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Long-term Alterations in Maternal Plasma Proteome after sFlt1-induced Preeclampsia in Mice

Egle Bytautiene, MD, PhD¹, Nataliya Bulayeva, PhD², Geeta Bhat, MS¹, Li Li², Kevin P Rosenblatt, MD, PhD², and George R Saade, MD¹

¹The University of Texas Medical Branch, Obstetrics & Gynecology, Galveston, TX

²The University of Texas Health Sciences Center at Houston, TX

Abstract

Objective—Preeclampsia (PE) is associated with long-term adverse maternal health, such as cardiovascular (CVD) and metabolic diseases. The objective of this study was to determine whether PE in a well characterized animal model induced by over-expression of sFlt-1 results in alterations in the maternal circulating proteome that persist long after delivery.

Methods—CD-1 mice at day 8 of gestation were injected with adenovirus carrying sFlt1 or the murine IgG2α Fc fragment as control. Depleted maternal plasma was analyzed 6 months after delivery by label-free liquid chromatography – mass spectrometry assay. The tandem MS data were searched against a mouse database and the resultant intensity data were used to compare abundance of proteins across disease/control plasma pool (MaxQuant/Mascot). Results were analyzed using Ingenuity Pathways Analysis (IPA). Right-tailed Fisher's exact test was used to calculate a *P*-value.

Results—Out of 150 proteins common for both groups, IPA determined 105 proteins ready for analysis. Diseases and disorders analysis showed significant enrichment of proteins associated with CVD. Within this cluster, the most abundant proteins were associated with vascular disease, atherosclerosis and atherosclerotic lesions. Other top disease clusters were inflammatory response, organismal injury and abnormalities, hematological and metabolic disease.

Conclusions—Exposure to sFlt1-induced preeclampsia alters multiple biological functions in mothers that persist later in life. Our results suggest that some of the long term adverse outcomes associated with preeclampsia may actually be a consequence of an underlying predisposition. If similar results are found in humans, developing preventive strategies for preeclampsia should also improve long term maternal health.

Keywords

maternal long-term health; preeclampsia; sFlt1; mice

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Corresponding author: Egle Bytautiene, MD, PhD 301 University Blvd, Galveston, TX 77555-1062 Phone: 409-747-5139, Fax: 409-772-2261, egbytaut@utmb.edu.

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INTRODUCTION

The increasing rates mortality and morbidity from cardiovascular diseases (CVD) in women warrants intensifying research into gender-specific mechanisms. The past decade saw an increase in studies reporting on the association between preeclampsia (PE) and later CVDs. Indeed, multiple studies and meta-analyses confirm that women, whose pregnancy was complicated by PE, have higher susceptibility to CVD later in life.

The pathogenesis and genetics of PE still needs to be elucidated. However, key pathophysiological factors – endothelial dysfunction, oxidative stress, inflammatory responses – are common to both PE and CVD. Moreover, both conditions share similar risk factors, such as obesity, diabetes, and dyslipidemia.¹

Whether PE directly influences the development of maternal CVD later in life or preeclampsia uncovers a pre-existing condition that would have led to CVD later in life anyway remains undetermined.²

To dissect the associations between PE and CVD, studies in animal models are needed. In our laboratory, we have established a mouse model of PE induced by overexpression of soluble fms-like tyrosine kinase (sFlt1). This model has been described elsewhere.³ Briefly, pregnant mice injected with sFlt1-carrying adenovirus on day 8 of gestation develop hypertension, endothelial dysfunction and other features characteristic of PE. We have also examined these mice 6 months postpartum and found that exposure to sFlt1 during pregnancy does not lead to increased blood pressure or vascular dysfunction later in life.⁴ These results favor the hypothesis that increased CVD in women after preeclampsia is the result of preexisting conditions common to both – PE and CVD. At 6 months postpartum, however, mice are still young adults and may not show direct evidence of CVD. Subtle changes that would lead to hypertension later than 6 months postpartum may still have occurred.

To further investigate consequences of PE on long term maternal health, our objective in the current study was to determine whether PE in a well characterized animal model induced by over-expression of sFlt-1 results in alterations of the maternal circulating proteome that persist long after delivery.

