INTRODUCTION

Cardiovascular disease complicates more than 2% of pregnancies, impacting both maternal and fetal health (**8**). In recent years, increasing evidence has suggested the existence of an intricate relationship between placental health and maternal and fetal cardiovascular pathology. Preeclampsia, a maternal syndrome of hypertension and proteinuria, affects 2-8% of pregnancies and is convincingly implicated in driving cardiovascular disease (**2**). Women with preeclampsia are at increased risk of peripartum maternal cardiomyopathy, and anti-angiogenic factors secreted by the placenta during preeclampsia have been shown to induce peripartum cardiomyopathy (**6**). Although the direct mechanisms driving preeclampsia pathophysiology are unclear, the developmental and molecular effects of preeclampsia are vast, involving dysfunctional spiral artery remodeling, placental ischemia, oxidative stress, an imbalance of pro- and anti-angiogenic factors, immunology, and inflammation (**5**).

While the effects of preeclampsia and placental health on maternal cardiovascular health have been more vigorously studied, knowledge of their impact on fetal cardiovascular development remains in its infancy. Early studies from the Helsinki Birth Cohort noted correlations between placental shape and risk for chronic disease in the fetus including cardiovascular disease and hypertension. These results gave rise to the concept of placental "programming" of the fetus (**11**). Further, early-onset preeclampsia and congenital heart defects were found to be strongly associated with an odds ratio of 7 (**1**). Due to limited availability of mouse models for preeclampsia, the study of mechanisms driving these associations are thought to be highly complex and have yet to be elucidated.

Angiogenic factors appear to play a significant role in both embryonic cardiac development and hypertensive disorders of pregnancy (**1**). Soluble *fms*-like tyrosine kinase-1 (sFlt-1) has been identified as an important anti-angiogenic factor in placental dysfunction. sFlt-1 is produced by trophoblasts often in the setting of placental ischemia and is a splice-variant of Flt-1, a transmembrane receptor for pro-angiogenic factors vascular endothelial growth factor (VEGF) and the structurally-similar platelet-derived growth factor (PlGF) (**3**). sFlt-1 binds to VEGF and PlGF, decreasing the bioavailability of these proteins, which leads to compromised endothelial integrity and increased oxidative stress (**4**). Early-onset preeclampsia is associated with higher levels of circulating sFlt-1 and increased fetal and maternal morbidity and mortality (**5, 10, 12**). Administration of recombinant human PlGF has been demonstrated to have a therapeutic effect on preeclampsia (**9**), while increased sFlt-1 levels were found to be associated with poor maternal cardiac function in patients with preeclampsia (**6**). Interestingly, systemic adenoviral overexpression of sFlt-1 in mouse models resulted in increased myocardial function reminiscent of diastolic dysfunction. However, in mouse models predisposed to peripartum cardiomyopathy through PGC1-alpha cardiac-specific deletion, nulliparous mice exposed to sFlt-1 overexpression developed profound dilated cardiomyopathy.

Despite the profound impact of sFlt-1 on maternal cardiovascular disease, the implications of sFlt-1 dysregulation on fetal cardiac development and function have been minimally studied. As sFlt-1 has been recognized as not only a biomarker for placental pathology in humans, but also as a driver of cardiac dysfunction, the effects of this protein on fetal cardiac development and neonatal heart regeneration are worthy of investigation.

HYPOTHESIS

Increased sFlt-1 is detrimental to mouse fetal cardiac development and cardiac function through dysregulation of VEGF/P1GF signaling.

APPROACH & INTERPRETATION

Specific Aim #1: To identify consequences of in utero sFtl-1 exposure on embryonic and postnatal cardiac development and function.

Fetal exposure to elevated levels of sFtl-1 will be simulated in an experimental group of mice by continuously infusing pregnant mice with recombinant sFlt-1 from the beginning of heart tube formation at E7.5 until birth. A negative control group of mice will receive isotonic infusions at the same gestational age. Umbilical venous serum and amniotic fluid will be assessed at delivery to confirm increased sFtl-1 level in the experimental group and normal sFtl-1 levels in controls. Pregnant mice will be sacrificed alongside negative controls and embryos will be harvested for histology at E11.5, E13.5, E15.5, and E18.5 to assess heart tube and endocardial cushion morphology as myocardial proliferation.

