Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2013 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 57. Role of mTOR in repair of UVB-induced DNA damage

7. **Start and End Date of Research Project:** 5/1/2013 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Lisa M. Shantz, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 93,436

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Sass-Kuhn, Suzanne	Research Support Assistant	100	$33,819 salary $11,389 fringe
Shantz, Lisa M	Associate Professor	5	$5633 salary $1888 fringe

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Feehan, Robert	Graduate Student	25

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Raptor and Rictor signaling in epithelial tumorigenesis	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	11/2013	$1,901,78 over 5 years	$ pending

Data was part of preliminary data in A1 application.

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

In the event that the above R01 is not funded, I am planning to include the data in this report as preliminary data for an R21 application designed specifically to address the role of mTORC2 in DNA repair.

12. Future of Research Project. What are the future plans for this research project?

We will continue to expand our mTORC2 DNA repair data, and apply for a grant as described above.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	
Female				
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				
Other				
Unknown				
Total			**1**	

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Funding for this project provided opportunities for valuable research training for a graduate student who will help to expand the base of highly qualified research scientists in years to come. In addition, the CURE funding enabled me to obtain preliminary data that will hopefully help to make a recent grant application to NIH be competitive for external support which will further strengthen the base for health research at Penn State Hershey.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a

performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Research project overview (from Strategic Plan):

Ultraviolet (UV) radiation is the major risk factor for developing skin cancer, the most prevalent cancer worldwide. Several recent studies from our lab and others indicate that mammalian target of rapamycin (mTOR) signaling is activated by UVB and may play an important role in skin tumorigenesis. mTOR exists in two functionally and compositionally distinct protein complexes: the rapamycin-sensitive mTOR complex 1 (mTORC1) and the rapamycin-resistant mTOR complex 2 (mTORC2). The goal of the proposed studies is to investigate the role of the two mTOR complexes in the repair of DNA damage, specifically global-genome nucleotide excision repair (GG-NER), in keratinocytes exposed to UVB. Direct absorption of UVB irradiation by DNA within the epidermis causes damage through the formation of covalent linkages that result in products such as cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts (6-4PPs), which are repaired by GG-NER. Several reports in the recent literature have suggested that the tumor suppressor PTEN and putative tumor suppressor AMPK promote GG-NER in the skin by decreasing levels of the xeroderma pigmentosum group C (XPC) protein. Both PTEN and AMPK are key negative regulators of mTOR signaling, with loss of either PTEN or AMPK leading to mTOR activation.

Using HaCaT cells, an established human keratinocyte cell line, as a model, Aim 1 will address the hypothesis that activation of mTOR impairs efficient GG-NER of UVB-induced DNA damage. We will use the specific mTORC1 inhibitor rapamycin and the mTOR kinase inhibitor Torin-2 to determine whether mTOR inhibitor protects UVB-treated cells from accumulating mutations by maintaining efficient GG-NER. The rate of repair will be evaluated by measuring removal of both CPD and 6-4PP lesions. To more accurately assess the contribution of the two mTOR complexes to GG-NER, we will use HaCaT cells with knock down of either Raptor or Rictor, which are essential for activity of mTORC1 and mTORC2, respectively.

A possible mechanism by which uncontrolled stimulation of mTOR signaling in the skin might impact the survival of UVB-treated cells will be addressed in Aim 2. These experiments will use HaCaT cells with knockdown of PTEN, a common early occurrence in

skin carcinogenesis, to induce mTOR-dependent signaling. PTEN loss is associated with XPC downregulation in keratinocytes, and exposure of cells to rapamycin or Torin-2 followed by measurement of XPC in chromatin-bound and soluble fractions will test the hypothesis that mTOR activation is necessary for this downregulation. If keratinocyte GG-NER is enhanced when mTOR activity is limited, this would not only provide a strong rationale for using mTOR inhibitors in skin cancer prevention, but also provide evidence of a new pathway contributing to regulation of GG-NER in the skin that has not been previously proposed.

