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):** 01/01/2010 – 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:** #2 - Relationships among PPAR gamma Activity, Hypoxia, and Differentiation in Human Placenta

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

8. **Name of Principal Investigator for the Research Project:** Tianjiao Chu, 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:

   $ $165,153.00

   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
Chu	PI	40%	$45,512.81
Goldin	Data Administrator	100%	$21,082.17
Zhu	Research Associate	100%	$15,469.19

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
Sadovsky	Collaborator	2%

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_____ 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_____ 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: _____)		$	$
	☐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_____ No___X___

If yes, please describe your plans:

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

We will continue the study of the effect of hypoxia on human placenta, and its relation to fetal growth restriction. Our study will expand to include microRNA expression analysis and proteomics data, with a focus on the roles played by microRNAs under hypoxic stress.

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.

This project trained one bioinformatician and one programmer. The codes and procedures developed for this project will be available to use by other researchers in Magee-Womens Research Institute for projects involving similar type of data analysis tasks.

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.

Fetal growth restriction (FGR) has been shown to result in fetal morbidity and mortality, as well as later health complications for adult survivors [1,2]. One of the main risk factors of FGR is believed to be hypoxia, which may prevent trophoblast differentiation, enhance trophoblast apoptosis, and contribute to fetal growth restriction (FGR) [3-5]. We hypothesize that there are sets of genes whose expression patterns could explain both the close relationships and the dissimilarities among these in vitro and in vivo processes. In particular, there is a typical course of hypoxia during later stages of gestation that is responsible for most of the observed fetal growth restriction.

In this study, we conducted an exploratory study of the relationships among the transcriptomes of undifferentiated trophoblast, differentiated trophoblast (under normal condition), trophoblast

under hypoxia, and placentas from normal and FGR pregnancies, using both supervised and unsupervised statistical learning procedures. Transcriptional analysis of the trophoblast treated with PPAR ligands was also performed, given the potential link between PPAR gamma and trophoblast differentiation [6,7].

This project has two special aims. In the first aim, using historical microarray gene expression data, we examined the gene expression profiles of primary human trophoblast (PHT) cells during differentiation, under hypoxia, treated with PPAR gamma ligands, as well as the gene expression of placentas from pregnancies with fetal growth restriction (FGR), and performed gene ontology analysis to identify the main functions for the genes differentially expressed under various treatment conditions. In the second aim, using new qPCR gene expression data, we conducted an extensive study of the relations among primary trophoblast cells treated with different patterns of hypoxic conditions, primary trophoblast cell cultured at different normoxic conditions, as well as normal placentas and placentas from pregnancies with fetal growth restriction.

Aim 1. Comparing the gene expression profiles of primary trophoblast cells treated by hypoxia, PPAR gamma ligands, normal differentiation, as well as normal and FGR placenta, and identify:
a): Sets of genes whose expression patterns undergo significant change and either agree to disagree among the in vitro hypoxia, trophoblast differentiation, PPAR gamma ligand sample, and the in vivo fetal growth restriction samples.
b): The genomic functional groups overrepresented in each set of genes identified in aim 1a.

Differential expression analysis and gene ontology analysis

Historic microarray gene expression data were obtained from the following four experiments:

1. PPAR gamma experiment: PHT cells were treated with PPAR gamma agonist GW 7845, PPAR gamma agonist troglitazone respectively. One sample from each condition is divided into 5 technical replicates, and each technical replicate is hybridized to a set of Affymetrix Human U95 A,B,C,D,E chips.
2. Differentiation experiment: Four samples of PHT cells were collected at 0, 24, 48, and 72 hours respectively during the normal differentiation. Each sample is hybridized to a set of Affymetrix Human U95 A,B,C,D,E chips.
3. Hypoxia experiment: Each of the three PHT samples was divided into two parts, treated with 20% oxygen and 0% oxygen respectively. All samples were collected after 48 hours' exposure to the treatment, and hybridized to Affymetrix Human U95A/v2 chips.
4. FGR experiment: Three normal and three FGR placentas were collected, and hybridized to Affymetrix Human U95Av2 chips.

