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):** 01/01/12-12/31/12

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:** 4100057668

6. **Project Number and Title of Research Project:** Project 1: In Vivo Analysis of Human C19MC MicroRNAs in a Transgenic Mouse Model

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

8. **Name of Principal Investigator for the Research Project:** Jean-Francois Mouillet, PhD

9. **Research Project Expenses**.

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

   $340,852.40

   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
Mouillet	PI	40%	$30,440.18
Chu	Co-investigator	30%	$25,765.15
Yeniterzi	Programmer	50%	$13,132.50
Shen	Bioinformatician	95%	$40,664.37
Sadovsky, E	Research assistant	50%	$15,148.02
Jennings	Research assistant	50%	$2,225.60
Sun	Research assistant	15%	$6,779.87
Hilko	Research assistant	100%	$3,420.00

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, Y	Co-investigator	As needed

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_____ 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:
None	☐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:

Although we have not yet found evidence of genes silenced by the C19MC transgenic miRNAs, expression of the transgenic C19MC in the mouse appears to be very similar to the endogenous human gene. As such it provides an interesting model for dissecting the molecular determinants governing the highly tissue-specific expression pattern of this unique family of miRNAs.

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

While expression of the C19MC cluster is normally restricted to the placenta, increasing amounts of evidence indicate that it can be ectopically expressed in various tissues in humans. This aberrant expression is usually associated with the development of tumors and cancer and is of clinical importance. The mechanisms underlying the abnormal expression of the C19MC miRNAs is currently unknown. However, recent experimental data indicate that epigenetic regulatory mechanisms play an important role in the control of this locus. Our mouse model provides a unique opportunity to study the control of C19MC expression pattern. We plan to use this mouse model and cellular models to dissect the mechanisms directing the specific expression pattern of the C19MC miRNAs.

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				
Female			1	
Unknown				
Total			**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			**1**	

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

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.

Our research enhanced technologies that address the role of miRNAs and their regulation. As

other researchers in our institute seek to utilize these techniques, our approaches will be instrumental for their pursuits.

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 recently shown that the C19MC miRNAs have the ability to increase resistance to viral infection in human cells. Now we are investigating the possibility that these miRNAs may enhance viral resistance of mouse transgenic embryos. This research has bolstered a new collaboration with William B. Klimstra from the Center for Vaccine Research at the University of Pittsburgh.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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. <u>Provide detailed results of the project.</u> 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.

Introduction

The placenta is an autonomous and transient organ that regulates the exchange between the maternal and fetal circulations. It also produces hormones for the maintenance of the pregnancy and plays an essential role in the establishment of maternal immunotolerance to the fetus. Placental dysfunction can have disastrous consequences for the fetus and continues to be one of the leading causes of maternal and perinatal morbidity and mortality. Despite its post–delivery accessibility, the placenta remains an understudied organ, and molecular mechanisms underlying placental insufficiency are poorly understood.

MicroRNAs (miRNAs) are short, noncoding RNAs that regulate gene expression by binding and repressing mRNAs of protein-coding genes. Their wide distribution and their ability to influence a multitude of target genes allow them to regulate a broad range of physiological and pathological processes. Global surveys of miRNA expression have shown that they are abundant in the human placenta where they likely contribute to the regulation of placental development and function. Recently, it was discovered that representative miRNAs from the C19MC locus are among the species that are the most highly expressed in the human placenta. This primate-specific locus harbors the largest cluster of miRNAs and contains 46 miRNA genes producing 59 mature miRNAs that are almost exclusively expressed in the placenta although they were recently detected in stem cells and in several types of cancers. Interestingly, during pregnancy, a significant number of placental C19MC miRNAs is released in the maternal circulation, raising the possibility that they act on distant maternal organs. The function of this family of miRNAs in the placenta or beyond remains unknown although we recently discovered that they are involved in the high resistance of placental trophoblasts to viral infection.

To explore the potential of C19MC miRNA actions, we developed a mouse model recapitulating the expression pattern of these miRNAs in humans. Transgenic mice were produced by pronuclear microinjection of a bacterial artificial chromosome (BAC) plasmid containing the entire C19MC cluster. This humanized mouse model was used to test the hypothesis that C19MCs regulate discrete mRNA and protein targets in the maternal organs. To test this hypothesis we designed experiments with the following aims:

> Specific Aim # 1: Analyze the ectopic expression of the human C19MC miRNAs in mouse organs and characterize their phenotype.
>
> Specific Aim # 2: Examine gene expression profiles in transgenic organs and their wild-type counterparts using microarrays and determine the cellular pathways impacted by these miRNAs.

Summary of Research Completed

The C19MC family of miRNAs is uniquely expressed in primates, and no equivalent sequences are found in the mouse genome. We therefore microinjected into one-cell embryos a BAC plasmid (BAC #RP11-1055O17) containing the entire human C19MC locus, including approximately 60 kb of sequence upstream of the first miRNA gene. A total of four independent transgenic lines passing the transgene to their offspring were established and used in the analyses performed during the funding period of this project. Interestingly, the four lines express the transgenic C19MC miRNAs in a very similar pattern and in a manner comparable to that of humans, with a high expression detected in the placenta. We also determined that the transgenic miRNAs are released from mouse placentas into the maternal circulation as in pregnant women. However, despite the high expression of the C19MC miRNAs in transgenic placentas we have not detected any phenotypic abnormalities or alterations at the level of gene expression.

Specific Aim # 1: Analyze the ectopic expression of the human C19MC miRNAs in mouse organs and characterize their phenotype.

Following the microinjection of BAC DNA into the pronucleus of fertilized eggs, we identified 10 founders, from which 4 independent transgenic lines were derived. Expression of the transgene in the different lines was then assessed by real-time PCR, using primers specific to several members of the C19MC family of miRNAs. Transgene expression was analyzed in transgenic adult mouse tissues and compared with expression in transgenic mouse placentae dissected from pregnant females at18.5 days *post coitum* (E18.5) (Fig. 1A). Real-time PCR data show that the transgenic C19MC miRNAs are predominantly expressed in the placentas of transgenic embryos as expected.

