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The opposing roles of anti-angiogenic factors in cancer and preeclampsia

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1. ABSTRACT

Hypertensive disorders of pregnancy such as preeclampsia present an increasing source of concern during gestation and accumulating evidence suggests there are long-term effects on the subsequent health of the mother and child. While formerly preeclamptic women have increased risk for later cardiovascular disease, they appear to have decreased risk of some cancers. Recent investigations have revealed exciting insights into potential mechanisms underlying the pathogenesis of preeclampsia and some of these findings may bear relevance to the attenuated cancer risk reported in the literature. Placental ischemia, regarded as a primary initiating factor in preeclampsia, results in elevated levels of factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin/CD105 (sEng) that generate profound effects on the vascular endothelium and cardiovascular function, Further, these factors may also influence development of susceptible organs such as the mammary. Moreover, recent evidence suggests these molecules may be regulated by factors derived from cigarette smoke. Taken together, elucidating mechanisms linking placental ischemia, endothelial function and subsequent cancer risk is an important step towards identifying novel therapies for cancer.

2. INTRODUCTION

A relationship between occurrences during pregnancy and subsequent incidence of several types of diseases in later life has been recognized for a number of years (1-19). Specifically, cardiovascular disease, metabolic disorders and several forms of cancer, such as breast and ovarian, seem to be influenced by events during pregnancy (1-4,6,8-10,12,14,15,20-23). Moreover, recent studies have shown both mother and offspring may be influenced by these pregnancy associated risks and benefits related to the incidence of cancer (2,10,22,24,25). While it appears pregnancy may impart a short-term increase in breast cancer, protective long-term effects are found in women with first parity before 30 years of age, and full term parity after 30 years is associated with increased breast cancer risk (26-28). In addition, multiparity results in decreased breast cancer risk regardless of age (29). Likewise, the protective effects of pregnancy towards breast cancer development are also well-established in rodent models (30).

While pregnancy has long been associated with reduced incidence of mammary cancer (26-28), only recently has a relationship between pregnancy complications such as preeclampsia and cancer incidence been reported (2-4,9,20,22,23). Although studies largely

support a protective effect of preeclampsia with respect to incidence of cancer, it should be noted there have been several dissenting reports (13,31-33). Reports indicating breast cancer is increased in preeclamptic women are not without limitations and include marginal statistical significance as well as a homogenous population that is predisposed to well-known genetic markers for cancer (31,33). Further, the meta-analysis by Bellamy *et al.* did not include an exhaustive review of the literature showing a reduction of breast cancer following preeclampsia (13).

There are likely a variety of factors underlying the range of observations regarding the relationship between preeclampsia and breast cancer, including age, genetic predispositions of specific populations, study inclusion and exclusion criteria, the nature of the control groups employed, early- versus late-onset preeclampsia, and the inability to control for numerous environmental factors that may influence cancer outcomes beyond that observed with a preeclamptic pregnancy. Hence, a difficulty in interpreting these previous studies is teasing out the respective roles of underlying genetic factors that may be related to both cancer risk and preeclampsia vis-à-vis factors that are produced subsequent to placental dysfunction observed in preeclamptic pregnancies. Moreover, the notion that factors from the ischemic placenta may play prominent roles in sequelae following complicated pregnancies has become especially intriguing considering the increasing evidence supporting the role of anti-angiogenic factors in the pathogenesis of preeclampsia (14,34-40).

The female endocrine milieu undergoes considerable changes during pregnancy, with alterations in a wide variety of circulating factors evident across gestation. Some of these changes, such as an increase in soluble fms-like tyrosine kinase-1 (sFlt-1) and decreases in placental growth factor (PlGF) and vascular endothelial growth factor (VEGF) during the latter half of pregnancy, are exaggerated during preeclampsia (41). Since pregnancy is an important period for the final phase of mammary gland development, gaining a better understanding of the mechanisms underlying the role of PlGF and VEGF in mammary cell differentiation and development may provide insights regarding the association between preeclampsia and cancer risk. Recent work has identified numerous circulating factors that are increased during a preeclamptic pregnancy compared to normal pregnancy, many of which derive from the poorly perfused ischemic placenta (35-40). While several of these factors (sFlt-1, VEGF, PlGF) are increased in the pregnant compared to the nonpregnant state, only sFlt-1 is typically increased further in preeclampsia (38). In contrast, free VEGF and PlGF are decreased in preeclamptic women despite increased total VEGF and PlGF (38). Elucidating the mechanisms by which factors elaborated by the ischemic placenta appear to inhibit cancer growth and development is an important endeavor towards developing innovative approaches, diagnostic tools and new therapeutic regimens for cancer patients. Although a variety of factors including genetic, immunological, behavioral, and environmental influences have been implicated in the pathogenesis of preeclampsia (42), placental ischemia remains a central feature of the syndrome (14).

The primary goal of this review is to contrast the opposing roles of angiogenic and related factors that are associated with both preeclampsia and cancer. To this end, we aim to summarize the putative mechanisms by which these molecules may alter endothelial function and mammary cell differentiation during a period of significant development and influence the progression of preeclampsia and later incidence of breast cancer.

3. CLINICAL SIGNIFICANCE OF PREECLAMPSIA

Preeclampsia is a pregnancy specific syndrome that affects approximately 5-8% of all pregnancies and is characterized by new-onset hypertension and proteinuria usually presenting after 20 weeks of gestation (42). While the past several decades have seen a rise of nearly 40% in the incidence of preeclampsia, this is largely thought to be due to increases in the number of higher order pregnancies (multiple births), and the age at onset of pregnancy and rate of obesity (43). The preeclamptic syndrome may progress to a point which culminates with injury to the blood vessels of major organs such as the liver and the brain as well as to the glomeruli of the kidney. In addition, the only known cure for preeclampsia is delivery, after which symptoms typically resolve within 48–72 hours. Consequently, the preeclamptic syndrome remains a considerable obstetric problem and a significant source of maternal and neonatal morbidity and mortality (42). Despite the recent attention paid to the preeclamptic syndrome and the efforts to characterize the suite of contributing factors (14,42,44), the underlying mechanisms of this condition remain unclear.