Summary of the Research Completed from 5/1/2013 - 12/31/2013:

Using treatment with the mTOR complex 1 (mTORC1) inhibitor rapamycin, and mice with conditional deletion of mTOR in the skin, we showed previously that UVB activates signaling downstream of both mTOR complexes, mTORC1 and mTORC2. Inhibition of mTORC1 with rapamycin effectively blocked the hyperproliferation response that occurs with UVB exposure, while keratinocytes were sensitized to UVB-induced apoptosis when both mTORC1 and mTORC2 activities were deleted [1], suggesting separate but complementary roles for the two mTOR complexes in prevention of photocarcinogenesis. The overall goal of the CURE studies was to determine whether mTOR inhibition also protects surviving UVB-treated cells from accumulating DNA damage.

Figures 1, 2, and 3 describe experiments designed to address Aim 1. Repair of DNA damage was assessed by measuring removal of CPD lesions after UVB exposure in HaCaT cells treated with either rapamycin to inhibit mTORC1 or Torin-2 to inhibit both mTOR complexes. This was followed by performing the same experiment in Rictor$^{-/-}$ mouse embryo fibroblasts (MEFs), to directly assess the effect of blocking mTORC2 activity. Surprisingly, rather than suggesting increased repair of surviving cells after UVB, the results showed that DNA damage increased over time when mTORC2 was inhibited. Western blot analysis of checkpoint proteins showed that treatment of cells with Torin-2 delayed the phosphorylation of Chk-1, Chk-2 and p53 in response to UVB, while rapamycin had no effect. Phosphorylation of histone H2.AX, which localizes to sites of DNA damage, was also delayed in Torin 2-treated cells. Aim 2 experiments, which are shown in Figures 4, 5 and 6, showed that in the presence of PTEN knockdown, XPC levels were decreased. Treatment with rapamycin, Torin-2 or the PI 3-kinase inhibitor LY294002 returned XPC to levels seen in control cells, suggesting the change in XPC protein levels observed with PTEN knockdown may be mediated by mTORC1.

Methods used:

Cell culture and drug treatment: HaCaT keratinocytes (obtained from The German Cancer Research Center) or Rictor$^{+/+}$ and Rictor$^{-/-}$ mouse embryo fibroblasts (a generous gift of Dr. Mark Magnuson, Vanderbilt University) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals), and penicillin/streptomycin (100µg/ml, Invitrogen), and cultured for less than 20 passages at 37°C with 5% CO_2. Drug treatments were added 1 hour before UVB exposure at a concentration of 50 nM for Rapamycin (Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD) and Torin 2 (Tocris Bioscience, Bristol, UK) and 1 µM for LY 294002 hydrochloride (LY; Tocris Bioscience, Bristol, UK). Vehicle dimethyl

sulfoxide (DMSO) was added as a control.

UVB Treatment: Cells were cultured until 70% confluence on 12 well cell culture plates (Greiner Bio-One) under normal culture conditions. Before UVB treatment, cells were washed twice with PBS, and then exposed to UVB (FS20 UVB bulbs, National Biological) emitting light between 290nm and 320nm in minimal PBS at dose described in the figure legends. A UVB 500C meter (National Biological) was used to monitor irradiation intensity. Following UVB irradiation, PBS was removed and replaced with the saved media containing the drugs treatments. For DNA damage assays, cells were incubated overnight in low serum (2%) prior to UVVB to minimize cell cycle progression. When inhibitors were used, cells were pre-incubated for 2 h before UVB at concentrations described in the figures.

DNA Damage Repair Assay: Cells were collected at different time points post-UVB and DNA was isolated using a QIAamp DNA Mini Kit (Qiagen). DNA concentration was calculated from the absorbance at 260 nm. The presence of CPDs in DNA was quantified by ELISA using a monoclonal antibody (TDM-2, COSMO BIO) according to the manufacturer's instructions. The absorbance of colored products derived from o-phenylene diamine (Sigma) was measured at 492 nm. For examining repair kinetics, the percentage (%) of repair was calculated by comparing the absorbance at the indicated time to that of the corresponding absorbance at time=0 when there was no opportunity of repair and 100% of the CPDs were present post-UVB.

siRNA Transfection: HaCaT cells were transfected with siRNA (On-TARGETplus SMARTpool, Dharmacon) targeting PTEN or with 1x siRNA Buffer as a negative control. The procedure was carried out according to the 12 well plate format in the Thermo Scientific DharmaFECT 1 siRNA transfection protocol for HEK293 cells.

