REVIEW ARTICLE

Placental biomarkers – A mechanistic review of their pathophysiology and their association with bronchopulmonary dysplasia

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ABSTRACT

Placental biomarkers have been studied extensively for their role in development and progression of preeclampsia. Two biomarkers in particular, soluble fms-like tyrosine kinase (sFlt-1) and placental growth factor (PlGF), are part of the angiogenic profile of preeclampsia and serve as a useful measure for placental dysfunction. Alterations in their level are responsible for the pathophysiology of preeclampsia, and their measurements can help predict and predate clinical signs and symptoms of preeclampsia, allowing for risk assessment and escalation in care. Given that these biomarkers measure placental dysfunction, it is reasonable to suspect that they may be useful in the prediction of other placentally derived disease processes, such as fetal growth restriction and adverse neonatal outcomes. Recent studies have shown that low plasma PlGF in maternal serum is more reliable in distinguishing fetal growth restriction secondary to placental dysfunction versus constitutionally small fetuses when compared to typical ultrasound indicators such as abdominal circumference and umbilical artery doppler studies. Altered PlGF has predicted adverse neonatal outcomes, such as development of bronchopulmonary dysplasia and even perinatal deaths. High cord blood levels of sFlt-1 have been shown to correlate with degree of neonatal thrombocytopenia, indicative of endothelial disruption in the newborn. In the era of personalized and precision medicine, placental biomarkers may assist in triaging fetal surveillance and delivery, as well as neonatal care. This review covers the known pathophysiology of these biomarkers, our current understanding of their signaling pathways and downstream effects and includes recent research in their association with a significant complication of prematurity - bronchopulmonary dysplasia.

Introduction

Placental biomarkers have been studied extensively for their role in development and progression of preeclampsia[1]. Two biomarkers in particular; soluble fms-like tyrosine kinase (sFlt-1), and placental growth factor (PlGF) are part of the angiogenic profile of preeclampsia and serve as a useful measure for placental dysfunction. Alterations in their level are responsible for the pathophysiology of preeclampsia, and their measurements can help predict and predate clinical signs and symptoms of preeclampsia, allowing for risk assessment and escalation in care[2]. First approved in Europe in 2009, and recently in the United States by the FDA, the biomarker ratio has found its way into clinical care as an adjunct to current testing for preeclampsia.

Given that these biomarkers measure placental dysfunction, it is reasonable to suspect that they may be useful in the prediction of other placentally derived disease processes such as fetal growth restriction (FGR) and adverse neonatal outcomes. Benton et al.[3] noted that low plasma PlGF (<5th percentile for gestational age) in maternal serum was more reliable in distinguishing FGR secondary to placental dysfunction versus constitutionally small fetuses when compared to typical ultrasound indicators such as abdominal circumference and umbilical artery doppler studies. Griffin et al.[4] in the UK utilized the 'PELICAN' cohort study to show low PlGF levels outperformed ultrasound and 46 other biomarkers for the prediction of delivery of a small for gestational age (SGA) newborn less than the 3rd percentile.

Regarding neonatal outcomes, Parchem et al. [5] as part of a secondary analysis of the PETRA (Preeclampsia Triage by Rapid Assay Trial) data noted that a low PlGF value was independently associated with an adverse neonatal outcome (aRR 17.2, 95% CI 5.2–56.3) with a sensitivity of 95.8% and a negative predictive value of 99.2%. All perinatal deaths in their study were predicted with a low PlGF.

While maternal biomarkers may have a role in predicting poor neonatal outcomes, they are not currently used to risk assess or triage delivery for suspected FGR. It is also unclear whether neonatal serum elevation of these markers after delivery has an impact on long term adverse outcomes. This review will focus on the pathophysiology of these particular biomarkers and their known interactions with the developing fetus and potential for downstream adverse effects, in particular bronchopulmonary dysplasia (BPD).

