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 717-783-2548.

1. **Grantee Institution:** University of Pittsburgh- of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 01/01/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Margaret C. McDonald, Ph.D.

4. **Grant Contact Person's Telephone Number:** 412-383-7474

5. **Grant SAP Number:** 4100047655

6. **Project Number and Title of Research Project:** 02 - Alterations in Bioenergetics and Mitochondrial Function in Tumor Cells

7. **Start and End Date of Research Project:** 01/01/2009 – 12/31/2010

8. **Name of Principal Investigator for the Research Project:** Bennett Van Houten, 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:

   $ 1,261,943.50

   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	Position Title	% of Effort on Project	Cost
Van Houten	Professor	50%	$248,841
de Moura	Postdoctoral Associate	87.5%	$ 84,985
Qian	Research Instructor	100%	$ 97,545
Wang	Postdoctoral Associate	100%	$131,150
Roginskaya	Lab Manager	100%	$123,086

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	Position Title	% of Effort on Project
None		

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:
BVH Pilot project: "Metabolic symbiosis: a new therapeutic target in head and neck tumors." On P50CA097190 (Grandis)	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Jan 2011	$34,653	$34,653
BVH Pilot project: "Bioenergetics: A new therapeutic target in metastatic melanoma". P50CA121973 (Kirkwood)	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Feb 2011	$35,000	$ 35,000

The preliminary data generated in this project were helpful in procuring these funds.

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:

We are planning on submitting grants to NCI. Specifically we are interesting in inhibiting tumor growth by directly attacking metabolism.

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

The future plans of this project are to understand bioenergetics in living tumor cells using fluorescence imaging approaches. These studies will combine multi-modal imaging, including fluorescence, MRI, and CT-PET.

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	1
Female			1	1
Unknown				
Total			**2**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			2	2
Unknown				
Total			**2**	**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White			2	1
Black				
Asian				1
Other				
Unknown				
Total			**2**	**2**

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

    Yes__X_____ No_____

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

    Amy Furda, BS, Graduate Student from Bethany College, WV

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.

    These funds helped develop and characterize a strong platform for the analysis of cellular bioenergetics at the University of Pittsburgh Cancer Institute. These studies greatly enhanced

our collaborative research (described below). Without these funds this research would not have been possible.

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_X_____ No_____

If yes, please describe the collaborations:

Dr. Shivendra Singh (University of Pittsburgh Cancer Institute)- role of natural products on cancer growth inhibition:

X. Xiao, A. A. Powolny, M. Barbi de Moura, A. Bommareddy, E.R. Hahm, B. Van Houten, and S. V. Singh. (2010). Phenethyl isothiocyanate inhibits oxidative phosphorylation to trigger reactive oxygen species-mediated death of human prostate cancer cells. J. Biol. Chem. 285(34):26558-69. PMCID: PMC2924093

E-R. Hahm, M. B Moura, E. E. Kelley, B. Van Houten, S. Shiva, and S.V. Singh (2011). Withaferin A-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species. PLOS One. 6(8):e23354. PMCID: PMC3154436

Drs. James A. Graves and Edward Prochownik (University of Pittsburgh Cancer Institute)- role of c-myc in tumor metabolism:

J. A. Graves, K. Rothermund, T. Wang, W. Qian, B. Van Houten, and E. V. Prochownik. (2010). Point mutations in c-Myc uncouple neoplastic transformation from multiple other phenotypes in rat fibroblasts PLoS One 5(10):e13717. PMCID: PMC2965668

Dr. Maria Jasin (Memorial Sloan-Kettering Cancer Center) – role of DNA ligase III in mitochondrial function:

Simsek, A. Furda, Y. Gao, J. Artus, E. Brunet, A. Hadjantonakis, B. Van Houten, S. Shuman, P. J. McKinnon & M. Jasin. (2011). Crucial roles for DNA ligase III in mitochondria but not in XRCC1-dependent repair. Nature Mar 10;471(7337):245-8. PMCID: PMC3261757

Dr. Gerald Schatten (University of Pittsburgh) – energy metabolism in stem cells:

S. Varum, A.S. Rodrigues, M.B. Moura, O. Momcilovic, C.A. Easley 4th, J. Ramalho-Santos, B. Van Houten , G. Schatten G. (2011). Energy metabolism in human pluripotent stem cells and their differentiated counterparts. PLoS One. 6(6):e20914. PMCID: PMC3117868.