We analyzed all the microarray data following a three-step procedure: We first used Robust Multi-array Analysis (RMA) algorithm [8] to normalize and summarize the probe set intensity values. Then empirical Bayesian algorithm, implement in the R package "limma" [9,10], was applied to test, for each gene, if it was differentially expressed between groups. The limma algorithm assumes an inverse Chi-square prior distribution for the within group variances of the genes, estimates the hyper parameters of the prior using the empirical Bayesian method, then performs a moderated student's t test using the posterior mean of the within group variance and

moderated degrees of freedom. Finally, the Storey's qvalue method was used to calculate the adjusted p values to control the false discovery rate [11].

One exception to the above procedure is the analysis of the differentiation experiment. Where for each group there was only a single sample of the PHT cells with no technical replicate. Given that the samples in the PPAR gamma experiment were very similar to the samples in the differentiation experiment, we decided to include the 5 technical replicates of the control group from the PPAR gamma experiment in the analysis of the differentiation experiment. The within group variances needed for the moderated t test were estimated from the 5 technical replicates borrowed from the PPAR gamma experiment.

With the false discovery rate controlled at 5%, for the PPAR gamma experiment, we found 26 genes that are down regulated both when the PHT cells were treated with GW 7845, and when treated with troglitazone, while 17 genes were up regulated. For the differentiation experiment, we found 1651 genes that are up regulated at 48 hours of differentiation, and their observed signals at 24 hours and 72 hours were higher than at 0 hour. We also found 1373 genes down regulated after 48 hours of differentiation, and their observed signals at 24 hours and 72 hours were lower than at 0 hour. For the hypoxia experiment, we found 40 genes up regulated under hypoxic condition, and 110 genes down regulated under hypoxic condition. For the FGR experiment, with false discovery rate controlled at 5%, we found no genes significantly differentially expressed.

Based on the results from the above analysis, for each experiment, we further identified the gene ontology terms overrepresented in the genes differentially expressed. We used one-tail Fisher's exact tests to identify the gene ontology terms in the biological processes (BP), molecular functions (MF), and cellular components (CC) categories. With the cutoff p value set at 0.0001, for the PPAR gamma experiment, we found 1 BP term overrepresented in genes up regulated both when treated by GW7845 and by troglitazone, and 4 BP terms overrepresented in genes down regulated both when treated by GW7845 and by troglitazone. For the differentiation experiment, we found 38 BP terms, 5 MF terms, and 15 CC terms overrepresented in genes that were up regulated at 48 hours of differentiation, and with observed signals at 24 hours and 72 hours being higher than at 0 hour. We also found 5 BP terms and 3 CC terms overrepresented in genes down regulated after 48 hours of differentiation, and their observed signals at 24 hours and 72 hours were lower than at 0 hour. For the hypoxia experiment, we found 1 BP term, 4 MF terms, and 1 CC term overrepresented in genes up regulated under hypoxic condition, and 1 BP term overrepresented in genes down regulated under hypoxic condition. For the FGR experiment, because no gene was differentially expressed when the FDR was controlled at 5%, we performed the gene ontology analysis respectively on the 33 and 71 genes with p values <= 0.01 and observed expression levels elevated/depressed in the FGR samples. When the p value cutoff was set to 0.0001 as for the previous 3 experiments, we found no overrepresented gene ontology terms.

<u>Relations among PPAR gamma agonists, differentiation, hypoxia, and FGR</u>

The main goal of our study was to analyze the relations among PPAR gamma, differentiation, hypoxia, and FGR. For this purpose, for each pair of experiments, we identified the genes

differentially expressed in both experiments (for the FGR experiment, we used the genes with p values <= 0.01), then created a 2 by 2 contingency table for these genes with two factors: The first factor is whether a gene is up or down regulated in the first of the two experiments, the second factor is whether a gene is up or down regulated in the second of the two experiments. We then used two-tail Fisher's exact test to test if the probability that a gene is up regulated in one experiment is dependent on whether it is up regulated in the other experiment.