Pathophysiology

sFLT-1 and PlGF are involved in signaling via VEGF (Vascular Endothelial Growth Factor) and its receptor family, which play an essential role in angiogenesis and vascular maturation. Null mutations of VEGF receptors are known to be embryonically lethal[6,7]. The balance of pro (PlGF) and anti-angiogenesis (sFLT-1) factors are essential for normal vascular development and tissue growth, with alterations in their balance responsible for dysregulation and a host of pathologies including cancer and preeclampsia[8].

Shibuya[7] first described the VEGF receptor-1 (VEGFR-1) in 1990, designating it as Fms-like tyrosine kinase-1 (Flt-1) due to similarities with a previously known tyrosine kinase receptor. It was later found that VEGF as a ligand bound to and activated Flt-1[9], solidifying its function in angiogenesis regulation. Further research has shown that the VEGF system has three receptors (VEGFR 1,2 and 3), with VEGFR-1 and 2 present in vascular endothelial cells and VEGFR-3 seen primarily in the lymphatic endothelium[8]. VEGFR-2 also known as Flk-1 and KDR in older studies is shown to have much lower affinity for VEGF but significantly higher biologic activity[10] (see Figure 1).

PlGF is a member of the VEGF family and while primarily expressed by the human placenta[11] is also seen in other microvascular environments[12]. PlGF is a homolog of VEGF sharing 44% of the amino acid sequence and has significant structural similarity to VEGF[13] allowing it to bind to VEGFR-1, but not to VEGFR-2. PlGF influences endothelial cell chemotaxis and proliferation and potentiates the pro-angiogenic effects of VEGF (possibly by displacing

VEGF from VEGFR-1 and allowing it to activate VEGFR-2)[10]. PlGF is produced by the placental villous cytotrophoblasts and synctiotrophoblasts, with maternal serum levels peaking around 30 weeks gestation in normal pregnancies[14]. It appears to play a vital role in placental vasculogenesis and angiogenesis and its production has been shown to correlate with oxygen tension, with PlGF expression significantly down-regulated by hypoxia[15,16].
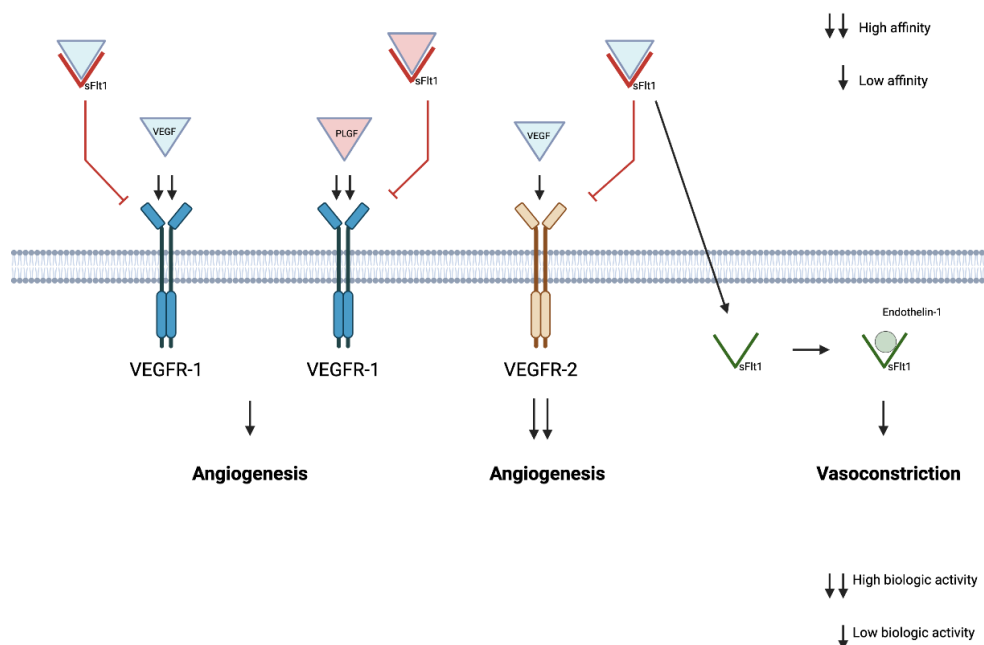