MATERIALS AND METHODS

Animals

The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch, Galveston, Texas. The animals were housed separately in temperature- and humidity-controlled quarters with constant 12:12-h light-dark cycles and were provided with food and water ad libitum. Female and male CD-1 mice were obtained from Charles Rivers Laboratories (Wilmington, MA) and bred in our facility. The animals were sacrificed using CO₂ inhalation according to the Animal Care and Use Committee and the American Veterinary Medical Association guidelines.

Study design

At day 8 of gestation, pregnant mice were randomly divided into two groups and injected via the tail vein with adenovirus carrying sFlt1 (AdsFlt1, 10⁹ plaque-forming units in 100 µL, n=6) or adenovirus carrying the murine IgG2α Fc fragment as the adenovirus control (AdmFc, 10⁹ plaque-forming units in 100 µL, n=6). The procedure to produce AdsFlt1 and AdmFc is described elsewhere.³ No other procedures were performed during pregnancy (blood / urine collection) in order not to intervene with gestation and delivery. Pregnant

mice were allowed to deliver. Pups were weaned from their respective mothers 3 weeks after delivery.

Food / drink intake, animal care and other activities were not regulated throughout the study. Experiments were performed in mothers at six months postpartum. We have started with 6 mice per group, however, one animal exposed to sFlt1 died before reaching 6 months postpartum. Mice were anesthetized with a mixture of ketamine (Ketalar, Parke-Davis, Morris Plains, NJ) and xylazine (Gemini, Rugby, Rockville Center, NY) and telemetric blood pressure transducers (PA-C20 model, Data Sciences, St. Paul, MN) were implanted. Arterial blood pressure (BP) was monitored in the unrestrained conscious mice as described previously.⁴ After a rest period of 3 days, blood pressure was recorded continuously for 4 consecutive days using Dataquest A.R.T. data acquisition system (DSI, St Paul, MN). Then, the mice were sacrificed, and blood was collected via heart puncture at the time of sacrifice in microtubes containing ethylenediaminetetraacetic acid (K₂EDTA) and was spun down at 4°C for 20 min at 10,000 rpm. Plasma was removed and stored at -80°C until analysis. The carotid arteries were dissected for *in vitro* vascular reactivity studies. Two-millimeter segments of carotid arteries were mounted in a wire myograph (Model 410A, J.P. Trading I/S, Aarhus, Denmark) using 25 µm tungsten wires. The preparations were bathed in physiological salt solution maintained at 37°C, pH ~7.4. A mixture of 95% O₂ and 5% CO₂ were bubbled continuously through the solution. The force was continuously recorded by an isometric force transducer and analyzed using PowerLab system and Chart 5 data acquisition and playback software (AD Instruments, Castle Hill, Australia). After stabilization of the tone, the vessels were contracted twice with 60 mM KCl for 10 min to enhance reproducibility of responses. Vascular reactivity was assessed to vasodilator acetylcholine (ACh, 10⁻⁹–10⁻⁵ M) and after pre-contracting vessels with phenylephrine (PE, 10⁻³–10⁻⁷ M).

Blood sFlt1 Measurements—Blood was collected via heart puncture at the time of sacrifice and was spun down. The sFlt1 level in the blood was measured using mouse soluble VEGF R1 immunoassay (R&D systems, Minneapolis, MN) according to the manufacturer's instructions.

Plasma preparation for Mass Spectrometry

Plasma was analyzed for each mouse separately. Whole plasma (10 µL) was depleted with Seppro Mouse Spin columns (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. Protein concentration was detected by Bradford assay (Bio-Rad, Hercules, CA). Plasma was denatured and reduced by 6M Urea with 20mM dithiothreitol in 150mM Tris buffer (pH=8.2) with subsequent alkylation by iodoacetamide (40mM). Samples were diluted with Tris buffer (50mM, pH=8.2), and Trypsin (1 µg/µL) was added at a 20:1 substrate:enzyme ratio. Digestion was carried out for 16h at 37°C and stopped by acidification. Samples were desalted with C18 columns (Waters, Milford, MA) according to the manufacturer's instructions and lyophilized.

