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:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 01/01/11- 6/30/12

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl A. Richards, MBA

4. **Grant Contact Person's Telephone Number:** 412-641-8932

5. **Grant SAP Number:** 4100054859

6. **Project Number and Title of Research Project:** 2 - miR-210 Regulation of Mitochondria Function

7. **Start and End Date of Research Project:** 01/01/11-12/31/11

8. **Name of Principal Investigator for the Research Project:** Xin Huang, PhD

9. **Research Project Expenses**.

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

   $293,290

   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
Huang	Principal Investigator	25%	$26,197.14
Zuo	Postdoctoral Fellow	100%	$42,686.18
Brozik	Technician	50%	$35,353.54

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:
The Mechanism of miR-210 Regulating Cellular Metabolism and Tumorigenesis	☐NIH ☐ Other federal (specify:_____) ☒Nonfederal source (specify: American Cancer Society)	October, 2011	$736,276	$720,000

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:

After we submit our manuscript for publication, we plan to submit a NIH grant application to further explore the mechanistic study on how miR-210 regulates glycolysis.

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

We plan to further investigate the mechanism of miR-210 regulation of aerobic glycolysis.

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____X_____ No_____

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

    We expanded our institute's research scope to include cancer metabolism research. By establishing the capacity of studying metabolism and mitochondrial function, we entered this highly competitive but active research field. Currently, metabolism has been recognized again as a critical component of tumorigenesis after its significance was discovered almost 100 years ago by German biochemist Otto Warburg. Our new line of research on ovarian cancer metabolism regulated by miR-210 identified a new player in this important process.

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:

We have established collaborations with Dr. Ben Van Houten at the University of Pittsburgh Cancer Institute (UPCI) to expand our research using their Seahorse system, and simultaneously to provide them our expertise on tumor hypoxia.

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 application's strategic plan). 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.

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death in women in the United States. Currently, there is no effective therapy for advanced-stage EOC. Because tumor hypoxia is correlated with poor prognosis in cancer patients and contributes significantly to more aggressive tumor phenotypes, such as metastasis and chemoresistance, a clear understanding of the mechanism of tumor hypoxia gene regulation and function of hypoxia-inducible genes will enable us to combat cancer by targeting tumor hypoxia. miR-210 is the most consistently induced microRNA under hypoxia. Thus, we plan to study miR-210 function with a focus on its role in regulating cellular metabolism. miR-210 and its target gene identified in this study may provide novel therapeutic targets for EOC treatment. We proposed the following three specific aims:

> Specific Aim 1: Determine the correlation of miR-210 copy number and expression in ovarian cancer cell lines.
> Specific Aim 2: Identify novel miR-210 target genes involved in cellular metabolism in ovarian cancer cells and their biological functions.
> Specific Aim 3: Delineate the correlation between miR-210 target genes expression and tumor hypoxia in banked ovarian cancer specimens.

We will summarize our findings to achieve each aim first and present our data in detail.

Specific Aim 1: We found that miR-210 is robustly induced under 0.5% oxygen for 24 hours in all 12 ovarian cancer cell lines studied by real-time PCR. In addition, we performed fluorescence *in situ* hybridization (FISH) with a bacteria artificial chromosome (BAC) probe that covers miR-210 genomic locus in these cells and determined their miR-210 genomic copy number status. Interestingly, we found that miR-210 is frequently deleted (61%) in ovarian cancer cell lines, despite the majority of cells only have one copy deleted. In addition, by analyzing the ovarian cancer TCGA data, we found that miR-210 is also frequently deleted in primary ovarian tumor samples. But miR-210 expression did not decrease correspondingly, which is also consistent with our findings in cell lines. Our data suggest that miR-210 is not the deletion target on 11p15 and miR-210 is essential to ovarian tumors because tumor cells maintained miR-210 expression when its copy number is deleted. We have successfully completed the Aim 1.

Specific Aim 2: We identified NDUFA4 as a novel miR-210 target by 3' UTR reporter assay, Western blotting, and locked nucleic acid (LNA) knockdown of miR-210. NDUFA4 is robustly suppressed by miR-210 and under hypoxic conditions. Identification of NDUFA4 as

a miR-210 target is significant since this provides a novel mechanistic explanation how HIF1α regulates mitochondrial oxidative phosphorylation under hypoxic conditions. We further demonstrated that NDUFA4 is remarkably repressed under hypoxic conditions by Western blotting and immunofluorescence staining. Functionally, we demonstrated that miR-210 overexpression and NDUFA4 knockdown can lead to a series of phenotype that are expected from NDUFA4 function. We found that miR-210 expression/NDUFA4 knockdown can decrease mitochondrial respiration, decrease mitochondrial ATP production, and increase glycolysis. We have successfully completed the Aim 2.