Dr. Michael Lotze (University of Pittsburgh Cancer Institute) – role of high-mobility group box 1 protein in mitochondrial function:

D. Tang, R. Kang, K.M. Livesey, G. Kroemer, T.R. Billiar, B. Van Houten , H.J. Zeh 3rd, and M.T. Lotze. (2011). High-mobility group box 1 is essential for mitochondrial quality control. Cell Metab. 13(6):701-11. PMCID: PMC3293110

Dr. Sankar Mitra (University of Texas Medical Branch, Galveston, Tx) – the role of endoG on mitochondrial function:

Publications in preparation

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.

Objectives

Rapidly growing tumor cells have unique bioenergetic and metabolic demands. Tumor cells have deregulated mitochondrial function and a higher reliance on glycolysis for the production of adenosine triphosphate (ATP). Altered mitochondria can generate reactive oxygen species (ROS), and it has been proposed that these ROS can help drive tumor development toward more highly metastatic cancers. ROS can also drive mitogenic responses, cause DNA damage, and elicit somatic mutations. Mitochondria contain their own 16.5 kbp genome encoding 13 proteins that are essential for electron transport and ATP synthesis through oxidative phosphorylation. Mitochondrial DNA (mtDNA) is more prone to oxidative damage than nuclear DNA and is therefore a critical target of ROS. This project tests the hypothesis that, as tumors develop, repeated waves of ROS arise from microscopic bouts of ischemia reperfusion injury within the growing tumor mass. These ROS trigger mitochondrial DNA damage and mitochondrial mutations, which in turn produce mitochondrial dysregulation and dependence on glycolysis.

Prior to our work, analysis of oxidative phosphorylation through oxygen consumption and of glycolysis through lactate production was performed in separate assays on a large number of tumor cells. We used a newly developed assay that directly and simultaneously measures oxygen consumption and proton production as lactate on relatively small numbers of cells. This novel approach allowed screening of a large number (n = ~ 30) of established tumor lines. In addition, this project studied mtDNA damage and mutations with mitochondrial deregulation, ROS production, and increased glycolysis. There were three specific aims:

<u>Aim 1</u>: Analyze alterations in mitochondrial function, including increased mitochondrial generation of ROS, the production of subsequent damage (direct damage or mutations) to either mitochondrial or nuclear DNA, and increased dependence upon glycolysis

We studied the bioenergetics of 17 breast cancer cell lines representing luminal, HER2-enriched, basal-like, and claudin-low subtypes, and they were compared to explants of breast epithelial cells from four breast reduction mammoplasty surgeries. Results showed that luminal cell lines displayed the highest levels of oxidative phosphorylation (OXPHOS), whereas basal and claudin-low had the lowest. Surprisingly, glycolysis rates were similar in the four subtypes and steady-state ATP levels correlated to levels of OSPHOS and glycolysis combined. Levels of pyruvate dehydrogenase (PDH) and complex Vα & β correlated well with levels of OXPHOS.

To better understand these data, we developed a regression model that predicts OXPHOS levels from the expression level of nine selected genes. These nine genes belong to two main clusters, which are highly associated with high and low OXPHOS levels and are relevant to cancer, cell cycle growth, respiration and metabolism (Table 1). These data indicated a direct association between gene expression signatures and OXPHOS levels in different breast cancer subtypes [(de Moura MB, Santana-Santos L, Janjic BM, Latimer JJ, Benos PV, and Van Houten B. Systems level approach identifies oxidative phosphorylation as an important driver in breast cancer subtypes. Submitted to *Cell Metabolism*].

Table 1 – Genes selected by the HITON-MB algorithm that can best discriminate between low and high OXPHOS breast cancer cell lines.