We did not detect any expression in placentae from wild-type embryos collected in transgenic females. Transgene expression was low in adult organs of transgenic mice, with the exception of testes, in which a robust expression was detected. Therefore, despite the absence of a homologous region, the mouse is able to correctly interpret the human genetic signal carried by the transgene and to process the transcript(s) into mature miRNAs. We also detected a substantial signal for the C19MC miRNAs in the plasma of mice carrying transgenic embryos

(Fig 1B). This observation is reminiscent of the situation in humans in which placental miRNAs are released in the systemic circulation. In mice, the placental origin of these miRNAs was confirmed by their detection in the plasma of wild-type females pregnant with transgenic pups but not in the plasma of non-pregnant transgenic females. Surprisingly, we did not observe correlation between the number of transgenic embryos and the level of C19MC miRNAs in the plasma. Because circulating C19MC miRNAs originate from the placenta, we expected to see higher concentrations in mice carrying the larger number of transgenic embryos.

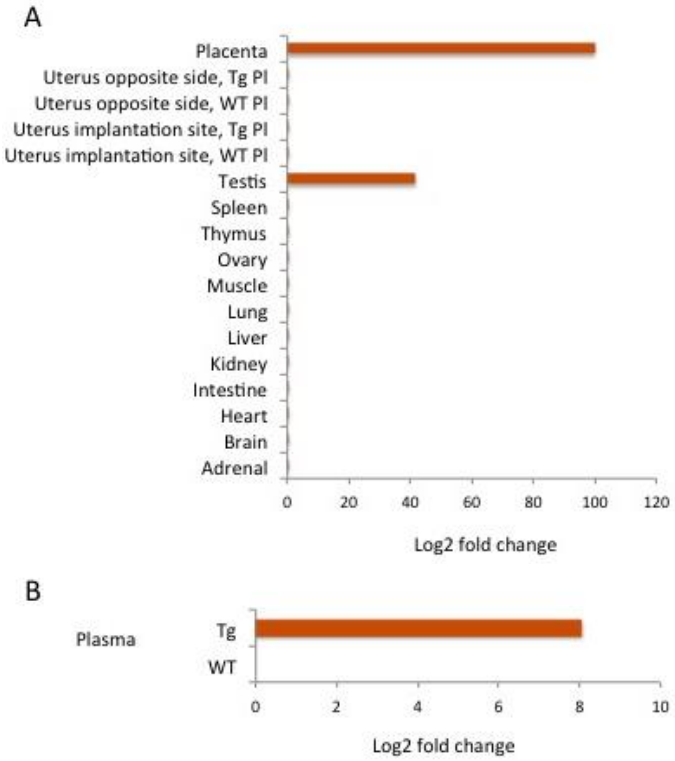

Fig. 1: Expression of transgenic C19MC miRNAs in mouse adult tissues. Total RNA samples were analyzed by real-time PCR of miR-517a using the miScript PCR System (Qiagen). (A) Relative expression level of miR-517a in tissues from adult transgenic mice. Bars represent the Log2 fold change in miR-517a levels in different tissues, taking the liver as reference. Tg Pl: transgenic placenta; WT Pl: wild-type placenta. (B) Relative miR-517a levels in plasma samples from pregnant transgenic (Tg) and wild-type (WT) females. Bars represent the Log2 fold change of miR-517a level in samples from transgenic mice, taking wild-type plasma samples as reference.

We also partially investigated expression levels of the transgene in fetal tissues. Detection of the transgene expression was initially performed in homogenized samples of whole embryos at a unique late stage (E18.5). C19MC miRNAs were detected but remained at low levels in transgenic whole embryos and were below the detection limit in their wild-type counterparts. The low abundance of miRNAs in whole embryos represents only a fraction of what was detected in the corresponding placenta. A few tissues were then explored, including the brain, liver, kidney (Fig. 2A), and fetal plasma (Fig.2B). The transgene expression was low in these tissues although it was clearly expressed in the fetal brain. Of note, low expression levels of C19MC miRNAs have been detected in some parts of the human brain.

Detection of C19MC miRNAs was confirmed in the plasma of transgenic embryos. Interestingly, plasma samples of wild-type embryos were completely negative for the detection of C19MC miRNAs although a relatively high concentration of these miRNAs was detected in the maternal circulation. This observation indicates that circulating maternal miRNAs did not cross the placental barrier. It can also be inferred that the C19MC miRNAs in the fetal circulation are exclusively from fetal origin without maternal contribution. However, at this stage of the study,

we do not know whether these plasma-circulating C19MC miRNAs were released from a subset of fetal cells or whether they were transferred from the placenta. Recently, we observed the presence of C19MC miRNAs in human fetal blood by sampling human fetal cord blood. Again the embryonic or extraembryonic origin of these circulating miRNAs has not been established. Our mouse model should allow us to find a definitive answer to the question of the origin of the plasma C19MC miRNAs in the fetus.

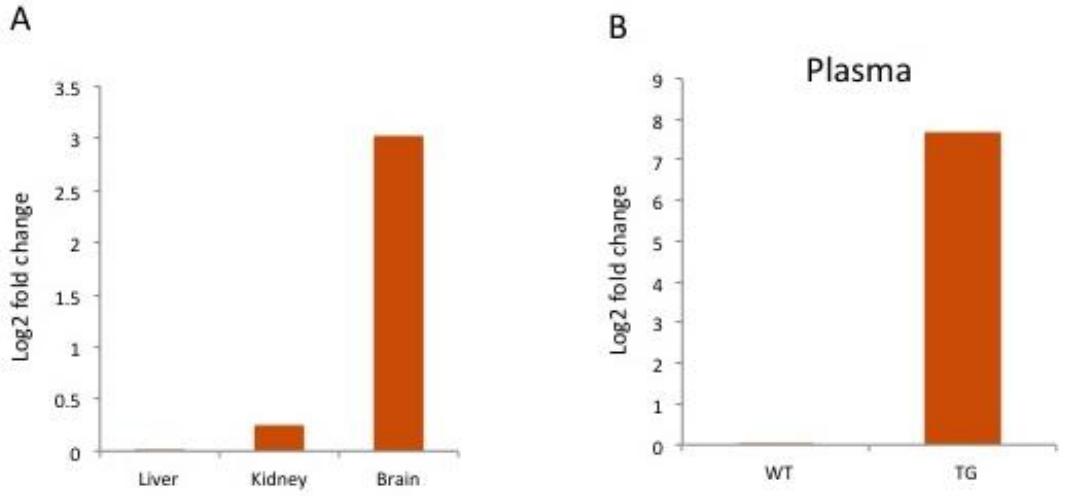

Fig. 2: Expression of transgenic C19MC miRNAs in mouse embryo tissues. Total RNA samples were analyzed by real-time PCR of miR-517a using the miScript PCR System (Qiagen). (A) Relative expression level of miR-517a in tissues from adult transgenic mice. Bars represent the Log2 fold change in miR-517a levels in various tissues, taking the liver as reference. (B) Relative miR-517a levels in plasma samples from transgenic (Tg) and wild-type (WT) embryos. Bars represent the Log2 fold change of miR-517a level in samples from transgenic mice, taking wild-type plasma samples as reference.