Specific Aim 3: We performed immunohistochemistry on 60 primary ovarian tumors samples on a tissue microarray. We found that localization of NDUFA4 is localized outside of hypoxic regions as we predicted. However, because of the difficulty in collecting enough material using laser capture microdissection (LCM), we were not able to measure miR-210 expression in these samples. As an alternative, we analyzed the recently published ovarian cancer TCGA data set that includes 506 high grade primary serous ovarian cancer samples. We found that miR-210 genomic locus is frequently deleted in TCGA samples, but miR-210 expression is maintained. In addition, miR-210 expression is highly correlated with classic hypoxia inducible genes, such as *GLUT1* and *LDHA*, suggesting that miR-210 is regulated by tumor hypoxia *in vivo* and homeostasis of miR-210 expression level is important for ovarian tumor cells. Thus, we have successfully completed the Aim 3.

Below is the detailed project report:

miR-210 is deleted in high frequency in ovarian cancer cells

We determined miR-210 copy number in 13 ovarian cancer cell lines using a BAC clone, RPCI11-496I9 that include the entire genomic region of miR-210. We labeled the BAC DNA in red and chromosome 11 centromere probe with green and performed fluorescence in situ hybridization (FISH) to measure miR-210 copy number. We observed miR-210 loss in at least 30% of cell population in eight of thirteen ovarian cancer cell lines examined (61%). Interestingly, the vast majority of miR-210 copy number loss is single copy loss and no homozygous deletion was detected (Fig. 1A), suggesting that miR-210 may not be a target of chromosome 11p15.5 deletion. In order to confirm this, we also performed RT-qPCR to estimate miR-210 baseline expression or hypoxia induction in 12 ovarian cancer cell lines. No direct correlation between miR-210 copy number and miR-210 baseline expression or hypoxia induction was observed (Fig. 1B).

NDUFA4 is a miR-210 target

Because miR-210 is highly induced under hypoxic conditions, we searched for its target genes that may be involved in cellular response to hypoxic stress. It's well known that under hypoxic conditions, cells up-regulate glycolysis by HIF1α to meet cellular energy demand due to diminished oxygen availability. However, how cells down-regulate oxidative phosphorylation (OXPHOS) is less clear. We found that NDUFA4, a subunit of mitochondria ETC Complex I, is a potential miR-210 target gene, which may explain how cells down-regulates OXPHOS. We cloned the 3' UTR of NDUFA4 into a luciferase reporter vector and performed reporter assay

with co-transfected miR-210 expressing construct. As shown in Fig. 2A, the luciferase activity can be specifically down-regulated by over-expressing miR-210, indicating NDUFA4 is a miR-210 target. We have also overexpressed miR-210 in SKOV3ip.1 cells and observed down-regulation of endogenous NDUFA4 protein (Fig. 2B). When miR-210 is knocked down under 2% oxygen, NDUFA4 repression by hypoxia can be partially rescued (Fig. 2C), further supporting our conclusion that NDUFA4 is a miR-210 target.

NDUFA4 is a specific mitochondria target of hypoxia

Because miR-210 is highly induced under hypoxia, we asked whether NDUFA4 expression is repressed under hypoxic condition. We collected a variety of cell lines from different origins and performed Western blotting. Strikingly, NDUFA4 expression is essentially completely repressed in all cell lines examined (Fig. 3A), suggesting NDUFA4 regulation under hypoxia is a general mechanism for cells to down-regulate OXPHOS. In order to examine whether hypoxic regulation of NDUFA4 is specific for Complex I subunits, we randomly selected three other subunits of Complex I for Western blotting analysis. Fig. 3B indicates that only NDUFA4 is specifically repressed by hypoxia, further suggesting that NDUFA4 may be the target of hypoxia regulation of mitochondria function. When we performed immunofluorescence staining in SKOV3 cells, NDUFA4 protein expression is completely repressed under 0.5% oxygen (Fig. 3C), confirming our Western blotting results.

miR-210/NDUFA4 regulate mitochondrial respiration

Because NDUFA4 is a subunit of mitochondrial ETC Complex I, we expected that oxygen consumption will be inhibited when miR-210 is overexpressed or NDUFA4 is knocked down by siRNA. As predicted, SKOV3 cells that over-express miR-210 or were transfected with siRNA targeting NDUFA4 demonstrated reduced oxygen consumption compared to parental cells (Fig. 4), indicating attenuated mitochondrial respiration.

