THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

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Eleni Z. Tsigas
Preeclampsia Foundation
6905 N. Wickham Road, Suite 302
Melbourne, FL 32940-7552

Re: 2016 Vision Grant Award Final Report

Dear Ms. Tsigas and Vision Grant Committee,

I am writing to inform you of my progress on the project entitled, "Metformin in a catechol-O-methyltransferase deficiency model of preeclampsia." The goal of this project is to understand the potential role of metformin in preeclampsia prevention by investigating its effects on placental metabolism. We have utilized both a human cell culture system and an established mouse model of preeclampsia (catechol-O-methyltransferase (COMT) knockout) to investigate the effects of metformin on placental trophoblast function and to study the association between preeclampsia and metabolism.

In the latter half of 2017, we continued our in vitro studies using HTR-8/SVneo extravillous cytotrophoblast cells (derived from first trimester human tissue; ATCC) to study the mechanism of metformin action on placental trophoblasts. Early in pregnancy, normal placentation requires trophoblast proliferation and invasion, which are regulated by oxygen (O_2) tension. Defects in this process are central to the pathophysiology of preeclampsia. Therefore, we have tested the effects of metformin and combination metformin/aspirin (given the current use of aspirin for preeclampsia prevention in high-risk women), on trophoblast proliferation and ATP production (proxy for mitochondrial function). Our results suggest that metformin alone, or in combination with aspirin, promotes trophoblast proliferation under hypoxic conditions mimicking the first trimester uterine environment (**Figure 1**). Cellular ATP levels also differ in metformin-treated compared with untreated control cells. These results demonstrate the impact of treatment on trophoblast function, and suggest a possible mechanism by which metformin might mitigate the process of poor placental invasion in women predisposed to severe preeclampsia. An abstract of these results will be presented in poster format at the Society for Maternal-Fetal Medicine Annual Meeting in Dallas, Texas on February 1, 2018.

With regards to our mouse studies, we have performed further characterization of the COMT knockout preeclampsia model to investigate how placental metabolism is affected in these mice. Since we hypothesize that metformin functions in vivo by modulating placental metabolism, it was critical to characterize the metabolic defect in COMT knockout mice, as this is suspected to contribute to the pathophysiology of the disease. Placental histology revealed significantly altered levels of glycogen, an

important energy/glucose source for the fetus, in knockout compared with wild type control placentas (**Figure 2**). We have previously shown that treatment of COMT knockout mice with 2-methoxyestradiol (2-ME), a product of COMT enzyme activity, ameliorates the preeclampsia-like phenotype in these mice. Consistent with this, 2-ME also rescued the placental glycogen phenotype (**Figure 2**). Placental glycogen has been shown to be increased in the context of preeclampsia. These findings provide additional evidence for the relationship between preeclampsia and metabolic syndromes, such as diabetes and obesity which are known risk factors for preeclampsia, and further support the biologic plausibility of a metabolism modifying agent, such as metformin, as a prevention strategy. These mouse data were incorporated into a broader story investigating the role of placental growth factor (PlGF) in preeclampsia and placental metabolism, and resulted in the submission of a manuscript entitled, "Loss of PlGF ameliorates the preeclampsia phenotype in COMT-deficient mice". The manuscript was submitted in December and is currently under review.

I want to thank the Foundation for supporting this work. We have had a productive year and will acknowledge the Vision Grant in all presentations and manuscripts that result from the work performed over the past year. On a personal note, the support has helped me in my transition from Fellow to Attending/Assistant Professor of Maternal-Fetal Medicine, a critical juncture of my career. I will continue to study preeclampsia with the goal of contributing to our fundamental understanding of the disease and potential interventions, and will apprise you of updates regarding the above submissions.

Best wishes in 2018!

Sincerely,

Jacqueline Parchem, MD
Kalluri Lab, Department of Cancer Biology
University of Texas MD Anderson Cancer Center

Department of Obstetrics, Gynecology & Reproductive Sciences
McGovern Medical School, University of Texas Health Science Center at Houston (UTHealth)