The C19MC cluster harbors 46 genes that can give rise to 59 mature miRNAs. Past studies in our laboratory have shown that miRNAs from this cluster constitute the most abundant pool of miRNA expressed in the human placenta. However, expression levels for individual species are very different, with some species highly expressed while others are barely detectable.

To capture the behavior of the whole C19MC cluster in the context of the mouse genome, we profiled the C19MC miRNAs in transgenic mouse placentae using a Human miRNA Microarray from Agilent, which contains probes for the C19MC miRNAs. The miRNA microarray data were summarized, normalized, and Log2–transformed. Then we applied a two-way analysis of variance (ANOVA) to the processed data that includes two independent factors: the strains of mice and the treatment (transgenic or wild-type) of the mice. We estimated the main effect of the treatment factor, which represents the Log2 fold change of the expression of miRNAs between the transgenic mice and the wild type mice. For comparison, we plotted the main effect of the transgenic treatment against the Log2 expression level of the C19MC miRNAs in human trophoblast cells that were cultured for 48 hours under standard conditions. The graph shown in figure 3 clearly shows a robust correlation ($r = 0.8507$, p value = 7.194E-14) between the two

sets of values, which indicate that, despite the profound differences in the genomic environment, the C19MC miRNAs are expressed with a similar pattern in the two conditions. This result again confirms that the genomic DNA fragment in the BAC is likely to contain most of the regulatory element required for directing the correct expression of the C19MC miRNAs.

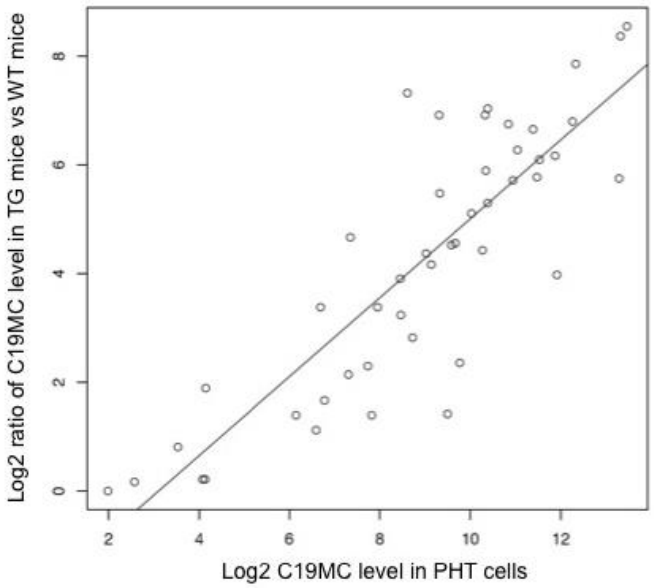

Fig. 3: Comparison between C19MC miRNAs expression patterns in transgenic mice and primary human trophoblasts (PHT). The Log2 ratio of C19MC in transgenic mice compared to wild-type mice was plotted against the mean Log2 expression of C19MC in PHT cells under standard conditions for 48 hours. The line represents the slope of the regression.

In humans, the C19MC locus is imprinted, and is exclusively expressed, from the paternally inherited allele. The epigenetic mechanisms regulating imprinting of this region have not yet been deciphered, but a differentially methylated region (DMR) has been identified, and its role in imprinting has been established. This domain, located 17 kb upstream of the first miRNA gene in the C19MC cluster, is included in the BAC vector used to generate the transgenic mice. We investigated the possibility that expression of the transgene was subject to a parent-of-origin effect that could reveal imprinting in the mouse model.

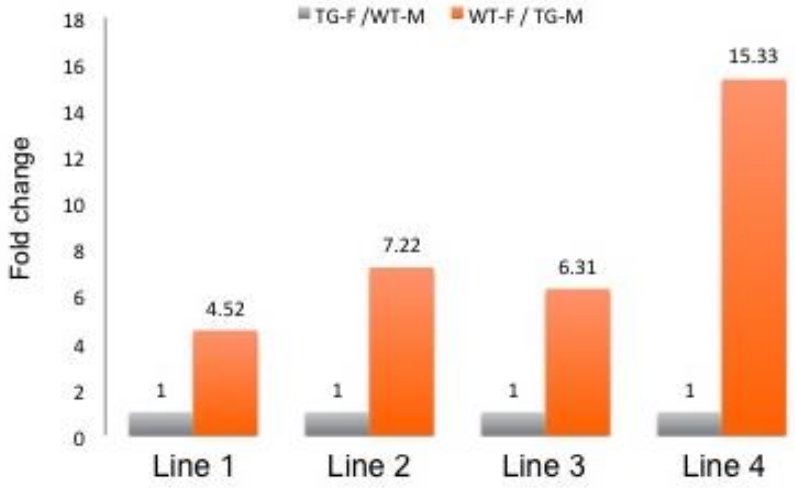

Fig. 4: Parent-of-origin effect on C19MC transgene expression. Relative miR-517a expression in the four transgenic lines was analyzed by real-time PCR in placentae from offspring which had inherited the transgenic locus paternally (orange bars) or maternally (gray bars). Expression in the offspring with maternal transmission was set at one for comparison. TG-F/WT-M: Transgenic female crossed with wild-type male. WT-F/TG-M: Wild-type female crossed with transgenic male.

Timed pregnancies consisting in different combinations of mating pairs, including a dam transgene positive crossed with a negative sire and a dam negative crossed with a transgenic sire, were set up, and expression of the transgenic C19MC miRNAs was analyzed in the progeny. Our data show that embryos that received the transgene from the male expressed a higher level of C19MC miRNAs compared to embryos that inherited the transgene from a female (Fig. 4). While this observation suggests imprinting, expression of the C19MC miRNA in transgenic mice is obviously not restricted to the paternal allele. It will be interesting to determine whether it is possible to detect differential methylation imprints of the DMR in our transgenic mice deriving from the different crossings.