miR-210/NDUFA4 regulate aerobic glycolysis

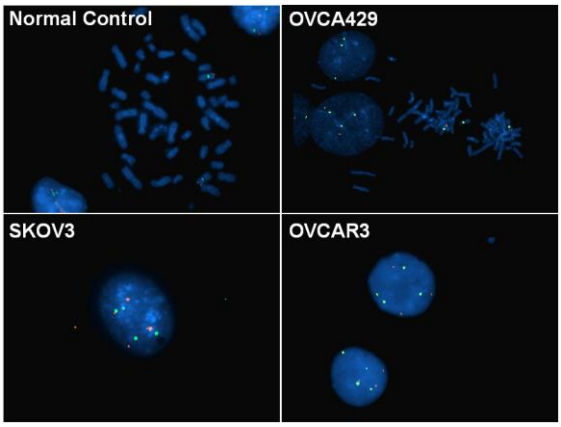
When we observed attenuated mitochondrial respiration, a direct prediction is that cellular ATP level will be affected since the majority of ATP is produced through oxidative phosphorylation (OXPHOS). We compared cellular ATP levels in parental cells and cells expressing miR-210 (210E) or with NDUFA4 knockdown (shNDU). As expected, ATP level is lower in 210E and shNDU cells when compared to parental controls (Fig. 5). We then reasoned that when cellular ATP level is decreased, cells may try to compensate for the lost ATP level by other means, one of which is glycolysis. We then measured cellular lactate production, the final product of glycolysis. Interestingly, lactate levels in both 210E and shNDU cells are increased (Fig. 6), suggesting that miR-210 regulates a feedback mechanism that can down-regulate OXPHOS while enhancing aerobic glycolysis. Currently, we are investigating the mechanism of this enhanced aerobic glycolysis.

miR-210 expression in primary ovarian tumors

The Cancer Genome Atlas (TCGA) project is intended to comprehensively analyze tumor samples using multiple platforms, such as gene expression, miRNA expression, promoter methylation, DNA copy number etc. We analyzed the ovarian cancer data that include tumors from 506 high grade serous ovarian cancer patients. Despite chromosome 11p15.5, where miR-210 is located, is frequently lost, miR-210 expression level is still compared to that of normal ovary controls (Fig. 7A), suggesting that miR-210 likely resides in a genomic location of heterozygous loss, and that other mechanisms may up-regulate miR-210 to compensate for the loss of miR-210 copy number. This also suggests that maintaining cellular miR-210 levels may be important for EOC development. Since miR-210 is robustly induced under hypoxic conditions, we examined the correlation between miR-210 expression and a hypoxic gene expression signature, consisting of 9 well-characterized hypoxia-responsive genes (Fig. 7B). The highly significant correlation of miR-210 expression with that of classic HIF1α target genes, such as genes in the glycolytic pathway, *SLA2A1* (*GLUT1*) and *LDHA* (Fig. 7C; p=0 for both genes), strongly support the notion that miR-210 expression in primary ovarian tumors is regulated by tumor hypoxia and tumor hypoxia may be the mechanism responsible for upregulating miR-210 expression in EOC to compensate for its copy number loss. We then analyzed NDUFA4 expression in primary ovarian cancer tumors using a tissue microarray (TMA) consisting of 60 primary tumor samples by immunohistochemistry (IHC). Interestingly, NDUFA4 is found localized exclusively with a classic tumor hypoxia marker, CAIX (Fig. 8), further confirming our analysis from ovarian cancer TCGA data, that miR-210/NDUFA4 is regulated by tumor hypoxia *in vivo*.

Figures

A.



	Normal	Loss	Gain
OVCA420	4%	96%	
OVCA429	2%	98%	
OVCA432	49%	51%	
OVCA433	2%	98%	
A2780	93%	3%	4%
CP70	92%	5%	3%
OVCAR3	2%	98%	
SKOV3	95%	5%	
MESOV	93%	3%	4%
IGROV1	69%	31%	
OVCAR8	27%	73%	
HEYA8	69%	31%	
ES2	75%		25%

B.

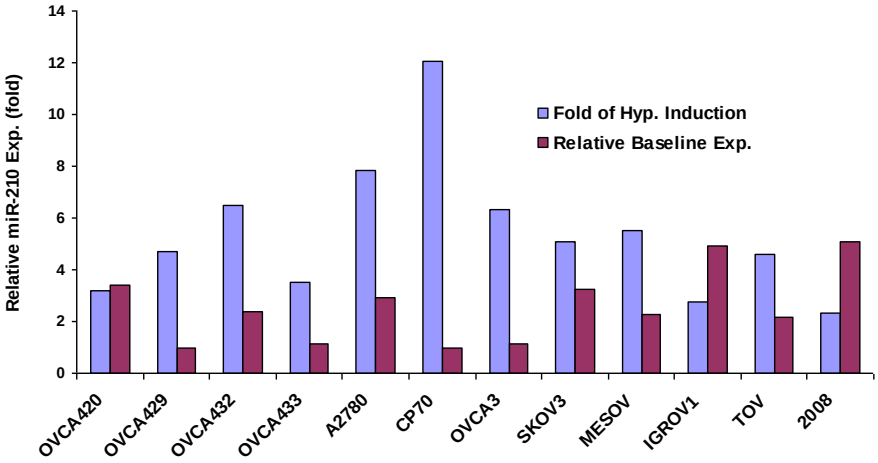


Figure 1**.** miR-210 copy number and expression is not correlated in ovarian cancer cells. (A) miR-210 is lost at high frequency in ovarian cancer cells. Left panel, representative images of miR-210 FISH; Right panel, summary of miR-210 FISH results in 13 ovarian cancer cell lines. (B) miR-210 baseline expression and hypoxia induction is robust and does not correlate with its copy number.