Out of the 4 experiments, we made 6 pair-wise comparisons, and created 6 contingency tables. Only in Fisher's exact test for the contingency table for the differentiation and hypoxia experiments (Table 1), we obtained a significant p value (<= 2.2E-16). Table 1 shows that among genes differentially expressed in both experiments, genes up-regulated at 48h differentiation are very likely to be down regulated under hypoxia, and genes down-regulated at 48h differentiation are very likely to be up-regulated under hypoxia. This suggests an extremely strong negative relation in gene expression dynamics in PHT cells between the differentiation and hypoxia.

Table 1. Genes differentially expressed in both differentiation and hypoxia experiments

Number of Genes	Up regulated under hypoxia	Down regulated under hypoxia
Up regulated at 48h Differentiation	0	96
Down regulated at 48h Differentiation	27	1

Using a similar approach as used in the previous section, for each pair of experiments, we also identified the BP terms over-represented in genes differentially expressed in both experiments (for the FGR experiment, we used the genes with p values <= 0.01), with the p value cutoff for one tail Fisher's exact test used to identifying overrepresented BP terms set at 0.001. We then created a 2 by 2 contingency table for these BP terms with two factors: The first factor is whether a BP term is overrepresented in genes up or down regulated in the first of the two experiments, the second factor is whether a BP term is overrepresented in genes up or down regulated in the second of the two experiments. We then used two-tail Fisher's exact test to test if the probability that a BP term is overrepresented in genes up regulated in one experiment is dependent on whether it is overrepresented in genes up regulated in the other experiment.

Out of the 4 experiments, we made 6 pair-wise comparisons, and created 6 contingency tables. None of them show significant dependency. However, the result for the comparison of the differentiation and hypoxia experiments is consistent with the negative dependency between hypoxia and differentiation shown in Table 1. Table 2 gives the BP terms overrepresented both in genes up/down regulated at 48h differentiation, and in genes down/up regulated under hypoxia.

We noticed that none of the BP terms overrepresented in genes differentially expressed between normal and FGR placentas are overrepresented in genes differentially expressed in any of the other three experiments. This further confirms our finding in the study of the aim 2 of this project that the expression profile of PHT cell in vitro is very different than the expression profiles of either normal or FGR placenta in vivo.

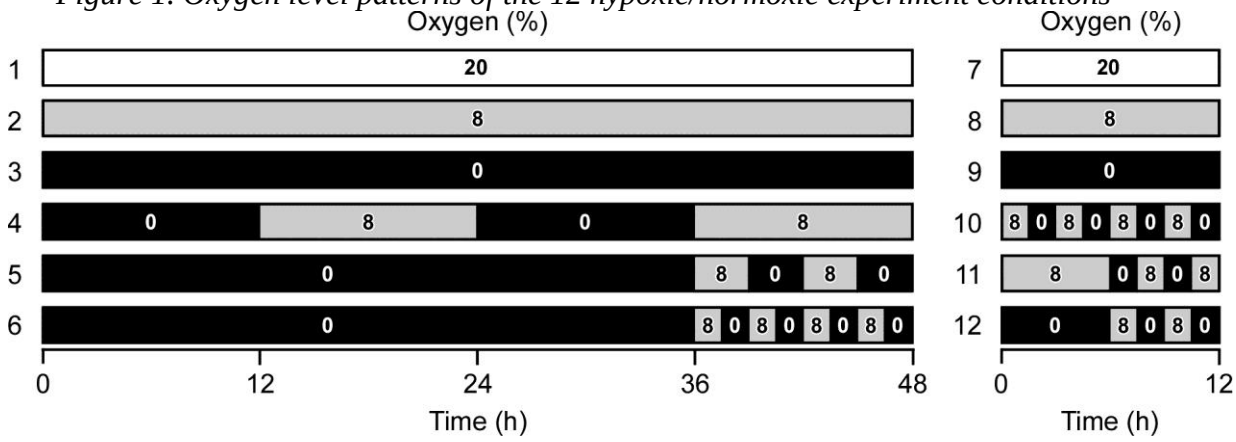
Table 2. BP terms overrepresented in genes differentially expresseed at 48h differentiation and under hypoxia

BP terms overrepresented in genes up-regulated at 48h differentiation and down-regulated under hypoxia	BP terms overrepresented in genes down-regulated at 48h differentiation and up-regulated under hypoxia
female pregnancy	taxis
	angiogenesis
	positive regulation of leukocyte migration
	regulation of viral genome replication
	regulation of locomotion
	signaling
	response to chemical stimulus
	viral infectious cycle

Aim 2. Analyzing the expression profiles of a selected set of 34 genes, and determine
a): For each gene, if it is consistently up/down regulated in vitro in hypoxia compared to normal condition.
b): For each gene, if it is differentially expressed in vivo between normal and FGR placentas.
c): Among all different schemes of hypoxia in vitro, which scheme generates a gene expression pattern closest to the gene expression pattern of the FGR placentas.