We next sought to better characterize the spatial expression of the transgenic miRNAs in mouse tissues using *in situ* hybridization (ISH). Experimental evidence indicates that the C19MC miRNAs are expressed in the trophoblast layer in the human placenta. However, to date there is no detailed study of C19MC expression in the various trophoblast cell lineages found in the human placenta. The mouse placenta is morphologically different from the human placenta but shows significant similarities in its organization. We were particularly interested in determining whether the C19MC miRNAs are produced in the trophoblast lineage as in humans and whether some subpopulations of trophoblasts show differences in the level of expression of these miRNAs. In the past, we have successfully used this approach to analyze the expression of several miRNA species at the cellular level in human placenta and mouse placenta sections. However, despite multiple attempts with varying experimental conditions, we were not able to obtain a reliable specific staining of any of the C19MC miRNAs tested. Both frozen sections and paraffin-embedded sections were used, in combination with various post-fixation treatments and hybridization conditions, but without success. The C19MC miRNA-LNA probes used were also

tested on human placenta tissue sections without additional success. The reason for the failure to detect these miRNAs is not known but appears to be specific for these probes. In spite of the widespread use of ISH to study the cellular distribution of miRNAs and the long history of ISH in general, it appears that the technique is still relatively inefficient at detecting some transcripts. A recent study from the Tuschl laboratory (Rockefeller University, NY) reported that only 37% of the miRNA ISH probes were functional in a large experiment in which they tested a set of 130 different probes (Pena et al. *Nature Methods* 6:139-141 2009).

As an alternative to ISH, we are currently working on an RNA–fluorescent ISH (FISH) approach to uncover the details of cellular expression of these miRNAs. A method using a Cy3-labelled DNA oligonucleotide probe complementary to repeated sequences in the expressed primary C19MC transcript was recently developed in the Cavaille laboratory (Toulouse University, France). This approach allows the detection of primary C19MC miRNAs at the site of transcription. We tested this method on primary human trophoblasts and observed a nice nuclear staining that revealed transcription at the C19MC locus. In contrast, no staining was detected in HTR-8/SVneo cells, a laboratory-transformed cell line derived from first trimester trophoblasts with the peculiarity of not expressing the C19MC miRNAs. Adapted for the staining of placental sections, this method would allow for the precise identification of the cells that express the C19MC miRNAs in human and mouse sections. Finally, we plan to use laser capture microdissection in combination with real-time PCR to finely analyze expression of the C19MC miRNAs in a limited subset of cells isolated from placental tissue.

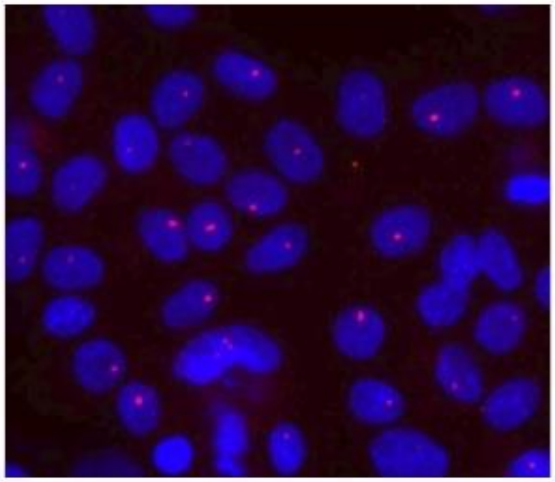

Fig. 5: RNA-FISH analysis of expression at the C19MC locus in primary human trophoblast cells. A Cy3-labeled DNA oligonucleotide probe (red), detecting sequences from the primary C19MC transcript, was used. Nuclei were counterstained with DAPI (blue). Note that most cells exhibit a single RNA-FISH signal, indicating monoallelic expression of C19MC in human trophoblasts.

The strong and specific expression of C19MC miRNAs in the placenta suggests that these miRNAs are involved in placental development and function. Several studies have also reported alterations of their expression patterns in cases of placental insufficiency. However, phenotypic analysis of C19MC transgenic mice has not revealed any abnormalities caused by the forced expression of these human miRNAs. Transgenic embryos were born at the expected rate from wild-type or transgenic females and did not exhibit obvious phenotype. A slight difference in the weight of the transgenic placentae that was originally noticed was not confirmed by subsequent analysis of larger numbers of placentae (Fig.6).

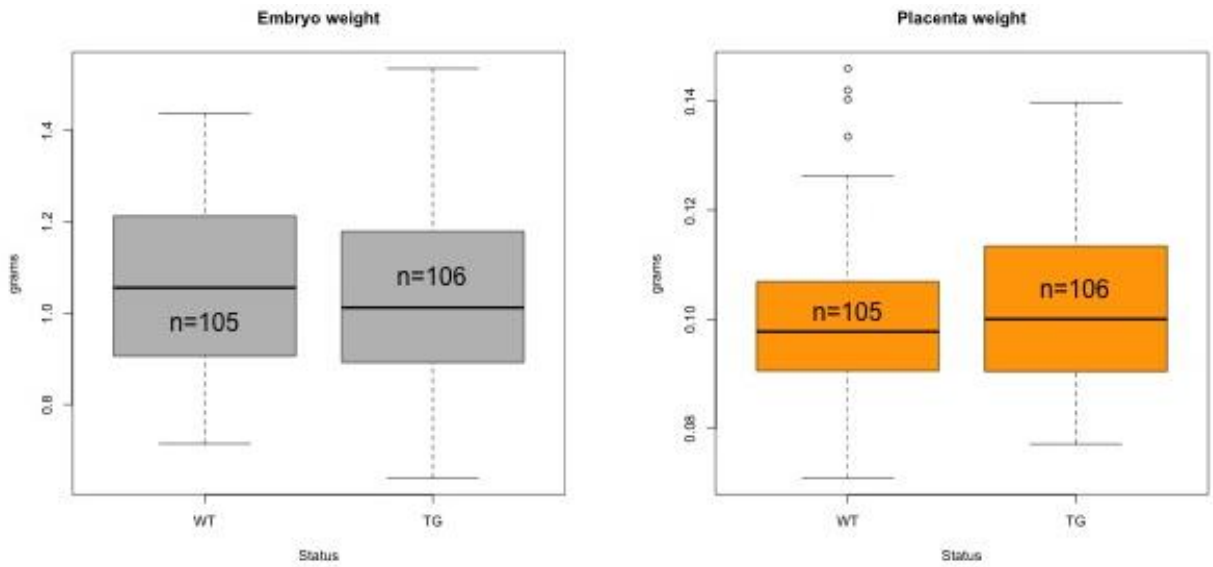

Fig. 6**:** Embryo weight and placental weight in C19MC-transgenic mice (TG) and wild-type mice (WT). No significant differences between WT mice and TG mice were found. Embryos and placentas were collected at 18.5 days *post coitum*.

