AWARD NUMBER: W81XWH-17-1-0410

TITLE: The Role of p53 Synthetic Lethality in Increased Chemosensitivity to DNA-Damaging Agents Conferred by the Exercise Myokine Irisin

PRINCIPAL INVESTIGATOR: Helen J. Hathaway, PhD

CONTRACTING ORGANIZATION: University of New Mexico
Albuquerque, NM 87131-0001

REPORT DATE: October 2019

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 2019	Annual	15 Sep 2018 - 14 Sep 2019

4. TITLE AND SUBTITLE
The Role of p53 Synthetic Lethality in Increased Chemosensitivity to DNA-Damaging Agents Conferred by the Exercise Myokine Irisin

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0410

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Helen J. Hathaway, PhD

E-Mail: hhathaway@salud.unm.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of New Mexico
Albuquerque, NM 87131- 0001

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
The goal of this project is to address the overarching challenge to revolutionize treatment regimens by replacing them with ones that are more effective and less toxic. Specifically, we are testing the idea that the exercise myokine Irisin can synergize with DNA damaging chemotherapeutics to induce cytotoxicity with less toxic concentrations of the chemotherapeutic. In year 2 we built upon our in vitro observations from year 1, indicating that irisin may attenuate breast cancer progression by reducing inflammation. Orthotopic xenograft experiments in which MDA-MB-231 tumor-bearing mice were treated with vehicle, doxorubicin (Dox), or Dox + Low or High Irisin concentrations, demonstrated that tumor growth could be reduced by Dox + Low Irisin below that of Dox alone. Moreover, mice treated with Dox + Irisin combinations showed less Doxorubicin-mediated cytotoxic weight loss compared to Dox alone. Finally, mice treated with Irisin-Dox combination had reduced white adipose tissue depots and lower expression of inflammation markers in both adipose tissue and skeletal muscle. These results suggest that Irisin may attenuate aggressive breast tumor growth when combined with Dox, and concomitantly, irisin may contribute to overall metabolic health by maintaining body weight, possibly through improved muscle mass and reduced white adipose tissue. Attenuation of inflammation may contribute to both positive outcomes.

15. SUBJECT TERMS
Breast cancer, Irisin, Doxorubicin, combination therapy, cytotoxicity, p53, migration, inflammation

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	15	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. Introduction

The goal of this project is to address the overarching challenge to revolutionize treatment regimens by replacing them with ones that are more effective and less toxic. Specifically, we are testing the idea that the exercise myokine Irisin can synergize with DNA damaging chemotherapeutics to induce cytotoxicity with less toxic concentrations of the chemotherapeutic. In year 2 we built upon our in vitro observations from year 1, indicating that irisin may attenuate breast cancer progression by reducing inflammation. Orthotopic xenograft experiments in which MDA-MB-231 tumor-bearing mice were treated with vehicle, doxorubicin (Dox), or Dox + Low or High Irisin concentrations, demonstrated that tumor growth could be reduced by Dox + Low Irisin below that of Dox alone. Moreover, mice treated with Dox + Irisin combinations showed less Doxorubicin-mediated cytotoxic weight loss compared to Dox alone. Finally, mice treated with Irisin-Dox combination had reduced white adipose tissue depots and lower expression of inflammation markers in both adipose tissue and skeletal muscle. These results suggest that Irisin may attenuate aggressive breast tumor growth when combined with Dox, and concomitantly, irisin may contribute to overall metabolic health by maintaining body weight, possibly through improved muscle mass and reduced white adipose tissue. Attenuation of inflammation may contribute to both positive outcomes.

2. Keywords

Breast cancer, Irisin, Doxorubicin, combination therapy, cytotoxicity, p53, migration, inflammation

3. Accomplishments

- **Major goals of the project -** For year 2 of the project, as stated in the SOW, the goals were to conduct in vivo therapeutic assays using breast cancer cell lines, orthotopically implanted, with varying p53 status (wild type vs mutant). Analysis of the in vivo assays were to include tumor growth endpoints, assessment of metastasis, complete histopathological analyses, and assessment of cell cycle/apoptosis assays through immunodetection.

- **Accomplishments –** The majority of our efforts in year 2 have been to create and analyze in vivo models of breast tumorigenesis. We have been focusing the majority of our efforts on the MDA-MB-231 orthotopic model, because irisin has been shown to affect the proliferation and migration of these cells. Moreover, the MDA-MB-231 orthotopic model readily metastasizes to the lungs of NOD-*scid*-gamma (NSG) mice (Jackson Labs stock #005557, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ), with 100% of mice exhibiting lung metastases by 53 days following cell inoculation (Iorns et al, 2012), allowing us to evaluate irisin's effects on metastasis. Thus far our observations have yielded encouraging and intriguing results, described below.

