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. Questions? Contact Health Research Program staff at 717-231-2825.

1. **Grantee Institution:** Trustees of the University of Pennsylvania

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 – 12/31/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Gearline Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100054874

6. **Project Number and Title of Research Project:** 7-The Unfolded Protein Response in Cancer

7. **Start and End Date of Research Project:** 7/1/2011 – 6/30/2012

8. **Name of Principal Investigator for the Research Project:** J. Alan Diehl, BS, 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:

   $ 159,877.44

   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
Chitnis, Nilesh	Postdoc	41% Yr 2	19,535
Bhattachariya, S.	Postdoc	50% Yr 1	18,460
Zheng, Hui	Postdoc	50% Yr 1	7,384

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
Diehl	Professor	5
Fuchs	Professor	5
Koumenis	Professor	5

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 awarded:
The UPR and Cancer	XNIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	9-2012	$7,140,970	$5,515,407

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

Yes_____ No__X___

If yes, please describe your plans:

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

Continue work on funded P01.

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_____ No___X_

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

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

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

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

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_____ No___X___

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

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.

Project 1 (Diehl)

Through micro-RNA profiling, we have identified micro-RNAs whose expression are regulated following ER stress. MiR211 was found to be strongly-induced in a PERK-dependent manner following induction of the UPR. We *hypothesize that miR211 functions as a rheostat to moderate CHOP accumulation and provide cells an opportunity to adapt to stress prior to committing to apoptosis.* To address this hypothesis, we proposed the following Specific Aims, execution of which has provided key data for submission of an initial manuscript and the foundation for future extramurally funded work.

Aim #1 determine whether PERK mediates miR-211 induction in response to micro-environmental signals (hypoxia, amino acid deprivation, glucose restriction) associated with ER stress in a PERK-dependent manner; Aim #2, determine the mechanism whereby miR-211 regulates CHOP accumulation; Aim #3, determine whether miR-211 accumulation in primary tumors is dependent upon PERK and will inversely correlate with CHOP expression. We have made outstanding progress on all aims resulting in submission of both a manuscript and a P01 application. A summary of the work performed and supported by this grant is summarized briefly below.

The research performed and presented in our manuscript, which is under review as a revised manuscript at *Molecular Cell*, has several novel findings that directly stem from experiments performed to address each aim proposed and whose importance impacts ER stress, tumor biology, and the role of micro-RNAs in cellular biology and chromatin structure. First, we have identified miRNAs that are regulated by ER stress (Integrated Stress Response or Unfolded Protein Response). Second, miR-211 the most robustly induced, has a transient profile of induction suggesting that its regulatory function is restricted to early points of ER stress. Importantly, PERK, and its downstream transcription factor effector, ATF4 are necessary and sufficient for miR-211 expression (Aim 1). The data observed support the induction of miR-211 and suggest a model of PERK-dependent induction of miR-211 functioning to prevent premature accumulation of *CHOP/GADD153*.

Project 2 (Koumenis).

We have previously shown that that hypoxia/anoxia and nutrient deprivation activate the PERK-eIF2α-ATF4 arm of the UPR. Tumor cells with inactivating mutations/deletions in this pathway display reduced resistance to these stresses. When amplified in tumor cells, the proto-oncogene c-Myc increases substantially the rates of protein synthesis by upregulating gene products involved in translation, including ribosomal proteins. We hypothesized that c-Myc activation would impose an increased burden on ER folding pathways and activate the UPR in transformed cells. Four aims were proposed that specifically addresses this hypothesis;

Aim #1, Determine the requirement for eIF2α phosphorylation and Nrf2 activation in c-Myc-induced transformation; Aim #2, elucidate the mechanism of cytoprotection afforded by UPR activation in the context of oncogenic transformation by Myc; Aim #3, investigate whether c-Myc overexpression induces activation of the other two major UPR pathways; Aim #4, determine the role of PERK activation in c-Myc induced lymphomagenesis using a transgenic

mouse model. Completion of the experiments proposed within these aims has resulted in the submission of a manuscript that is currently under revision at the *Journal of Clinical Investigation*. In addition the data generated through the execution of these aims serves as the basis of a P01 proposal. A summary of the work performed and supported by this grant is summarized briefly below.