3.1. Risk factors for preeclampsia

Most women who develop preeclampsia do not have clinical risk factors that can be readily identified, but about 1/3 will (45). The two most significant risk factors for developing preeclampsia are personal history of preeclampsia and having antiphospholipid antibodies. History of preeclampsia increases the risk by seven times that of an age, parity, ethnicity matched pregnant control. Interestingly, while having antiphospholipid antibodies (anticardiolipin, antibodies and/or lupus anticoagulant) increases the risk, women with preeclampsia were no more likely to have these antibodies than their matched counterparts without preeclampsia. Pre-existing insulin dependent diabetes and a pre-pregnancy body mass index (BMI) over 35 quadruple the risk for developing preeclampsia. The risk of preeclampsia triples with nulliparity, family history of preeclampsia, and multiple pregnancy while a BMI $\geq$ 35 when presenting to the clinic for the first obstetric visit, maternal age over 40, and pre-existing vascular disease (such as hypertension) double the risk (46). Younger age (teenage) and race have also been indicated as risk factors for preeclampsia as women of African American descent possess the highest ethnicity risk. *In vitro* fertilization also is a risk factor for preeclampsia, possibly due to the formation of the chorion while *in vitro*. The risk is even higher in women using donor eggs for *in vitro* fertilization (45).

Although several factors have been suggested to be protective against the development of preeclampsia, the data is less conclusive regarding these protective factors and many are difficult to sort out independently given other maternal characteristics. Physical activity during pregnancies is regarded to be protective against development of preeclampsia (47,48), however this relationship is strongest when BMI is less than 30. The beneficial role of physical activity on blood pressure during a hypertensive pregnancy has also been recently reported in two different animal models of preeclampsia (49,50). Indeed, both the animal studies and studies in women have reported a beneficial effect of exercise during pregnancy on angiogenic balance during pregnancy (47,49-51). How this relates to subsequent incidence of cancer and cardiovascular disease remains to be seen. While diet also may play a role, whether this is due to the effects of altering BMI is not clear. One study has reported chocolate intake may decrease the risk for preeclampsia, however the manner in which this may occur remains unclear (52). The effects of cigarette smoking on contributing to preeclampsia are currently under investigation, but early studies have shown that cigarette smoking may actually lower the risk of developing preeclampsia, however these protective effects are not seen in women with chronic hypertension, in these women with chronic hypertension, cigarette smoking is an independent risk factor for developing preeclampsia (53).

3.2. Risk factors for cancer

Risk factors for uterine cancer include age greater than 50, family history, and the largest risk being estrogen exposure (54). Estrogen exposure includes nulliparity or low parity (pregnancy provides higher progesterone to estrogen ratios), anovulation (decreased progesterone to estrogen levels), late menopause (increased time of exposure to estrogens), diabetes mellitus (activation of the insulin-like growth factor pathway and impaired regulation of endogenous sex hormones), Tamoxifen use or history of unopposed (no progesterone) exogenous estrogen use. Obesity is also implicated as a risk factor for developing uterine cancer. A woman who is greater than 50lbs overweight increases her risk of endometrial cancer 10 times the relative risk (55). This is presumably due to the increased sex hormone production by adipose cells. Using birth control pills, maintaining a healthy weight and using progesterone with estrogen replacement decreases the risk of uterine cancer (55).

Although most women who get ovarian cancer do not have many risk factors, there are a few worth mentioning. Age is the largest risk factor for ovarian cancer, about 90% of women who get ovarian cancer are over the age of 40 (56). Other risk factors include family history of ovarian cancer, personal history of breast, colon or uterine cancer, being of Eastern European (Ashkenazi) Jewish background, nulliparity or low parity, and having endometriosis (55,57). In addition, BRCA1/BRCA2 gene mutations are also associated with increased risk of ovarian and breast cancer (58).

Of the three mostly female dependent cancers, ovarian, breast and endometrial, breast cancer is the most studied and publicized, and for a good reason. Breast cancer remains the most common invasive cancer in women worldwide and is the leading cause of death of women in mid-life (59). The American Cancer Society estimated that over 200,000 new cases of breast cancer were diagnosed in 2010 (60). This accounts for 28% of all new cancer cases and 15% of cancer deaths. Uterine cancer accounts for 6% of new cases with 3% of cancer deaths while ovarian accounts for 3% of new cases and 5% of cancer deaths (60). As will be discussed further in this review, many of the risk factors that contribute to these female cancers, estrogen exposure, advanced age, lack of physical activity and obesity, are also on the rise.

The primary risk factors for breast cancer are biological sex and age. Indeed, a 45 year old woman is 35% more likely to die of breast cancer than ischemic heart disease (61). About 7/100 women will get breast cancer by the age of 70 (55,62). Other risk factors that slightly increase the risk of developing breast cancer include longer exposure to estrogens as seen in early age of menarche and later age of menopause, nulliparity, older age for first pregnancy, personal history of breast cancer or non-cancerous breast disease, family history of breast cancer, radiation to the breast or chest wall, being overweight, low physical activity level, long term use of estrogen/progesterone hormone replacement therapy, using oral contraceptives, and drinking more than one drink of alcohol per day (63). Although the majority of women who develop breast cancer will not have one of these risk factors, the cumulative risk for breast or ovarian cancer in female carriers of BRCA1/BRCA2 gene mutations is estimated to be greater than 80% by the age of 70 (58,64). The only protective factors against breast cancer seem to be increased level of activity, healthy body weight, breast feeding, and in the case of gene mutations bilateral mastectomies. Review of dietary modifications and environmental exposures are inconclusive at this time (61).