The reproductive performance of the transgenic mice was apparently not affected by the expression of the transgenic miRNAs. In humans, ectopic expression of C19MC miRNAs is usually associated with the development of tumors, but transgenic mice that were bred for a period of at least 1 year did not show any sign of pathology; in particular, there was no sign of cancer. We recently showed in our laboratory that the C19MC miRNAs participate in the elevated resistance of primary human trophoblasts to infection by a wide range of viruses. We also showed that a high resistance can be induced in other cell types upon transfection of the C19MC miRNAs. In the light of these exciting results, we are currently investigating whether the C19MC transgenic embryos might be more resistant to viral infection than their wild-type counterparts.

Aim 2: Examine gene expression profiles in transgenic organs and their wild-type counterparts using microarrays and determine the cellular pathways impacted by these miRNAs.

In this section of the project we sought to investigate the impact of the transgenic C19MC miRNAs on the expression pattern of mouse placental genes with the specific goal of identifying target genes for these miRNAs. As outlined in our research plan, we conducted preliminary studies based on predicted human miRNA targets and showed that a number of these potential targets are sufficiently conserved in the mouse to expect a functional interaction with the C19MC miRNAs. Although transgenic mice do not exhibit an observable phenotype, which rules out the possibility of a strong effect on a large set of genes, there is still the possibility that subtle differences exist that can only be revealed in specific conditions, such as stress conditions or metabolic challenges. Microarray technology allows in-depth survey of transcriptional activity and could help reveal a molecular phenotype when there is no morphological phenotype.

To test this idea, we extracted total RNA from 12 wild-type placentae and 12 transgenic placentae from our different transgenic lines. RNA samples were labeled and hybridized to the Agilent SurePrint G3 Mouse GE 8x60K microarray. The data for 24 mouse placentas were first Log2-transformed and then normalized using the cyclic loess normalization method. The normalized data were then analyzed using two-way ANOVA, with two factors: the Treatment factor, representing whether the placenta is transgenic or wild-type; and the Strain factor, representing the four transgenic lines. Moderated F-test, as implemented in the R package limma, was performed for the Treatment factor to identify the genes that are differentially expressed between the transgenic placenta and wild-type placenta. The q-value method was used to adjust the p values of the moderated F-test to control for the false discovery rate. Because no genes were found to have significant adjusted p values at the 0.05 level, we retained the genes satisfying one of the following criteria: (1) the Log2 fold change between the wild-type and the transgenic placenta is at least 1 and the p value of the moderated F-test is no greater than 0.001, or (2) the Log2 fold change between the wild-type and the transgenic placenta is at least 2 and the p value of the moderated F-test is no greater than 0.01, or (3) the adjusted p value of the moderated F test is no greater than 0.1.

Using this approach, we identified 56 transcripts that appear to be differentially regulated between wild-type mouse placentae and those expressing the transgenic C19MC miRNAs. Among these transcripts, 41% code for protein-coding genes, 27% correspond to large intergenic non-coding RNA (lincRNA), and the rest is made up of unannotated transcripts. Moreover, only 10 of the protein-coding genes were actually downregulated in transgenic placentae, and not all of the human orthologs contain predicted target sites for at least one of the C19MC miRNAs. We also performed a hierarchical cluster analysis through dendrogram using Ward's method to compare the data from the 24 microarrays (Fig.7). This analysis shows that the datasets from the 24 microarrays tend to cluster by mouse line rather than by the presence, or not, of the transgene.

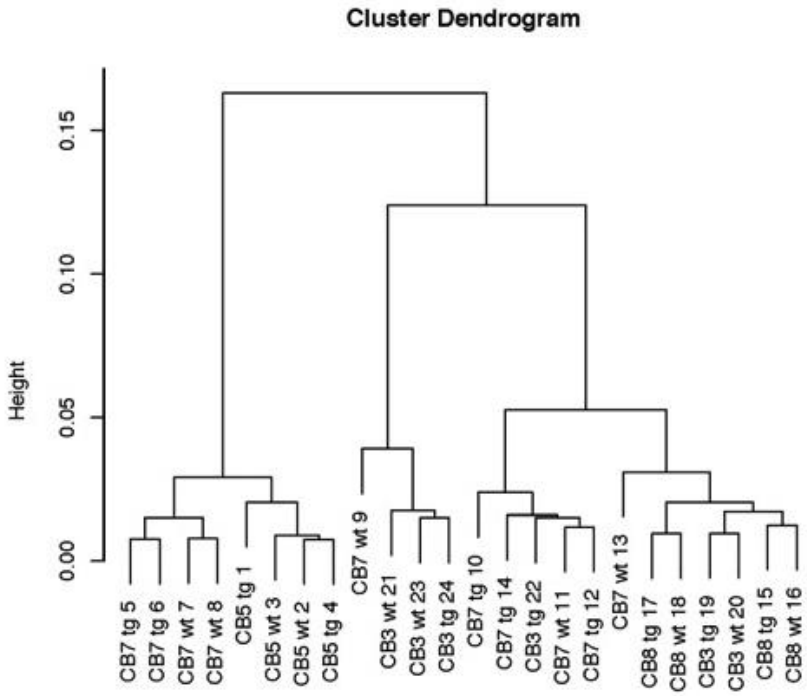

Fig. 7**:** Hierarchical cluster analysis of the 24 arrays using Ward's method, based on Euclidean distance. The distance between two arrays is defined as 1-the Pearson's correlation between the expression levels of the genes that are above the median expression level in the two arrays. Microarrays 1 to 24 were performed using samples from the different transgenic lines (CB3, CB5, CB7, and CB8).