Gene Symbol	Gene Name	10-fold cross-validation HITON-MB
AGR2	Anterior gradiente 2 homolog (*Xenopus laevis)*	4
AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
CAPG	Capping protein (actin filamento), gelsolin-like	1
IGF2BP2	Insulin-like growth fator 2 mRNA binding protein 2	10
MSN	Moesin	2, 3, 4, 6, 8, 10
PDP1	Pyruvate dehydrogenase phosphatase catalytic subunit 1	7
RHOB	Ras homolg gene family, member B	1, 2, 3, 6, 8, 9, 10
SGK1	Serum/glucocorticoid regulated kinase 1	8
XBP1	X-box binding protein 1	1, 5, 6, 7, 9, 10

<u>Aim 2</u>: Define the role of specific cancer genes in altering energy balance, ROS production, and subsequent genomic insult

Mitochondria are organelles centrally important for bioenergetics as well as regulation of apoptotic death in eukaryotic cells. High-mobility group box 1 (HMGB1), an evolutionarily conserved chromatin-associated protein that maintains nuclear homeostasis, is also a critical regulator of mitochondrial function and morphology. In collaboration with Dr. Michael Lotze's laboratory (UPCI), we demonstrated that the heat shock protein beta-1 (HSPB1 or HSP27) is the

downstream mediator of this effect. Disruption of the HSPB1 gene in embryonic fibroblasts with wild-type HMGB1 recapitulated the mitochondrial fragmentation, deficits in mitochondrial respiration, and ATP synthesis observed with targeted deletion of HMGB1. Forced expression of HSPB1 reversed this phenotype in HMGB1 knockout cells. Mitochondrial effects mediated by HMGB1 regulation of HSPB1 expression served as a defense against mitochondrial abnormality, enabling clearance and autophagy in the setting of cellular stress. These findings reveal an essential role for HMGB1 in autophagic surveillance with important effects on mitochondrial quality control (Figure 2) [Tang D, Kang R, Livesey KM, Kroemer G, Billiar TR, Van Houten B, Zeh HJ 3rd, and Lotze MT. (2011). High-mobility group box 1 is essential for mitochondrial quality control. Cell Metab. 13(6):701-11. PMCID: PMC3293110].

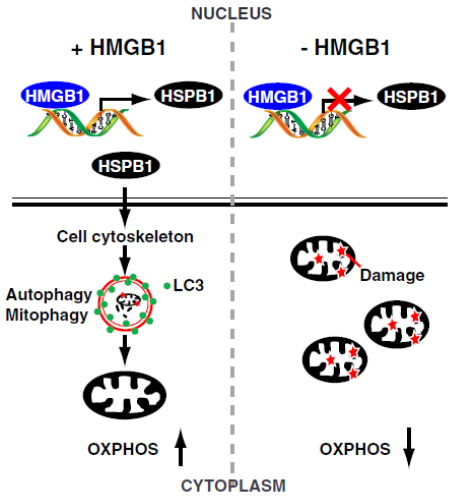

Figure 2. Schematic of the mechanism by which HMGB1 Modulates Mitochondrial Respiration and Morphology by Sustaining Mitophagy
In wild-type cells, HMGB1 functions as a transcriptional regulator of HSPB1 gene expression. Phosphorylation of HSPB1 is necessary to regulate the actin cytoskeleton, which affects the cellular transport of autophagy and mitophagy in response to mitochondrial injury. Loss of either HMGB1 or HSPB1 results in phenotypically similar deficient mitophagy typified by mitochondrial fragmentation, decreased aerobic respiration, and subsequent ATP production.

***Aim 3*: Assess whether stem cells have altered energy balance**

We collaborated with Dr. Gerald Schatten's laboratory (University of Pittsburgh) to examine induced pluripotent stem cells (IPSCs), because many cancer types have been reported to display metabolic modifications with features that might be similar to stem cells. Understanding the metabolic properties of human pluripotent stem cells as compared to human embryonic stem cells (hESCs) and their differentiated counterparts is thus of crucial importance.