Lung cancer is the 2nd most common form of cancer behind breast cancer for women and prostate cancer for men (60). Over 200,000 new cases of lung cancer were estimated in the year of 2010 along with over 150,000 estimated deaths from lung and bronchus cancer (60). The most obvious and prevalent carcinogen leading to lung cancer is tobacco smoke. Almost 80% of lung cancer deaths in women and 90% in men are due to smoking, the Center for Disease Control place cigarette smoking as the number one risk factor for lung cancer. While contributing greatly to lung and bronchus cancers, smoking is also linked to larynx, esophageal, bladder, kidney, pancreatic, cervical, gastric, and blood (leukemia) cancers (65). Other carcinogens leading to lung cancer include radon, asbestos, arsenic, silica and chromium.

There are conflicting and inconsistent reports regarding the effects preeclampsia may have on the prevalence of cancer after the preeclamptic pregnancy. One case control study from the Medical Birth Registry and the Cancer Registry in Norway, showed a 19% decreased risk for breast cancer in women who were diagnosed with preeclampsia and/or hypertension in their first pregnancy (66) while another study cohort in Jerusalem showed an

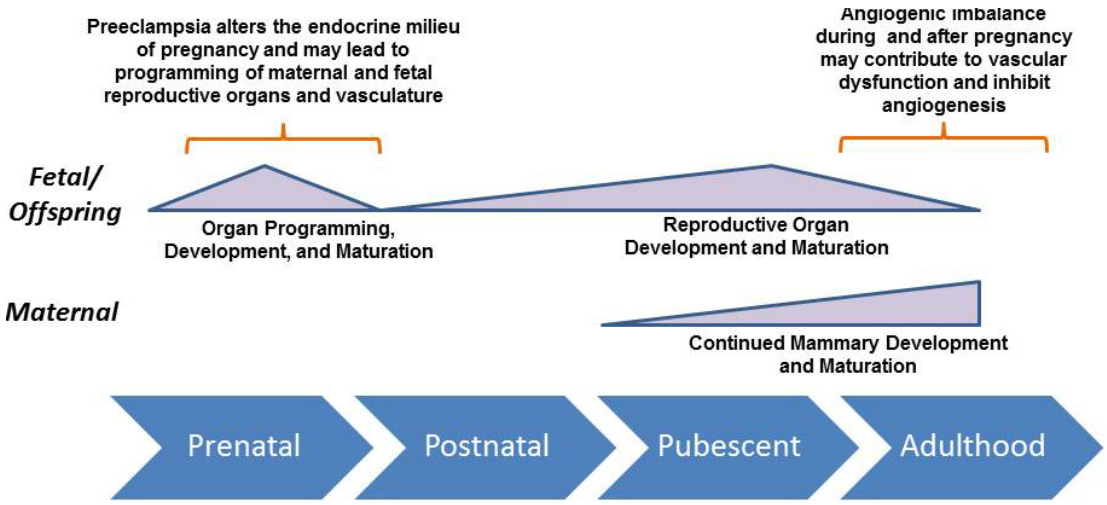

Figure 1. Maternal and fetal organ development and maturation can be influenced by alterations in the endocrine milieu at different discrete periods of fetal and post-natal life. These critical windows for programming later cell function occur during fetal life, pubescence and during pregnancy. Alterations in maternal physiology due to pregnancy itself or the pregnancy environment due to factors such as preeclampsia or smoking may impact mammary development or vascular health in later life.

overall increased incidence of cancer as well as site specific cancers such as ovarian, breast and stomach following a preeclamptic pregnancy (67). Lastly, while preeclampsia has been linked to a lower lifetime risk of breast cancer in the female offspring from preeclamptic births (4), further studies are need to verify this observation.

4. RELATIONSHIPS BETWEEN PREECLAMPSIA AND LATER DISEASE

4.1. Cardiovascular diseases

The relationship observed between events during pregnancy and disease incidence in later life falls into one of two categories: 1) alterations in maternal physiology (such as those associated with preeclampsia) that associate with disease risk, and 2) perturbations of fetal development that have long term consequences for the offspring. These are summarized in Figure 1. Previous studies support the idea that women who have had a preeclamptic pregnancy are more likely to have elevations in markers of inflammation such as C-reactive protein (CRP), dyslipoproteinemia, and are at increased risk for end stage renal disease and cardiovascular disease (5,7,11,13,14,16). While elevations in factors such as CRP suggest the presence of a persistent mild inflammatory state in formerly preeclamptic women, the mechanisms by which this occurs remain unclear. Further, recent work also suggests that, not only are alterations in circulating factors and inflammatory markers (e.g. sFlt-1, sEng, cytokines) likely to be responsible for much of the preeclamptic syndrome, they may also predispose the maternal cardiovascular system to subsequent endothelial dysfunction as the mother ages (5,7,11,13,16,68-71).

4.2. Cancers

A growing body of epidemiological evidence indicates that a preeclamptic pregnancy provides a protective effect against development of several types of cancer, including breast cancer, in post-partum gravidas and their offspring in later life (2,4,9,20,72-75). While the vast majority of epidemiological studies report a decreased risk of cancer (2,4,9,20,72-75), it should be noted that there have been several studies that have described no such associations between preeclampsia and cancer incidence (13,31,32). Other reproductive factors such as placental size and function, age at first pregnancy and alterations in a variety of gestational hormones have all been proposed as possible factors (9,10,32,76). While the exact links between preeclampsia and reduced risk of breast cancer remains unknown, it is also unclear if genetics, environmental factors, or something specific to preeclampsia such as placental ischemia plays a prominent role in these findings. In addition, the timing of preeclampsia whether early- or late- onset, may play a role, especially since early-onset preeclampsia is more often associated with placental ischemia and fetal growth restriction when compared to late-onset preeclampsia. Moreover, the duration of exposure to inflammatory cytokines is much longer in the early-onset *vs.* late-onset preeclampsia patients. Taken together, the endocrine milieu that occurs during preeclampsia may influence subsequent breast cancer risk.