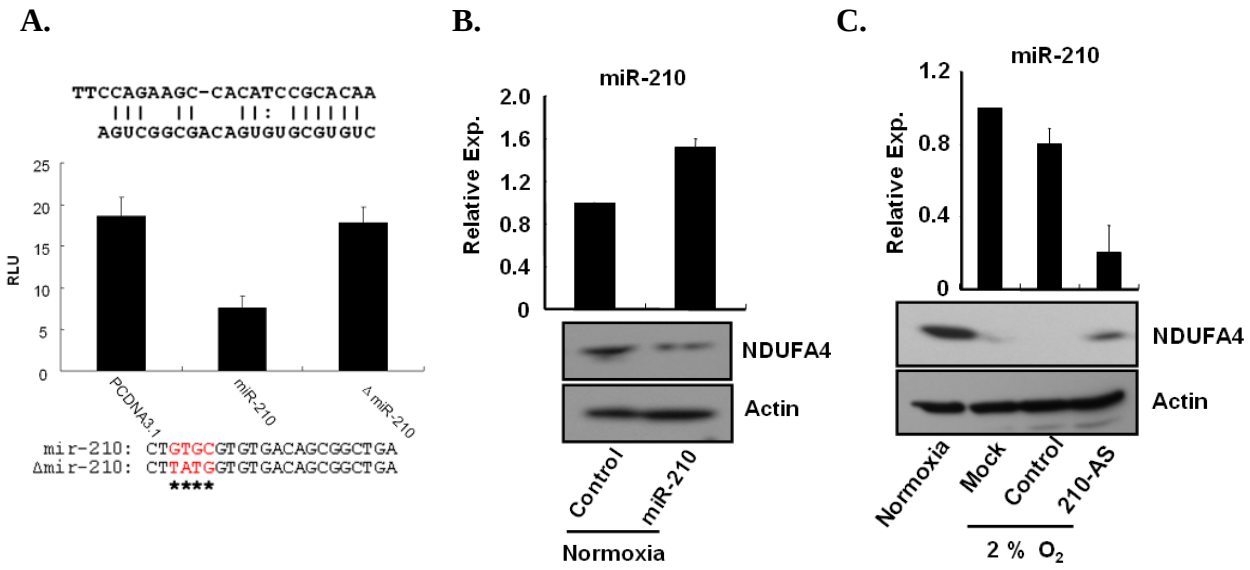


Figure 2. NDUFA4 is a miR-210 target. (A) miR-210 can repress luciferase reporter activity by interacting with NDUFA4 3' UTR cloned at downstream of the luciferase reporter gene. When miR-210 "seed region" is mutated, the repression is abolished. Top panel, the putative binding between miR-210 and NDUFA4 3' UTR. Bottom panel, the mutation of miR-210 "seed region" in the miR-210 expression construct. (B) Ectopic expression of miR-210 can down-regulate endogenous NDUFA4 expression. (C) Knockdown of miR-210 expression at 2% oxygen by locked nucleic acid (LNA) oligo against miR-210 can partially rescue NDUFA4 expression.