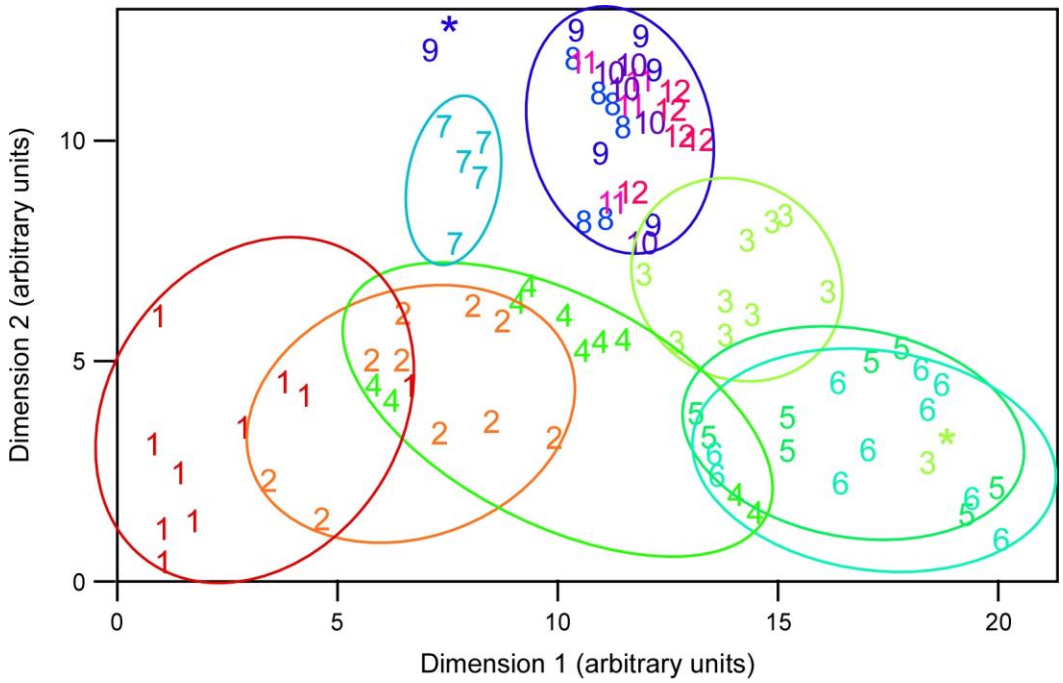
In the study of the second aim of the project, we analyzed the gene expression data from PHT cells treated under various normoxic and hypoxic conditions, as well as normal and FGR placentas. PHT cells were collected from five normal human placentas. PHT cells from all 5 placentas were treated under 6 different conditions for 48 hours. PHT cells from 3 of the 5 placentas were treated under another 6 different conditions for 12 hours. These 12 conditions as shown in Figure 1, differ in their duration and the change of oxygen level during the course of treatment. Placental tissue samples were obtained from pregnancies complicated by FGR (n = 4) and gestational-age matched controls (*n* =4). The data set consists of the qPCR measurement of the expression of 34 genes, which are known to be regulated in placentas from pregnancies complicated by FGR, in the PHT cell samples and placentas samples.

Figure 1. Oxygen level patterns of the 12 hypoxic/normoxic experiment conditions



To directly visualize changes in patterns of gene expression among the experimental conditions, we first interrogated the data using multidimensional scale (MDS) analysis [12], based on Euclidian distance. Fig. 2 is a two dimensional representation of the expression levels of the 34 genes resulting from the MDS analysis. In addition to the clear separation between conditions representing long (conditions 1-6) or short (conditions 7-12) periods in culture, we found that extended periods in O_2=0% (conditions 3,5,6) resulted in the greatest distance from standard culture conditions (O_2=20%), with O_2=8%, (condition 2) or intermittent 8%/0% (condition 4) representing intermediate value.