Figure 1 – VEGF – Vascular Endothelial Growth Factor, VEGFR-1 (Flt-1), sFLt-1 (soluble version of Flt-1) , VEGFR-2 (Flk1, KDR). PLGF can bind to VEGFR-1 but not to VEGFR-2, and likely modulates VEGF activity by displacing it from VEGFR-1. sFLT-1 binds to circulating VEGF and PLGF blocking its ligand activity.

Conversely sFLT-1 expression is increased with hypoxia, and is a major anti-angiogenic factor produced by the placenta during pregnancy, especially in the setting of preeclampsia[1,8,17,18]. The Flt-1 gene is known to encode for two mRNA's – the full length transmembrane receptor (Flt1, VEGFR-1) and a truncated version that lacks the cell membrane anchoring domain and the intracellular tyrosine kinase[19,20] (see Figure 2 and Figure 3). This soluble version of the Flt-1 receptor (sFlt-1) acts a decoy molecule by binding to VEGF and PLGF with high affinity and acting as a competitive inhibitor. It also binds directly with VEGFR-1 and 2 and acts as a potent inhibitor of angiogenesis in vivo[8,19,20]. sFLT-1 is primarily produced by the trophoblast and its expression is significantly upregulated in hypoxic conditions, possibly via the hypoxia-inducible factor (HIF) 1[21]. It has also been shown to be expressed in vascular endothelium and in monocytes[19]. Because it lacks anchoring domains to the cell membrane it is free to circulate and is known to induce endothelial dysfunction by adhering to and antagonizing VEGF and PlGF receptors altering angiogenesis and vascular maturation[1]. (see figure 1)

sFLT-1 levels are known to be elevated in healthy pregnancies compared to non-pregnant controls (mean serum levels of 1762 vs. 85 pg/mL)[22]. In normal pregnancy, sFLT-1 levels remain fairly stable till 33 to 36 weeks gestation where they begin to rise by ~145 pg/mL per week till delivery. However in pregnancies complicated with preeclampsia the levels are noted to steeply increase about five weeks prior to overt symptomatology, and are markedly elevated compared to non-preeclamptic controls (8150 vs. 2467 pg/mL)[18]. Conversely PlGF concentrations rise during early pregnancy, peaking at ~32 weeks and decrease nearing term gestation. Patients who developed preeclampsia had decreased levels of PlGF as early as 13 weeks compared to controls

and remained lower at time of delivery (73 vs. 370 pg/mL). VEGF levels did not starkly vary with gestational age, but did show reduction in preeclamptic patients[18].
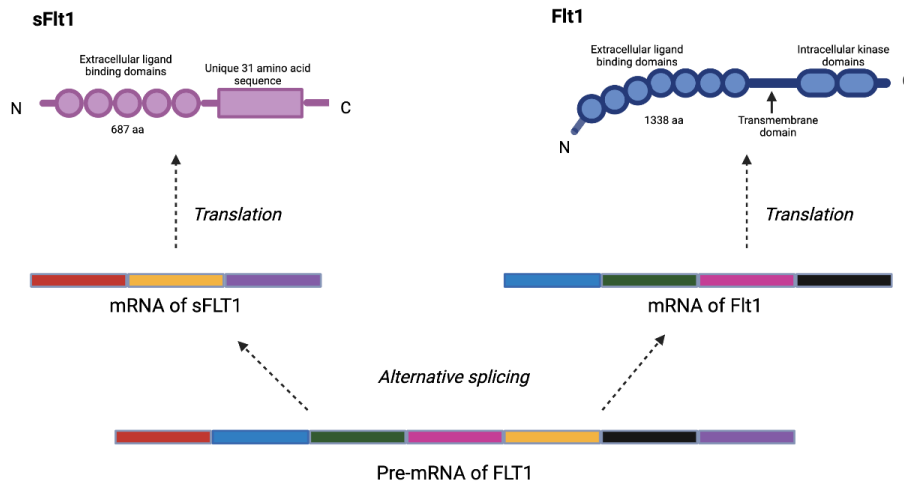