Mass Spectrometry

After reconstitution in 2% (v/v) acetonitrile 0.1% (v/v) formic acid samples were analyzed on a LTQ Orbitrap XL (Thermo-Fisher Scientific, Bremen, Germany) interfaced with an Eksigent nano-LC 2D plus ChipLC system (Eksigent Technologies, Dublin, CA). About 0.5 µg of sample was loaded onto a ChromXP C₁₈-CL trap column (200 µm i.d. x 0.5 mm length, 3 µm particle size) at a flow rate of 3 µl/min. Reversed-phase C₁₈ chromatographic separation of peptides was carried on a ChromXP C₁₈-CL column (75 µm i.d x 10 cm length, 3 µm) at 300 nL/min, with the column temperature controlled at 60°C. Solvent A with 0.1 % formic acid in water and solvent B with 0.1% formic acid in acetonitrile were used for

HPLC gradient. Gradient conditions were: 3%-8% B for 5 min; 8%-33% B for 120min; 33%-90% B for 10 min; 90% B held for 10 min; 90% -3% B for 5 min. The total run time was 150 min. The LTQ Orbitrap was operated in the data dependent mode to simultaneously measure full scan MS spectra in the Orbitrap and the five most intense ions in the LTQ by CID, respectively. In each cycle, MS1 was acquired at target value 1E6 with resolution $R=100,000$ (m/z 400) followed by top 5 MS2 scan at target value 3E4. The mass spectrometric setting was as follows: spray voltage was 1.6 KV, charge state screening and rejection of singly charged ion were enabled. Ion selection thresholds were 8000 for MS2; 35% normalized collision energy; activation Q was 0.25, and dynamic exclusion was employed for 30 seconds. Each sample was analyzed in triplicate.

Label-free analysis

Data analysis was performed with MaxQuant software, supported by Mascot as a database search engine for peptide identification. Average LFQ intensity values were used to calculate sFlt1/mFc protein ratio.

Ingenuity Pathways Analysis (IPA)

Data were expressed as spectra intensity ratio sFlt1 group over mFc group (sFlt1/mFc). Molecules with ratio outside the range of 0.8 to 1.2 were included in the final analysis. We used IPA to determine whether any peptides can be mapped to different biological or disease functions (Ingenuity Systems, www.ingenuity.com). For the final analysis, we used the IPA content version 14197757 released on August 11th 2012. The dataset was filtered for species (mouse) and confidence (experimentally observed) and included molecules with direct and indirect relationships. The ratio between the two groups was converted to fold increase / decrease by IPA. Our objective was to identify the biological functions affected by exposure to sFlt1 during pregnancy.

The Functional Analysis identified the biological functions and/or diseases in Ingenuity's Knowledge Base that were specific to our data set. IPA has three primary categories of functions: Molecular and Cellular Function; Physiological System Development; and Diseases and Disorders. There are 85 high-level functional categories that are classified under the primary categories. Lower level functions and specific functions are classified within the 85 high-level functions and each may be under multiple high-level categories. In other words, each function is not discrete. However, IPA ranks associated functions based on their significant differences and gives an overall assessment of primary function. If a higher order disease/disorder category contains two or more specific functions that reach statistical significance, the software displays the most statistically significant value on the y-axis of a bar graph. Right-tailed Fisher's exact test was used to calculate a probability value (P) determining the probability that each biological function and/or disease assigned to that data set is due to chance alone. A P value of 0.05 was considered statistically significant.

Using IPA, we have also generated networks from our data set. A data set containing gene identifiers and corresponding ratio values was uploaded into the application. Each identifier was mapped to its corresponding object in Ingenuity's Knowledge Base. Using a range outside 0.8 and 1.2 for the ratio, molecules with significantly differentially regulated expression were identified. These molecules, called Network Eligible molecules, were overlaid onto a global molecular network developed from information contained in Ingenuity's Knowledge Base. Networks of Network Eligible Molecules were then algorithmically generated based on their connectivity.

Canonical pathways analysis identified the pathways from the IPA library of canonical pathways that were most significant to our data set. The significance of the association

between the data set and the canonical pathway was measured in 2 ways: 1) A ratio of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway is displayed. 2) Fisher's exact test was used to calculate a *P*-value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone.

The *P*-value associated with functional analysis for a dataset is a measure of the likelihood that association between a set of functional analysis molecules in our experiments and a given process or pathway is due to random chance. The smaller the *P*-value the less likely that the association is random, meaning the more significant the association. *P*-values were very small in our study, thus IPA converted it into more illustrative number $-\log(P\text{-value})$.