  In pilot experiments, we first identified an appropriate dose of Doxorubicin (Dox) for use in our combination studies. Dox mechanism of action involves inhibition of DNA replication, and because the NSG mice have a blunted DNA repair mechanism, they are more sensitive to the side effects of Dox (Barve et al, 2018). Dox was administered to non-tumor-bearing NSG female mice at 0.5, 1.0, or 1.5 mg/kg, once a week for 4 weeks by IP injection, which mimics the planned Dox treatment regimen. PBS vehicle served as negative, non-drug treated control. As shown in

Fig. 1, vehicle- and 0.5 mg/kg-treated mice steadily gained weight whereas mice receiving 1.0 mg/kg maintained their weight until day 21, after which they began to lose a modest percentage of body weight. By day 42, weight loss in the 1.0 mg/kg group was on average less than 12%, and despite the weight loss this group maintained other signs of good health, including normal activity and grooming. However, the 1.5 mg/kg-treated group demonstrated substantial body weight loss by day 18 (loss of 25% body weight is a criteria for euthanasia), and all mice in that cohort required euthanasia by day 32. That endpoint is prior to the planned experimental endpoint of approximately 49 days from tumor inoculation, a desirable endpoint because we expect it will permit an assessment of metastasis to the lungs. Based on these results, we reasoned that 1.0 mg/kg Dox, administered weekly for 4 weeks by IP injection, would allow us to resolve any effects of irisin in combination therapeutic trials.

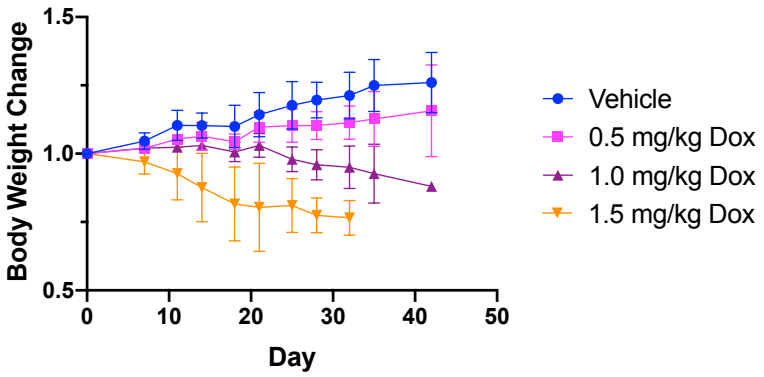

Figure 1. Evaluation of Dox dose effects on body weight. Non-tumor-bearing NSG female mice were injected IP with the indicated doses of Dox once a week for 4 weeks (days 0, 7, 14, and 21). Mice were weighed on the indicated days. Data are expressed as fold change in body weight compared to day 0. $p < 0.0001$ by two way ANOVA.

To evaluate Dox + Irisin combination therapy, NSG female mice were inoculated with 2.5 x 10^6 MDA-MB-231 cells into the left inguinal mammary fat pad. Once tumors became palpable they were measured with calipers twice weekly, and when tumors reached a volume of 75 – 450 mm^3 (about 21 days following inoculation), treatments were begun. The six treatment groups included: 1) vehicle; 2) Dox alone; 3) Low dose Irisin; 4) High dose Irisin alone; 5) Dox + Low dose Irisin; 6) Dox + High dose Irisin. Two doses of irisin were chosen based on a literature review, and represent a 50-fold difference between low (20 μg/kg/day over 28 days) and high (1 mg/kg/day over 28 days) doses. Specifically, it was shown that a low dose of irisin (100 μg/kg by injection, once/week for 4 weeks) is insufficient to induce white adipose browning, while improving bone density (Colaianni et al, 2015), while high doses (1 mg/kg/day by injection for 6 days) exacerbate bone loss and induce white adipose browning (Kim et al, 2018). Although our treatment regimen, consisting of a continual release of Irisin over 28 days via osmotic minipump inserted subcutaneously, does not exactly mimic either referenced regimen, there is as yet no consensus for in vivo Irisin treatment in the field, and as a result we attempted to approximate the highest and lowest doses that produced measurable, but distinct, bioactivities, while minimizing the stress of daily treatments.

In this study, MDA-MB-231 tumor size steadily increased in vehicle-treated mice, and 1.0 mg/kg Dox (weekly x 4) had no effect on tumor growth (Fig. 2a), although average Dox-induced body weight loss was less than 10% compared to vehicle control-treated mice at the end of the experiment and did not interfere with study completion (Fig. 3a). Dox + High Irisin co-treatment also did not affect tumor growth, however tumor size in the Dox + Low Irisin co-treatment group trended smaller compared to vehicle control or Dox alone by Study Day 42 (Fig. 2a). At necropsy, tumors were excised and volumes were calculated, demonstrating a significant difference in tumor volume between the groups (Fig. 2b). In contrast, Irisin alone at either low or high concentration did not abrogate tumor growth compared to vehicle control (Fig. 2c & d). The effect of Dox + irisin in this study are modest, likely due to large variability in the Dox-treated arm, and the small cohort size, however we have increased the cohort size in an ongoing, repeat experiment in order to more rigorously test the hypothesis.