Figure 2. MDS plot of the gene expression profiles of PHT cells under 12 hypoxic/normoxic conditions



We used the linear mixed effect model method implemented in the R package lme4 [13] to identify genes differentially expressed between any two conditions, with the sample as the random effect. The numbers of differentially expressed genes between pairs of long term conditions are shown in Table 3. The results confirm that extended exposure to O_2=0% (conditions 3,5,6) resulted in the highest number of significantly up- or down-regulated genes when compared to exposure to either O_2=20% or O_2=8% (conditions 1, 2).

Table 3. Number of genes differentially expressed between 6 long term conditions

Number of genes significantly up-regulated \ Number of genes significantly down-regulated	1	2	3	4	5	6
1		11	12	13	17	20
2	14		11	10	24	25
3	17	13		19	29	31
4	8	2	9		21	24
5	8	3	2	5		11
6	8	3	2	4	0	

Linear mixed effect model was also used to determine whether the overall expression level of the 34 genes is up- or down-regulated between any two conditions, with the genes used as a random effect, in addition to the sample. The p values were adjusted using Benjamini and Hochberg's method [14]. The results for the 6 long term conditions are shown in Table 4, where we see that conditions 5 and 6 have significantly lower overall expression level than conditions 1 to 4.

Table 4. Adjusted p values for comparisons of overall expression level among 6 long term conditions

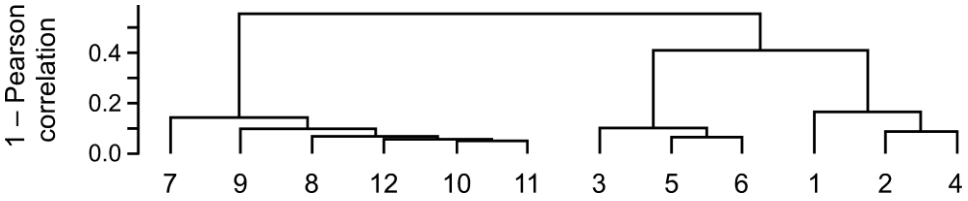
	Cond1	Cond2	Cond3	Cond4	Cond5	Cond6
Cond1	1	1	0.741	0.092	5.27E-06	2.59E-07
Cond2	1	1	0.741	0.054	2.73E-06	4.13E-07
Cond3	0.741	0.741	1	0.085	5.33E-06	2.42E-07
Cond4	0.092	0.0543	0.085	1	1.24E-06	2.42E-07
Cond5	5.27E-06	2.73E-06	5.33E-06	1.24E-06	1	0.0254
Cond6	2.59E-07	4.13E-07	2.42E-07	2.42E-07	0.0254	1

The difference in the number of differentially expressed genes between each pair of conditions in the extended culture conditions (1-6, Fig. 1) was then compared to the corresponding short

culture conditions (7-12, Fig. 1) using Fisher's exact test, with p values adjusted by Benjamini and Hochberg's method to control the false discovery rate. Nine of the 15 comparisons between short and long conditions showed significantly greater changes in the long term culture ($p<0.05$ after adjusting for false discovery rate), and none showing greater changes in the short term culture.