Finally, this analysis suggests that ectopic expression of C19MC miRNAs does not have a significant effect on gene expression in the mouse placenta. While it is possible that the absence of results indicate that C19MC miRNAs, in the mouse context, do not have any silencing capacity, we are still investigating the details of the experimental design. For example, we have observed a fairly high variability at the level of gene expression between the placentae from the different lines that may be a consequence of different transgene insertions, with local perturbations of gene activity. These elements may contribute to elevating the noise and masking some subtle changes in gene expression. Most miRNAs exert only a modest effect on individual mRNA targets, and detection of these alterations can be challenging when the variability of the data is high. Also, because C19MC miRNAs are exogenous in the mouse, the constant miRNA-target interactions that usually take place throughout evolution did not occur to potentially increase their repression efficiency. Therefore, even if they are functional in the mouse, they may have reduced repression strength on most targets that are not perfectly conserved beyond the seed sequence. We are currently re-analyzing the data and are also considering running another hybridization using placenta samples from single transgenic lines in order to decrease the variability and increase our chance of detecting differentially expressed target genes.

Conclusion

The highly restricted expression of C19MC miRNAs in the placenta of higher primates constitutes a hurdle to the study of their function and regulation. We therefore decided to generate a transgenic mouse model recapitulating the expression of the human C19MC cluster. Several transgenic lines were established and analyzed during the period of funding of this project. These mice were shown to primarily express the transgenic C19MC miRNAs in the placenta and, thus, provide a useful model for analyzing the regulatory signals that control their targeted expression. However, to date no alterations of the phenotype at the morphological, behavioral, and biochemical levels were observed. Although better characterization of these mice and refined analyses of our microarray screen may still reveal subtle changes, it seems that, despite their abundance, transgenic C19MC miRNAs do not influence gene expression in the context of mouse placenta. Recent studies have postulated that coevolution is an important element in the establishment of functional interactions between miRNAs and their targets and that this could explain the absence of effect in this model. Yet it is intriguing that the overexpression of so many heterospecific miRNAs does not cause any adverse effect, considering, especially, that some of them share identical seed sequences with endogenous mouse miRNAs.

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:

The data collected during this period are interesting preliminary data though insufficient for publication in this form. However, we plan to pursue our efforts on deciphering the mechanisms that control the expression of the C19MC miRNAs and gather enough data that will serve as a basis for a future publication.

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	POSITION TITLE
Jean-Francois Mouillet, PhD	Assistant Professor of OBGYN & Reproductive Sciences

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Universite de Bourgogne, Dijon, France	BS	1988	Cellular biology
Universite de Bourgogne, Dijon, France	MS	1989	Animal physiology
Universite de Bourgogne, Dijon, France	PhD	1996	Molecular biology
Swiss Federal Institute of Technology, Zurich, Switzerland	Postdoctorate	1997-1999	Molecular biology
Washington University School of Medicine	Postdoctorate	1999-2005	Reproductive biology

A. Personal statement

Dr. Mouillet is a research scientist skilled in molecular biology, particularly in the area of regulation of gene expression. He has several years of experience in research on placental microRNAs. He also has knowledge and experience in generation of mouse mutant models, including "knockout" mice and transgenic mice using modified BAC plasmids.

B. Positions and honors

2005-2007	Staff Scientist, Department of OBGYN, Washington University
2007-2009	Research Instructor, Department of OBGYN and Reproductive Sciences, Univ of Pittsburgh
2009-2012	Instructor, Department of OBGYN and Reproductive Sciences, Univ of Pittsburgh
2012-present	Assistant Professor, Department of OBGYN and Reproductive Sciences, Univ of Pittsburgh

C. Selected publications

1. Lezzi M, Bergman T, Mouillet JF, Henrich VC. The ecdysone receptor puzzle. *Arch Insect Biochem Physiol* 1999;41:99-106.
2. Mouillet JF, Bousquet F, Sedano N, Alabouvette J, Nicolai M, Zelus D, Laudet V, Delachambre J. Cloning and characterization of new orphan nuclear receptors and their developmental profiles during *Tenebrio* metamorphosis. *Eur J Biochem* 1999;265:972-81.
3. Ou Q, Mouillet JF, Yan X, Dorn C, Crawford PA, Sadovsky Y. Steroidogenic factor 1 is a monomeric orphan, but does not work alone. *Endocr Res* 2000;26:1003-4.
4. Ou Q, Mouillet JF, Yan X, Dorn C, Crawford PA, Sadovsky Y. The DEAD box protein DP103 is a regulator of steroidogenic factor-1. *Mol Endocrinol* 2001;15:69-79.
5. Mouillet JF, Henrich VC, Lezzi M, Vogtli M. Differential control of gene activity by isoforms A, B1 and B2 of the Drosophila ecdysone receptor. *Eur J Biochem* 2001;268:1811-9.
6. Yan X, Mouillet JF, Ou Q, Sadovsky Y. A novel domain within the DEAD-box protein DP103 is essential for transcriptional repression and helicase activity. *Mol Cell Biol* 2003;23:414-23.
7. Mouillet JF, Sonnenberg-Hirche C, Yan X, Sadovsky Y. p300 regulates the synergy of steroidogenic factor-1 and early growth response-1 in activating luteinizing hormone-□ subunit gene. *J Biol Chem* 2004;279:7832-39.
8. Dorn C, Mouillet JF, Yan X, Ou Q, Sadovsky Y. Insulin enhances the transcription of luteinizing hormone-□ gene. *Am J Obstet Gynecol* 2004;191:132-7.
9. Donker RB, Mouillet JF, Nelson DM, Sadovsky Y. The expression of Argonaute2 and

related microRNA biogenesis proteins in normal and hypoxic trophoblasts. *Mol Hum Reprod*, 2007;13:273-9.
10. Mouillet JF, Yan X, Ou Q, Jin L, Muglia L, Crawford PA, Sadovsky Y. DP103 (Ddx20) is essential for early embryonic development and modulates ovarian morphology and function. *Endocrinology* 2008;149:2168-75. PMC2329271
11. Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT, and Sadovsky Y. The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. Placenta 2010;31:781-4. PMC3204658
12. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. miR-205 silences MED1 in hypoxic primary human trophoblasts. *FASEB J* 2010;4:2030-9. PMC2874470
13. Mouillet JF, Chu T, and Sadovsky Y. Expression patterns of placental microRNAs. Expression patterns of placental microRNAs. Birth Defects Res A Clin Mol Teratol 2011;91:737-43.
14. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod. 2012;18:417-24. PMC3389496.

D. Research support
Ongoing

NIH/NICHD R21 HD071707 (PI: Sadovsky, Co-I: Mouillet) 12/1/11 – 12/31/13
Small RNAs at the placental maternal communication interface
This project will use next generation deep sequencing technology to comprehensively interrogate maternal-placental-fetal trafficking of microRNAs, and analyze dynamic changes in microRNA in pregnancies complicated by clinically relevant placental injury.

