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

PRINCIPAL INVESTIGATOR: Gregory Tochtrop

CONTRACTING ORGANIZATION: Case Western Reserve University

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6. AUTHOR(S)
Gregory Tochtrop
Liraz Levi

E-Mail:

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Case Western Reserve University

10900 EUCLID AVE
CLEVELAND OH 44106-1712

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14. ABSTRACT

We propose that inhibition of FABP5 represents a novel approach to diverting endogenous RA from pro-proliferative (PPAR δ) 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:CRABP-II expression.

15. SUBJECT TERMS

NONE LISTED

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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 as was detailed in the previous reports.

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

These activities have been completed as was detailed in the previous reports.

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

These activities have been completed as was detailed in the previous reports.

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 as was detailed in previous reports.

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

Since Report 1 we have begun migrating most of our metabolism work to cultured hepatocytes in lieu of perfused organ systems. Specifically, we have begun our investigations of furanyl-FAs in the following culture cell lines: AML12, IHH, and PH5CH8. Based on our data reported in reporting period 1, we know that F_a proceeds through beta oxidation with clear evidence of this molecules going through three cycles to afford a molecule with a three carbon spacer to the furan functionality. Subsequent initial work in cultured hepatocytes with a protected form of the furanyl-FA (shown below as PgO) indicate that derivitized versions of these molecules can also undergo omega-oxidation followed by one round of beta oxidation. This has led us to propose a novel ten-carbon metabolic end-product of furanyl-FAs *in vivo*. If confirmed this result would represent an exciting new direction moving forward.

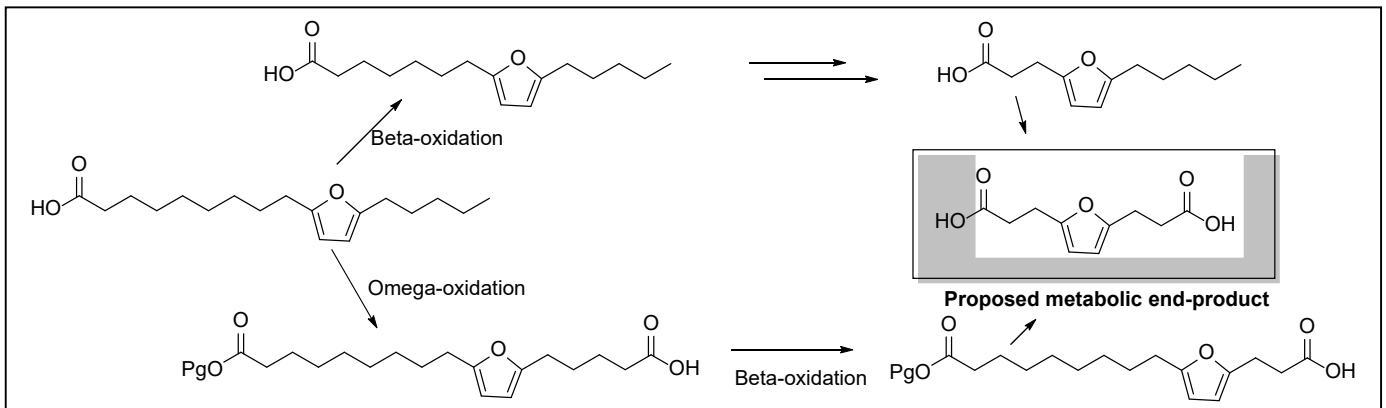

Figure 3: Proposed metabolic end-product for furanyl-FA F_a

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

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?

- During the time period between the previous reporting period and the current reporting period minimal opportunities were available due to effects corresponding to COVID-19. For the period of March 2020 until August 2020 access to research laboratories was minimal, and since August 2020 opportunities have remained at approximately 30% of normal.

How were the results disseminated to communities of interest?

- During the time period between the previous reporting period and the current reporting period minimal opportunities were available due to effects corresponding to COVID-19. For the period of March 2020 until August 2020 access to research laboratories was minimal, and since August 2020 opportunities have remained at approximately 30% of normal.

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

We are currently migrating much of our work towards more common dietary fatty acids, and drug metabolites and their ability to inhibit FABP5 and shift RA signaling. We plan to further validate the specificity of these molecules for 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. These common dietary fatty acids and drug metabolites will be integrated into the studies outlined for 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.

Further, we have been able to show that these molecules will undergo beta oxidation, but that rate is greatly attenuated compared to standard fatty acids.

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.

We predict that the results we describe here will be important for the fields of organic synthesis and metabolism. In terms of synthesis, orthogonal alkylation of furan is important, and could be widely used. In terms of metabolism, it is generally not known what the fate of furan is in mammalian systems.

What was the impact on technology transfer?
As reported before, results established so far for the ability of FABP5 inhibitor to suppress growth of TNBC cells have led to provisional patent application describing the use of AM404 and another small

molecule FABP5-inhibitor as novel treatment approach for TNBC (U.S. Provisional Patent Application No. 62/881,978, CWRU Ref. No. 2020-3687).

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, and metabolic byproducts of common drugs. For example, we have been able to show that palmitate, stearate, and a metabolic byproduct of acetaminophen display similar effects as compared to the reported data for 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: 2
Contribution to Project: Project oversight, and direct supervision of Ms. Stewart, Ms. Folkwein-Kennehan, 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:Heather Folkwein-Kennehan
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.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Partnering PI Levi will be leaving CWRU as of 1/2021

What other organizations were involved as partners?
Nothing to Report