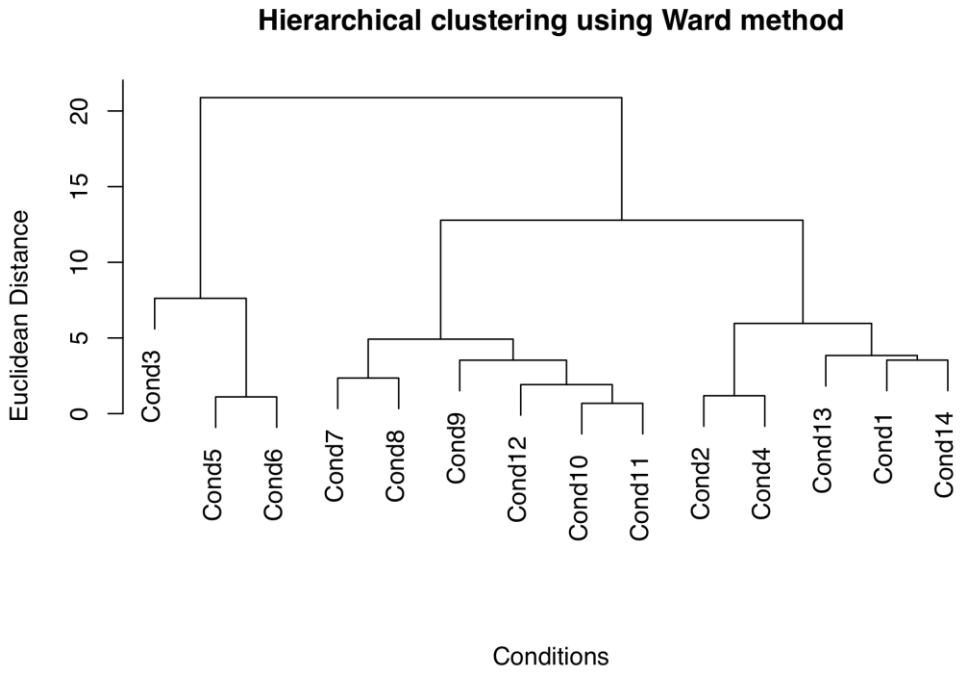
To further define the relatedness of our experimental paradigms, we deployed a hierarchical clustering using 1 minus average Pearson's correlation between samples from two conditions as the dissimilarity between the two conditions. Fig. 3 depicts the distance between the experimental conditions, corroborating our finding using MDS. Importantly, our data showed that clustered conditions 1, 2, and 4, exhibit gene expression patterns that are distinct from the clustered conditions 3, 5, and 6.

Figure 3. Clustering of 12 in vitro conditions using expression levels of 34 genes



From the PCR data, we found that most of the genes are significantly differentially expressed between in vivo and in vitro. Using linear mixed effect model to identify 13 genes not significantly differentially expressed between in vivo and in vitro. We clustered the 14 in vivo and in vitro conditions by the median expression levels of these 13 genes using hierarchical clustering algorithm with ward method, and found that among the in vitro conditions, condition 1 is the closest to the two in vivo conditions, and it is closer to the normal in vivo condition than to the FGR in vivo condition.

Figure 4. Clustering of 12 in vitro conditions using expression levels of 13 genes



<u>Summary</u>

From the analysis of the historic microarray data of the gene expression dynamics of PHT cells when treated PPAR gamma agonists, 48 hours differentiation, 48 hour hypoxia, as well as placenta from FGR pregnancies, we found that the effect of hypoxia on the gene expression pattern of the PHT cells is almost the opposite of the effect of differentiation (under normal condition). We also noticed that in vivo gene expression patterns of normal and FGR placentas are not very close to the in vitro expression patterns of PHT cells.

From the analysis of the qPCR data in the second aim, we found that the profile of gene expression in mild hypoxia (O_2=8%) clustered with patterns in O_2=20% rather than with patterns seen in O_2=0%. Alternating atmospheric oxygen between 8% and 0% resulted in gene expression patterns that are closer to O_2=8% rather than O_2=0%. The addition of alternating oxygen between 8% and 0% after an extended period in O_2=0% had a relatively small impact on gene expression patterns compared to continuous exposure to O_2=0%. Not surprisingly, all changes in gene expression patterns among the different exposure conditions were minimized during a short exposure period of 12 h. These findings will be important for the understanding of the strength and limitations of the studying placenta hypoxic injuries using PHT cells under in vitro conditions.

Reference

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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. The timing and duration of hypoxia determine gene expression patterns in cultured human trophoblasts	S. Oh, T. Chu, and Y. Sadovsky	Placenta	Jan, 2011	☒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_____ No____X _____

If yes, please describe your plans:

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

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.