We compared the energy metabolism of stem cells and their somatic counterparts. Focusing on mitochondria, we tracked organelle localization and morphology. Furthermore, we performed gene expression analysis of several pathways related to the glucose metabolism, including glycolysis, the pentose phosphate pathway, and the tricarboxylic acid (TCA) cycle. In addition, we determined oxygen consumption rates (OCR) using a metabolic extracellular flux analyzer, as well as total intracellular ATP levels using high performance liquid chromatography (HPLC). Finally, we explored the expression of key proteins involved in the regulation of glucose metabolism.

Our results demonstrated that, although the metabolic signature of IPSCs is not identical to that of hESCs, nonetheless they clustered with hESCs rather than with their somatic counterparts. ATP level, lactate production, and OCR revealed that human pluripotent cells rely mostly on glycolysis to meet their energy demands. Furthermore, our work pointed to some of the strategies that human pluripotent stem cells might use to maintain high glycolytic rates, such as high levels of hexokinase II and inactive pyruvate dehydrogenase (PDH) (Arum S, Rodrigues AS, Moura MB, Momcilovic O, Easley CA 4th, Ramalho-Santos J, Van Houten B, Schatten G. Energy metabolism in human pluripotent stem cellsand their differentiated counterparts. *PLoS One*. 2011;6(6):e20914. PubMed PMID: 21698063; PubMed Central PMCID: PMC3117868).

The other part of this aim was to assess the effect of mitochondrial DNA damage on tumor cell function. We were able to collaborate with two different groups (Dr. Maria Jasin, Memorial Sloan-Kettering Cancer Center, New York, New York, and Dr. Sankar Mitra, University of Texas Medical Branch, Galveston, Texas) to show that the integrity of mitochondrial DNA is essential for viable cell growth. Together these two studies clearly showed that strand break in mitochondrial DNA was sufficient to cause cell death.

Mammalian cells have three ATP-dependent DNA ligases that are required for DNA replication and repair. Homologues of ligase I (Lig1) and ligase IV (Lig4) are ubiquitous in eukarya, whereas ligase III (Lig3), which has nuclear and mitochondrial forms, appears to be restricted to vertebrates. Lig3 is implicated in various DNA repair pathways with its partner protein Xrcc1. Deletion of Lig3 results in early embryonic lethality in mice, as well as apparent cellular lethality, which has precluded definitive characterization of Lig3 function. In the collaboration with Dr. Jasin, we used preemptive complementation to determine the viability requirement for Lig3 in mammalian cells and its requirement in DNA repair. Various forms of Lig3 were introduced stably into mouse embryonic stem (mES) cells containing a conditional allele of Lig3 that could be deleted with Cre recombinase. With this approach, we found that the mitochondrial, but not nuclear, Lig3 was required for cellular viability (Figure 3). Although the catalytic function of Lig3 was required, the zinc finger (ZnF) and BRCA1 carboxy (C)-terminal-related (BRCT) domains of Lig3 were not. Remarkably, the viability requirement for Lig3 could be circumvented by targeting Lig1 to the mitochondria or expressing Chlorella virus DNA ligase, the minimal eukaryal nick-sealing enzyme, or *Escherichia coli* LigA, a NAD1 (nicotinamide adenine dinucleotide)-dependent ligase1. Lig3-null cells were not sensitive to several DNA-damaging agents that sensitize Xrcc1-deficient cells. Our results established a role for Lig3 in mitochondria, but distinguish it from its interacting protein Xrcc1 [Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis A, Van Houten B, Shuman S, McKinnon PJ, and Jasin M. (2011). Crucial roles for DNA ligase III in mitochondria but not in XRCC1-dependent repair. Nature Mar 10;471(7337):245-8. PubMed PMID: 21390132; PMCID: PMC3261757].