We have also found that eIF2α is required for survival of c-Myc transformed cells. We have expressed the MycER construct in eIF2αser51ala cells, which harbor a non-phosphorylatable allele of eIF2α Induction of Myc in the Wt cell resulted in increased proliferation, clonogenic survival and modest apoptosis; however, expression of c-Myc in eIF2αser51ala cells resulted in massive apoptosis 48h after induction. These results firmly establish that eIF2α is downstream of PERK in the Myc-induced transformation process. We are currently investigating the role of eIF2α in Myc-induced cytoprotective autophagy (Aim 1).

We have further established that Myc activation in lymphoma cells and immortalized MEFs leads to activation of cytoprotective autophagy. Specifically, we have now inhibited autophagy both pharmacologically (with spautin-1 and bafilomycin) and genetically (knockdown of ULK01 and use of Atg5-/- MEFs). In all cases, compromising the ability of cells to induce autophagy resulted in increased cell death. These results strongly support a cytoprotective role of c-Myc-induced, UPR-dependent autophagy in Myc-dependent transformation (Aim 2).

Through our collaboration with Dr. Diehl, we have investigated the consequences of c-Myc expression in PERK-proficient and PERK-deficient hematopoietic progenitor cells. Specifically, Dr. Diehl's group isolated bone marrow hematopoietic cells, expressed c-Myc from a constitutive promoter and excised PERK by adding 4-HT in ERCre:PERK$^{fl/fl}$ cells. Cells lacking functional PERK (80% efficient knockout) demonstrated significantly reduced ability to form colonies in methylcellulose and exhibited significantly increased apoptosis compared to PERK proficient cells (no 4-HT added). These results suggest that PERK is required for mouse lymphocyte transformation with Myc (Aim 4).

Hart LS, Cunningham JT, Datta T, Lehman SL, Li Y, Qiu B, Zhang H, Bi M, Liu H, Li C, Thomas-Tikhonenko A, Koong A, Fuchs SY, Perl AE, Mills IG, Diehl JA, Ruggero D, and Koumenis C. Endoplasmic Reticulum Stress-Mediated Autophagy Promotes Myc-Dependent Transformation and Tumorigenesis. *JCI 2012.*

In Project 3, we *<u>hypothesized</u> that activation of ISR during mammary and pancreatic tumorigenesis helps to evade anti-proliferative and anti-survival effects of IFNα/β signaling through stimulated degradation of IFNAR1 and that these events play an important role in growth and progression of breast and pancreatic carcinomas and Myc-expressing lymphomas.*

Three Aims were proposed to test this overall hypothesis.
Aim 1. Determine how inducers of ISR accelerate the degradation of IFNAR1 and determine the role of this pathway in suppression of cellular responses to IFNα/β; Aim 2. Delineate the signaling pathways that are stimulated by inducers of ISR and that mediate phosphorylation and accelerated degradation of IFNAR1 and suppression of IFNα/β signaling; Aim 3. Determine the role of ISR-stimulated IFNAR1 degradation in tumorigenesis.

We have carried out experiments to address the stated hypotheses of all three aims. As a direct result of this work, we have submitted a manuscript to *Oncogene* describing these results. Initially, our studies demonstrated that inducers of ISR including hypoxia, amino acid deficit, and diverse inducers of PKR robustly accelerate IFNAR1 degradation. We further found that constitutively active PKR expressed in human breast cancer cells also plays a key role in maintaining the low levels of IFNAR1 in these cells. Our additional experiments clearly demonstrated that activation of ISR and ensuing downregulation of IFNAR1 has rendered human tumor cells (melanoma and breast cancer cells) poorly sensitive to signaling and anti-proliferative effects elicited by IFNα/β (Aim 1).

Subsequently, we performed experiments utilizing pharmacological and genetic approaches to test whether the effects of ISR induction on IFNAR1 stability and signaling are mediated by phosphorylation of eIF2α on Ser51 by either PERK or PKR or GCN2. We found that activation of p38α stress-activated protein kinase downstream of eIF2α is essential for subsequent phosphorylation of IFNAR1 on priming site (S532) followed by phosphorylation of its degron (on S535 by CK1) leading to the recruitment of βTrcp E3 ubiquitin ligase and subsequent IFNAR1 ubiquitination and degradation. These data along with previously published results established the importance of these mechanisms in IFNAR1 downregulation in response to ISR (Aim 2).