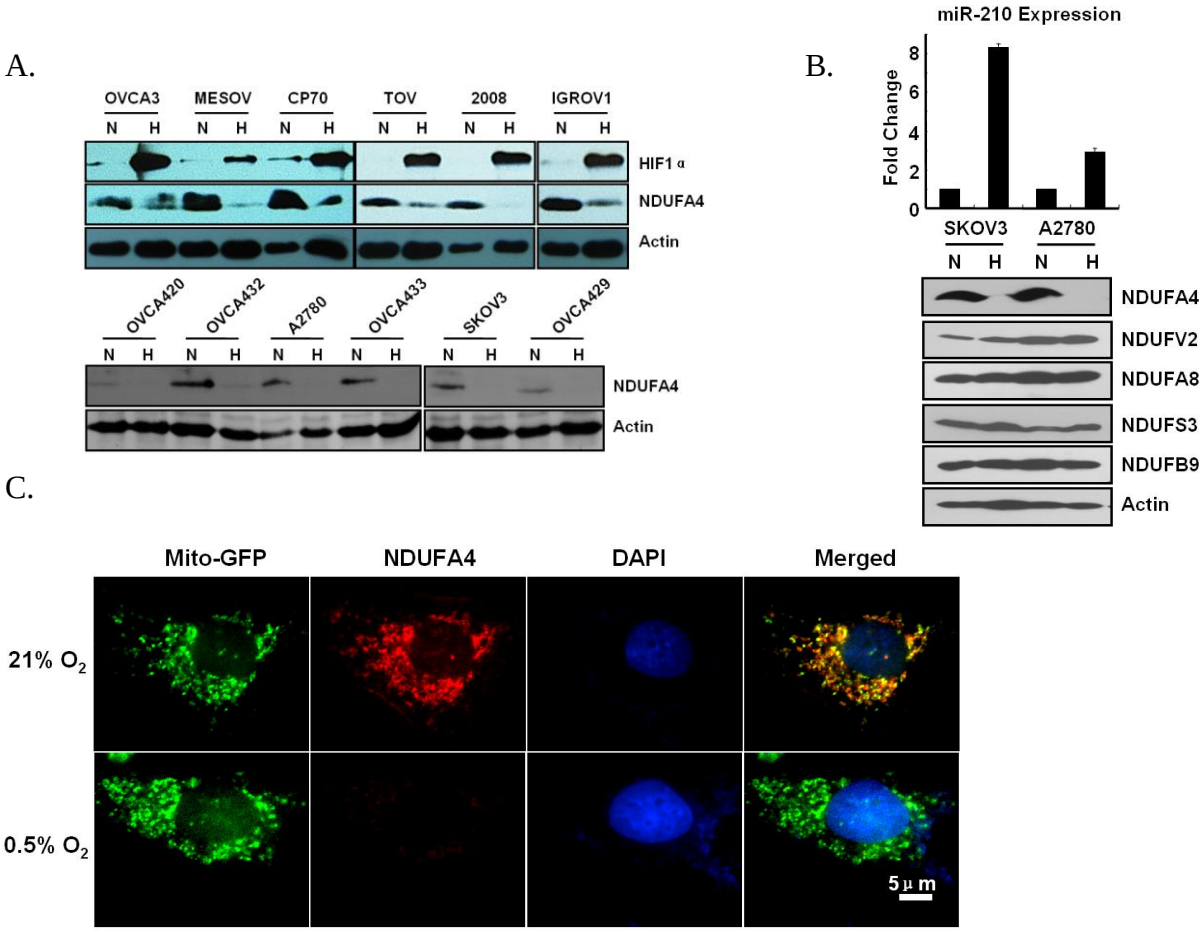


Figure 3. NDUFA4 expression is repressed by hypoxia. (A) NDUFA4 expression is repressed by hypoxia in ovarian cancer cell lines. (B) NDUFA4 is a mitochondria ETC Complex I subunit that is specifically repressed by hypoxia. (C) Immunofluorescence staining of NDUFA4. NDUFA4 is located in mitochondria where its expression is completely abolished when treated under hypoxia. Mito-GFP, a GFP protein that is localized in mitochondria, is used as a control.

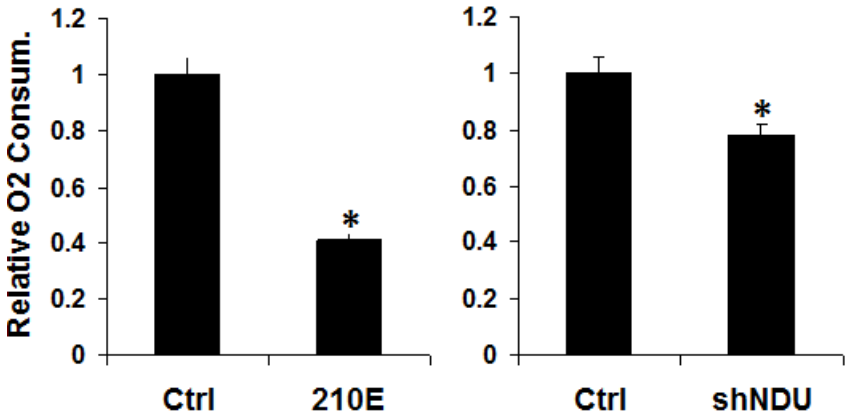


Figure 4. Ectopic expression of miR-210 (210E) and knockdown of NDUFA4 (shNDU) in SKOV3 cells lead to decreased oxygen consumption when compared to parental control cells.