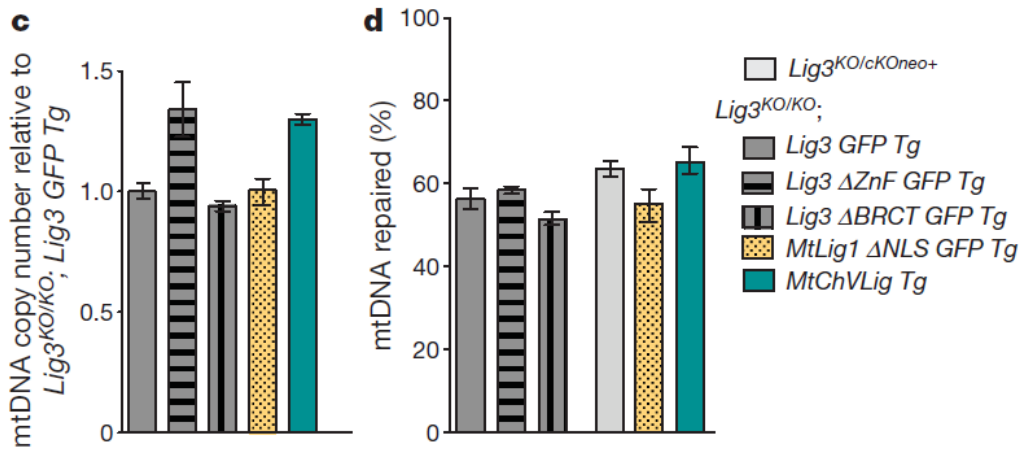

Figure 3. Mitochondrial DNA ligase activity can be provided by a variety of DNA ligases. A, Cells expressing exogenous DNA ligases are competent to replicate and maintain mtDNA. mtDNA copy number was quantified by qPCR by amplifying a 117 base pair (bp) fragment from mtDNA. Values are presented relative to levels of mtDNA in Lig3KO/KO; Lig3 GFP Tg cells. Data represent the mean of two biological repeats each determined twice by qPCR6s.e.m. **B,** Cells expressing exogenous sDNA ligases showed similar capacities for mtDNA repair after oxidative damage. Cells were treated with 175 microM hydrogen peroxide for 15 min and allowed to recover for 1.5 h. To measure repair, a 10-kb mtDNA fragment was amplified after damage and quantified by qPCR. Values were normalized to the amplification of a 117-bp mtDNA fragment. Data represent the mean of two to four determinations on multiple clones with each qPCR performed twice +/-s.e.m.

A. **B.**

Reactive oxygen species (ROS), continuously generated as byproducts of respiration, inflict more damage on the mitochondrial (mt) than on the nuclear genome because of the nonchromatinized nature and proximity to the ROS source of the mitochondrial genome. Such damage, particularly single-strand breaks (SSBs) with 5-blocking deoxyribose products generated directly or as repair intermediates for oxidized bases, is repaired via the base excision/SSB repair pathway in both nuclear and mt genomes. In collaboration with Dr. Mitra's group, we showed that EXOG, a 5'-exo/endonuclease and unique to the mitochondria (unlike FEN1 or DNA2, which, like EXOG, had been implicated in the removal of the 5'-blocking residue), was required for repairing endogenous SSBs in the mt genome. EXOG depletion induced persistent SSBs in the mtDNA, enhanced ROS levels, and caused apoptosis in normal cells but not in mt genome-deficient rho0 cells (Figure 4). Thus, these data show for the first time that persistent SSBs in the mt genome alone could provide the initial trigger for apoptotic signaling in mammalian cells [Tann AW, Boldogh I, Meiss G, Qian W, Van Houten B, Mitra S, and Szczesny B. (2011) Apoptosis induced by persistent single-strand breaks in the mitochondrial genome:Critical role of EXOG (5' EXO/Endonuclease) in their repair. J Biol Chem.286(37):31975-83. PMCID: PMC3173182].