Finally, we have used established human cancer cell lines to determine the role of ISR-mediated downregulation of IFNAR1 in tumorigenic properties of these cells. Our studies demonstrated that, under ISR conditions, human breast cancer and malignant melanoma cells receive an additional impetus for invasion and increased cell motility. Whereas RNAi-mediated knockdown of relevant ISR kinases (PKR or GCN2) impede this increased motility/invasiveness, these characteristics could be restored upon concurrent knockdown of IFNAR1. Taken together with observed ISR-induced desensitization of cancer cells to suppression of cell viability by IFN, these studies provide a strong foundation for future direct examination of the role of IFNAR1 degradation in tumorigenesis using mouse models in vivo (Aim 3).

1. Bhattacharya S, HuangFu W-C, Dong G, Qian J, Baker DP, KararJ, Koumenis C, Diehl JA, and Fuchs SY. Anti-tumorigenic effects of Type 1 interferon are subdued by integrated stress responses. *Oncogene 2013.*

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. Anti-tumorigenic effects of Type 1 interferon are subdued by integrated stress responses.	Bhattacharya S, HuangFu WC, Dong G, Qian J, Baker DP, Karar J, Koumenis C, Diehl JA, Fuchs SY.	Oncogene	2013	☐Submitted ☐Accepted X Published

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

Yes_____ No_X__

If yes, please describe your plans:

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.

The work performed is mechanistic in nature and at this time, it is to early to assess clinical impact.

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.

No major discoveries

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

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
J. Alan Diehl	Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
diehlj	

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
NC State University	B.S.	1990	Biochemistry
University of Missouri-Columbia	Ph.D.	1995	Biochemistry
St. Jude Children's Research Hospital	Postdoc	1995-1999	Tumor Cell Biology

A. Personal Statement

The overarching goal of the research conducted within my laboratory is the discovery of molecular mechanisms that contribute to uncontrolled cell proliferation and decreased cell death, which are collectively associated with neoplastic growth. With this as a goal, one area of focus is cell survival and lipid signaling pathways utilized during tumorigenesis to facilitate tumor growth and expansion. This direction of research centers on the endoplasmic reticulum associated kinase, PERK, a noted mediator of cellular adaptive pathways in cells exposed to environmental stresses. PERK functions as a cellular sensor of nutrient availability and under conditions of nutrient limitation, functions to limit cellular growth and expansion with nutrient availability. Our focus has been to establish key signaling events downstream of PERK that mediate its physiologic functions. Our approach is multifaceted, in that we use cell biology, molecular biology and animal models of disease to address the functional relevance of the UPR and PERK to tumor progression. The focus of our current work centers on the regulation of cell survival by PERK during tumor expansion. In summary, our work has resulted in the identification of novel pathways that contribute to both tumor initiation and tumor progression. The second area of research focus my lab is the elucidation of mechanisms whereby growth-signaling pathways regulate the mitogenically responsive D-type cyclins and more specifically, how these pathways regulate accumulation of an active, nuclear cyclin D1-dependent kinase. My laboratory has made seminal contributions to our understanding of post-translation regulation of cyclins and their deregulation in cancer. Among these is the revelation that regulated cyclin D1 localization functions as a key mechanism whereby cells maintain normal growth characteristics. The temporal localization of cyclin D1 determines access to a key negative regulatory E3 ligase that we have identified; SCF^{Fbx4}. The identification of the SCF^{Fbx4} E3 ligase was supported directly by this grant. In addition to our work on post-translational regulation of cyclin D1 and its importance to various forms of human cancer (lymphoma, breast, melanoma, and esophageal), we have also been intensely investigating the molecular underpinnings of D1-driven neoplastic growth. During the previous 4 years, we have firmly established that aberrant nuclear accumulation of active cyclin D1/CDK4 during S-phase generates double strand DNA breaks and increases the frequency of chromosomal translocations which contribute directly to cellular acquisition of pro-oncogenic second hits and cancer development. A key effector of pro-oncogenic cyclin D1 activities is the PRMT5/MEP50 arginine methyltransferase. The latter finding provides a direct, mechanistic link between cyclin D1 and a vast literature suggesting pro-transcriptional activities for cyclin D1. Our demonstration that cyclin D1 can influence transcription via targeting an epigenetic regulator provides innovative and significant insights with regard to its pro-neoplastic activities. My laboratory continues to investigate the contribution of PRMT5/MEP50 to neoplastic pathologies associated with dysregulation of cyclin D1 (Li et al, Cancer Discovery *In Press* 2015) to assess both its molecular contributions to tumorigenesis and also the possibility that PRMT5/MEP50 may represent an important and novel therapeutic target in cancers, such as MCL, for which there are currently no effective therapeutic strategies.