A network is a graphical representation of the molecular relationships between molecules. Molecules are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). All edges are supported by at least 1 reference from the literature, from a textbook, or from canonical information stored in the Ingenuity Pathways Knowledge Base. Human, mouse, and rat orthologs of a gene are stored as separate objects in the Ingenuity Pathways Knowledge Base, but are represented as a single node in the network. The intensity of the node color indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product.

Data analysis for blood pressure, vascular reactivity, and blood sFlt-1 levels

Blood pressured and vascular reactivity data passed Shapiro-Wilk normality and Equal Variances tests. The BP data obtained from telemetry system were plotted as mean values over 24 hour period, expressed as mean $\pm$ SEM and analyzed using t-test ($P<0.05$). In the vascular reactivity PE precontraction was used to obtain the percentage of relaxation induced by the Ach. Results were expressed as mean $\pm$ SEM. The area under the concentration response curve (AUC) and means at different concentrations were compared using t-test ($P<0.05$).

The sFlt-1 levels were calculated with a standard curve that was derived from known concentrations of the recombinant protein. Data passed Shapiro-Wilk normality test, but not the Equal variance test, thus comparisons between sFlt1 and mFc groups were made using Mann-Whitney Rank Sum Test ($P<0.05$). When nonpregnant age-matched female mice were added to the analysis Kruskal-Wallis One Way Analysis of Variance on Ranks ($P<0.05$) was employed.

RESULTS

There was no difference in maternal weight between the sFlt1 and mFc groups on day 1 of gestation, at delivery and at 6 months postpartum (data not shown). Adiposity – a percent of adipose tissue from total body weight – was also not different between the groups at 6 months postpartum (mFc group $8.16 \pm 1.9\%$ vs sFlt1 group $7.3 \pm 1.9\%$; $P=0.8$). There was no statistical difference in the levels of sFlt1 between the two groups of mice (Table 1). The levels of sFlt 1 were similar to age-matched nonpregnant mice (1.5 ± 0.1 ng/mL).. Blood pressure and carotid artery responses to acetylcholine reflecting endothelium function were not different between the two groups as well (Table 1). While SEM for blood pressure results are large, results presented in Table 1 had passed the Equal Variance test with $P=0.27$ for SBP and $P=0.56$ for DBP. The power of the performed test was below the desired power of 0.800. Less than desired power indicates that we are less likely to detect a difference when one actually exists. Thus, negative results should be interpreted cautiously. However,

we have already observed the same results in a different set of animals, in the study which have been published.⁴

We identified a total of 150 peptides expressed in plasma of mice previously exposed to sFlt-1-induced preeclampsia and controls. Of these, IPA determined 105 molecules that were ready for analysis. These peptides were spread among 75 high-level functional categories (out of 85 available). The top five high-level functional categories associated with exposure to preeclampsia were CVD, Protein Synthesis, Hematological System Development and Function, Organismal Functions, and Tissue Morphology (Figure 1).

The top biological function category was CVD. Out of 56 diseases/disorders in this cluster found in our data set (Figure 2), the top five were vascular disease (22 molecules), atherosclerosis (14 molecules), atherosclerotic lesion (12 molecules), size of atherosclerotic lesion (9 molecules), and area of atherosclerotic lesion (7 molecules).

For the Protein Synthesis category, 7 biological functions were identified. Of these, the most significant (Figure 3) were quantities of protein lipid complex (11 molecules), HDL cholesterol (10 molecules), LDL cholesterol (7 molecules), and protein in blood (17 molecules), as well as lipoprotein metabolism (4 molecules). Changes in 53 biological functions belonging to the Hematological System Development and Function category were identified. The top 5 in this category (Figure 4) were blood coagulation (12 molecules), quantity of phagocytes (15 molecules), cell movement of leukocytes (17 molecules), quantity of macrophages (9 molecules) and blood platelets aggregation (7 molecules). The fourth most significant category was Organismal Functions (Figure 5) with changes in blood coagulation (12 molecules) and flow (4 molecules), wound healing (6 molecules) and infarct healing (1 molecule). Sixty biological functions specific to changes associated with exposure to sFlt1-induced preeclampsia belonged to the Tissue Morphology category. Of these, the top most significant (Figure 6) were size of lesion (14 molecules), size of atherosclerotic lesion (9 molecules), area of atherosclerotic lesion (7 molecules), quantity of phagocytes (15 molecules), and quantity of macrophages (9 molecules).