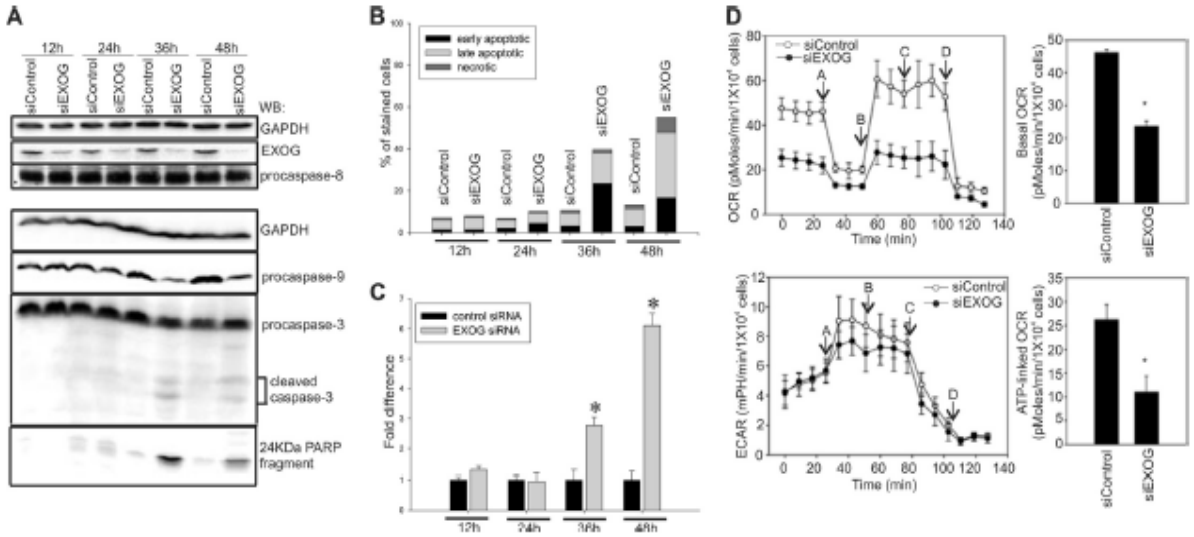

Figure 4. EXOG depletion induces mitochondrial dysfunction and programmed cell death. *A*, Western blot (*WB*) analysis of EXOG depletion and caspase activation at various times after transfection of HeLa cells with EXOG-siRNA compared with control siRNA. GAPDH was used as a loading control. Proteins separated on 12.5% SDS-PAGE were probed with antibodies as indicated. *B*, quantitation of early apoptotic, late apoptotic, and necrotic cell populations in HeLa cells at 12, 24, 36, and 48 h post-transfection with EXOG siRNA. The mean result of three independent experiments is shown. *C*, level of superoxide anion was measured by MitoSOX Red staining in EXOG siRNA-transfected HeLa cells compared with control. The mean results ± S.E. of three independent experiments is shown. * indicates $p < 0.05$ compared with control. *D*, OCR and extracellular acidification rate (*ECAR*) were measured 48 h after EXOG-siRNA transfection of MCF7 cells. The inhibitors were injected sequentially at indicated time points as follows: *A*, oligomycin (1 μg/ml); *B*, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (0.3 μM); *C*, 2-deoxyglucose (100 mM); *D*, rotenone (1 μM). The data are representative of two independent experiments performed in replicates of five. Basal OCR is the mean of first four time points prior to injection A. ATP-linked OCR is the basal OCR (mean of points 1–4) minus the oligomycin level of OCR (mean of points 5–7). Student's *t* test was used to compare mean values, $p < 0.001$.

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two

publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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. Mitochondrial dysfunction in neurodegenerative diseases and cancer.	M.B. Moura, L. S. Santos and B. Van Houten.	Environmental and Molecular Mutagenesis 51(5):391-405	November 2009	☐Submitted ☐Accepted **X** Published
2. Energy metabolism in human pluripotent stem cells and their differentiated counterparts.	S. Varum, A.S. Rodrigues, M.B. Moura, O. Momcilovic, C.A. Easley 4th, J. Ramalho-Santos, B. Van Houten , G. Schatten G.	PLoS One. 6(6):e20914	January 2011	☐Submitted ☐Accepted **X** Published
3. Crucial roles for DNA ligase III in mitochondria but not in XRCC1-dependent repair.	Simsek, A. Furda, Y. Gao, J. Artus, E. Brunet, A. Hadjantonakis, B. Van Houten, S. Shuman, P. J. McKinnon & M. Jasin.	Nature 471(7337):245-8.	April 2010	☐Submitted ☐Accepted **X** Published
4. High-mobility group box 1 is essential for mitochondrial quality control.	D. Tang, R. Kang, K.M. Livesey, G. Kroemer, T.R. Billiar, B. Van Houten , H.J. Zeh 3rd, and M.T. Lotze.	Cell Metabolism 13(6):701-11.	September 2010	☐Submitted ☐Accepted **X** Published
5. Apoptosis induced by persistent single-strand breaks in the	A.W. Tann, I. Boldogh, G. Meiss, W. Qian, B. Van Houten, S. Mitra,	Journal of Biological Chemistry	December 2010	☐Submitted ☐Accepted **X** Published

mitochondrial genome: Critical role of EXOG (5' EXO/Endonuclease) in their repair.	and B. Szczesny. (2011)			

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:

As mentioned in publication 5, we have submitted a large work-up of breast cancer cell lines, which was part of Aim1.