None

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	
NAME Tianjiao Chu, PhD	POSITION TITLE Assistant Professor of OBGYN and Reproductive Sciences
eRA COMMONS USER NAME	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Shandong University, Jinan, China	BA	1989	Chinese Lang. & Lit
University of Toledo, Toledo, OH	MA	1997	Philosophy
Carnegie Mellon University, Pittsburgh, PA	MS	2001	Data Mining
Carnegie Mellon University, Pittsburgh, PA	PhD	2003	Logic & Computation

A. Personal statement

My research interest and experience have been mainly in the developing the statistical models and computational algorithms for the analysis of biological datasets, especially the high throughput microarray and next generation sequencing data.

B. Positions and employment

3-2005	Research Scientist, Institute for Human and Machine Cognition, Univ. of West Florida
2005-2007	Research Scientist, JustSystems Evans Research, Pittsburgh, PA
2007-	Assistant Professor, Dept. of OBGYN and Reproductive Sciences, University of Pittsburgh

Honors

2008-2010	Program committee member of the 24th, 25th, and 26th Conferences on Uncertainty in Artificial Intelligence
2010	Prenatal Diagnosis 2009 Malcolm Ferguson-Smith Young Investigator Award

2010 Best paper on next generation sequencing in *Bioinformatics*

C. Selected publications –

Refereed articles

1. Chu T, Glymour C, Scheines R, Spirtes P. A statistical problem for inference to regulatory structure from associations of gene expression measurement with microarrays. *Bioinformatics* 2003;19:1147-52. PMID: 12801876
2. Chu T. Limitations of statistical learning from gene expression data. *Interface 2004: Computing Science and Statistics* 2008;36:266-85.
3. Chu TJ, Peters DG. Serial analysis of the vascular endothelial transcriptome under static and shear stress conditions. *Physiol Genomics* 2008;34:185-92. PMID: 18505769
4. Chu T, Glymour C. Search for additive nonlinear time series causal models. *Journal of Machine Learning Research* 2008;9:967-91.
5. Chu T, Bunce K, Hogge WA, Peters DG. Statistical model for whole genome sequencing and its application to minimally invasive diagnosis of fetal genetic disease. *Bioinformatics* 2009;15;25:1244-50. PMID: 19307238
6. Chu T, Burke B, Bunce K, Surti U, Allen Hogge W, Peters DG. A microarray-based approach for the identification of epigenetic biomarkers for the noninvasive diagnosis of fetal disease. *Prenat Diagn* 2009 Nov;29(11):1020-30. PMID: 19650061
7. Mouillet JF, Chu T, Hubel C, Nelson DM, Parks WA, Sadovsky Y. Mir-205 Silences MED1 in Hypoxic Primary Human Trophoblasts. *FASEB* 2010;24(6):2030-9. PMID: 20065103
8. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. *Placenta* 2010;31(9): 781-4. PMID:20667590
9. Chu T, Bunce K, Hogge WA, Peters DG. Statistical considerations for digital approaches to non-invasive fetal genotyping. *Bioinformatics* 2010;26(22):2863-6. PMID: 20870643
10. Chu T, Bunce K, Hogge WA, Peters DG. A novel approach toward the challenge of accurately quantifying fetal DNA in maternal plasma. *Prenat Diagn* 2010;30(12-13):1226-9. PMID:21072784

Books and book chapters

1. Wimberly F, Danks D, Glymour C, Chu, T. Problems for Structure Learning: Aggregation and Computational Complexity. In: *Computational Methods in Gene Regulatory Networks*. Das, Welch, Caragea, Hsu (ed.). IGI Global, in press.

D. Research support

Ongoing

NIH (PI: Aleksandar Rajkovic) 10/01/09–09/30/11
Homeodomain Regulated Genetic Pathways in Oogenesis and Ovarian Failure

NIH (PI: Yoel Sadovsky.) 08/05/10-05/31/15
Placenta Injury and microRNA

PA Department of Health (PI: T. Chu) 01/01/11–12/31/11
(Research Formula Funds)
Analysis of small RNAs in the fetal placental maternal interface

Completed

PA Department of Health (PI: T. Chu) 01/01/09–12/31/09
(Research Formula Funds)
Integration Study of the Target Genes of PPAR gamma in Human and Mouse Placenta

PA Department of Health (PI: T. Chu) 01/01/10–12/31/10

(Research Formula Funds)
Relationships among PPAR gamma activity, hypoxia, and differentiation in human placenta