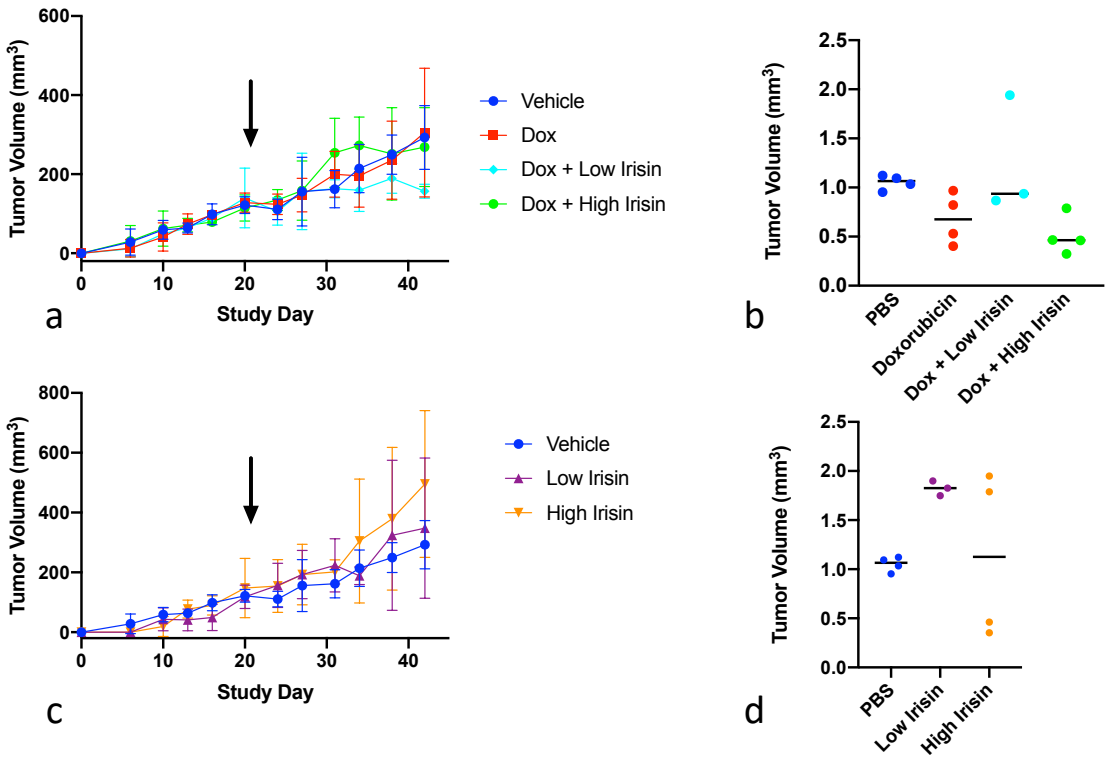

Figure 2. Dox-Low Irisin combination trends toward tumor growth inhibition. NSG female mice bearing orthotopic MDA-MB-231 tumors were treated with 1 mg/kg Dox by IP injection once a week for 4 weeks, 28-day slow-release osmotic minipumps releasing 20 μg/kg/day irisin (Low Irisin) or 1 mg/kg/day irisin (High Irisin), or Dox-Irisin combinations, starting on day 21. Control mice received IP injections containing PBS vehicle and PBS minipumps; Dox-only treated mice also received PBS minipumps. For clarity, Vehicle compared to Dox alone, or Dox + Irisin combination treatments are shown in panels **a** and **b**, while Vehicle compared to Irisin alone are shown in panels **c** and **d**, although treatments for all groups were conducted simultaneously. Day 0 refers to the day of tumor inoculation; black arrow indicates the start of treatments. Panels **a** and **c** show tumor growth trends throughout the assay, while panels **b** and **d** depict tumor volumes at necropsy. For panel **b**, p = 0.03 by One-Way ANOVA.

These results indicate that Irisin alone has no a anti-tumorigenic activity, but they suggest that Irisin may enhance the tumor-directed cytotoxicity of Dox in an in vivo scenario, as

hypothesized. We deliberately chose a Dox concentration that could be tolerated by the mice for the duration of the experiment, and that we reasoned would allow us to detect any synergism with Irisin co-treatment. The mechanism by which Irisin synergizes with Dox remains to be established, and studies are underway to analyze harvested tumors to evaluate histopathology, proliferation, apoptosis, and vascularization, all of which have been shown to be influenced by Irisin (Bostrom et al, 2012; Gannon et al, 2015).

We found that Irisin-Dox co-treatment had an unexpected benefit in MDA-MB-231 tumor-bearing mice, in that Irisin partially abrogated the Dox-induced body weight loss. High Irisin dose (1 mg/kg/day, 28 days) improved body weight to a statistically significant extent, whereas body weight in mice treated with Dox and Low Irisin dose (20 μg/kg/day, 20 days) trended toward improved body weight (Fig. 3a). Irisin alone at either dose did not affect body weight, indicating that Irisin-Dox co-treatment counteracted the negative effects of Dox on body weight loss.