Western blot: Cells were subjected to Western blot analysis as described previously [1]. Briefly, cells were harvested and lysed using 1x SDS sample buffer. Samples were boiled and run on either a 7.5% or 10% polyacrylamide gel and transferred to a PVDF membrane. Imaging of proteins was performed by customary western blot procedure. Antibodies utilized in the assay comprised of anti-XPC (H-300) (Santa Cruz), anti-GAPDH (New England Biolabs), anti-alpha/beta-tubulin, anti-Phospho-Akt (Ser473) and total Akt, anti-Phospho-p70 S6 Kinase (Thr389) and total p70 S6 kinase, anti-Phospho-Chk1 (Ser345), anti-Phospho-Chk2 (Thr68), anti-Phospho-p53 (Ser15), anti-Phospho-Histone H2A.X (Ser139), and anti-rabbit IgG, HRP-linked Antibody (all from Cell Signaling).

Results:

Aim 1

Measurement of DNA damage in HaCaT cells exposed to UVB

Much of the mutagenic and carcinogenic effects of UV irradiation are a consequence of DNA damage in the form of cyclobutane pyrimidine dimers (CPDs). To assess the effect of mTOR inhibition on repair of CPDs after exposure to UVB, HaCaT cells (human keratinocytes) were pre-incubated in vehicle or 50 nM Torin-2, a TOR kinase inhibitor that blocks pathways downstream of both mTORC1 and mTORC2, then exposed to either a low, non-apoptotic (5

mJ/cm^2) or higher, apoptotic (25 mJ/cm^2) dose of UVB (Figure 1). As expected, HaCaT cells exposed to low dose UVB completely repaired UVB-induced DNA damage within 24 h, as measured by decreased CPDs remaining (Figure 1), indicating efficient GG-NER. Significant repair was also seen at higher dose UVB (Figure 1). Contrary to our original prediction that mTOR inhibition would protect surviving cells from DNA damage, inhibition of mTOR with Torin-2 significantly reduced the repair of CPDs at 6h post-UVB in cells exposed to either a sub-apoptotic or apoptotic UVB dose. Interestingly, in cells exposed to the higher, apoptotic dose of UVB, treatment with Torin-2 actually accentuated DNA damage by almost 40% at 6 h post-UVB compared to cells at zero time (Figure 1).

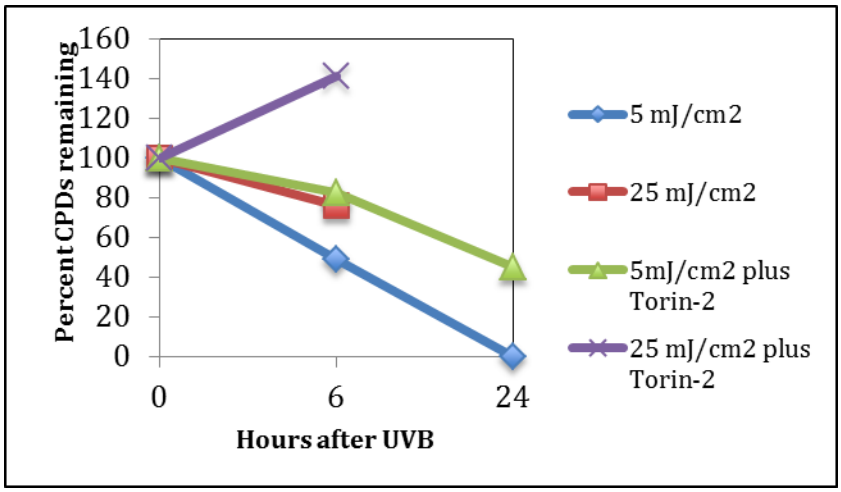

Figure 1. Treatment of HaCaT cells with Torin-2 reduces repair of DNA damage by GG-NER in response to UVB. Cells were exposed to either sub-apoptotic or apoptotic doses of UVB with or without preincubation with Torin-2 and repair of CPDs was measured over 24 h.