Figure 2 - -alternative splicing of Flt-1 mRNA producing the soluble fms-like tyrosine kinase (sFLT-1)
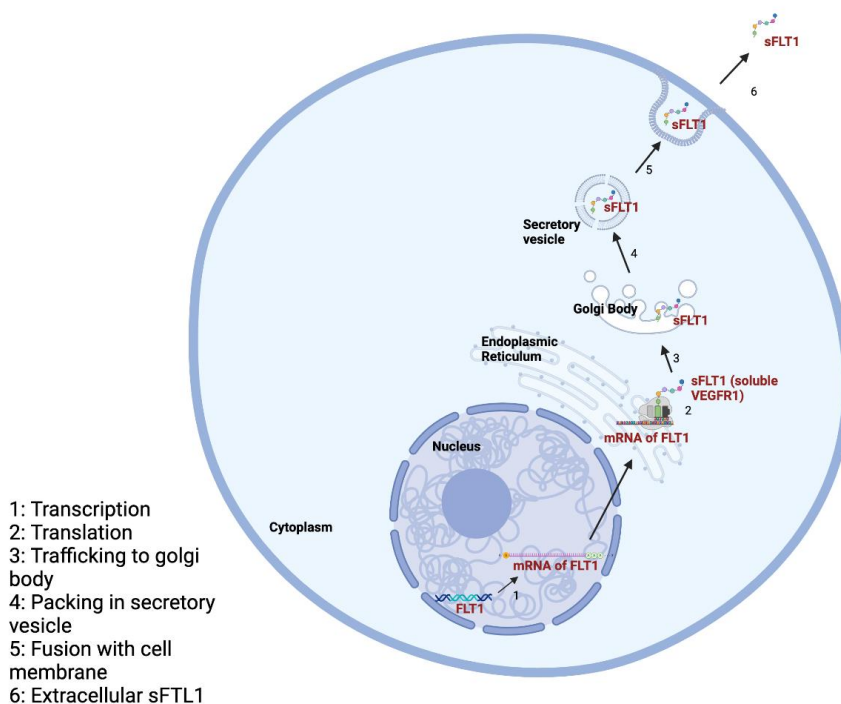

Figure 3 – cellular trafficking of sFLT-1

Biomarkers and Bronchopulmonary Dysplasia

Bronchopulmonary dysplasia (BPD) is a common complication of prematurity, with significant neonatal impact and healthcare costs. Characterized as a chronic lung disorder secondary to abnormal alveolarization and pulmonary vascular development, it affects almost 50% of extremely preterm newborns born at less than 28 weeks gestational age with long term respiratory and neurodevelopmental impairment[23,24]. Infants with BPD suffer from long term respiratory insufficiency, pulmonary hypertension, exercise intolerance, frequent lung infections and recurrent hospitalization[25].

Multiple epidemiologic studies have noted an increased risk of BPD in preterm newborns born to preeclamptic mothers[26–28]. The underlying hypothesis being that preeclampsia is a known anti-angiogenic state, and that lung development similar to vascular formation requires an appropriate balance of pro and anti-angiogenic factors[27,29].

BPD is characterized by aberrant lung development, with histological studies noting a pattern of decreased alveolarization (fewer and larger alveoli with fewer septae), decreased capillary density and overall dysmorphic pulmonary vasculature[25,29]. VEGF is known to be expressed in the distal airspace and it's signaling effects are essential to develop the alveolar structure of the lungs[30–34]. Given the proximity of the airways and blood vessels in the embryonic lung, it is hypothesized that angiogenesis plays an important role in normal alveolarization, with VEGF intricately linked to this process[35,36].

