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):** 1/1/2010 – 12/31/2010

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:** 4100050901

6. **Project Number and Title of Research Project:** 3 **-** Hypoxia Inducible miR-210 as a Potential Therapeutic Target in Renal Cell Carcinoma

7. **Start and End Date of Research Project:** 1/1/2010 – 12/31/2010

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:

   $ 158,500.00

   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%	$25,507.84
Zuo	Post-doctoral Fellow	100%	$25,210.33
Tan	Post-doctoral Fellow	100%	$30,826.28

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

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

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____X____ No_____

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

Clinical & Translational Science Institute at University of Pittsburgh - $15,000.

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_____ No____X____

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:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

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 R01 grant application to further explore the potential of targeting miR-210 in renal cell carcinoma and other hypoxic tumors.

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

We plan to further investigate the mechanism of phosphor-p53 (S15) upregulation by miR-210 knockdown in RCC4 cells and expand our observations into other renal cell carcinoma cell lines and other tumor cells under hypoxic conditions.

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				2
Female				
Unknown				
Total				**2**

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

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

Jianhong Zuo, M.D., Ph.D., University of California at San Francisco (UCSF)

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 renal cell carcinoma research. By performing next generation sequencing, we also gained experience on this cutting edge technology, which will be helpful for other investigators in the institute to utilize this powerful technology to address different research and clinical questions.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. 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.

Hypoxia Inducible miR-210 as a Potential Therapeutic Target in Renal Cell Carcinoma

Clear cell renal cell carcinoma (ccRCC) is among the tumors most resistant to radiation- and chemo-therapies. Currently, there is no effective treatment for this devastating disease. Our project intends to address the function of miR-210 in ccRCC apoptosis and survival and elucidate the mechanism of suppression of cell proliferation caused by miR-210 inhibition.

Based on the data we have collected during this project, miR-210 seems to be an attractive target for developing novel therapies against ccRCC. We proposed the following three specific aims:

Specific Aim 1:	Determine the miR-210 function in ccRCC with *VHL* mutation
Specific Aim 2:	Identify miR-210 target gene in ccRCC
Specific Aim 3:	Elucidate the mechanism of cell death caused by miR-210 inhibition in ccRCC

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

Specific Aim 1: We found that miR-210 is essential for ccRCC cell proliferation. We used locked nucleic acid (LNA) technology to knockdown miR-210 expression. We also attempted a miR-sponge approach, but without success. Knockdown of miR-210 in ccRCC cells results in significantly decreased cell proliferation, but not apoptosis.

Specific Aim 2: We established cell lines ectopically expressing the HA-tagged Argonaute 2 (AGO2) protein, a major component of microRNA functioning machinery, RNA induced silencing complex (RISC). By immunoprecipitation of HA-AGO2, miR-210 was significantly enriched and the RNA was extracted for next generation sequencing. The Genomic and Proteomic Core Laboratory at University of Pittsburgh successfully performed the next generation sequencing using a SOLiD platform. We obtained approximately 60 million reads per sample. However, due to the extremely large amount of data collected, the data analysis is still ongoing.

Specific Aim 3: We found that when miR-210 is inhibited in ccRCC cells cell proliferation, but not cell death, is affected. Thus, we focused on elucidating the mechanism of attenuated cell proliferation following miR-210 knockdown. We found that when miR-210 is inhibited in ccRCC cells, the p53 pathway is activated, resulting in elevated p27, thus providing a mechanistic link between elevated miR-210 expression and cellular proliferation in ccRCC. Because hypoxia can upregulate p53, our findings suggest that upregulation of miR-210 due to stabilized HIF1α in ccRCC may suppress otherwise active p53 response to constitutive active HIF1α pathway. Currently, we are investigating how knockdown of miR-210 activates the p53 pathway.