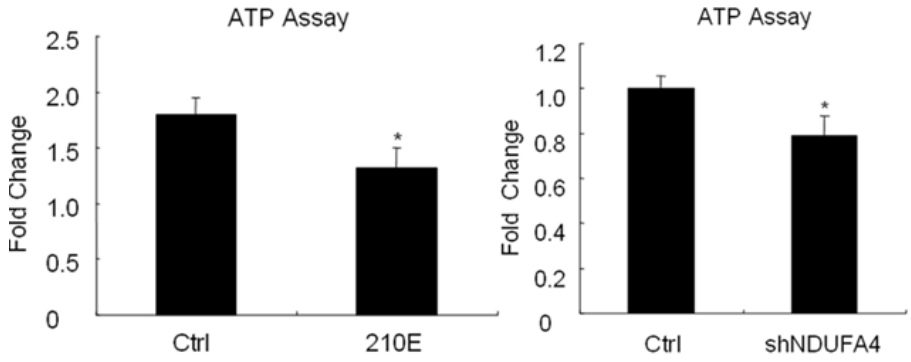


Figure 5. Ectopic expression of miR-210 (210E) and knockdown of NDUFA4 (shNDU) in SKOV3 cells lead to decreased cellular ATP levels when compared to parental control cells.

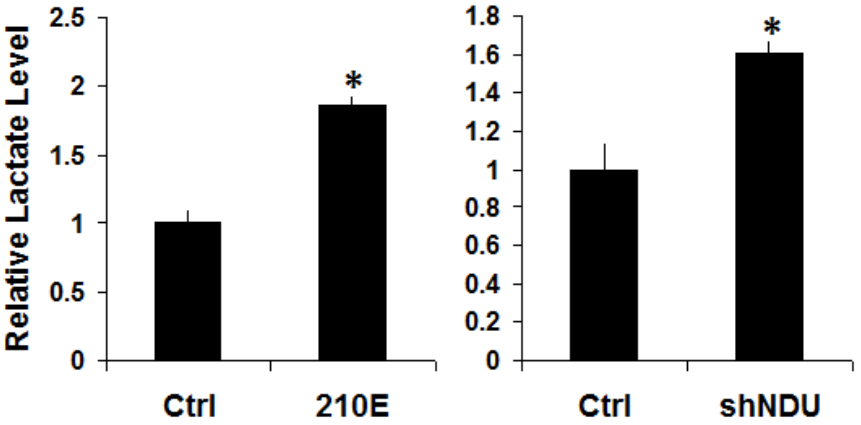


Figure 6. Ectopic expression of miR-210 (210E) and knockdown of NDUFA4 (shNDU) in SKOV3 cells lead to increased lactate production when compared to parental control cells, indicating enhanced glycolysis.

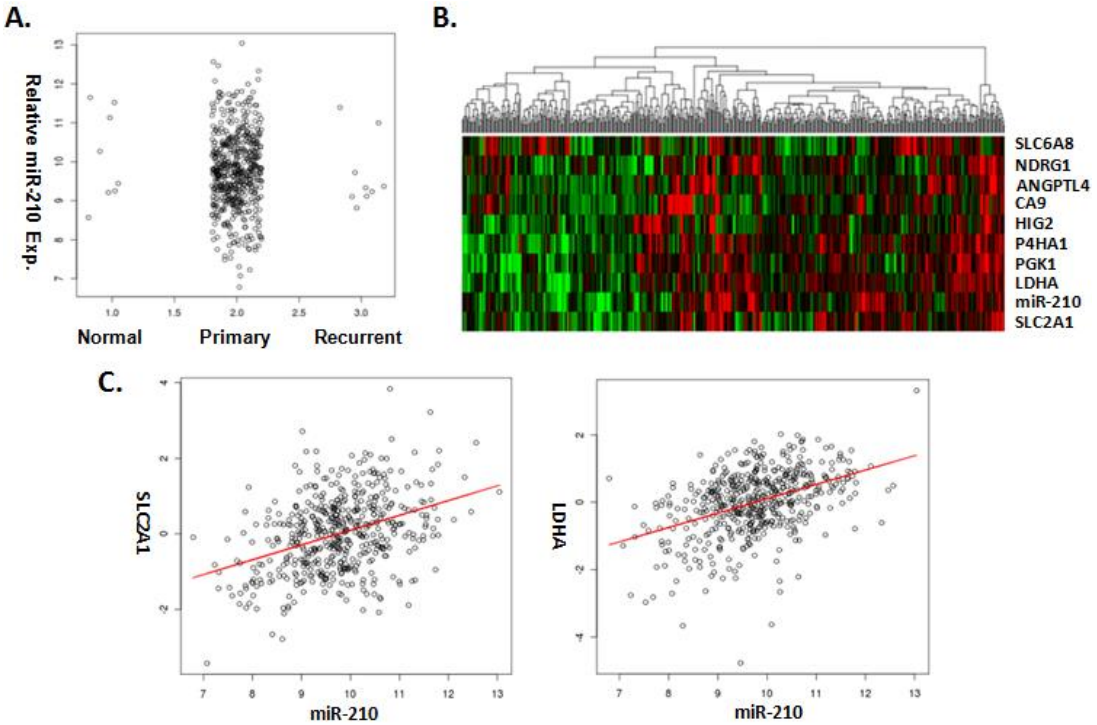


Figure 7. miR-210 in primary EOC. A) miR-210 expression in TCGA ovarian cancer and normal control samples. B) In 506 TCGA samples, miR-210 expression is clustered with that of classic hypoxia inducible genes; each column represents one patient. C) miR-210 expression is highly correlated with that of *SLC2A1* and *LDHA*. Left panel, *SLC2A1*; right panel, *LDHA*.