Assessment of DNA damage signaling checkpoints in HaCaT cells treated with Torin-2

A very recent study has shown that, in addition to its inhibition of mTOR, Torin-2 exhibits inhibitory activity against the mTOR-related PIKK family kinases ATM and ATR, major regulators of the DNA damage response in cells exposed to UVB [2].

We therefore analyzed the effect of Torin-2 treatment on UVB-induced checkpoint function. DNA damage-mediated growth arrest acts through phosphorylation and activation of the checkpoint kinases Chk-1 and Chk-2, followed by p53 accumulation and activation [3]. Exposure of cells to 20 mJ/cm^2 UVB induces phosphorylation of

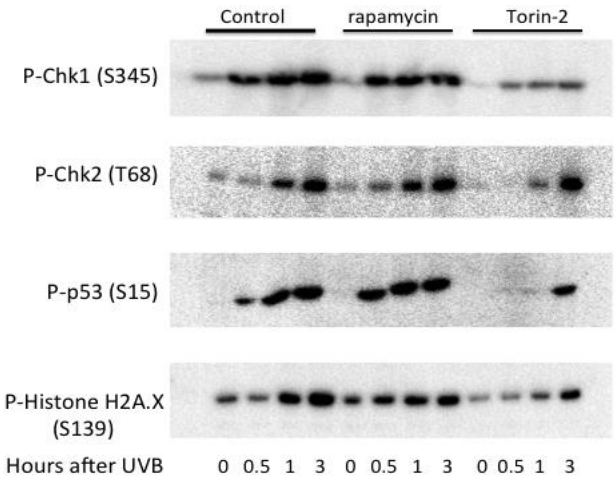

Figure 2. Western blot analysis of DNA damage response checkpoint proteins in UVB-treated cells with and without inhibitors of mTOR. Cells were exposed to 20 mJ/cm^2 UVB and harvested at the indicated time points. For inhibitor treatments, rapamycin (50 nM) and Torin-2 (50 nM) were pre-incubated for 2h before UVB.

Chk-1 by ATR and Chk-2 by ATM, as well as p53 phosphorylation, in a time-dependent manner (Figure 2). Phosphorylation of all three proteins was markedly inhibited in cells exposed to Torin-2. In contrast, exposure to rapamycin had no effect (Figure 2). Similarly, phosphorylation of histone H2A.X, which upon phosphorylation localizes to the site of DNA damage, was also delayed in Torin-2 treated cells but not in cells treated with rapamycin. These data imply that the increased DNA damage response seen with Torin-2 treatment may be the result of ATM/ATR inhibition rather than changes in mTOR activity. However, they do not rule out a role for mTORC2 in the DNA damage response.

Measurement of DNA damage repair in mouse embryo fibroblasts with deletion of Rictor
To directly address the question of whether inhibition of mTORC2 affects the DNA damage response, we assayed CPD repair in MEFs with deletion of Rictor expression, and wild-type controls (Figure 3). The results show that absence of Rictor augments DNA damage in the form of CPDs after UVB exposure, and this effect occurs at both low and high dose UVB. Thus, inhibition of mTORC2 causes increased sensitivity to UVB-induced DNA damage, providing a mechanism for the increased apoptosis seen in our earlier studies [1]. These results suggest that mTORC2 (or Rictor) plays an important role in regulating cell survival under DNA damaging conditions.