Below is the detailed project report:

miR-210 is highly expressed in ccRCC cells

ccRCC patients have high frequency of the *VHL* gene mutation (~57-80%). *VHL* is an E3 ubiquitin ligase functioning as a tumor suppressor gene in renal cells by degrading HIFα. We selected two ccRCC cell lines with an identical genetic background, RCC4 and RCC4/VHL, to study miR-210 expression. The RCC4/VHL cell line was derived from the RCC4 cell line by re-introducing a wild-type *VHL* gene into the RCC4 cells. Thus, the RCC4 and RCC4/VHL cells are genetically identical other than the status of the *VHL* gene. We confirmed by Western blotting that HIF1α is stabilized in RCC4 cells as a result of mutating the *VHL* gene. Reconstituting the wild-type VHL gene in RCC4/VHL could efficiently degrade the HIF1α protein (Figure 1, upper panel). We then performed microRNA Northern blotting to detect miR-

210 expression in RCC4 and RCC4/VHL cells. Our results clearly demonstrated that miR-210 is highly expressed in RCC4 cells compared to RCC4/VHL cells, reflecting that stabilized HIF1α could up-regulate miR-210 in ccRCC (Figure 1, lower panel).

Down-regulating miR-210 expression reduces RCC4 cell proliferation

Since miR-210 is highly up-regulated in RCC4 cells with the mutated *VHL* gene, we utilized locked nucleic acid (LNA) technology to knock down miR-210 expression in RCC4 and RCC4/VHL cells in order to investigate miR-210 function. As shown by TaqMan real-time PCR assay in Figure 2A, we efficiently down-regulated miR-210 expression by antisense LNA against miR-210 in both cell lines. Interestingly, when miR-210 was knocked down, cellular proliferation was significantly reduced in RCC4 cells (Figure 2B). We further investigated the mechanism of reduced cell proliferation after knocking down miR-210 expression. Because apoptosis is a critical factor in regulating cell proliferation, we stained the RCC4 cells with FITC conjugated Annexin V antibody and propidium iodide (PI) after miR-210 knock down and performed flow cytometry analysis. Surprisingly, we only observed slightly increased RCC4 cell apoptosis after miR-210 knock down (Figure 2C), suggesting that apoptosis alone cannot explain the significantly reduced cell proliferation after miR-210 knock down in RCC4 cells. To dissect the mechanism further, we performed clonogenic cell survival assays to examine cell survival after miR-210 knock down. RCC4 cells were re-plated into 4 plates with 300 or 3000 cells for 24 hours post transfection with LNA against miR-210 or a scramble LNA. The cells were then kept growing for two weeks and then the cells were stained by crystal violet and the colonies were counted. Interestingly, the number of colonies formed in cells transfected with LNA against miR-210 was significantly less than that of cells transfected with the scramble LNA. The difference in colony formation nicely explained the difference we observed in the cell proliferation assay. Because the reduced colony formation may contribute to apoptosis, cell cycle arrest (including senescence), and autophagy, we are currently performing experiments to determine the mechanisms responsible for the phenotype.

Down-regulating miR-210 expression induces phospho-p53 (S15) and p27 expression

The *TP53* gene is a major tumor suppressor gene in the human genome. The protein product of *TP53*, p53, is a master regulator of cell cycle progression and apoptosis. We therefore reasoned that p53 may be responsible for the significantly slower cell proliferation after miR-210 knockdown. Since ATM and ATR kinases can phosphorylate p53 at serine 15 (S15), which stabilize p53 and leads to cell cycle arrest, we examined the status of phospho-p53 (S15). As expected, phospho-p53 (S15) is highly upregulated after miR-210 knockdown in RCC4 cells (Figure 3). We also examined two major downstream targets of p53 involved in cell cycle regulation, p21 and p27. No change in p21 expression was detected. However, a significant increase of p27 was detected by Western blotting. Currently, we are performing cell cycle and microarray experiments after miR-210 knockdown to further elucidate the mechanism of miR-210 function in RCC4 cells.