NIH/NICHD R01 HD065893 (PI: Sadovsky, Co-I: Mouillet) 8/15/10–5/31/15
Placental injury and microRNA
The main objective of this proposal is to mechanistically decipher the action of miRNAs that are regulated during trophoblast adaptation to injury.

NIH/NICHD R01 HD071707 (PI: Sadovsky, Co-I: Mouillet) 09/01/12-05/31/17
Primary human trophoblasts and the transfer of viral resistance
The long-term goal of this research is to identify the mechanisms underlying the high resistance to virus infection of human primary trophoblasts (PHT) and how conditioned PHT medium can confer viral resistance to non-placental cells.

BIOGRAPHICAL SKETCH	
NAME Tianjiao Chu, PhD	POSITION TITLE Assistant Professor of OBGYN & Reproductive Sciences

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/medical datasets, especially the high throughput genomic and proteomic data. Recently I have been working on the developing of statistical procedures for non-invasive prenatal diagnosis of aneuploidy, as well as algorithms studying the relations among miRNA, mRNA, and proteins in human placenta.

Positions and employment

2003-2005 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

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. Ning W, Chu T, Li CJ, Choi A, Peters D. Genome Wide Analysis of the Endothelial Transcriptome under Short-Term Chronic Hypoxia. *Physiol Genomics* 2004;18:70-8. PMID: 15100389
3. Peters DG, Kudla DM, Deloia JA, Chu TJ, Fairfull L, Edwards RP, Ferrell RE. Comparative gene expression analysis of ovarian carcinoma and normal ovarian epithelium by serial analysis of gene expression. *Cancer Epidemiol Biomarkers Prev.* 2005 Jul;14(7):1717-23.
4. Peters DG, Ning W, Chu TJ, Li CJ, Choi AMK. Comparative SAGE analysis of the response to hypoxia in human pulmonary and aortic endothelial cells. *Physiol Genomics* 2006;26:99-108. PMID: 16595741
5. Chu T. Limitations of statistical learning from gene expression data. *Interface 2004: Computing Science and Statistics* 2008;36:266-85.
6. 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
7. Chu T, Glymour C. Search for additive nonlinear time series causal models. *Journal of Machine Learning Research* 2008;9:967-91.
8. 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
9. 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
10. 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

11. Sheng Y, Lin CC, Yue J, Sukhwani M, Shuttleworth JJ, Chu T, Orwig KE. Generation and characterization of a Tet-On (rtTA-M2) transgenic rat. *BMC Dev Biol.* 2010 Feb 16;10:17.
12. 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
13. 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
14. 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
15. Ochalski ME, Shuttleworth JJ, Chu T, Orwig KE. Thalidomide treatment attenuates chemotherapy-induced gonadal toxicity. *Fertil Steril.* 2011 Feb;95(2):819-22.
16. Rajakumar A, Chu T, Handley DE, Bunce KD, Burke B, Hubel CA, Jeyabalan A, Peters DG. Maternal gene expression profiling during pregnancy and preeclampsia in human peripheral blood mononuclear cells. *Placenta*. 2011;32(1):70-8. PMID: 21075447
17. Chu T, Handley D, Bunce K, Surti U, Hogge WA, Peters DG. Structural and Regulatory Characterization of the Placental Epigenome at Its Maternal Interface. *PLoS One* 2011;6(2): e14723. doi:10.1371/journal.pone.0014723
18. Mouillet JF, Chu T, Sadovsky Y. Expression patterns of placental microRNAs. *Birth Defects Res A Clin Mol Teratol.* 2011 Aug;91(8):737-43. doi: 10.1002/bdra.20782. Epub 2011 Mar 21.
19. Budiu RA, Mantia-Smaldone G, Elishaev E, Chu T, Thaller J, McCabe K, Lenzner D, Edwards RP, Vlad AM. Soluble MUC1 and serum MUC1-specific antibodies are potential prognostic biomarkers for platinum-resistant ovarian cancer. *Cancer Immunol Immunother.* 2011 Jul;60(7):975-84. Epub 2011 Apr 2. PMID: 21461842
20. Peters D, Chu T, Yatsenko S, Hendrix N, Hogge WA, Surti U, Bunce K, Dunkel M, Shaw P, Rajkovic A. Non-Invasive Prenatal Diagnosis of a Fetal Microdeletion Syndrome. *NEJM*. Accepted
21. Oh SY, Chu T, Sadovsky Y. The timing and duration of hypoxia cluster changes gene expression in cultured primary human trophoblasts. *Placenta*. 2011 Oct 8. Epub
22. Hermann BP, Sukhwani M, Salati J, Sheng Y, Chu T, Orwig KE. Separating spermatogonia from cancer cells in contaminated prepubertal primate testis cell suspensions. *Human Reproduction*. 2011 Oct 20. Epub
23. Suzuki H, Ahn HW, Chu T, Bowden W, Gassei K, Orwig K, Rajkovic A. SOHLH1 and SOHLH2 coordinate spermatogonial differentiation. *Developmental Biology*, 2011 Oct 26. . Epub
24. Bunce K, Chu T, Surti U, Hogge WA, Peters DG. Discovery of epigenetic biomarkers for the non-invasive diagnosis of fetal disease. *Prenat Diagn*. 2012:32(6):542-9. Epub 2012 April 19.
25. Himes KP, Handley D, Chu T, Burke B, Bunce K, Simhan HN, Peters DG. Comprehensive analysis of the transcriptional response of human decidual cells to lipopolysaccharide stimulation. *J Reprod Immunol*. 2012 Jan;93(1):17-27
26. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. *Mol Hum Reprod*. 2012;18(8):417-24. Epub 2012 Feb 29
27. Shalom-Barak T, Zhang X, Chu T, Schaiff WT, Reddy JK, Xu J, Sadovsky Y, Barak Y. Placental PPARgamma Regulates Spatiotemporally Diverse Genes And A Unique Metabolic Network. *Developmental Biology* 2012 Aug 29

C. Research support

Ongoing

NIH (PI: Donald Scott) 08/15/11-05/31/13
Epigenomic regulation of gene expression in diet induced obesity
NIH (PI: David Peters) 05/15/11-01/31/16
Non-invasive detection of fetal aneuploidy by next-generation DNA sequencing
NIH (PI: Yoel Sadovsky) 08/05/10-05/31/15
Placenta Injury and microRNA

Yoel Sadovsky, MD

Dr. Sadovsky is the Director, Magee-Womens Research Institute and a Professor of OBGYN, Microbiology and Molecular Genetics, and CTSI, University at Pittsburgh School of Medicine. Dr. Sadovsky's research centers on feto-placental development and trophoblast function. He seeks to decipher the molecular mechanisms underlying placental dysfunction, which impacts pregnancy and impedes fetal growth. Using primary human trophoblasts, human placental biopsies, and genetically altered mice, his lab examines the homeostatic adaption of trophoblasts to cellular stressors. Employing computation-intensive, high-throughput transcriptomic (RNAseq and arrays) and proteomic methodologies, Dr. Sadovsky examines the role of microRNAs and other small RNAs in placental pathways that govern the adaption of human trophoblasts to altered micro-environment.