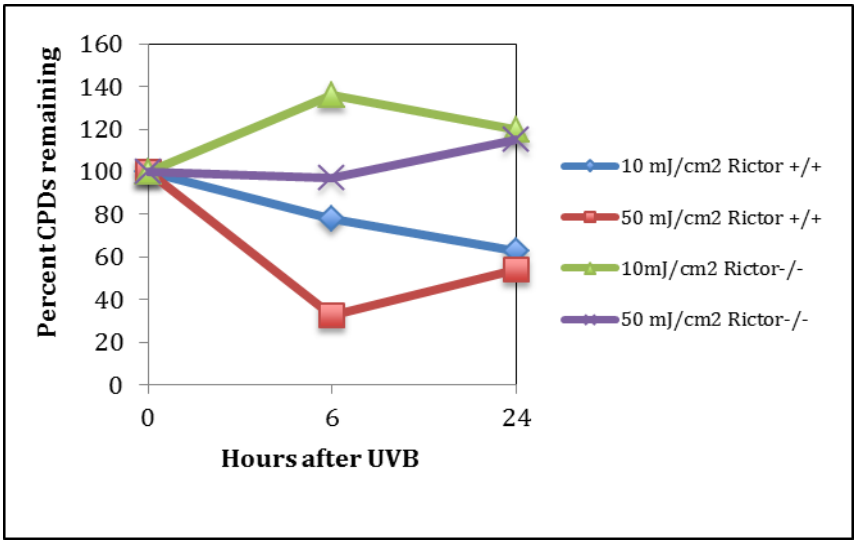

Figure 3. Deletion of Rictor reduces repair of DNA damage by GG-NER in response to UVB. Cells were exposed to either sub-apoptotic or apoptotic doses of UVB and repair of CPDs was measured over 24 h.

Aim 2
Knockdown of PTEN expression in HaCaT cells by siRNA.
To establish the necessary dosage of siPTEN needed for the successful knockdown of PTEN in HaCaT cells, a dose response was performed using 3 concentrations of siPTEN (25µM, 50µM, & 100µM) transfected into HaCaT cells. The cells were incubated with the siRNA for 72 h and levels of PTEN expression were measured using western blot analysis. It was determined that 100nM of siPTEN was sufficient to successfully knockdown PTEN expression in HaCaT cells (Figure 4), and this concentration was used for subsequent experiments.

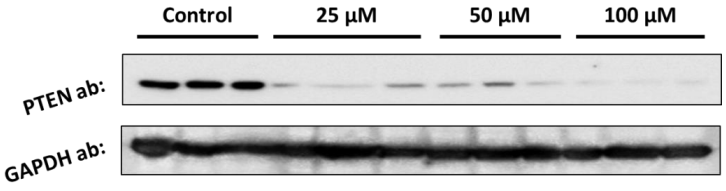

Figure 4. siRNA PTEN dose response in HaCaT cells. PTEN expression in HaCaT cells transfected with 25µM, 50µM, or 100µM siRNA targeting PTEN following 72 h of incubation. GAPDH used as a loading control.

Verification of the inhibitory effects Rapamycin, Torin 2, & LY on control cells and cells with PTEN knockdown.

HaCaT cells were treated with each inhibitor as described in the Methods and Western blot analysis was performed on each group to evaluate the levels of phosphorylated AKT-Ser473 (p-AKT (473)) and phosphorylated p70 S6 Kinase (p-S6K) as a measure of mTORC1 and mTORC2 activity, respectively, in the presence or absence of PTEN knockdown. Figure 5 shows that, without inhibitor treatment, siPTEN resulted in increased phosphorylation of both AKT and S6K. Torin 2 and LY both lowered P-AKT (S473) levels while rapamycin, Torin 2, and LY all blocked phosphorylation of S6K expression (Figure 5). Inhibition occurred in both the presence and absence of PTEN knockdown.