Down-regulating miR-210 expression sensitizes RCC4 cells to chemotherapeutic drugs

Since ccRCC is among the tumors most resistant to radiation and chemotherapy, we tested whether inhibition of miR-210 could sensitize RCC4 cells to cell death induced by common chemotherapeutic agents, such as Doxorubicin and Camptothecin. We added Doxorubicin and Camptothecin to RCC4 cells 24 hours post miR-210 antisense LNA or scramble LNA

transfections. Interestingly, inhibition of miR-210 significantly sensitized RCC4 cells to Doxorubicin and Camptothecin induced cell death (Figure 4), indicating that mir-210 is a novel therapeutic target for ccRCC.

Identifying miR-210 target genes

To better understand the mechanism of miR-210 in sensitizing RCC4 cells to chemotherapeutic drugs, identification of miR-210 targets is essential. We utilized argonaute protein immunoprecipitation (RNP-IP) followed by the SOLiD next generation sequencing (NGS) to comprehensively identify miR-210 target genes. NGS has a unique advantage in identifying miRNA targets since it does not require prior knowledge of the target genes to be sequenced and thus, could potentially identify novel miR-210 targets that otherwise may be missed by conventional microarray experiments. We first established RCC4 and RCC4/VHL cell lines stably expressing a HA-tagged AGO2 protein (Figure 5A), which is the major functional component of the RNA induced silencing complex (RISC) where the miRNAs' inhibitory function on their target genes is carried out. We then performed a RNP-IP assay using an anti-HA antibody to pull-down the AGO2 protein and associated mRNAs. By comparing the immunoprecipitated mRNA profiles between RCC4 and RCC4/VHL cells, we will be able to comprehensively identify miR-210 targets. The experimental strategy is illustrated in Fig. 5B. The immunoprecipitated mRNA samples have been sequenced at the University of Pittsburgh Genomics and Proteomics Core Facility (GPCL). More than 60 million reads have been collected for each IPed sample. However, due to the extremely large volume of data acquired by SOLiD sequencing, the data analysis is still ongoing at the Bioinformatics Core at GPCL.