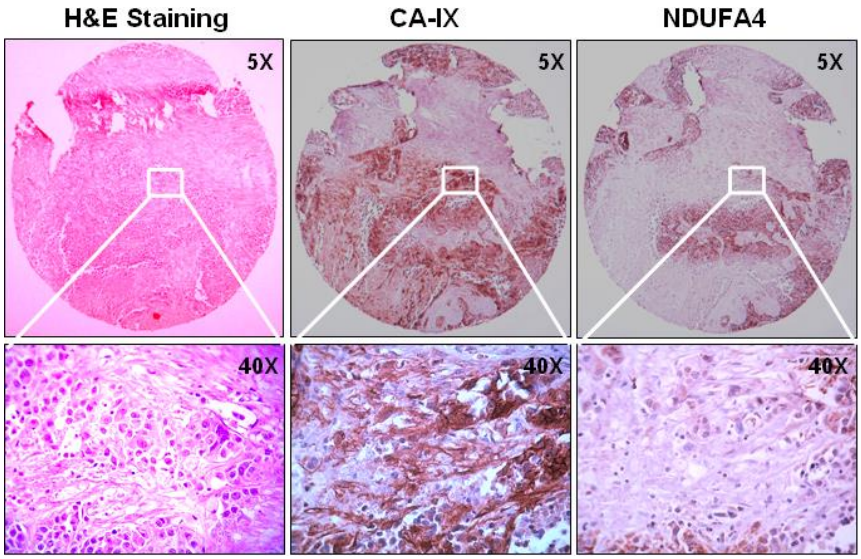


Figure 8. NDUFA4 is localized outside of hypoxic regions in primary EOC tumors. Left panel, H&E staining; Middle panel, CAIX staining; Right panel, NDUFA4 staining. Serial sections were used to ensure the same tumor areas are stained. The top panel is a low magnitude (5X) view of the staining. The bottom panel shows the enlarged areas of the top panel at a higher magnitude (40X).

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. 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:

We plan to submit our research to Cell Metabolism in 2-3 months.

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.

We uncovered a novel mechanistic link between miR-210 expression and repressed mitochondrial oxidative phosphorylation function under hypoxic conditions. It is well known that HIF1α can enhance glycolysis under hypoxic conditions by up-regulating transcription of genes in the glycolytic pathway. However, how mitochondrial function is suppressed under hypoxia is much less clear. Our finding suggest that up-regulation of miR-210 under hypoxia can down-regulate mitochondrial function by inhibiting NDUFA4, a subunit of mitochondrial electron transport chain Complex I, providing a mechanistic explanation on this unanswered question.

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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the 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 Xin Huang	POSITION TITLE Assistant Professor of OB/GYN
eRA COMMONS USER NAME HUANGX2	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Sichuan University, Chengdu, China	B.S.	1994	Microbiology
Fudan University, Shanghai, China	M.S.	1997	Genetics
University of Pittsburgh, Pittsburgh, PA	Ph.D.	2002	Human Genetics
University of Pittsburgh Cancer Institute, Pittsburgh, PA	Postdoc	2002-2005	Cancer Genetics
Stanford University, Stanford, CA	Postdoc	2005-2008	Radiation Oncology

A. Personal Statement

I am interested in understanding the mechanism of tumor hypoxia in ovarian cancer initiation, progression, and metastasis, particularly on hypoxia-inducible microRNA function. The long term goal of my lab is to identify novel therapeutic targets for ovarian cancer treatment.

B. Positions and Honors

Professional Appointments

2002-2005 Postdoctoral Fellow, University of Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA; Advisor: Tony E. Godfrey, Ph.D.

2005-2008 Postdoctoral Scholar, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA; Advisors: Amato J. Giaccia, Ph.D. and Quynh-Thu Le, M.D.

2008-2009 Research Associate, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA

2009-2010 Research Assistant Professor of OB/GYN, Department of OB/GYN and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA

2010-present Assistant Professor of OB/GYN, Department of OB/GYN and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA

Professional Memberships:

2002 – present Member, American Society for Cancer Research (AACR)

C. Selected Peer-Reviewed Publications:

Most relevant to the current application

1. Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT, Giaccia AJ. Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. Mol Cell. 2009, 35(6):856-67. PMCID: PMC2782615
2. Huang X, Le QT, Giaccia AJ. MiR-210 - micromanager of the hypoxia pathway. Trends Mol Med. 2010, 16(5):230-7. PMCID: in process.