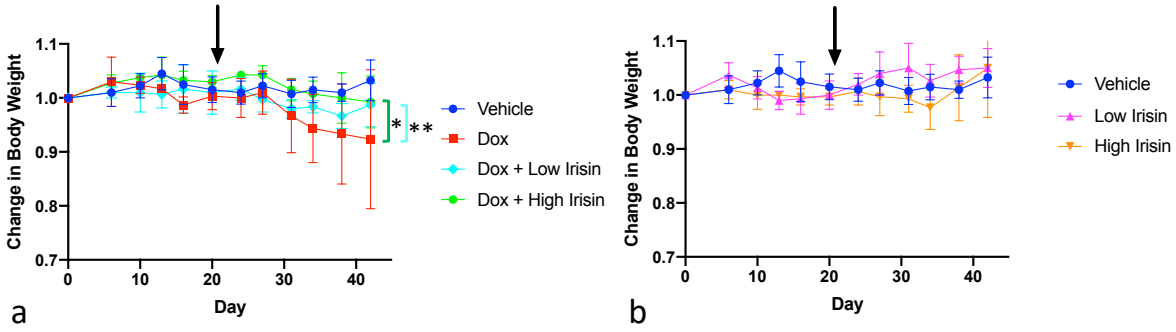

Figure 3. Irisin partially abrogates Dox-dependent body weight loss. NSG female mice bearing orthotopic MDA-MB-231 tumors, treated with vehicle, Dox, and Low or High Irisin concentrations as indicated in Fig. 2, were monitored for body weight. For clarity, Vehicle compared to Dox alone, or Dox + Irisin combination treatments are shown in panel **a**, while Vehicle compared to Irisin alone are shown in panel **b**. Day 0 refers to the day of tumor inoculation; black arrow indicates the start of treatments. *, p = 0.02; ** p = 0.3 by two way ANOVA.

This result was somewhat surprising, since Irisin has been shown to reduce body weight in obese mice, which has been attributed to increased thermogenesis and browning of white fat in a PPARγ coactivator-1α (PGC-1α)-dependent manner (Boström et al, 2012). PGC-1α, a transcription factor, is upregulated in muscle during exercise and regulates the expression of a number of genes related to energy metabolism, including Fndc5, whose cleavage produces the secreted myokine Irisin (Boström et al, 2012).

To begin to address this unexpected observation, in addition to tumor size and body weight measures we evaluated and harvested a number of tissues at the termination of the in vivo cohort described above, including skeletal and cardiac muscle, brown and white adipose tissues (BAT and WAT), and bone. We observed a dramatic reduction in the size of the gonadal WAT depots in mice treated with Dox + High Irisin, compared to Vehicle (Fig. 4a & b). Inguinal WAT also appeared to be reduced in size (not shown). In this cohort we did not quantitatively harvest WAT, however we processed the tissues for RNA, protein, and formalin-fixation/paraffin-

embedding. Hematoxylin & eosin-stained sections of gonadal WAT demonstrates that Irisin treatment reduced adipocyte size (Fig. 4c-f), as previously reported (Boström et al, 2012). Quantification of adipocyte size is ongoing. BAT was quantitatively excised, and the differences in BAT weights between the groups were not significantly different (Fig. 4g).