When biological functions were analyzed at the lower level, the top five most significant (Figure 7) were vascular disease (22 molecules), atherosclerosis (14 molecules), quantity of protein lipid complex in blood (11 molecules), quantity of HDL cholesterol in blood (10 molecules), and coagulation of blood (12 molecules).

IPA identified 107 canonical pathways involving molecules from our dataset. The top 5 pathways (Figure 8) were Liver X Receptor/Retinoid X Receptor (LXR/RXR) Activation (29 molecules from our dataset out of 117 involved in this pathway), Acute Phase Response Signaling (30/163), Coagulation System (12/35), Complement System (10/29), and Atherosclerosis Signaling (14/119).

Some of the top upregulated and downregulated molecules are shown in Table 2. The most upregulated was glycosylation dependent cell adhesion molecule 1 with fold change in expression value of 38.5, and the most downregulated was Complement component 9 (fold change in expression value -120.7).

IPA mapped 8 networks associated with our data set (Table 3). The highest number of molecules (28) was clustered in the network containing Protein Synthesis, Cardiovascular Disease, and Lipid Metabolism functions (Figure 9).

COMMENT

In this study, we explored the plasma protein profile in mice 6 months after sFlt1-induced PE. We found that sFlt1-induced PE alters expression of proteins implicated in many biological functions which persist later in life. The top impaired biological function was found to be cardiovascular, top lower level function – vascular, top canonical pathway – LXR/RXR activation. The most molecules were clustered in the network associated with protein synthesis, CVD, and lipid metabolism. A few limitations of our study need to be noted – only one age group was included in the study and we did not control for food intake. Also, the sample size is small, thus we could not completely reject the null hypothesis regarding differences in blood pressure/vascular reactivity between groups.

The significance of our study is that we demonstrate that exposure to sFlt1-induced preeclampsia alters protein levels associated with multiple biological functions in mothers even before such obvious characteristics such as high blood pressure or endothelial dysfunction manifest. We found that alterations occur not only in canonical pathways – well established and classically characterized signaling pathways, but also in network related pathways, which are representation of relationship between molecules.

Based on discovery nature of our experiments, further studies could be performed to establish biomarkers, which could identify mothers early in postpartum period at risk for developing CVD later in life. For example, one of the up-regulated molecules in our data set was apolipoprotein C-I (APOC1). The APOC1 is responsible for the activation of esterified lecithin cholesterol with an important role in the exchange of esterified cholesterol between lipoproteins and in removal of cholesterol from tissues. It is found to be increased in plasma of obese mice and humans.^{5,6} Moreover, there is evidence in the literature that this molecule is a predictive marker for CVD.⁷ Our mice were not obese, however, we have determined an increase in APOC1 in mothers exposed to sFlt1-overexpression-induced preeclampsia. The other possible biomarker was Apolipoprotein A-I (APOA1) – a major component of the high-density lipoprotein complex (“good cholesterol”) and helps to clear cholesterol from arteries. APOA-1 infusion reduces arterial cholesterol and myocardial lesions in a rat model of cardiac dysfunction and insulin resistance.⁸ APOA1 was downregulated in mothers after sFlt1-induced preeclampsia. Thus, our study is a first step to establish early biomarkers in mothers, whose pregnancy was complicated by preeclampsia.

Finally, our results revealed possible preventive applications. For example, we found that the top modified canonical pathway after exposure to sFlt1 was activation of LXR/RXR. The LXR/RXR is involved in the regulation of lipid metabolism, inflammation, and cholesterol to bile acid catabolism, and modulators of LXR are being considered for the treatment of CVDs.⁹ The molecules involved in LXR/RXR pathway include lipoproteins among others – HDL and LDL. It has been shown that gestational hypertension and preeclampsia were associated with lower HDL cholesterol 18 years after index pregnancy.¹⁰ Increased plasma levels of very low density lipoprotein and triglycerides had also been determined in former preeclamptic women.¹¹ Moreover, metabolic syndrome was more prevalent in women post preeclampsia.¹² Hence, again – our study by identifying altered biological functions and pathways is a first step in establishing prevention measures against CVDs in mothers after preeclampsia. Overall, we propose that treating preeclampsia, i.e. early implementing prevention, before the symptoms of CVD appear, could be of significant importance in preventing the later maternal disease.