J. Alan Diehl
B. Positions and Honors

Positions and Employment

06/95-05/99	Research Associate, Howard Hughes Medical Institute, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN.
07/99-08/01	Assistant Professor, Eppley Institute for Research in Cancer and Allied Diseases
08/01-06/05	Assistant Professor, Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA.
08/01-6/05	Assistant Investigator, Abramson Family Cancer Research Institute, UPenn, Philadelphia, PA.
07/05-06/09	Associate Professor, Department of Cancer Biology, UPenn, PA.
07/05-06/14	Investigator, Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA.
07/07-06/14	Director, Cancer Cell Biology, Abramson Family Cancer Research Institute
07/09-06/14	Professor, Department of Cancer Biology, UPenn.
2014-Present	Associate Director for Basic Science, Hollings Cancer Center, Medical University of South Carolina.
2014-Present	Endowed Professor of Lipodomics and Pathobiology, Department of Biochemistry and Molecular Biology, Medical University of South Carolina.

Other Experience and Professional Memberships

2003-Present	American Association for Cancer Research (Member)
2003-Present	American Society for Biochemistry and Molecular Biology (Member)
2003-Present	American Society for Microbiology (Member)
2004-2010	American Cancer Society: Cell Cycle and Growth Control Committee (Member 2004-2007, Vice-Chair 2008, Chair 2009-2010)
2007-2011	Molecular Oncology, National Cancer Institute (Charter Member, Vice-Chair 2010-2011)
2012-2013	Ad Hoc Member of CSRS
2012-present	NCI omnibus R21 and R03 Review Committee
2013	ZRG1 CB-G Special Emphasis Panel; Chair

Honors

1995	Who's Who in America Universities and Colleges
1995	Outstanding Doctoral Student Majoring in Biochemistry by The American Institute of Chemists and the St. Louis Institute of Chemists
2000	*V* Scholar
2004	American Cancer Society Research Scholar
2006	Leukemia and Lymphoma Society Scholar
2009	Faculty of 1000, Biology
2013-2014	J. Samuel Staub Endowed Professor, UPenn
2014-present	Endowed Professor of Lipidomics and Pathobiology, Medical University of South Carolina

NAME Constantinos Koumenis	POSITION TITLE Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) ckoumeni	

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
Aristotle University, Thessaloniki, Greece	B.S.	1989	Pharmacy
University of Houston, Houston, TX	Ph.D.	1994	Biochemistry
Stanford Univ. School of Medicine, Stanford, CA	Postdoctoral	1994 – 1999	Radiation Oncology