An alluring hypothesis that derives from these findings speculates that factors such as these may interact with differentiating mammary gland cells during pregnancy and alter their phenotype towards a less cancerous lineage. In support of such a hypothesis, we have recently observed that serum from pregnant rats with placental ischemia and preeclamptic-like symptoms inhibit proliferation of several human and murine breast cancer cell lines *in vitro* (19,77-79). In addition, pharmacological inhibitors of VEGF and PlGF are reported to reduce cancer cell proliferation and tumor growth *in vivo* (80) and *in vitro* (81). Nevertheless, further studies are needed to identify the exact connections

between cancer cell proliferation and preeclampsia. We have proposed that factors associated with placental ischemia and preeclampsia may contribute to altered risk of breast cancer in the mother and in the offspring. Alterations in the trajectory of fetal development have long been associated with increased risk for cardio-renal and metabolic disorders (82,83), however, the fetal origins hypothesis has recently been extended to later development of cancer (84-86). Indeed, we have recently observed that rat offspring from hypertensive pregnancies due to placental ischemia appear to be much more susceptible to the development of mammary tumors following exposure to the carcinogen NMU at 21 days of age (87).

The protective effect of pregnancy regarding incidence of breast cancer may occur through alterations in the process of differentiation and changes within the epithelial cell population during the development of the mammary gland. It is thought that exposure to a variety of circulating factors that are either pregnancy or preeclampsia specific may underlie this process. Pregnancy induces differentiation of mammary terminal end buds which in turn may reduce susceptibility to later tumor development. Moreover, the condition of preeclampsia is particularly interesting as it encompasses changes during an especially important time of mammary gland development during pregnancy where differentiation and changes within epithelial and stromal populations of cells are occurring in adulthood. Hence, studying the developmental windows illustrated in Figure 1 and identifying factors that may reduce breast cancer risk by regulating the differentiation and proliferation of mammary progenitor cell populations could provide excellent targets for preventative therapies.

4.3. Factors linking preeclampsia and cancer risk

Although the pathophysiology of preeclampsia remains unclear, placental ischemia/hypoxia is widely regarded as a key factor (88,89). The initiating event in preeclampsia is thought to be reduced placental perfusion, which in turn leads to secretion of circulating factors that lead to widespread dysfunction of the maternal vascular endothelium. Inadequate trophoblast invasion leading to incomplete remodeling of the uterine spiral arteries is considered to be a primary cause of the placental ischemia (89). Hence, a poorly perfused and hypoxic placenta is thought to synthesize and release increased amounts of factors such as sFlt-1 and sEng (35,37,38,40). Figures 2 and 3 illustrate pathways in which circulating factors associated with preeclampsia or placental ischemia that induce widespread activation/dysfunction of the maternal vascular endothelium may also afford protection from subsequent development of mammary cancer by altering neoplastic stem cell differentiation in the mother during pregnancy. Alternatively, if angiogenic imbalance is present in the fetal compartment these same effects may occur *in utero* as well. Furthermore, since the hypertension and increased levels of circulating factors associated with preeclampsia largely remit after delivery, it appears likely that the placenta plays a major role in these observations. Interestingly, Wolf *et al.* have shown that sFlt-1 is significantly, albeit moderately elevated up to one year post-partum (71). Hence, persistent mild elevations of factors such as sFlt-1 may contribute to inhibition of tumor development in later life.

Although several lines of evidence support the hypothesis that in preeclampsia the ischemic placenta contributes to endothelial cell dysfunction in the maternal vasculature by excess production of anti-angiogenic factors, the mechanisms by which factors such as sFlt-1 and sEng are increased by the ischemic placenta remain unclear. Moreover, further studies are necessary to determine if these factors contribute to protection from subsequent development of cancer afforded to women and offspring that have endured the preeclamptic syndrome.

While the maternal vascular endothelium appears to be the primary target of factors that are secreted in response to placental ischemia/hypoxia, it remains unknown which target cells are important in the cancer inhibition afforded by preeclampsia. Moreover, it remains unclear which factor(s) are responsible for the epidemiological observations. Interestingly, the observations that a variety of markers for endothelial dysfunction appear to correlate well with the occurrence of preeclampsia, also fit well with relationships to decreased breast cancer incidence. While there are a number of other factors ranging from genetics to lifestyle factors that may influence incidence of both cancer and preeclampsia, the following sections will focus on the roles of a variety of factors that are secreted or affected by the ischemic placenta and may mediate either directly or indirectly the altered susceptibility to cancer in preeclamptic women.

5. THE ROLES OF ANGIOGENIC FACTORS IN PREECLAMPSIA

5.1. HO-1 in preeclampsia

Inducible heme oxygenase (HO-1), one of the three isoforms of heme oxygenase, is an enzyme responsible for catalyzing the degradation of heme into carbon monoxide (CO), ferrous iron, and biliverdin, quickly converted by biliverdin reductase to bilirubin. HO-1 is mostly expressed in the liver and spleen, but also found in vascular endothelium, smooth muscle cells, and many other tissues. Also known as heat-shock protein (Hsp) 32, HO-1 is induced by a variety of factors including cellular stress, its substrate heme, or hypoxia depending upon the tissue and species (90-92). HO-1, often coined a stress-responsive protein, is involved in several protective actions including anti-apoptotic responses, attenuated inflammatory response, and protection against oxidative stress and vasoconstriction (93-98).