To evaluate postnatal effects of in utero exposure to sFlt-1, embryos in both treated and untreated groups will be taken to term and sacrificed at P1, P8, P14, and P30 to evaluate for changes in cardiac morphology, cardiomyocyte proliferation, vascularization, and cellular composition using immunohistochemical studies. Additionally, these animals will be evaluated functionally by echocardiography prior to death. Assuming viability, cardiomyocyte structural morphology will be evaluated at P30 by electron microscopy (EM) following tissue fixation. Further, cardiac tissue will be harvested at corresponding embryonic and postnatal time points to evaluate for transcriptional changes in critical cardiac transcription factors, angiogenic factors, and structural transcripts associated with cardiomyocyte maturation by Realtime PCR.

Specific Aim #2: To characterize the effect of in utero sFlt-1 exposure on postnatal cardiac stress.

To characterize the cardiac effects of in utero sFlt-1 exposure following myocardial stress, offspring of mothers treated with sFlt-1 during pregnancy and age-matched controls will be exposed to both physiologic and pathologic stress. At two months of age, experimentally treated sFlt-1 animals and age-matched controls will undergo echocardiography followed by 4 weeks of voluntary running. One week following completion of running protocol, mice will undergo post-run echocardiography and sacrificed for histologic analyses. To model two different forms of pathologic stress, experimental and age-matched controls will be subjected to either transaortic constriction (TAC) or myocardial infarction (MI) by LAD ligation. Four weeks following, TAC/MI mice will undergo echocardiography and be sacrificed for histologic analyses.

Specific Aim #3: To define the mechanism of sFlt-1 in myocardial dysfunction.

Prior studies have demonstrated the role of sFlt-1 in inducing mitochondrial dysfunction in endothelial cells. Immunohistochemical studies and subcellular fractionation experiments in sFlt-1 overexpression will characterize localization patterns of sFlt-1. Mitochondria will be isolated from both P1 sFlt-1-treated and control neonatal cardiomyocytes. Utilizing Seahorse technology, measures of mitochondrial structure and function including oxidation rates in interfibrillar mitochondria and subsarcolemmal mitochondria, respiration and acidification rates, and mitochondrial membrane potential will be evaluated. RNA-seq analyses will also be performed on nuclear and mitochondrial DNA to identify whole transcriptome differences

between the experimental and control groups. Binding partners for sFlt-1 will be assessed in cardiac tissue at the time of the most pronounced phenotype by overexpression by tandem affinity purification in HL-1 cardiac cell lines. Lastly, rescue of the observed phenotypes secondary to sFlt-1 overexpression with exogenous VEGF/P1GF will be performed and assessed through echocardiographic, histological, and transcriptomic analyses.

SIGNIFICANCE

The placenta is the site of selective communication between two different cardiovascular systems; it is no wonder then that this organ could have lasting impacts on either the maternal or fetal heart despite the temporary nature of its existence. Studies in the last few decades have demonstrated a strong relationship between placental disease and maternal life-long cardiovascular disease risk. However, less is known about how placental disease changes cardiovascular function in the offspring. Factors affecting fetal cardiovascular development are complex given the constant changes that both placenta and heart undergo in the course of embryological and fetal growth.

It is uncertain how much sFlt-1 is responsible for preeclampsia or if it is a byproduct with potent effects. However, sFlt-1 has proven to be a strong and reliable candidate for use as a clinical marker for preeclampsia diagnosis and prognostication. sFlt-1 also appears to be deeply involved in several drivers of cardiac disease pathology: oxidative stress, endothelial dysfunction, inflammation, and immune system activation. Expression of sFlt-1 is highest in early-onset preeclampsia and is associated with increased risk for maternal cardiovascular disease. Since strong associations between sFlt-1 and maternal cardiovascular disease in preeclampsia have already been demonstrated, it is imperative to consider the impact of sFlt-1 anti-angiogenesis on the developing fetal heart. Importantly, dysregulation of anti-angiogenic factors may play a role in limiting the regenerative window of the neonatal heart. Even though sFlt-1 is suspected to be a byproduct of the preeclampsia pathology, mounting evidence suggests that it is a potent driver of human cardiovascular disease. Understanding of the mechanisms that dictate how sFlt-1 impacts cardiac function is vital and would help to advance the field in mitigating the effects of placental disease on cardiovascular health.

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