A. Personal Statement

My research interests are primarily focused on the study of the tumor microenvironment with particular emphasis on the effect of hypoxia on tumor progression and resistance to therapy. Our group was the first to demonstrate that hypoxia activates a translational control program known as the Integrated Stress Response (ISR), which adapts cells to endoplasmic reticulum (ER) stress. Inactivation of ISR signaling by mutations in the ER kinase PERK and the translation initiation factor eIF2α, impair cell survival under hypoxia. Tumors derived from these mutant cell lines are smaller and exhibit higher levels of apoptosis in hypoxic areas compared to tumors with an intact ISR. Moreover, we demonstrated that the ISR target proteins ATF4 and CHOP are highly expressed in hypoxic areas of human tumor samples. More recently, with the help of the PI, Dr. Diehl, we have expanded these findings to include another eIF2α kinase, GCN2, which is activated by nutrient (amino acid) deprivation. We have also initiated small molecule screens to identify inhibitors or the UPR or compounds that preferentially kill tumor cells through overactivation of ER stress, with the ultimate goal to inhibit tumor cell adaptive processes to improve therapeutic outcome. Our research has been continuously funded by the NIH in the last 10 years, and we have published extensively in the above-mentioned fields. Since 2009, I have been serving as the co-leader of the Radiobiology & Imaging Program of the Abramson Comprehensive Cancer Center at the Univ. of Pennsylvania, which has 32 full-time members. The goal of the proposed P01 application is to develop an innovative and highly interactive program encompassing the labs of Dr. Fuchs, Dr. Diehl, and my own. My own research has benefited substantially by collaborating with these co-Investigators. For example, in addition to the exchange of relevant information and ideas, the Diehl lab has provided novel transgenic models of PERK deficiency, and the Fuchs lab has provided expertise in the use of proteasome inhibitors as hypoxia-targeting agents based on our UPR studies. Therefore, I believe that our collective research expertise, interests, and common reagents and animal models will enable us to make significant advancements towards the successful completion of the studies described in this P01 application.

B. Positions and Honors

Positions and Employment

1994-1999: Postdoctoral Fellow, Dept. Radiation Oncology, Stanford University School of Medicine, Stanford, CA.

1999-2005: Assistant Professor, Dept. Radiation Oncology, Comprehensive Cancer Center, Wake Forest Univ. School of Medicine, Winston-Salem, NC.

2003-2006: Assistant Professor, Dept. of Neurosurgery, WFUSM, Winston-Salem, NC.

2005-2006: Associate Professor, Depts. Radiation Oncology, and Neurosurgery, Comprehensive Cancer Center, Wake Forest Univ. School of Medicine, Winston-Salem, NC.

2006-present: Associate Professor (Tenured), Dept. of Radiation Oncology, University of Pennsylvania School of Medicine, Philadelphia, PA.
2009-present: co-Leader, Radiobiology and Imaging Program, Abramson Comprehensive Cancer Center, University of Pennsylvania School of Medicine, Philadelphia, PA.

Other Experience and Professional Memberships

2004 NIH Special Emphasis Panel ZRG1, GGGE 02. (Ad-hoc Reviewer), October '04.
2004-2005 NIH Special Emphasis Panel, Innovative Molecular Analysis Technologies (IMAT) ZCA1 SRRB-C J1S, November '04, June-July '05, May 2010.
2005 Congressionally Directed Medical Research Program (CDMRP)-Breast Cancer Research Program Pathobiology-3, August '05.
2006 Department of Veterans Affairs, Merit Review Subcommittee, ONC-A (Ad-Hoc)
2006 NIH, Drug Discovery and Molecular Pharmacology (DMP) study section (Ad-Hoc)
2007 NSF, Ad hoc reviewer
2005-2007 NIH, Basic Mechanisms of Cancer Therapeutics (BMCT) study section (Ad-hoc reviewer)
2006-2010 NIH, Basic Mechanisms of Cancer Therapeutics (BMCT) study section standing member.
2008-present: Editorial Board Member, *J Clin Inv.*, *BMC Cancer*, *Cancer Biol & Ther*.

Honors

1997-1999: National Research Service Award (NRSA), NCI (F-32).
1994-1996: Dean's Postdoctoral Fellowship, Stanford University School of Medicine.