The breakdown products of heme have also been shown to have effective cytoprotective properties. Although capable of cellular damage, free iron, along with the interactions of HO, generates ferritin which serves as an antioxidant (99). Biliverdin and its breakdown product, bilirubin, also act as strong antioxidants (100), as well as being active in the reduction of inflammatory response (101) and the prevention of cellular necrosis (102). CO, a cellular messenger and the subject of much research, has been shown to act as an anti-inflammatory substance,

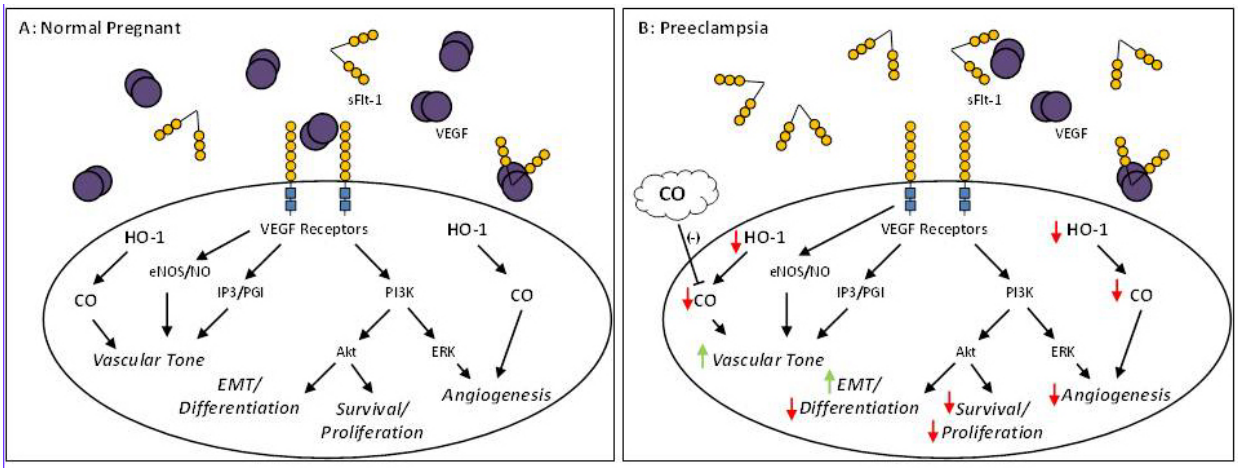

Figure 2. Cellular function and/or fate may be affected by the angiogenic balance between vascular endothelial growth factor (VEGF) and its receptor, soluble Fms-like tyrosine kinase-1 (sFlt-1) during normal pregnancy (panel A), and preeclampsia (panel B). During normal pregnancy (panel A), the existence of a pro-angiogenic balance (VEGF/PlGF > sFlt-1) favors the maintenance of proper vascular tone and endothelial function. During preeclampsia the existence of an angiogenic imbalance (VEGF/PlGF < sFlt-1) favors endothelial dysfunction and the development of manifestations of the preeclamptic syndrome. Alterations of angiogenic balance may also affect differentiation of pluripotent mammary and endothelial cells and subsequent function of these cell types. Induction of heme oxygenase-1 (HO-1) and cigarette smoke may increase factors such as carbon monoxide (CO) and influence VEGF/PlGF signaling and vascular tone.

reduce cellular apoptosis, and regulate vascular smooth muscle cell proliferation (103-105). HO-1 and its breakdown products, especially CO, are also believed to play important roles in pregnancy, particularly in the pathogenesis of preeclampsia, as well as cancer. Figure 2 illustrates how CO may play a role in altering vascular tone during pregnancy and preeclampsia.

Although we and others have reported decreased expression of HO-1 in the placenta during preeclampsia (36,98,106), not all studies are in agreement (107,108). Moreover, whether reduced expression of HO-1 contributes to the oxidative stress observed in preeclampsia remains unclear. A recent study has also shown the induction of HO-1 results in the attenuation of placental ischemia-induced hypertension and normalizes the angiogenic balance of sFlt-1 to VEGF (109), two molecules that play important roles in the pathophysiology of preeclampsia. Other studies have shown that the HO pathway inhibits the release of sFlt-1 as well as soluble endoglin (sEng) (110,111) and suggest that HO-1 may be an important regulator of various factors believed to contribute to the development of preeclampsia and have therapeutic potential for this disorder.

5.2. VEGF/sFlt-1

Perhaps the most prominent molecule proposed to play a key role in the pathogenesis of preeclampsia is sFlt-1. Considerable clinical evidence has accumulated indicating that preeclampsia is strongly linked to an imbalance between pro-angiogenic PlGF and VEGF and anti-angiogenic (sFlt-1) factors in the maternal circulation (38,112-118). Although VEGF and PlGF are widely recognized for potent angiogenic and mitogenic effects, both have also been recognized as important contributors to cell homeostasis, in particular with respect to the balance of oxidative stress (119,120).

Both maternal plasma and amniotic fluid concentrations of sFlt-1 are increased in preeclamptic patients, as well as placental sFlt-1 mRNA expression (38,118,121-125). In addition, higher levels of sFlt-1 are associated with a drop in circulating levels of free VEGF and PlGF in women with preeclampsia (116). The soluble and endogenously produced sFlt-1 largely originates from the placenta due to alternative splicing and may disrupt VEGF/PlGF signaling either by binding VEGF or by forming heterodimers that may block access to the other VEGF/PlGF receptor, KDR (VEGF-R2, Flk) (126).