Kasahara[30] noted that chronic blockade of VEGF receptors induced apoptosis in alveolar cells, and emphysema in a rat model, highlighting the importance of the VEGF signaling pathway in normal airway development and maintenance. Hasan et al[37]. noted low levels of VEGF along with elevated levels of sFLT-1 in the tracheal aspirate of preterm infants that developed BPD, concluding that their levels could be utilized as biological predictors for the development of this disorder. Bhatt[38] showed disrupted pulmonary vasculature and decreased VEGF in newborns dying from BPD concluding that impaired angiogenesis likely contributed to their pathology. Thebaud et al. not only showed that VEGF blockade in newborn rats impaired alveolar development mimicking BPD, but that postnatal intra-tracheal VEGF gene therapy (to induce overexpression of VEGF) was able to preserve and restore normal alveolarization[29].

Utilizing umbilical cord blood, Yang et al.[23] noted that <u>elevated</u> PlGF levels could be used as a predictor for development of bronchopulmonary dysplasia (BPD) in preterm infants. The mechanism of this is unclear at this time, but in a prior paper they also showed that PlGF overexpression caused emphysematous change in a mice model, and that PlGF could inhibit proliferation of pulmonary Type II epithelial cells in vitro [39]. As previously noted, PlGF is known to modulate VEGF activity by displacing VEGF from VEGFR-1 allowing more binding of VEGFR-2, and there is some initial research noting a larger role for PlGF in the intra molecular receptor cross talk[40].

To study the possible mechanistic impact of maternal preeclampsia on fetal lung development, Tang et al[25]. Injected sFLT-1 versus saline into the amniotic sac of pregnant Sprague-Dawley rats during the late canalicular and early saccular stage of their fetal lung development (gestational age 20 days, term being 22 days). They noted that the rats injected with intraamniotic sFLT-1 showed decreased alveolar number and reduced pulmonary vessel density consistent with BPD and also evidence of right and left ventricular hypertrophy. They hypothesized that in-utero exposure to high amniotic fluid levels of sFLT-1 secondary to maternal preeclampsia increases the risk of abnormal lung development by impeding VEGF signaling.

Conclusion

In summary, there is now abundant evidence that proper angiogenesis is crucial to early lung development with the VEGF signaling pathway playing an important role. Given the known interaction of sFLT-1 and PlGF with VEGF/VEGF-R, it is reasonable to expect alterations in their levels to influence neonatal adverse outcomes. Maternal and cord blood levels of these biomarkers have shown predictive value for the development of maternal and neonatal adverse outcomes[14,23,41–45], but further research is needed to evaluate if these biomarkers persist in neonatal bood and quantify their complex downstream effects.

The possible future impact of this research is that maternal serum level of these biomarkers may influence optimal delivery timing, especially in pregnancies complicated by preeclampsia or fetal growth restriction. Given the potential deleterious effects of abnormal placental signaling on the

developing fetus and neonate, earlier delivery may be protective in selected cases. Therapeutic interventions to mitigate adverse perinatal outcomes are also being studied. Early research in extracorporeal apheresis to reduce circulating sFLT-1 in pregnant patients affected with preeclampsia has been promising[46]. A study by McLaughlin et al. found the addition of low molecular weight heparin in the early second trimester could correct low circulating PlGF levels[47]. As an example of the future potential of precision medicine, Turanov et al. developed a short interfering RNA (siRNA) sequence that selectively silenced sFLT-1 mRNA in a preeclamptic animal model prolonging the pregnancy without adverse effects to the developing fetus[48].

While these above approaches are groundbreaking, the described molecular pathways of placental dysfunction remain incomplete, and further ongoing research is needed. A better understanding of the complex interplay between placenta, fetus and eventual neonate will help elucidate more targeted therapeutics and optimize clinical management of these serious maternal and neonatal conditions.

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