Training

1981	BS (Biomedical sciences), Hebrew Univ, Jerusalem Israel
1985	MD, Hebrew Univ, Jerusalem Israel
1986-90	Resident (OBGYN), Washington Univ, St. Louis
1990-92	Fellow (Maternal-fetal medicine), Univ California, San Francisco
1992-93	Postdoctoral fellow (Gene regulation), Univ California, San Francisco

Professional experience

1993	Asst Professor, OBGYN, Washington University
1997-07	Joint Appointment: Dept of Cell Biology and Physiology, Washington Univ
1997-07	Director, Maternal-Fetal Medicine Fellowship Program, Washington Univ
1998-07	Member, Division of Biology and Biomedical Sciences, Washington Univ
1999	Assoc Prof (tenure), OBGYN, Cell Biology and Physiology, Washington Univ
1999-07	Director, Div of Maternal-Fetal Medicine and Ultrasound, Washington Univ
2004-07	Professor (tenure), OBGYN, Cell Biology and Physiology, Washington Univ
2007-Present	Director, Magee-Womens Research Institute Univ Pittsburgh
2007-Present	Professor (tenure) and Vice Chair (research), OBGYN, Univ Pittsburgh
2007-Present	Elsie Hilliard Hillman Chair of Women's Health Research, Univ Pittsburgh
2009-Present	Professor of Microbiology and Molecular Genetics, CTSI, Univ Pittsburgh

Honors, awards, and national service

1998-00	NIH-NICHD study sections (*ad hoc* member)
2002-06	NIH-NICHD Maternal and Child Health Research Study Section
2005-06	Chair, NIH-NICHD OB and Maternal-Fetal Biology Research Study Section
2004	Society for Gynecologic Investigation (SGI) President's Achievement Award
2005-10	Steering Comm Chair, NICHD Genomic and Proteomic Network for Preterm Birth Research
2007-08	President, Perinatal Research Society
2009-Present	Council Member, Society for Gynecologic Investigation
2009-13	NICHD Council Member, National Advisory Child Health and Human Devpt
2011-12	NICHD Council Subcommittee on Planning and Policy member
2011-present	Chair, March of Dimes Prematurity Research Study Section
2011-present	Univ Kansas Institute for Reproductive Health and Regenerative Medicine Advisory Board
2012-present	Univ Colorado Center for Women's Health Research Advisory Board
2012-present	Editor, *Placenta*

2012-present NICHD Division of Intramural Research Review Panel
2012-present March of Dimes Scientific Advisory Council

Selected publications (from a list of 124)

1. Sadovsky Y, Crawford PA, Woodson KG, Polish JA, Clements MA, Tourtellotte LM, Simburger K, Milbrandt J. Mice deficient in the orphan receptor SF-1 lack adrenal glands and gonads, but express P450scc enzyme in the placenta, and have normal embryonic serum levels of corticosteroids. *Proc Natl Acad Sci USA* 1995;92:10939-43.
2. Lee SL, Sadovsky Y, Swirnoff AH, Polish JA, Goda P, Gavrilina G, Milbrandt J. Luteinizing hormone deficiency and female infertility in mice lacking the transcription factor NGFI-A (Egr-1). Science 1996;273:1219-21.
3. Mariani TJ, Budhraja V, Mecham BH, Gu CC, Watson MA, Sadovsky Y. A variable fold-change threshold determines significance for expression microarrays. FASEB J 2002;10.109.
4. Budhraja V, Spitznagel E, Schaiff WT, Sadovsky Y. Incorporation of gene-specific variability improves expression analysis using high-density DNA microarrays. BMC Biol 2003;1:1. PMC288513.
5. Yan X, Mouillet JF, Ou Q, Sadovsky Y. A novel domain within the DEAD-box protein DP103 is essential for transcriptional repression and helicase activity. Mol Cell Biol 2003;28:414-23.
6. Mouillet JF, Sonnenberg-Hirche C, Yan X, Sadovsky Y. p300 regulates the synergy of SF-1 and Egr-1 in activating LH gene. J Biol Chem 2004;279:7832-9.
7. Chen BS, Nelson DM, Sadovsky Y. NDRG1 modulates the response of term human trophoblasts to hypoxic injury. J Biol Chem 2006;281:2764-72.
8. Mouillet JF, Chu T, Nelson DM, Mishima T, Sadovsky Y. miR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J 2010;24:230-9.
9. Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WA, Sadovsky Y. The expression of hypoxia-regulated miRNAs in plasma of pregnant women with fetal growth restriction. Placenta 2010;31:781-4.
10. Mouillet JF, Chu T, Sadovsky Y. Expression patterns of placental microRNAs. Birth Defects Research A, 2011;91:737-43.
11. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod, 2012;18:417-2

Current research support

NIHR01ES11597/Regulation of Trophoblast Differentiation and Function (9/01–5/13); Sadovsky PI

NIHR01HD065893/Placental Injury and microRNA (8/10–5/15); Sadovsky, PI

NIHR21HD071707/Small RNAs at the Placental Maternal Communication Interface (12/11–12/13); Sadovsky PI

NIH P01HD069316/Molecular and Cellular Controls of Placental Metabolism (6/12–2/17); Sadovsky, PD

NIHR01HD075665/Primary Human Trophoblasts and the Transfer of Viral Resistance (9/12–6/17); Sadovsky, Co-PI

NIHK12HD063087/Magee-Womens Basic and Translational Reproductive Health Training Program (9/09–8/14); Sadovsky, Res Director

NIHK12HD043441/Building Interdisciplinary Research Careers in Women's Health (BIRCWH)

in Pittsburg (7/12–6/17); Sadovsky, Co-PI