Recently, Bridges *et al.* have reported a model of increased circulating sFlt-1 in pregnant rats using recombinant sFlt-1 delivered via osmotic mini-pump placed intraperitoneal and found that the dams are hypertensive, have smaller placentae and fetuses, are proteinuric, and show evidence of impaired vascular function in late gestation (127). While these *in vivo* studies have established the importance of sFlt-1 as a preeclamptic factor, further studies are needed to determine if there are long term effects on cancer incidence that may be related to increased exposure to this factor during pregnancy. Gilbert *et al.* have recently demonstrated that reduced uteroplacental perfusion pressure increased plasma and placental sFlt-1 and this is associated with increased blood pressure, proteinuria, and decreased free plasma VEGF and PlGF in the late gestation pregnant rat (35). Similar findings have been reported in a model of uteroplacental ischemia in the baboon that also results in hypertension, proteinuria and increased circulating sFlt-1 (37). Both of these models demonstrate that a focal reduction in placental

blood flow is a stimulus for increased production and secretion of anti-angiogenic factors during pregnancy (35,37). Data from these studies and others have revealed species specific differences with respect to which angiogenic factors are most abundant during pregnancy (35, 37, 38). In human pregnancy PlGF appears to be the most abundant circulating angiogenic factor whereas VEGF is more abundant in rat pregnancy. Nevertheless, these findings suggest that both of these models may be valuable systems for testing hypotheses regarding the effects of anti-angiogenic factors on cancer cells.

5.3. sEng/TGF-beta

The soluble form of endoglin (sEng) is another molecule recently proposed to play a key role in the pathogenesis of preeclampsia (40,128). sEng has also been shown to be anti-angiogenic as it is thought to impair TGF-beta binding to cell surface receptors (40,129). Recent studies investigating sEng during preeclampsia and in animal models have further established the role of anti-angiogenic factors. Venkatesha *et al*. have shown that sEng inhibits *in vitro* endothelial cell tube formation to a similar extent as sFlt-1. That group has also shown that increasing circulating sEng in pregnant rats by way of adenoviral infection results in many features of the preeclamptic syndrome (40). In addition, recent work has shown that sEng production is stimulated *in vitro* and *in vivo* by placental ischemia (36,130).

5.4. Insulin-like growth factors (IGFs)

While IGFs are thought to play a role in cardiovascular function (131-135), their exact roles in the pathophysiology of cardiovascular diseases such as preeclampsia remain unclear. Since IGFs are thought to increase production of NO in vascular tissue, it is appealing to propose that IGFs would be decreased in preeclampsia. Available evidence from clinical studies largely suggest that IGF-2 concentrations are increased (136-138), and that IGF-1 concentrations are not changed (136,138) in preeclamptic women. In contrast, several studies have reported decreased (139,140) IGF-1 concentrations in preeclamptic women. Very little work has been performed to evaluate IGF concentrations in animal models of preeclampsia. Our lab has reported that maternal plasma IGF-2 concentrations are decreased (87) in rats with placental ischemia induced hypertension (RUPP model) but that amniotic fluid concentrations of IGF-2 are increased (141) in late gestation. We have not observed changes in IGF-1 in this model (141). Nevertheless, the increased IGF-2 we have observed could play a role in altering the trajectory of mammary development *in utero* in this model. This hypothesis is supported by recent studies in our lab suggesting growth restricted female offspring from RUPP pregnancies have increased susceptibility to mammary tumors when exposed to NMU compared to progeny from normal pregnant rats (87).

6. THE ROLES OF ANGIOGENIC FACTORS IN CANCER

6.1. HO-1

HO-1 appears to have enigmatic roles in cancer biology and because of this it remains unclear how useful it may be as a therapeutic target in cancer treatment. HO-1 is known to have cytoprotective effects *in vivo* and *in vitro* (142-146) and these may work to help maintain cancer growth. HO-1 expression has been shown to be increased in AH136B hepatoma solid tumors in a rat model (143) as well as highly expressed in several tumor cell lines including squamous carcinoma cells and gastric cancer cell lines (147-149). Inhibition of HO-1 has also been associated with reductions in cell growth *in vitro* and the inhibition of tumor growth (149-153) while up-regulation of HO-1 reportedly has an opposite effect (151). Further, inhibition of HO-1 has been shown to decrease VEGF concentration, microvessel density and inhibit tumor growth in mice (154). In contrast there is a report that induction of HO-1 inhibits rat and human breast cancer cell proliferation (155). Viewed in concert it appears that the bulk of evidence suggests that inhibition of HO-1 may be the most useful target for cancer therapy.

6.2. VEGF/sFlt-1

In recent years, VEGF has been recognized as essential for the vascularization of cancers. The resulting vessels supply oxygen and nutrients as well as an escape route for metastasizing cancer cells. As one might expect, VEGF expression has been strongly correlated with vessel density and poor prognosis in breast, ovarian, lung, renal, brain, and gastrointestinal cancers (156-158). In addition to the role of VEGF in angiogenesis, VEGF is important in development, regulating proliferation, migration, cell fate and survival in endothelial cells. VEGF is mitogenic, stimulates migration, promotes epithelial mesenchymal transition, and promotes survival of many cell types including hematopoetic cells, epithelial, neuronal and muscle cells. Similar to endothelial cells, breast cancer cells express both VEGF and VEGF receptors. During mammary gland development and during cancer development and progression many sources of VEGF are present. VEGF can be produced by breast cancer cells and the surrounding mammary stroma, tumor associated fibroblasts and inflammatory cells during mammary gland development, early cancer development and the later invasive and metastatic stages (159). Tumor growth beyond 1-2 mm results in hypoxia as the tumor grows, which in turn stimulates increased VEGF expression. In addition hypoxia within stromal epithelial cells triggers epithelial mesenchymal transition, an important process in breast cancer initiation (160). Moreover, increased VEGF expression activates the expression of oncogenes such as Kras, Hras, v-src, EGFR/Her1, Her2, FOS, trkB, V-p3K, PTTg1 and Bcl-2 (161).