3. Ho AS,* Huang X*, Cao H, Christman-Skieller C, Bennewith K Le QT, Koong AC. Circulating miR-210 as a novel hypoxia marker in pancreatic cancer. Transl Oncol. 2010, 3(2):109-13. (* equal contribution) PMCID: PMC2847318.

Additional selected publications of importance to the field (in chronological order)

4. Saunders WS, Shuster M, Huang X, Gharaibeh B, Enyenihi AH, Petersen I, Gollin SM. Chromosomal instability and cytoskeletal defects in oral cancer cells. Proc Natl Acad Sci U S A. 2000, 97(1):303-8. PMCID: PMC26658
5. Huang X, Gollin SM, Raja S, Godfrey TE. High resolution mapping of the 11q13 amplicon and identification of a gene, TAOS1, that is amplified and over expressed in oral cancer cells. Proc Natl Acad Sci U S A. 2002, 99(17):11369-74. PMCID: PMC123263
6. Hsu LC, Huang X, Seasholtz S, Potter DM, Gollin SM. Gene amplification and over expression of protein phosphatase 1alpha in oral squamous cell carcinoma cell lines. Oncogene. 2006, 25(40):5517-26. PMCID: PMC16619035
7. Wu G, Xing M, Mambo E, Huang X, Liu J, Guo Z, Chatterjee A, Goldenberg D, Gollin SM, Sukumar S, Trink B, Sidransky D. Somatic mutation and gain of copy number of PIK3CA in human breast cancer. Breast Cancer Res. 2005, 7(5):R609-16. PMCID: PMC1242128
8. Wu G, Z Guo Z, Chatterjee A, Huang X, Rubin E, Wu F, Mambo E, Chang X, Osada M, Sook Kim M, Moon C, Califano JA, Ratovitski EA, Gollin SM, Sukumar S, Sidransky D, Trink B. Overexpression of glycosylphosphatidylinositol (GPI) transamidase subunits phosphatidylinositol glycan class T and/or GPI anchor attachment 1 induces tumorigenesis and contributes to invasion in human breast cancer. Cancer Res. 2006, 66(20):9829-36. PMCID: PMC17047043
9. Huang X, Godfrey TE, Gooding WE, McCarty KS Jr, Gollin SM. Comprehensive genome and transcriptome analysis of the 11q13 amplicon in human oral cancer and synteny to the 7F5 amplicon in murine oral carcinoma. Genes Chromosomes Cancer. 2006, 45(11):1058-69. PMCID: PMC16906560
10. Xi L, Lyons-Weiler J, Coello MC, Huang X, Gooding WE, Luketich JD, Godfrey TE. Prediction of lymph node metastasis by analysis of gene expression profiles in primary lung adenocarcinomas. Clin Cancer Res. 2005, 11(11):4128-35. PMCID: PMC2211271
11. Parikh RA*, White JS*, Huang X, Schoppy DW, Baysal BE, Baskaran R, Bakkenist CJ, Saunders WS, Hsu LC, Romkes M, Gollin SM. Loss of distal 11q is associated with DNA repair deficiency and reduced sensitivity to ionizing radiation in head and neck squamous cell carcinoma. Genes Chromosomes Cancer. 2007, 46(8):761-75. (* equal contribution) PMCID: PMC17492757
12. Le QT, Kong C, Lavori PW, O'byrne K, Erler JT, Huang X, Chen Y, Cao H, Tibshirani R, Denko N, Giaccia AJ, Koong AC. Expression and prognostic significance of a panel of tissue hypoxia markers in head-and-neck squamous cell carcinomas. Int J Radiat Oncol Biol Phys. 2007, 69(1):167-75. PMCID: PMC17707270
13. Bennewith KL, Huang X*, Ham CM*, Graves EE, Erler J, Kambham N, Feazell J, Yang GP, Koong A, Giaccia AJ. The role of tumor cell-derived connective tissue growth factor (CTGF/CCN2) in pancreatic tumor growth. Cancer Res. 2009, 69(3):775-84. (* equal contribution) PMCID: PMC2747032
14. Duvvuri U, Shiwarski DJ, Xiao D, Bertrand C, Huang X, Edinger RS, Rock JR, Harfe BD, Henson BJ, Kunzelmann K, Schreiber R, Seethala RS, Egloff AM, Chen X, Lui VW, Grandis JR, Gollin SM. TMEM16A induces MAPK and contributes directly to tumorigenesis and cancer progression. Cancer Res. 2012, 72(13): 3270-3281. PMCID: in process.

D. Research Support

Ongoing Research Support

American Cancer Society Research Scholar Grant (Huang, X., PI) 7/1/2012 – 6/30/2016
The mechanism of miR-210 regulating cellular metabolism and tumorigenesis