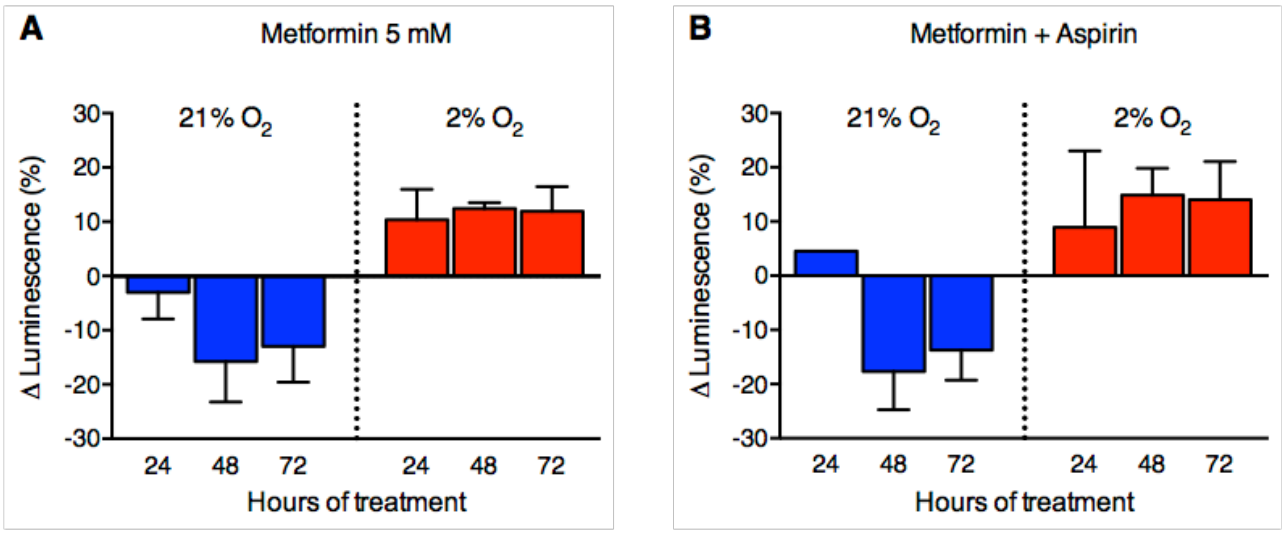

Figure 1. Metformin treatment promotes HTR-8 extravillous cytotrophoblast proliferation under hypoxic conditions. Cell viability measured by luminescent assay (CellTiter Glo, Promega) at 24-hour time points. Cells treated with metformin (**A**) or combination metformin and low-dose aspirin (**B**), and exposed to normal oxygen tension (21%) vs. hypoxia (2%). Data expressed as percent change in luminescence compared with untreated control at each time point.

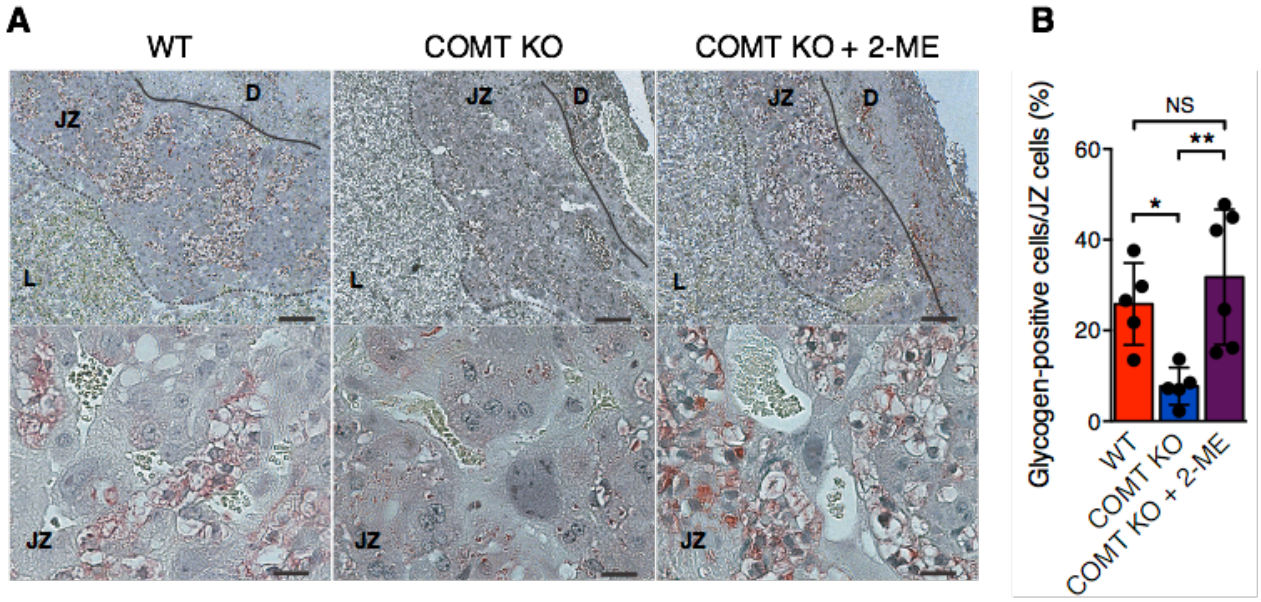

Figure 2. Placental glycogen content in COMT knockout preeclampsia model. (**A**) Representative images of the placenta (low-magnification, top panels) and of the junctional zone (high-magnification, lower panels) showing glycogen staining (Best's carmine, red) of E17 placentas from the indicated groups. Scale bar: 100 µm and 25 µm in low- and high-magnification images, respectively. (**B**) Ratio (%) of glycogen-positive cells over total junctional zone cells per visual field (n=2 litters, at least 2 placentas per group). 2-ME, 2-methoxyestradiol; COMT, catechol-O-methyltransferase; D, decidua; JZ, junctional zone; L, labyrinth. Results shown as mean +/- S.D. One-way ANOVA; NS, not significant, *$P < 0.05$, **$P < 0.01$.

Vision Grant Budget Summary 2017

Laboratory Supplies	Jan-June	July-Dec
Cell culture	$ 2,800	$ 3,200
Chemicals and reagents	$ 400	$ 1,000
Molecular biology supplies	$ 1,200	$ 1,200
General supplies	$ 3,200	$ 3,200
Animal Housing and Maintenance		
Housing and maintenance	$ 1,300	$ 2,000
Other		
Data analysis software	$ 500	
TOTAL	$ 9,400	$ 10,600

Laboratory supplies

- Cell culture: HTR-8 cell line, culture media, serum, media supplements, trypsin, plasticware, cryovials, nitrogen gas
- Chemicals and reagents: metformin, standard chemicals, buffers
- Molecular biology supplies: cell viability assays, mitochondrial function kit and reagents
- General supplies: microcentrifuge tubes, conical tubes, plates and flasks, pipet tips, gloves, Pipet-aid