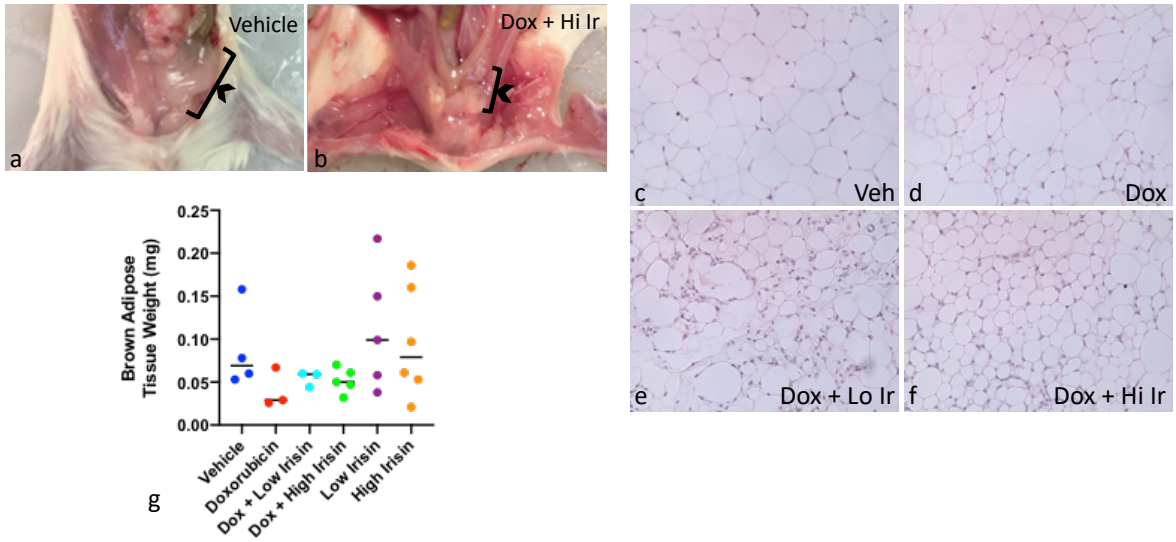

Figure 4. Irisin modifies adipose tissue profile in Dox-treated, tumor-bearing mice. Gonadal WAT and BAT were harvested from NSG female mice bearing orthotopic MDA-MB-231 tumors, treated with vehicle, Dox, and Low or High Irisin concentrations. Gonadal WAT from vehicle (PBS)-treated (**a**) and Dox + High Irisin-treated mice (**b**) are shown, with the brackets indicating the fat depots. In panels **c – f**, Gonadal WAT was formalin-fixed, paraffin embedded, and 5 μm sections were stained with hematoxylin & eosin. **c**, Vehicle-treated, **d**, Dox-treated, **e**, Dox + Low Irisin-treated, **f**, Dox + High Irisin-treated. In panel **g**, BAT depots were harvested and weighed. Differences were not statistically significant by one-way ANOVA ($p = 0.28$).

Our observations suggest that Dox + Irisin co-treatment may blunt the negative effects of Dox on muscle atrophy, possibly through a shift to a more thermogenic state. To address this, we examined the expression of genes altered during a thermogenic shift, including those that are known to be regulated through Fndc5/Irisin such as UCP1 (Boström et al, 2012; Colaianni et al, 2015). We found no difference in UCP1 expression in BAT (Fig. 5b), and moreover we saw a small but significant decrease in UCP1 expression in gonadal WAT (Fig. 5a), which was unexpected given the fact that Irisin is known to upregulate UCP1 in vivo, as part of its increased thermogenic and "white-to-brown fat" conversion. At the current time it is not clear if this effect is due to contradictory actions of Dox, which also slightly but significantly reduced UCP1 expression in gonadal WAT. Indeed, Dox treatment significantly downregulates UCP1 in cardiac muscle, leading to reactive oxygen species which is a significant cause of Dox-induced cardiotoxicity (Bugger et al, 2011). Comparison of adipose tissues treated with Irisin alone vs Dox + Irisin may help to resolve this finding.

Also of interest were Irisin-regulated genes involved in inflammation such as TNFα, since Irisin is known to attenuate inflammation (Askari et al, 2018; Mazur-Bialy et al, 2017) and our previously reported RNAseq results suggest that irisin downregulates specific pro-inflammatory

effectors, including TNFAIP8L1 and TNFRSF21 (Padmavathia et al, 2018; Wu et al, 2018). We found that TNFα expression was significantly reduced by Dox + Irisin co-treatment compared to vehicle control in both BAT and quadriceps skeletal muscle (SKM), while Dox treatment alone did not affect TNFα expression (Figs. 5c & d).

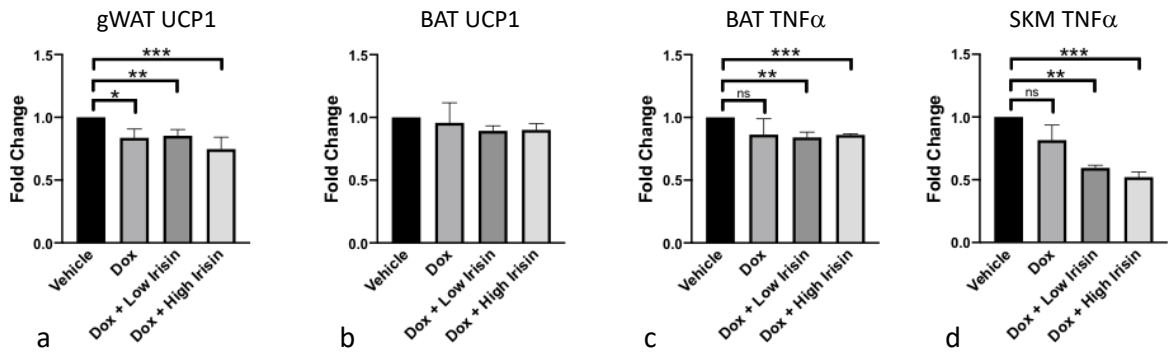

Figure 5. Tumor-bearing, Dox + Irisin-treated mice show evidence of attenuated inflammation. Reverse transcription RT-PCR was performed on RNA > cDNA prepared from gonadal WAT (gWAT), BAT, and quadriceps skeletal muscle (SKM). **a**, UCP1 expression in gWAT is reduced by Dox and Dox + Irisin treatment compared to vehicle control treatment. *, p = 0.017; **, p = 0.008; ***, p = 0.012 by t test. **b**, UCP1 expression in BAT is unchanged by Dox and Dox + Irisin treatment compared to vehicle. **c**, The inflammatory cytokine TNFα is significantly reduced in BAT by Dox + Irisin co-treatment, but not Dox alone. **, p=0.012; ***, p=0.006 by t test. **d**, TNFα is significantly reduced in SKM by Dox + Irisin co-treatment, but not Dox alone. **, p=0.006; ***, p=0.004 by t test.