Figures

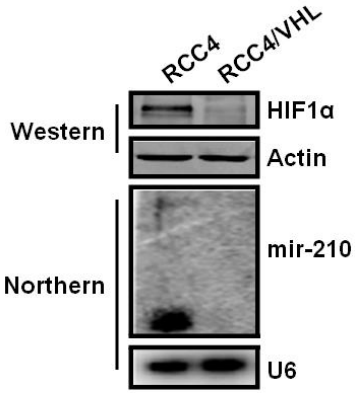


Figure 1. miR-210 is highly expressed in RCC4 cells

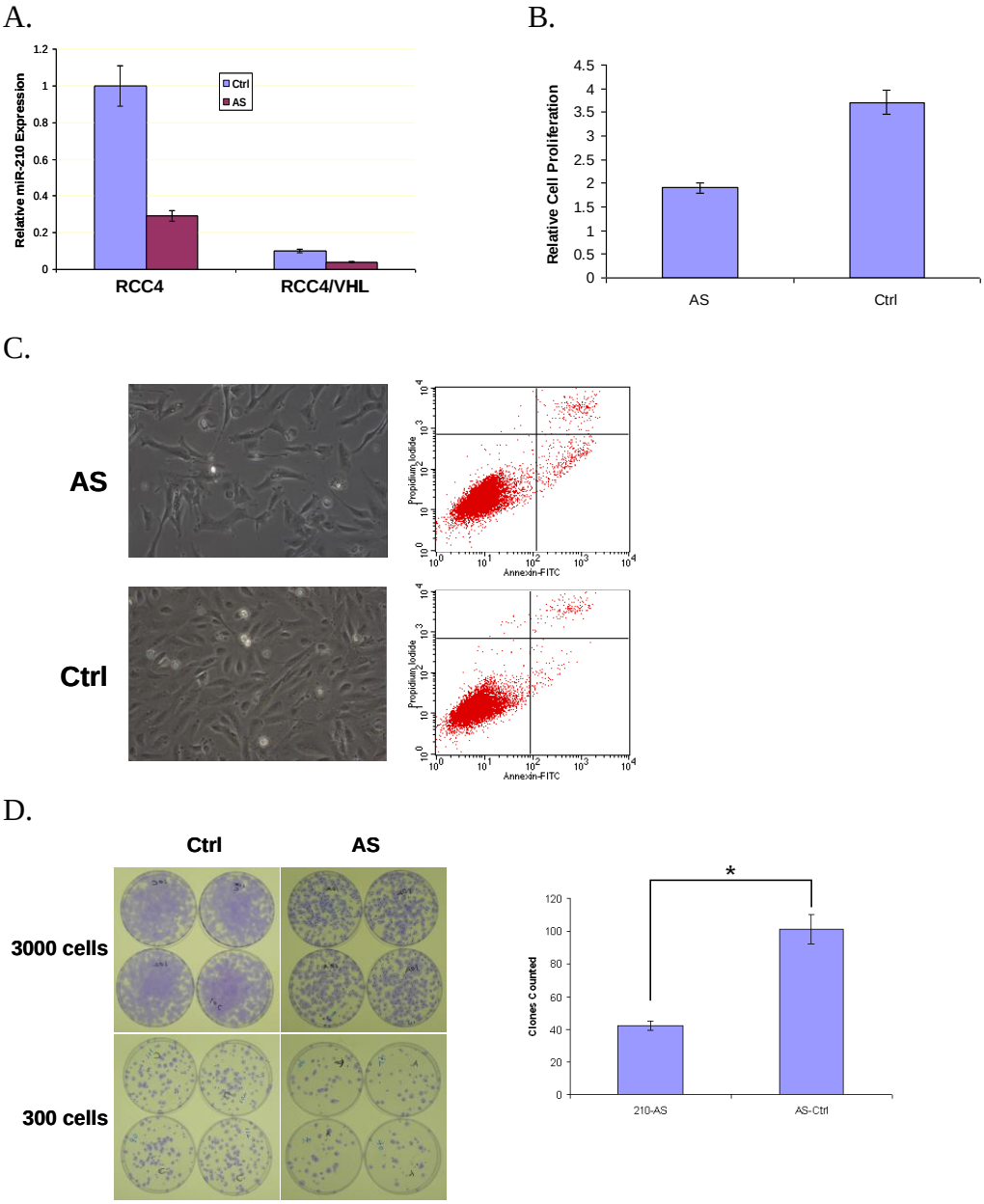


Figure 2. Inhibition of miR-210 results in decreased RCC4 cell proliferation. A. LNA antisense oligo (AS) efficiently knocked down miR-210 expression in RCC4 and RCC4/VHL cells compared to control scramble oligo (Ctrl), measured by RT-QPCR. B. Knockdown of miR-210 results in an approximately 2-fold decrease of cell proliferation. Cells were counted 48 hours after transfection. C. Slight increase in apoptosis is observed by Annexin V staining followed by flow cytometry analysis. D. RCC4 cell survival is significantly reduced after miR-210 knockdown as measured by clonogenic cell survival assay. Right panel, colony quantification for the 300-cell assay. * indicates $p < 0.05$ by two-tailed student's t-test.

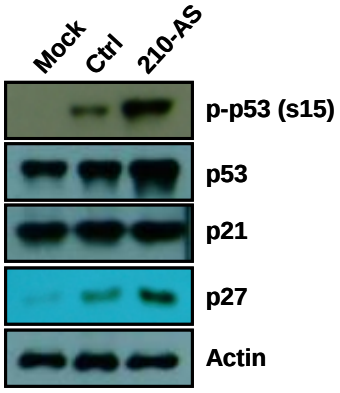


Figure 3. Inhibition of miR-210 in RCC4 cells results in increased phosphor-p53(S15) and p27 expression.