We propose that attenuation of VEGF/PlGF signaling, via factors such as sFlt-1, can modify the differentiation of stem cell and progenitor cells during mammary gland development, pregnancy and cancer (Figure 2). In addition, conditions that increase VEGF/PlGF production can be treated by sFlt-1, which will compete with Flt-1 receptor and may down regulate breast cancer stem cell survival as well as block angiogenic signals at tumor sites (Figure 2). Regulation or attenuation of VEGF signaling by sFlt-1 may provide unique therapeutic options, prevent or decrease cancer

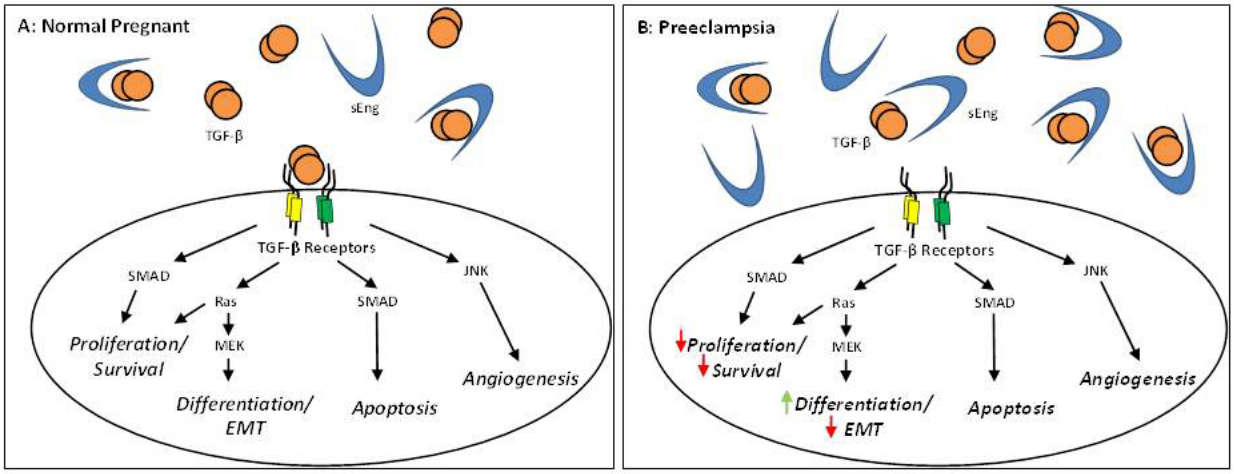

Figure 3. Cellular function and/or fate is affected by the angiogenic balance between transforming growth factor-beta (TGF-beta) and its co-receptor antagonist, soluble endoglin (sEng) during normal pregnancy (panel A), and preeclampsia (panel B). During normal pregnancy (panel A), the existence of a pro-angiogenic balance (TGF-beta > sEng) favors the maintenance of proper vascular tone and endothelial function. During preeclampsia the existence of an angiogenic imbalance (TGF-beta < sEng) favors endothelial dysfunction and the development of manifestations of the preeclamptic syndrome. Alterations of angiogenic balance may also affect differentiation of pluripotent mammary and endothelial cells and subsequent function of these cell types.

development or reduce the growth and metastasis of cancer. Considering that formerly preeclamptic women often have persistently increased levels of sFlt-1 and glucose intolerance long after parturition (70), this may be a factor in the reduced incidence of certain cancers in these women.

Clinical treatment with VEGF antibodies and VEGF/PlGF receptor inhibitors are on-going. Most solid tumors (breast, lung, renal, colorectal and liver) express high levels of VEGF due to hypoxic stress and activation of oncogenes (162). Anti-VEGF neutralizing antibody significantly decreases the growth of tumors in mice and human, specifically anti-VEGF antibody Bevacizumab has been successful increasing survival in advanced colorectal cancer (163,164). Non-squamous non-small cell lung cancer treatment with Bevacizumab has also been approved (165). In addition tyrosine kinase inhibitors that can target VEGF signaling have been approved for renal carcinoma treatment. Currently treatments and clinical trials are ongoing for VEGF inhibitors that are used to treat many solid tumors including breast and ovarian cancers (166).

Using a model of placental ischemia induced hypertension during pregnancy, we have recently observed that breast cancer cells exposed to sera from rats with placental ischemia and increases concentrations of sEng and sFlt-1 demonstrate decreased proliferative capacity (unpublished observations). This animal model of placental ischemia mimics many features of preeclampsia and may provide valuable insights regarding mechanisms of the observed anti-cancer effects associated with preeclampsia. We suggest that the effects of VEGF/PlGF with respect to breast cancer are not limited to the late stages of tumor development involving angiogenesis, and suggest VEGF/PlGF may also be important in the early stages of stem cell and progenitor fate decisions that occur during the development of the mammary gland. The prospect that the protective effect for breast cancer in preeclamptic women may be mediated at least in part through the increased expression of sFlt-1 and attenuation of VEGF/PlGF signaling is novel. Thus, sFlt-1, a regulator of VEGF/PlGF activity, may attenuate neoplastic signaling within the developing and/or remodeling mammary gland, in part mediated by VEGF/PlGF and this protective effect occurs during several discrete periods spanning from fetal life to adulthood in which differentiation and development in the mammary gland is active.

6.3. sEng/TGF-beta

Angiogenesis is essential for growth of tumor cells and the effects of TGF-beta on tumor angiogenesis is well-documented (168). TGF-beta directly targets key angiogenic factors including VEGF expression (168,169). sEng attenuates TGF-beta signaling by interfering with TGF-beta binding to receptors (170). Recently, Criswell *et al.* showed that knockdown of Eng in metastatic cancer cells impaired motility and invasion in a murine model (171). Likewise, anti-Eng cancer therapy has been successful in treating mammary carcinoma in mice (172).