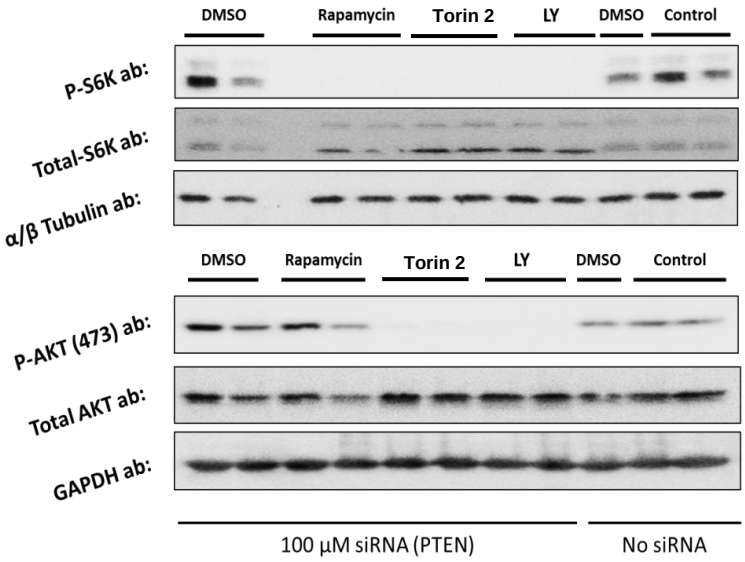

Figure 5. PTEN siRNA activates mTORC1 and mTORC2 signaling, which is repressed by inhibitor treatment. (Top) p-S6K/Total S6K expression and (Bottom) p-AKT (S473)/total AKT expression in vehicle- or drug-treated siPTEN HaCaT cells at 2 h post UVB irradiation (50 mJ/cm^2). α/β Tubulin (Top) and GAPDH (Bottom) were used as controls.

Inhibitor treatment restores baseline XPC expression in siPTEN HaCaT cells following UVB exposure.

To ascertain how the manipulation of the PTEN/AKT pathway affects levels of XPC following UVB exposure, the siPTEN HaCaT cells were subjected to 50 mJ/cm^2 UVB exposure after either DMSO, rapamycin, Torin 2, or LY were applied to the cells. Cells were harvested and Western blot analysis was performed to determine XPC protein levels (Figure 6). Levels of XPC in the siPTEN-DMSO group decreased at 2 h post UVB irradiation. The addition of rapamycin, Torin 2 or LY restored XPC expression to the levels seen in HaCaT cells without PTEN knockdown (Figure 6), suggesting that XPC expression in cells exposed to UVB may be under control of mTORC1 signaling pathways. These data extend previous findings characterizing regulation of DNA damage repair by PTEN [4].

Summary and conclusions.

Our data indicate that inhibition of mTORC2-dependent pathways accelerates accumulation of UVB-induced DNA damage, which ultimately may promote elimination of UV-damaged cells through apoptosis and

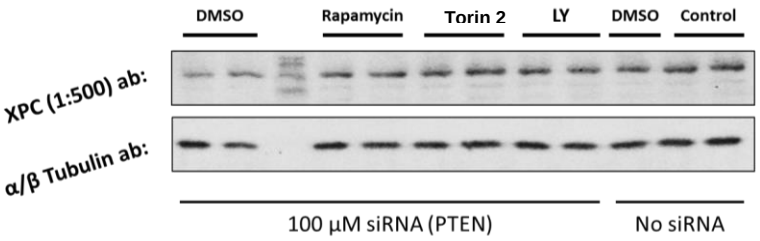

Figure 6. Rapamycin, Torin 2 and LY restore XPC expression in siPTEN HaCaT cells exposed to UVB. XPC expression in drug-treated siPTEN HaCaT cells 2 h post-UVB irradiation (50 mJ/cm^2). α/β Tubulin used as a control.

subsequent inhibition of skin cancer development. Further, our PTEN knockdown studies suggest that the synthesis of DNA repair proteins may be under control of mTORC1 in UVB-treated cells. Future studies will use these preliminary results to further define the roles of both TOR complexes in the DNA damage response to UVB.