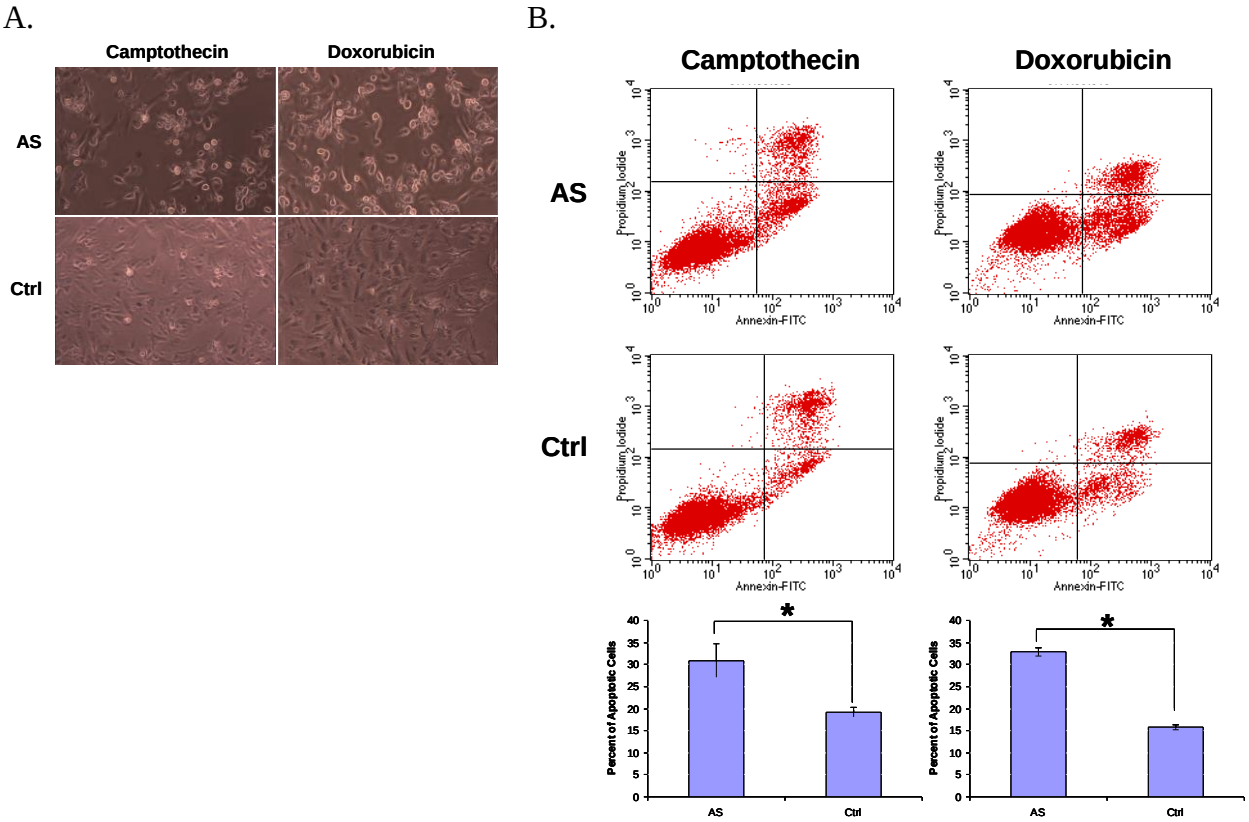
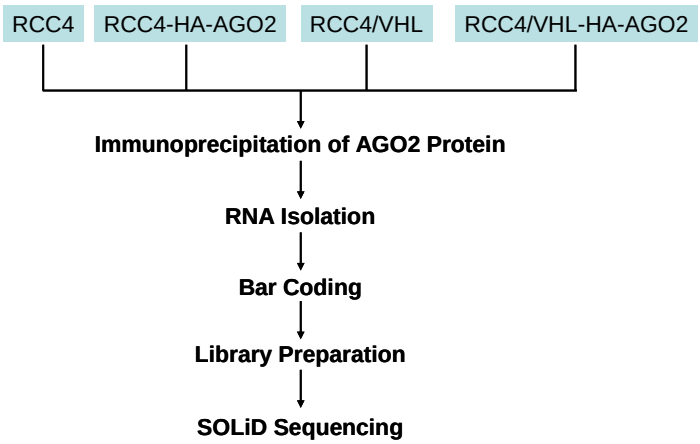