Intriguingly, it was recently shown that exercise can partially abrogate the cytotoxic effects of Dox on cardiac and skeletal muscle (Bredahl et al, 2016). Dox can upregulate the expression of forkhead-box O (FoxO1 and FoxO3) transcription factors, followed by elevated transcription of FoxO target genes (Kavazis et al, 2014). Conversely, exercise suppresses FoxO1 and FoxO3 and subsequently downregulates FoxO1 target genes, including genes that induce atrophy (Kavazis et al, 2014). PGC-1α has also been shown to reduce skeletal muscle atrophy through suppression of FoxO (Sandri et al, 2006). Since PGC-1α upregulates Fndc5 expression and Irisin secretion, collectively these observations suggest that Irisin may counteract Dox-dependent muscle atrophy and cytotoxicity by counteracting Dox-dependent FoxO upregulation. We plan to examine these pathways in tissues harvested from the previous and ongoing in vivo cohorts.

As described in the previous progress report, our results increasingly point to a role for Irisin acting as an anti-neoplastic agent in breast cancer through multiple pathways, including inflammation. Intriguingly, obesity is associated with both an inflammatory state, and with increased breast cancer risk (Argolo et al, 2018). Irisin may emerge as one possible link between these outcomes, since irisin reduces adipocyte deposition as well as its proposed role in attenuating cancer progression (Mazur-Bialy et al, 2017). Moreover, it has become apparent that wild type p53 is an important factor in suppressing inflammation in the tumor environment (Uehara and Tanaka, 2018), therefore it is possible that Irisin's anti-inflammatory actions in part compensate for the loss of p53, as in our MDA-MB-231 model. Finally, we are also investigating possible tumor cell-autonomous actions of Irisin, using MDA-MB-231 cells in a modified in vitro assay that includes collagen I substrates, which better mimics the in vivo tumor

microenvironment, rich in collagen. These studies are in progress and results are expected within the next few weeks.

In summary, we have made good progress related to Aim 3, to analyze Irisin synergy with Dox in in vivo assays. The results so far have suggested a modest effect on tumor growth, which we believe will become significant with a larger cohort of mice, currently underway. In addition, the in vivo assay has revealed an additional benefit of Irisin co-treatment with respect to attenuation of the cytotoxic effects of Dox. We are currently analyzing the molecular basis for this effect, and our observations thus far suggest that Irisin may counteract Dox cytotoxicity through anti-inflammatory mechanisms. Additionally, we are analyzing tissues harvested from the in vivo cohort to determine how Dox + Irisin limited tumor growth.

- References cited in Section 3, Accomplishments

1. Argolo DF, Hudis CA, Iyengar NM (2018) The impact of obesity on breast cancer. *Curr. Oncol. Rep*. 20:47. doi: 10.1007/s11912-018-0688-8.

2. Askari H, Rajani SF, Poorebrahim M, Haghi-Aminjan, H, Raeis-Abdollahi E, Abdollahi M (2018) A glance at the therapeutic potential of irisin against diseases involving inflammation, oxidative stress, and apoptosis: An introductory review. *Pharm. Res*. 129:44-55. doi:10.1016/j.phrs.2018.01.012.

3. Barve A, Casson L, Krem M, Wunderlich M, Mulloy JC, Beverly LJ (2018) Comparative utility of NRG and NRGS mice for the study of normal hematopoiesis, leukemogenesis and therapeutic response. *Exp. Hematol*., 67:18-31. doi:10.1016/j.exphem.2018.08.004.

4. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM (2012) A PGC1α-dependent myokine that drives browning of white fat and thermogenesis. *Nature* 481(7382):463–468. doi:10.1038/nature10777.

5. Bredahl EC, Pfannensteil KB, Quinn CJ, Hayward R, Hydock DS (2016) Effects of exercise on Doxorubicin-induced skeletal muscle dysfunction. *Med. Sci. Sports Exercise* 48(8):1468-1473. doi: 10.1249/MSS.0000000000000926.

6. Bugger H, Guzman C, Zechner C, Palmeri M, Russell KS, Russell RR (2011) Uncoupling protein downregulation in doxorubicin induced heart failure improves mitochondrial coupling but increases reactive oxygen species generation. *Cancer Chemother Pharmacol* 67(6):1381-1388. doi:10.1007/s00280-010-1441-7.

7. Colaianni G, Cuscito C, Mongelli T, Pignataro P, Buccoliero C, Liu P, Lu P, Sartini L, Di Comite M, Mori G, Di Benedetto A, Brunetti G, Yuen T, Sun L, Reseland JE, Colucci S, New MI, Zaidi M, Cinti S, and Grano M (2015) The myokine irisin increases cortical bone mass. *PNAS* 112(39):12157-12162. https://doi.org/10.1073/pnas.1516622112.