Literature Cited:

1. Carr, T.D., Digiovanni, J., Lynch, C.J. and Shantz, L.M. (2012) Inhibition of mTOR Suppresses UVB-Induced Keratinocyte Proliferation and Survival. *Cancer Prev Res*, **5**, 1394-1404.
2. Liu, Q., Xu, C., Kirubakaran, S., Zhang, X., Hur, W., Liu, Y., Kwiatkowski, N.P., Wang, J., Westover, K.D., Gao, P., Ercan, D., Niepel, M., Thoreen, C.C., Kang, S.A., Patricelli, M.P., Wang, Y., Tupper, T., Altabef, A., Kawamura, H., Held, K.D., Chou, D.M., Elledge, S.J., Janne, P.A., Wong, K.K., Sabatini, D.M. and Gray, N.S. (2013) Characterization of Torin2, an ATP-competitive inhibitor of mTOR, ATM, and ATR. *Cancer Res*, **73**, 2574-86.
3. Van Laethem, A., Garmyn, M. and Agostinis, P. (2009) Starting and propagating apoptotic signals in UVB irradiated keratinocytes. *Photochem Photobiol Sci*, **8**, 299-308.
4. Ming, M., Feng, L., Shea, C.R., Soltani, K., Zhao, B., Han, W., Smart, R.C., Trempus, C.S. and He, Y.-Y. (2011) PTEN Positively Regulates UVB-Induced DNA Damage Repair. *Cancer Research*, **71**, 5287-5295.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of

Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

A future article describing the role of mTORC2 in the UVB DNA damage response will include the data reported here.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

This is to our knowledge the first study to suggest a role for mTORC2 in the DNA damage response to UVB. Based on our previous results and the experiments reported here, our data fit a model where limiting the activity of mTORC2 results in the accumulation of DNA damage in cells treated with UVB, resulting in increased apoptosis. Thus, mTORC2 inhibition may promote elimination of UV-damaged cells and subsequent inhibition of skin cancer development. These data suggest that TORC2 is a relevant target in preventing or reversing UVB damage to the skin.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Shantz, Lisa M.	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
lshantz	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Scranton, Scranton, PA	B.S.	05/84	Chemistry
Johns Hopkins University, Baltimore, MD	Ph.D.	09/89	Pharm./Mol. Science
Pennsylvania State University College of Medicine, Hershey, PA	Postdoctoral	11/89 – 12/92	Cell. & Mol. Physiology

A. Personal Statement.

I have extensive experience in non-melanoma skin carcinogenesis studies using transgenic animal models. We were the first to show a link between induction of the Raf/MEK/ERK pathway and increased ornithine decarboxylase (ODC) in skin tumors. Importantly, we established that high ODC activity is a necessary component of MEK-induced skin tumor development, and both antizyme (AZ), which binds to ODC and causes it to be degraded, and DFMO, a suicide inactivator of ODC, are a powerful suppressors of these tumors. I also have a long-standing interest in the mechanism of protein synthesis regulation by mTOR signaling in both skin carcinogenesis and other models of neoplastic transformation. Current work uses mice with conditional deletion of mTOR components within the basal layer of the epidermis to explore the role of mTOR-dependent pathways in skin carcinogenesis in response to both chemical carcinogens and UV light.

B. Positions and Honors.

Positions

1993 – 2001 Assistant Professor, Department of Cellular and Molecular Physiology, The Milton S. Hershey Medical Center, Hershey, Pennsylvania

2001 – present Associate Professor, Department of Cellular and Molecular Physiology, The Milton S. Hershey Medical Center, Hershey, Pennsylvania

Honors

1980-84 – University Scholarship
1983 – Sigma Xi - Scientific Research Honor Society
1983 – Phi Lambda Upsilon - Chemistry Honor Society
1984 – Magna Cum Laude - BS from University of Scranton
1988 – Upjohn Travel Award, American Association for Cancer Research
1999 – Established Investigator Award; American Heart Association
2007 – Chair-Elect, Gordon Research Conference on Polyamines
2009, 2011 – Dean's Award for Excellence in Teaching; Penn State College of Medicine
2011 – Chair, Gordon Research Conference on Polyamines