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.

This work has led to an increased understanding of the role of bioenergetics in tumor cell growth. Surprisingly, we have found that mitochondrial function appears to be important for the maintenance of tumor cell growth, which contradicts the Warburg hypothesis. Future work is focused on understanding the relative role of glycolysis and oxidative phosphorylation in tumor growth, development, and metastasis.

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.

Major discoveries of this project include:
From Aim 1:

- Tumor cells show a broad dependence on both glycolysis and oxidative phosphorylation.
- Detailed analysis of breast cancer cell lines was able to show that while luminal and Her2 positive cells show high OXPHOS, basal and claudin-low cells showed lower levels of OXPHOS and no concomitant increase in glycolysis.

- Using gene expression profiles, we were able to predict levels of OXPHOS using a set of nine genes.

From Aim 2:

- Myc overexpression causes an increase in metabolic flexibility and, under the correct conditions, can cause an increase in both oxidative phosphorylation and glycolysis.
- P53 knock down causes a decrease in OXPHOS.
- HMGB1 knock down causes a decrease in OXPHOS.

From Aim 3:

- Induced pluripotent stem cells have lower OPXHOS and increased glycolysis as compared to their parental progenitor cells.

Additional discoveries supported by this grant:

- Loss of mitochondrial DNA Ligase activity is toxic to cells.
- Loss of ExoG, a major DNA metabolizing enzyme in mitochondria, causes increased mitochondrial DNA damage, loss of mitochondrial function, and cell death.
- Taken together, these last two findings indicate that mitochondrial DNA damage is sufficient to trigger cell death and, thus, makes mtDNA an interesting target for the treatment of cancer.

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 Bennett Van Houten, Ph.D.	POSITION TITLE
eRA COMMONS USER NAME	Professor of Pharmacology and Chemical Biology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	MM/YY	FIELD OF STUDY
Clarion State College, PA	B.Sc.	1976-1980	Biology
Oak Ridge School of Biomedical Sciences, Oak Ridge, TN	Ph.D.	1980-1984	Biomedical Sci./Genetics

Positions and honors

Positions and Employment

1988-1990 Research Assistant Professor, Departments of Pathology and Biochemistry, University of Vermont

1990-1994 Assistant Professor, Departments of Pathology, Biochemistry and Microbiology and Molecular Genetics, University of Vermont.

1994-1999 Senior Scientist, Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX

1994-1998 Associate Professor, Department of Human Biological Chemistry & Genetics, and Department of Pathology, UTMB

1998-1999 Professor, Department of Human Biological Chemistry & Genetics, and Department of Pathology, UTMB.

1999-2008 Chief, Program Analysis Branch, DERT, NIEHS, NIH and Senior Investigator, Laboratory of Molecular Genetics, DIR, NIEHS, NIH.

2008- Richard M Cyert Professor of Molecular Oncology, Department of Pharmacology and Chemical Biology; Leader, Program Molecular and Cellular Cancer Biology Program, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA

2009- Faculty member, Molecular Biophysics and Structural Biology Graduate Program, University of Pittsburgh and Carnegie Mellon University.

2009- Faculty member, Graduate Program in Biochemistry and Molecular Genetics, Univ. of Pittsburgh.

