MID-YEAR VISION GRANT PROGRESS REPORT

PROJECT TITLE: Regulation and functions of placental sFlt-1: relevance to preeclampsia.

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The main goal of this work was to understand the fundamental causes of the pregnancy complication preeclampsia. Experiments were designed to develop new methods for studying the roles of specific proteins in placental function and disease and for testing novel therapeutic approaches to preeclampsia using a mouse model system. One of the major goals was to specifically address the physiological role and regulation of a protein called sFlt-1, which is found at high levels in the blood of women with preeclampsia and which is believed to cause the maternal symptoms of the disease. Understanding this protein and why it accumulates will have a direct impact on the design of clinical therapies for preeclampsia.

The specific aims of the original proposal were to: 1) develop an inducible placenta-specific transgene expression system using the lentivrial “Tet-On system” and monitor induced transgene expression in placentae at different stages of pregnancy by bioluminescence imaging; 2) test the effects of different levels of VEGF overexpression in trophoblast cells at different stages of pregnancy; 3) define the physiological role of placental sFlt1. These aims have not been modified during the course of this study.

Summary of progress:

Development of methods for inducible placenta-specific gene expression and in vivo monitoring of transgene expression: We developed a significant new technique for the study of gene functions in the mouse placenta, as there is a long-standing absence of methods for expression of transgenes specifically in the placenta. The recent development of lentivirus-based placental gene expression methods has greatly helped, but these have significant limitations in both consistency and controllability. Most genes important for placental development or function exhibit highly stage-specific expression. Moreover, many of the most common and serious disorders of pregnancy, which can affect both maternal and fetal health, are associated with developmental defects in the placenta and abnormal stage-specific gene expression. For all these reasons, better methods for studying gene function in the placenta, particularly those that allow stage-specific expression, are urgently needed.

Our method for the inducible expression of genes specifically in the mouse placenta was based on the recently developed, third generation doxycycline-inducible gene expression system developed by Clontech, known as TetON 3G. This newest promoter-transactivator combination exhibits extremely low background and a greatly increased sensitivity to doxycycline (10-100-fold greater than other versions of dox-inducible transactivators), making it highly suitable for *in vivo* studies and making it the most sensitive dox-inducible transgenic mouse system now available. The greatly increased sensitivity is especially important for studies on early placental development, where high levels of dox have been shown to exhibit toxic effects. We have generated a transgenic mouse expressing the TetOn 3G protein ubiquitously and show that these mice are healthy and undergo normal pregnancy. We used a recently developed site-specific recombination system for the creation of our transgenic lines that has been engineered to lack cis sequences from the parent vectors that can interfere with expression *in vivo*. Using lentivirus-based transduction of the TetOn target promoter-reporter genes into the trophectoderm of mouse blastocysts, we demonstrated that the system has essentially no background in the absence of doxycycline, but that it is rapidly and strongly responsive to doxycycline both in blastocysts *in vitro* and developing and mature placenta *in vivo*. Transient induction in blastocysts allowed preselection of blastocysts for consistent levels of infection and transgene expression. After implantation into pseudopregnant mice lacking the TetOn 3G transgene, we demonstrated similar dose-response and decay rates at all stages of pregnancy using *in vivo* bioluminescence imaging. Reporter protein signals could be detected shortly after doxycycline administration by i.p. injection and lasted on the order of 48 hours, being undetectable at 60 hours post-injection.

We believe this system will be of broad interest to those studying not only molecular regulation of placental functions and pregnancy disorders, but also those wanting to understand early embryonic phenotypes. Furthermore, our TetOn 3G transgenic line, the most sensitive dox-inducible system now available in mice, will be broadly useful for inducible gene expression studies in other tissues. A manuscript resulted from this study has been accepted for publication in *Endocrinology*.

Physiological role of placental sFlt1: We investigated the function of placental sFlt1 in pregnant mice by knocking down placental expression by infection with a lentiviral vector expressing both a shRNA specific for sFlt1 and GFP (LV-shRNA.sFlt1-copGFP) using the placenta-specific transgene expression. This method was highly effective in significantly reducing sFlt1 production

in the placenta. A lentivirus expressing GFP alone was used as a control. Our initial results of sFlt1 knockdown in the mouse placenta are very encouraging, and we are in the process of preparing different constructs and lentiviruses for pregnancy stage-specific knockdown of sFlt1 using the above doxycycline-inducible placenta-specific gene expression system.

VEGF overexpression in the placenta: We developed a lentivirus expressing VEGF (LV-VEGF164) and successfully overexpressed VEGF in the mouse placenta using our placenta-specific gene delivery method. The initial data show dramatic adverse effects on pregnancy following VEGF over expression in the placenta, and we are in the process of further data analysis and modifications of the methods.

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Publications:

Fan, X., Pettit, M., Gamboa, M., Huang, M., Dhal, S., Druzin, M.L., Wu, J.C., Chen-Tsai, Y. and Nayak, N.R. (2012) Transient, inducible, placenta-specific gene expression in mice. Endocrinology, In press.