systems.*

These activities have been completed at the Tochtrop lab as was detailed in the previous report.

2.2. Examine the rate(s) of metabolism/catabolism in normal tissues, and cancer cell lines.

Nothing to report.

Specific Aim 3. Assess the effects of furanyl-FAs on mammary tumor development in vivo.
IACUC approval for mice protocol was established by Levi.

3.1. *Assess the efficacy of furanyl-FAs in inhibiting tumor development in xenograft mouse models of breast cancer.*
Nothing to report

3.2. *Test the ability of furanyl-FAs to prevent tumor formation in the transgenic MMTV-Neu/Erb-B2 model of mammary carcinogenesis.*

Nothing to Report

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

- Levi attended AACR meeting “Obesity and Cancer: Mechanisms Underlying Etiology and Outcomes”, 2018.
- Levi and Stewart attended The 2018 4th International FASEB Conference on Retinoids. Levi attended career development workshop at this meeting.
- Two undergraduate students were working on this project during the summer in the Levi lab. Students were training in cancer research under Levi and Stewart supervision.

How were the results disseminated to communities of interest?

- Levi presented a poster at the Obesity and Cancer: Mechanisms Underlying Etiology and Outcomes.
- Stewart presented poster at the 2018 retreat of Case Comprehensive Cancer Center.
- Stewart gave a talk and presented a poster at the 2018 4th International FASEB Conference on Retinoids.

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

We currently have few molecules that were validated by cell culture assays to inhibit FABP5 and shift RA signaling. We plan to further validate the specificity of the compounds to FABP5 by testing the effect of the compounds on stable cell lines (mentioned above) in which levels of FABP5 are manipulated. In addition, binding affinity to other FABP5 will be measured. Next, we aim in moving forward to Aim 2 and Aim 3.

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

We were able to demonstrate that indeed, our criteria for screening for FABP inhibitor are appropriate. Most important, we were able to demonstrate the dual activity of FABP5 inhibitor that not inhibits transcriptional activity of PPARδ but also shifts RA to activate RAR. We demonstrate that targeting FABP5 inhibit growth of cancer cells in culture.

What was the impact on other disciplines?

As FABP5 is highly expressed in many types of cancer, this approach for treatment and prevention of tumors can be useful for other cancers as well.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to Report.

CHANGES/PROBLEMS:
Changes in approach and reasons for change

We expanded the scope of fatty acids to consider in the role of cancer chemopreventive agents. This proposal was based on our strong preliminary data on furan-containing fatty acids, but meanwhile we also tested other common dietary fatty acids. For example, we have been able to show that palmitate and stearate displays similar effects as compared to F_a.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to Report.
Significant changes in use or care of human subjects
Nothing to Report.
Significant changes in use or care of vertebrate animals.
Nothing to Report.
Significant changes in use of biohazards and/or select agents.

Nothing to Report.
PRODUCTS:
Publications, conference papers, and presentations
Nothing to Report.
Report only the major publication(s) resulting from the work under this award.
Nothing to Report.
Website(s) or other Internet site(s)
Nothing to Report.
Technologies or techniques
Nothing to Report.
Inventions, patent applications, and/or licenses
Nothing to Report.
Other Products
Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?
Name:Gregory Tochtrop
Project Role: PI/Professor
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0003-2447-254X
Nearest person month worked: 3
Contribution to Project: Project oversight, and direct supervision of Ms. Stewart, Dr. Han, Ms. Shang, and completion of the metabolomics work reported here.

Name:Liraz Levi
Project Role: PI/Instructor/Research Scientist
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0001-7462-2396
Nearest person month worked: 12
Contribution to Project: Project oversight, direct supervision of Ms. Stewart, establishing stable cell lines, biochemical molecular and cells assays (Aim 1), data analysis.

Name:Elizabeth Stewart
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 12
Contribution to Project: Contributions to small molecule synthesis, and biological contributions to Aim 1.

Name:Yong Han
Project Role: Research Associate
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 5
Contribution to Project: Ms. Small molecule synthesis.

Name:Grace Shang
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 5
Contribution to Project: Small molecule synthesis.

Name:Monica Rolince
Project Role: Undergraduate Student
Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 3
Contribution to Project: Testing effect of compounds on carcinogenic properties of cells.

Name: Surya Gopal
Project Role: Undergraduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Screening of compounds.

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?
Nothing to Report