Honors

2012 Provost Inaugural lecture, Richard M. Cyert Chair in Molecular Oncology.

2008 Medal of the Slovak Academy of Sciences for Support of Science.

2005 NIH Award of Merit for evaluation activities in the Division of Extramural Research and Training

2003 NIH Award of Merit for exemplary performance in the management of the Toxicogenomics Research Consortium.

2002 NIH Director's Award for outstanding effort conceptualizing, initiating, and implementing the NIEHS Toxicogenomics Research Consortium

2002 NIH Award of Merit for outstanding efforts in conceptualizing, initiating, and implementing the NIEHS Scientific Publications Information Retrieval System (SPIRES)
2001 NIH Award of Merit for outstanding effort conceptualizing, initiating, and implementing the NIEHS Toxicogenomics Research Consortium
2001 NIH Award of Merit for praiseworthy service in performing programmatic services, analyses, and evaluations to the benefit of NIEHS and the scientific community
9/96-8/31/99 Burroughs Wellcome Fund Scholar Award in Toxicology
1986-1988 Individual National Research Service Award

Selected Publications

1. Simsek D, Furda A, Gao Y, Artus J, Brunet E, Hadjantonakis A, Van Houten B, Shuman S, McKinnon P.J. & Jasin M. (2011). Crucial roles for DNA ligase III in mitochondria but not in XRCC1-dependent repair. Nature 471:245-249. NIHMSID: NIHMS262666
2. Tang D, Kang R, Livesey K. M., Kroemer G, Billiar T. R, Van Houten B, Zeh H.J., Lotze M. T. (2011). HMGB1 [High Mobility Group Box 1] is Essential for Mitochondrial Quality Control. Cell Metabolism Jun 8;13(6):701-11. NIHMSID: NIHMS300250
3. Hahm E-R, Moura M. B, Kelley E. E., Van Houten B, Shiva S; Singh S.V. (2011). "Withaferin A-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species". PLOS One. 6(8):e23354. Epub 2011 Aug 10. PMCID: PMC3154436
4. Liu Y., Reeves D., Kropachev K, Cai Y, Ding S, Kolbanovskiy M, Kolbanovskiy A, Bolton J.L., Broyde S, Van Houten B, Geacintov N.E. (2011). Probing for DNA damage with β-hairpins: Similarities in incision efficiencies of bulky DNA adducts by prokaryotic and human nucleotide excision repair systems in vitro. DNA Repair (Amst). Jul 15;10(7):684-96. NIHMSID: NIHMS304066
5. Tann A.W., Boldogh I, Meiss G, Qian W, Van Houten B, Mitra S, Szczesny B. (2011). Apoptosis induced by persistent single-strand breaks in the mitochondrial genome:Critical role of EXOG (5' EXO/Endonuclease) in their repair. J Biol Chem.;286(37):31975-83. PMCID: PMC3173182
6. Yeh J.I., Levine A. S., Du S, Chinte U, Ghodke H., Wang H, Shi H., Hsieh C. L., Vu T., Conway J. F., Van Houten B, V. Rapić-Otrin (2012). Damaged DNA induced UV-DDB dimerization and its roles on chromatinized DNA repair . Proc. Natl. Acad. Sci. Jul 20 [Epub ahead of print] PMID: 22822215
7. J.H. Zhu, A.M. Gusdon, H. Cimen, B. Van Houten, E. Koc, C.T. Chu. (2012).Impaired mitochondrial biogenesis contributes to depletion of functional mitochondria in chronic MPP(+) toxicity: dual roles for ERK1/2. Cell Death Dis. 2012 May24; PMCID: PMC336080
8. J.A. Graves , Y. Wang, S. Sims-Lucas , E. Cherok, K. Rothermund , M.F. Branca, J. Elster, D. Beer-Stolz, B. Van Houten, J. Vockley, E.V. Prochownik. (2012). Mitochondrial Structure,Function and Dynamics Are Temporally Controlled by c-Myc. PLoS One. 2012;7(5):e37699. Epub 2012 May 21. PMCID: PMC3357432
9. M. Barbi de Moura, G. Vincent, S. L. Fayewicz, N. Bateman, B. Hood, M. Sun, J. Suhan, S. Duensing, Y. Yin, C. Sander, J. M. Kirkwood, D. Becker, T. P. Conrads, B. Van Houten, and S. J Moschos. (2012). Mitochondrial respiration is an important therapeutic target in melanoma PLoS One PMCID:PMC3422349