8. Gannon NP, Vaughan RA, Garcia-Smith, R, Bisoffi M, Trujillo, KA (2015) Effects of the exercise-inducible myokine irisin on malignant and non-malignant breast epithelial cell behavior *in vitro. Int J Cancer* 136:E197-E202. https://doi-org.libproxy.unm.edu/10.1002/ijc.29142

9. Iorns E, Drews-Elger K, Ward TM, Dean S, Clarke J, Berry D, El Ashry, D, Lippman M (2012) A new mouse model for the study of human breast cancer metastasis. *PLOS ONE* 7(10): e47995. https://doi.org/10.1371/journal.pone.0047995.

10. Kavazis AN, Smuder AJ, Powers SK (2014) Effects of short-term endurance exercise training on acute doxorubicin-induced FoxO transcription in cardiac and skeletal muscle. *J Appl Physiol* 117:223-230. doi:10.1152/japplphysiol.00210.2014.

11. Kim H, Wrann CD, Jedrychowski M, Vidoni S, Kitase Y, Nagano K, Zhou C, Chou J, Parkman VA, Novick SJ, Strutzenberg TS, Pascal BD, Le PT, Brooks DJ, Roche AM, Gerber KK, Mattheis L, Chen W, Tu H, Bouxsein ML, Griffin PR, Baron R, Rosen CJ, Bonewald LF, Spiegelman BM (2018) Irisin mediates effects on bone and fat via αV integrin receptors. *Cell* 175(7):1756-1768.e17. https://doi.org/10.1016/j.cell.2018.10.025.

12. Mazur-Bialy AI, Bilski J, Pochec E, Brzozowski T (2017) New Insight into the Direct Anti-Inflammatory Activity of a Myokine Irisin Against Proinflammatory Activation of Adipocytes. Implication for Exercise in Obesity. *J. Phys. Pharm*. 68:243-251.

13. Padmavathia G, Banika K, Monishaa J, Bordoloia D, Shabnama B, Arfusob F, Sethic G, Fane L, Kunnumakkaraa AB (2018) Novel tumor necrosis factor-α induced protein eight (TNFAIP8/TIPE) family: Functions and downstream targets involved in cancer progression. *Cancer Letters* 432:260-271. https://doi.org/10.1016/j.canlet.2018.06.017.

14. Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL, Spiegelman BM (2006) PGC-1α protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *PNAS* 103(44):16260-16265. doi:10.1073/pnas.0607795103.

15. Uehara I, Tanaka N (2018) Role of p53 in the Regulation of the Inflammatory Tumor Microenvironment and Tumor Suppression. *Cancers (Basel)*. 10(7):219. doi:10.3390/cancers10070219

16. Wu H, Pang P, Liu M, Wang S, Jin S, Liu F, Sun C, (2018) Upregulated miR-20a-5p expression promotes proliferation and invasion of head and neck squamous cell carcinoma cells by targeting of TNFRSF21. *Onc. Reports* 40(2):1138-1146. https://doi.org/10.3892/or.2018.6477.

- **Training and professional development opportunities -** Although the project is not specifically intended to provide training opportunities, we have been fortunate to host an undergraduate scholar of the Minority Access to Research Careers (MARC) Program, Ms. Shania Sanchez. The

MARC Program is an NIH-funded program to provide underrepresented minority (URM) undergraduate students training in STEM fields, to increase students' competitiveness for graduate programs, and to increase URM representation in STEM fields such as biomedical science. Ms. Sanchez's stipend is supported by the MARC Program, and she devotes approximately 15 hr/week to research in our lab. Ms. Sanchez is mentored by myself and the Research Specialist supported by this award, Mr. Brandon Phinney. Ms. Sanchez's efforts are integral to the project, and she will be co-author on manuscripts generated by these efforts in year 3.

- **Dissemination of results -** These results have been disseminated at the Society for Advancement of Chicano and Native Americans in Science (SACNAS) annual meeting from October 31 – November 2, 2019, by Ms. Shania Sanchez. These venues exposed members of groups underrepresented in biomedical science to cutting edge research.

- **Plans during the next reporting period to accomplish the project's goals -** We will complete the detailed molecular analysis of the in vivo MDA-MB-231 orthotopic study reported here, and we will complete similar detailed analysis of the ongoing study, with increased sample size. Analysis of Irisin's impact on metastasis is ongoing, but is a high priority based on the in vitro observations in year 1 suggesting effects on tumor cell migration. An additional high priority in our post-mortem analysis is inflammatory mediators. Moreover, based on our findings in the first in vivo study, we will expand the post-mortem collection and analysis to include quantitative harvest of WAT depots, skeletal muscle, and cardiac muscle, serum collection (to detect Irisin and other hormones/myokines in blood), and we will include collection and analysis of bone, since Irisin regulates cortical bone mass, although published studies find differing results depending on Irisin concentration and treatment course (Colaianni et al, 2015; Kim et al, 2018). We will also conduct similar studies using the MCF7 alternative cell line, with wild type p53, as described in the SOW.