NAME Serge Y. Fuchs	POSITION TITLE Professor
eRA COMMONS USER NAME (credential, e.g., agency login) syfuchs	

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
Yaroslavl State Medical Institute, Yaroslavl, Russia	M.D.	1987	Medicine
All-Union Cancer Research Center, Russian Academy of Medical Sciences, Moscow, Russia	Ph.D.	1992	Experimental Oncology
American Health Foundation, Valhalla, NY, USA	Postdoctoral Training	1996	Cancer Research and Cell & Molecular Biology

A. Personal Statement

My long-term research goals are to understand how ubiquitin-mediated turnover of key regulatory signaling proteins affects the outcome of signal transduction and how these processes might be altered and controlled in diseases, such as cancers. Specifically, in studies proposed here, we plan to determine how a signal transduction pathway induced by conditions of tumor microenvironment (hypoxia, amino acid starvation, etc.) accelerates the degradation of interferon alpha (IFNα) receptor (IFNAR1) and decreases the responsiveness of cells to endogenous (and, perhaps, therapeutic) IFNα/β. I have the expertise, leadership, and motivation necessary to successfully carry out the proposed work. I have a broad background in cell biology and biochemistry, with specific training and expertise in key research areas for this application (such as experimental oncology and signal transduction). As a postdoctoral fellow in 1994, I started the studies on protein ubiquitination and its role in transcriptional regulation. At the Mount Sinai School of Medicine, I expanded my research to include the characterization of novel E3 ubiquitin ligases and delineation of the role of protein ubiquitination and proteasomal degradation in signal transduction pathways altered in human diseases. As a PI on several previous university- and NIH-funded grants, I laid the groundwork for the proposed research by discovering the mechanisms by which phosphorylation-dependent ubiquitination regulates the abundance and function of receptors for cytokines (such as IFNα/β) and polypeptide hormones (such as prolactin and growth hormone). In addition, I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications from each project. As a result of these previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. My team has discovered the existence of novel ligand-inducible and ligand-independent pathways and demonstrated that activation of the latter pathway by UPR inducers affects the extent of cellular responses to IFNα/β. These studies were greatly aided by the ongoing close collaboration with the labs of Drs. J. Alan Diehl and Constantinos Koumenis. My current interests are rapidly expanding into the area of tumor biology, as per the role of identified mechanisms in the pathogenesis of cancers that activate Integrated Stress Responses (ISR). Our jointly proposed studies should enable us to gain comprehensive insights into the role of UPR and ISR in tumorigenesis and delineate the mechanisms that mediate this role. Whereas I have a demonstrated record of independent successful and productive research projects in an area of high relevance for human disease, the joint expertise and experience of my laboratory and the groups of Drs. Diehl and Koumenis will further elevate our ability to lead the proposed project and significantly contribute to this area of research.

B. Professional Experience

1996-1997 Associate Research Scientist, American Health Foundation, Valhalla, NY
1997-2000 Research Assistant Professor, Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, NY
2006-2006 Assistant Professor, Department of Animal Biology, University of Pennsylvania Philadelphia, PA
2006-2010 Associate Professor, Department of Animal Biology, University of Pennsylvania Philadelphia, PA
2007-present Director, Mari Lowe Center for Comparative Oncology Research, University of Pennsylvania, Philadelphia, PA

2010-present Professor, Department of Animal Biology, University of Pennsylvania Philadelphia, PA

Honors, Awards, Extramural Activities:

1983-86 Yaroslavl State Medical Institute Student Scientific Association Awards

1987 Medical Doctorate Summa Cum Laude, Yaroslavl State Medical Institute

2001-present Member, American Association for Cancer Research, American Society for Biochemistry and Molecular Biology, International Society for Interferon and Cytokine Research, The Endocrine Society

1997–present Ad Hoc reviewer for Science, Cell, Cancer Cell, The EMBO Journal, Journal of Biology, Molecular and Cellular Biology, Proceedings of National Academy of Sciences USA, Journal of Clinical Investigations, Journal of Biological Chemistry Journal of Immunology, Journal of Virology, Oncogene, EMBO Reports, Cancer Biology & Therapy, Molecular Carcinogenesis, International Journal of Cancer, Gene.

2004 – present NIH *Ad hoc* Study Section Member, BMCT (Basic Mechanisms in Cancer Therapy); Reviewer of grants for National Science Foundation, USA, Cancer Research UK Foundation, Israel Science Foundation

2006 – 2010 NIH Study Section Member, BMCT (Basic Mechanisms in Cancer Therapy)

2007 – 2010 Editorial Board Member, Signal Transduction and Therapy

2007 – present Editorial Board Member, Journal of Biological Chemistry, Cancer Biology & Therapy