A two-stage model has been suggested in the pathogenesis of preeclampsia: reduced placental perfusion followed by the maternal systemic manifestations.¹³ Several pathophysiological pathways have been implicated in the development of preeclampsia:

abnormal implantation,¹³ oxidative stress,¹⁴ inflammatory mechanisms,^{15;16} endothelial dysfunction,¹³ renin-angiotensin system,^{17;18} and activation of thrombosis.¹⁹ It is believed that inhibition of angiogenic factors or an angiogenic imbalance between pro-angiogenic factors and anti-angiogenic factors can result in preeclampsia.²⁰ Literature supports a role for abnormal angiogenesis in the development of preeclampsia.²¹⁻²³ In a prospective cohort, Levine and colleagues found that sFlt1 levels in women who develop preeclampsia rise above those in women who remain normotensive several weeks before the onset of the clinical syndrome.²⁴ High levels of circulating sFlt1 in early pregnancy are associated with development of preeclampsia weeks later.²¹⁻²³ The sFlt1 is the soluble form of the vascular endothelial growth factor (VEGF) receptor 1 which binds VEGF and other angiogenic factors in the circulation, thereby decreasing their action at the target organs. As further evidence for the role of dysregulation of angiogenesis in the causation of preeclampsia, animal models have been reported where a preeclampsia-like condition is induced with overexpression of sFlt1.²¹ Our study further confirms that animal models of sFlt1-overexpression-induced PE could be utilized to study long-term maternal outcomes.

Our data also are in line with epidemiological studies demonstrating associations between PE and metabolic syndrome later in life – greater insulin, proinsulin, triglycerides and lower HDL cholesterol.¹⁰ We found that HDL, LDL and lipoprotein metabolism molecules were highly associated with exposure to sFlt1-induced PE.

The association between PE and maternal long term health outcomes is well established in epidemiological studies. Investigations to determine if PE unmasks preexisting CVD risk factors or is a direct cause of CVD and other chronic diseases later in life are needed. Studies in humans to differentiate between these two pathways are not possible. Animal models are therefore critical to answer these questions. Our results suggest that some of the long term adverse outcomes associated with PE may be a consequence of hypertensive disorders during pregnancy.. Though a possibility that combination of predisposing factors and PE itself is responsible for long term maternal health could not be totally excluded. The results need to be evaluated with certain caution – sFlt1 is only one of the many factors involved in PE pathogenesis. However, if similar data will be obtained by analyzing plasma from former preeclamptic women , developing preventive strategies for preeclampsia should improve long term maternal health.

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CONDENSATION

In animal model exposure to sFlt1-induced preeclampsia alters multiple biological functions in mothers that persist later in life.

Figure 1. IPA function graph for top five functional categories. Each bar represents significantly increased or decreased molecules corresponding to that biological function.

Figure 2.

IPA function graph for top five diseases/disorders under Cardiovascular function. Each bar represents significantly increased or decreased molecules corresponding to that disease/disorder.

Figure 3. IPA function graph for top five functions under Protein Synthesis category. Each bar represents significantly increased or decreased molecules corresponding to that biological function.

Figure 4. IPA function graph for top five functions under Hematological System Development and Function category. Each bar represents significantly increased or decreased molecules corresponding to that biological function.

Figure 5. IPA function graph for top five functions under Organismal Functions category. Each bar represents significantly increased or decreased molecules corresponding to that biological function.

Figure 6. IPA function graph for top five functions under Tissue Morphology category. Each bar represents significantly increased or decreased molecules corresponding to that biological function.

Figure 7. IPA function graph for top five lower level biological functions. Each bar represents significantly increased or decreased molecules corresponding to that biological function.

Figure 8. IPA function graph for top canonical pathways. Each bar represents significantly increased or decreased molecules corresponding to that canonical pathway.

Figure 9.

IPA-mapped network graph containing molecules from Protein Synthesis, Cardiovascular Disease, and Lipid Metabolism functions. Molecules are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). The intensity of the node color indicates the degree of up- (red) or down- (green) regulation. Nodes are displayed using various shapes that represent the functional class of the gene product.