Figure 4. Inhibition of miR-210 sensitizes RCC4 cells to Camptothecin and Doxirubicin, two common chemotherapeutic drugs. A. Massive cell death was observed in RCC4 cells treated with the two drugs while miR-210 is inhibited, but no apparent cell death was observed in cells treated only with the drugs. B. Quantification of apoptosis by Annexin V staining followed by flow cytometry. There is an approximately two-fold increase of apoptosis when inhibition of miR-210 is combined with Camptothecin and Doxirubicin treatment compared to drug treatment alone. * indicates $p < 0.05$ by two-tailed student's t-test.

A.



B.

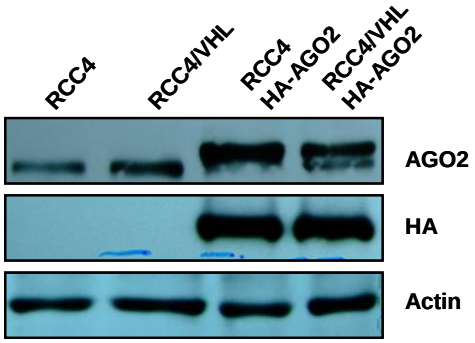


Figure 5. Strategy for identifying miR-210 target genes in RCC4 using AGO2 immunoprecipitation and next generation sequencing. A. Schematic illustration of the strategy to identify miR-210 targets in RCC4 cells. B. RCC4 and RCC4/VHL cell lines stably express HA-tagged AGO2 protein.

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

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, listed in the table, in a PDF version 5.0.5 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.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐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 findings to Cancer Research in the next 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 mechanistic link between elevated miR-210 expression and suppressed p53 pathway in clear cell renal cell carcinomas (ccRCC). Because hypoxia can upregulate p53, our finding suggest that upregulation of miR-210 due to stabilized HIF1α in ccRCC may suppress otherwise active p53 response to constitutive active HIF1α pathway.

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___X__ No_____

If yes, please describe your plans:

Once we complete our study, we plan to submit a patent for using miR-210 as a novel therapeutic target in renal cell carcinoma.

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

NAME	POSITION TITLE
Xin Huang	
eRA COMMONS USER NAME HUANGX2	Assistant Professor of OB/GYN & Reproductive Sciences

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

My postdoctoral research, carried out under the mentorship of Amato J. Giaccia at Stanford University School of Medicine, examined the mechanism of hypoxia regulation of miR-210 and miR-210's role in tumor growth initiation. I was among the first to demonstrate hypoxia regulation of microRNA expression and to fully characterize the mechanism of HIF1α regulation of miR-210 expression. I further developed a novel immunoprecipitation approach to experimentally identify miR-210 target genes and discovered that ectopically expressed miR-210 can repress tumor xenograft growth initiation by downregulating normoxic gene expression. I am interested in miR-210's function in a variety of tumor types, including renal cell carcinoma, ovarian cancer, and cervical cancer. We utilize genetic and biochemical approaches to investigate miR-210 function and identify miR-210 targets. Eventually, I like to develop novel therapeutics targeting miR-210 in human cancers.

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

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: in process.

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

1. 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
2. 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
3. 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
4. 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
5. 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
6. 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
7. 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
8. 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
9. 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
10. 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