C. Most relevant peer-reviewed publications (in chronological order; 15 of 47 total).

1. **Shantz, L.M.** and Pegg, A.E. Ornithine decarboxylase induction in transformation by H-Ras and RhoA. *Cancer Res.* **58**: 2748-2753, 1998. PMID: 9661886
2. Kimball, S.R., **Shantz, L.M.**, Horetsky, R.L. and Jefferson, L.S. Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF-4E and phosphorylation of ribosomal protein S6. *J. Biol. Chem.* **274**, 11647-11652, 1999 PMID: 10206976
3. Feith, D.J., **Shantz, L.M.** and Pegg, A.E. Targeted antizyme expression in the skin of transgenic mice reduces tumor promoter induction of ornithine decarboxylase and decreases sensitivity to chemical carcinogenesis. *Cancer Res*. **61**:6073-81, 2001. PMID: 11507056
4. **Shantz, L. M.,** Guo, Y., Sawicki, J. A., Pegg, A. E., and O'Brien, T. G. Overexpression of a dominant-negative ornithine decarboxylase in mouse skin: effect on enzyme activity and papilloma formation. *Carcinogenesis* **23**: 657-64, 2002. PMID: 11960919
5. **Shantz, LM** Transcriptional and translational control of ornithine decarboxylase during Ras transformation. *Biochem. J.* **377**: 257-264, 2004. PMCID: PMC1223852
6. Feith DJ, Bol DK, Carboni JM, Lynch MJ, Sass-Kuhn S, Shoop PL, **Shantz LM.** Induction of ornithine decarboxylase activity is a necessary step for mitogen-activated protein kinase kinase-induced skin tumorigenesis. *Cancer Res.* **65**:572-8, 2005. PMID: 15695401
7. Feith, DJ, Origanti, S, Shoop, PL, Sass-Kuhn, S, **Shantz, LM.** Tumor suppressor activity of ODC antizyme in MEK-driven skin tumorigenesis. *Carcinogenesis* **27**: 1090-1098, 2006. PMID: 16400186
8. Origanti, S and **Shantz, LM.** Ras transformation of RIE-1 cells activates cap-independent translation of ornithine decarboxylase: regulation by the Raf/MEK/ERK and PI3-kinase pathways. *Cancer Res.* **67**: 4834-4842, 2007. PMID: 17510413
9. Feith DJ, **Shantz LM**, Shoop PL, Keefer KA, Prakashagowda C, Pegg AE. Mouse skin chemical carcinogenesis is inhibited by antizyme in promotion-sensitive and promotion-resistant genetic backgrounds. *Mol Carcinog.* **46**: 453-465, 2007. PMID: 17219416
10. Nowotarski SL, **Shantz, LM**. Cytoplasmic accumulation of the RNA binding protein HuR stabilizes the ornithine decarboxylase transcript in a murine non-melanoma skin cancer model. *J. Biol. Chem.* **285:** 31885-31894, 2010. PMCID: PMC2951260
11. Nowotarski SL, Origanti S, **Shantz LM.** Posttranscriptional regulation of ornithine decarboxylase. *Methods Mol Biol.* **720**:279-92, 2011. PMCID: PMC3590796
12. Zhang Y, Cheng Y, Ren X, Hori T, J Huber-Keener K, Zhang L, Yap KL, Liu D, **Shantz LM**, Qin ZH, Zhang S, Wang J, Wang HG, Shih IM, Yang JM. Dysfunction of Nucleus Accumbens-1 Activates Cellular Senescence and Inhibits Tumor Cell Proliferation and Oncogenesis. *Cancer Res*. **72**: 4262-4275, 2012. PMCID: PMC3614094
13. Shi C, Cooper TK, McCloskey DE, Glick AB, **Shantz LM,** Feith DJ. S-adenosylmethionine decarboxylase overexpression inhibits mouse skin tumor promotion. *Carcinogenesis* **33**:1310-1318, 2012. PMCID: PMC3499050
14. Origanti S, Nowotarski SL, Carr TD, Sass-Kuhn S, Xiao L, Wang JY, **Shantz LM.** Ornithine decarboxylase mRNA is stabilized in an mTORC1-dependent manner in Ras-transformed cells. *Biochem J.* **442**:199-207, 2012. PMCID: PMC3495583
15. Carr, TD, DiGiovanni, J, Lynch, CJ, **Shantz, LM**. Inhibition of mTOR signaling suppresses UVB-induced cell proliferation and survival. *Cancer Prev. Res.* **5**: 1394-1404, 2012. PMCID: PMC3518591