Shapes: $\triangle$ transporter; $\diamond$ peptidase; $\circ$ enzyme; $\square$ phosphatase; $\square$ growth factor; $\bigcirc$ transmembrane receptor; $\bigcirc$ other; $\bigcirc$ complex / group. Relationships: $\text{A} \text{---} \text{B}$ direct interaction; $\text{A} \text{---} \text{B}$ indirect interaction; $\text{A} \text{---} \text{B}$ binding only; $\text{A} \rightarrow \text{B}$ acts on.

Abbreviations: AGT - angiotensinogen (serpin peptidase inhibitor, clade A, member 8); APOA1 -apolipoprotein A-I; APOA2 - apolipoprotein A-II; APOA4 - apolipoprotein A-IV; APOB -apolipoprotein B (including Ag(x) antigen); APOC3 - apolipoprotein C-III; APOD - apolipoprotein D; APOE - apolipoprotein E; APOM - apolipoprotein M; C8A - complement component 8, alpha polypeptide; CRP - C-reactive protein, pentraxin-related; ECM1 (includes EG:100332249) - extracellular matrix protein 1; F2 - coagulation factor II (thrombin); F12 -coagulation factor XII (Hageman factor); FN1 (includes EG:100005469) - fibronectin 1; HBB -hemoglobin, beta; HBD (includes EG:15130) - hemoglobin, delta; HDL – high density lipoprotein; IL1RAP - interleukin 1 receptor accessory protein; LDL – low density lipoprotein; Mbl1 - mannose-binding lectin (protein A) 1; NFkB (complex) - nuclear factor-kappaB; PLG – plasminogen; PLTP - phospholipid transfer protein; PON1 - paraoxonase 1; SERPINA3 - serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3; SERPINC1 - serpin peptidase inhibitor, clade C (antithrombin), member 1; SERPINF1 - serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; SERPINF2 - serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2; Vegf – vascular endothelium growth factor; VLDL – very low density lipoprotein; VTN – vitronectin.

Table 1

Blood levels of sFlt-1, blood pressure (BP), and carotid artery responses to Ach in sFlt1 and mFc groups at 6 months postpartum.

Parameter	mFc group n=6	sFlt1 group n=5	P-value
sFlt1 (ng/mL)	1.03 ± 0.22	1.8 ± 0.6	0.4
Systolic BP (mmHg)	129.6 ± 3.7	138.3 ± 24.7	0.7
Diastolic BP (mmHg)	110.9 ± 7.3	115.5 ± 23.83	0.9
Ach AUC (arbitrary units)	330.0 ± 22.0	343.5 ± 28.4	0.7

AUC – area under the curve

Table 2

Some of the highly upregulated or downregulated molecules in plasma from mice exposed to sFlt1-overexpression during pregnancy in comparison to controls.

UPREGULATED		DOWNREGULATED	
Entrez Gene Name - Symbol	Fold Change	Entrez Gene Name - Symbol	Fold Change
Glycosylation dependent cell adhesion molecule 1 (pseudogene) - GLYCAM1	38.5	Complement component 9 - C9	-120.7
protein Z, vitamin K-dependent plasma glycoprotein - PROZ	36.0	Orosomucoid 1 - Orm1 (includes others)	-58.3
Phospholipase C, epsilon 1 - PLCE1	34.5	Immunoglobulin heavy constant gamma 3 - Ighg3	-32.10
Neurobeachin - NBEA	26.8	Transthyretin - TTR	-16.69
Coagulation factor X - F10	9.4	Immunoglobulin heavy constant gamma 2A - Ighg2a	-10.7
Apolipoprotein C-I-APOC1	3.8	Apolipoprotein A-I – APOA1	-3.2

Table 3

Networks identified by IPA analysis.

ID	Focus molecules	Top Functions
1	28	Protein Synthesis, Cardiovascular Disease, Lipid Metabolism
2	18	Gastrointestinal Disease, Hepatic System Disease, Liver Steatosis
3	18	Endocrine System Disorders, Gastrointestinal Disease, Hereditary Disorder
4	13	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry
5	11	Humoral Immune Response, Protein Synthesis, Inflammatory Response
6	7	Dermatological Diseases and Conditions, Hereditary Disorder, Immunological Disease
7	1	Hematological Disease, Cardiovascular Disease, Organismal Injury and Abnormalities
8	1	Connective Tissue Disorders, Immunological Disease, Inflammatory Disease