4. Impact

- **Impact on the development of the principal discipline of the project -** Our first in vivo study has confirmed the hypothesis, in part, in that Irisin combined with Doxorubicin improves tumor outcomes, i.e., tumor growth is reduced, albeit modestly. With refined experimental protocols we believe that we will achieve meaningful results in repeat experiments. Moreover, we are highly encouraged by results that suggest that Irisin addition to Dox therapy may have additional benefits by attenuating weight loss associated with Dox cytotoxicity. This may be attributed to reduced inflammation and increased thermogenesis, conversion of WAT to BAT, and improved muscle metabolism, although ongoing molecular analysis of tissues harvested from treated mice will help to define molecular mechanisms. These goals will be accomplished in year 3.

- **Impact on other disciplines -** Nothing to report for this project period.

- **Impact on technology transfer** - Nothing to report for this project period.

- **Impact on society beyond science and technology** - Nothing to report for this project period.

5. Changes/Problems

- **Changes in approach and reasons for change -** As we described in the Year 1 progress report, our focus has changed somewhat since we observed more modest reductions in cell viability in with Dox/Irisin combinations against the sensitive cell line MDA-MB-231 than expected. Nevertheless Irisin combined with Dox in vivo does appear to provide benefits, in both tumor growth reduction and metabolic shifts that may counteract Dox cytotoxicity. Based on the Irisin literature and our in vitro results, and now results from an in vivo cohort, we are aggressively exploring altered inflammatory tumor microenvironment, a thermogenic shift enhanced by reduced inflammation, and reduced tumor cell migration as targets of Irisin bioactivity.

- **Actual or anticipated problems or delays and actions or plans to resolve them -** There are no current problems that will cause further delays.

- **Changes that had a significant impact on expenditures -** Nothing to report for year 2.

- **Significant changes in use or care of human subjects -** Not applicable.

- **Significant changes in use or care of vertebrate animals -** Our use of vertebrate animals has not changed from the approved IACUC protocol at this time. The in vivo portion of the project is underway as proposed. Based upon the outcome of the first experiments, the major changes in the in vivo assays involve post-mortem analysis, so they are not subject to IACUC approval. We do plan to include DEXA scanning of subsequent mice cohorts so that we can measure bone density and body composition. This procedure is non-invasive and involves anesthesia and live animal imaging, which takes a few minutes per mouse. We have an amendment submitted to the IACUC to add this procedure, and this will be approved before DEXA scanning will be performed.

- **Significant changes in use of biohazards or select agents -** Not applicable.

6. Products

- **Publications, conference papers, and presentations -**

    - **Journal Publications** - Nothing to report during this project period. A manuscript describing the in vivo findings is in preparation, and we expect to submit this manuscript by mid-2020.

    - **Books or other non-periodical, one-time publications** - Nothing to report during this project period.

    - **Other publications, conference papers, and presentations -**

        - Poster presentation - University of New Mexico Minority Access to Research Careers (MARC) Symposium - August 25, 2018. Presented by MARC Scholar Shania Sanchez

- Poster presentation - 2018 SACNAS National Diversity in STEM Conference, Oct. 31 – November 2, 2019, Honolulu, HI. Presented by MARC Scholar Shania Sanchez.
- Websites - N/A
- Technologies or techniques - N/A
- Inventions, patent applications, licenses - N/A
- Other products - N/A

7. Participants & Other Collaborating Organizations

- **Individuals working on the project -**

Name:	Helen Hathaway
Project Role:	PI
Researcher Identifier:	ORCID ID - 0000-0002-7879-5056
Nearest person month worked:	1
Contribution to Project:	Provide overall oversight and guidance; coordination of work; train and supervise technician and student assistant
Funding Support:	This award

Name:	Laurie Hudson
Project Role:	Co-I
Researcher Identifier:	TBD
Nearest person month worked:	1
Contribution to Project:	provide guidance in experimental design and data interpretation; consult regularly with PI; assist with experimental design, especially for drug combination studies
Funding Support:	This award

Name:	Brandon Phinney
Project Role:	Research Specialist
Researcher Identifier:	N/A
Nearest person month worked:	6
Contribution to Project:	Performs experiments, collates data, presents data to PI and Co-I's, provides day-to-day training to student
Funding Support:	This award

Name:	Shania Sanchez
Project Role:	Undergraduate Research Assistant
Researcher Identifier:	N/A
Nearest person month worked:	4.5
Contribution to Project:	Performs in vitro experiments, cell culture

Funding Support:	Minority Access to Research Careers (MARC) Program - T34 GM008751 (NIH)

- **Any change in active other support of PD/PI or senior/key personnel** - Nothing to report for this project period.

- **Other organizations involved as partners** - Nothing to report for this project period.

8. **Special Reporting Requirements** - N/A

9. **Appendices** - N/A