In the cancer environment, TGF-beta's multipotent actions are evident as TGF-beta acts as a tumor suppressor of cancer growth in early stages while in later stages it promotes cell growth, invasion and metastasis (173-177). TGF-beta is also an inducer of apoptosis and can regulate the formation of breast cancer through apoptosis (178,179). However, as with many of TGF-beta's actions, this depends on cell type, differentiation state and the location within the mammary gland. TGF-beta is an important factor in mammary epithelia differentiation. TGF-beta induced epithelial mesenchymal transition has been suggested to be an important process in breast cancer stem cell progression. Indeed as the mammary epithelium differentiates through the onset of

pregnancy, TGF- beta induces epithelial mesenchymal transition resulting in both normal and cancerous mammary epithelial cells acquiring stem cell properties (180). Therefore epithelial mesenchymal transition that is mediated by TGF-beta could increase tumorgenicity as well as self-renewal traits in breast cancer (181). Figure 3 illustrates the potential role for sEng in the modification of cancer risk associated with preeclamptic pregnancy.

A regulatory factor such as sEng that decreases the population of progenitor cells and attenuates TGF-beta signaling, and results from the broad expression and production of TGF-beta during times of malignancy and metastasis may elucidate new mechanisms of therapeutic treatment for both preventing and treating breast cancer. Hence, it is possible that factors in preeclamptic serum regulate TGF-beta signaling in undifferentiated breast cancer cells and by epithelial mesenchymal transition induced formation of mammary epithelial stem-like cells. Taken together this suggests sEng could be an exciting therapeutic target to pursue as it can modulate both early and late transformation events.

6.4. IGF

The importance of insulin-like growth factors (IGFs) in the development and progression of cancers is a rapidly expanding area of study as IGF's are becoming increasingly recognized for their role in various types of cancers (183). IGFs are considered mammotropic and it has been proposed that unusual exposures to IGFs in early life may predispose to later occurrence of breast cancer (183). This is due in part to observations that IGF-1 correlates strongly with circulating levels of hematopoietic stem and progenitor cells and this is thought to be a strong predictor of subsequent cancer development (184). Recent work by Baik *et al.* revealed increased cancer risks through stem cell potential being positively associated to the intrauterine levels of growth hormones, IGF-1 and IGFBP-3, present in human cord blood samples (185). In addition, recent evidence has provided a link between cord blood IGF-1 levels at term of pregnancy and birth weight that is concordant with incidence of breast cancer in tow distinct populations (186).

7. SUMMARY AND PERSPECTIVES

Breast cancer is the second leading cause of cancer in women. Although risk reduction is an essential strategy in preventing the development of breast cancer, progress to date in this area has been limited. The recent epidemiological studies report that formerly preeclamptic women have a reduced incidence of breast cancer, however the mechanisms underlying this observation remain unknown. Preeclampsia often involves impairment of placental perfusion, which in turn leads to placental ischemia and widespread maternal endothelial dysfunction. However, studies in recent years have made great strides in identifying factors that are involved in mediating the hypertension associated with preeclampsia. Hence, it is possible that these newly identified factors may play additional important roles and we eagerly await further research in this area.

Despite the epidemiological evidence indicating pregnancy provides a protective effect against breast cancer, the mechanisms that mediate this effect are not clear. While these studies may provide some insight into what factors may provide this protective effect, the timeline of these signals is not clear. The proposed mechanisms for the protective effects on breast cancer include altered hormone levels and responsiveness to hormones, epithelial cell differentiation toward a less neoplastic potential, and reduction of mammary stem cells and subsequent decrease in neoplastic progenitor cells. Because of the complexity of interactions within whole organisms, there is an increasing need to move beyond cell culture studies and make use of animal models such as the RUPP rat or other rodent models of preeclampsia that have strong similarities with human mammary gland development, pregnancy associated protective effects and breast cancer.

The observed increases in sFlt-1 and sEng during preeclampsia provide intriguing targets both with respect to the protective effect of pregnancy and the protective effect attributed to preeclampsia. While circulating sFlt-1 and sEng are increased in the pregnant compared to the non-pregnant state, they are increased to a greater degree in preeclampsia. In contrast, free VEGF and PlGF are decreased in preeclamptic women despite an increase in total VEGF and PlGF (38). The inhibitory effect of VEGF antagonism on angiogenesis in cancer is clear as evidenced by the many clinical trials that use VEGF inhibitors to block invasion and metastasis. Further, it is unclear if the effect of VEGF/PlGF and the regulation of VEGF/PlGF signaling during mammary gland differentiation, in particular during pregnancy mediates this protective effect. This is particularly interesting in light of the known effect of VEGF on the epithelial mesenchymal transition during differentiation and neoplastic transformation. In addition, recent reports that sEng is another important factor increased during preeclampsia presents another exciting therapeutic target. sEng can regulate TGF-beta signaling such as differentiation of stem cells, and epithelial mesenchymal transition all of which make sEng an intriguing target. Furthermore, alterations in other mammotropic hormones like IGF may prove to be important regulators of cancer development and progression. Studies on the mechanisms of breast cancer are particularly difficult given that mammary gland development occurs across three distinct periods (fetal development, puberty and pregnancy). Hence, developing animal model systems that recapitulate each of the critical periods of human mammary development is essential. Experimental data investigating gestational factors and the development of breast cancer are limited because of the difficulties inherent to performing mechanistic studies in pregnant women. Further studies in models such as these may provide additional insights regarding the factors involved in breast cancer protection.

While many uncertainties remain regarding the mechanisms underlying the development of breast cancer, several strong associations have emerged indicating that factors during pregnancy provide a protective effect against several forms of cancer. With recent studies providing

strong support for the role of anti-angiogenic factors in the pathogenesis of preeclampsia, the possibility that these molecules play a role in modifying the susceptibility to cancer development has become increasingly reasonable. The increasing number of animal models for preeclampsia research, including transgenic models, knockout models, infusion models, and RUPP may provide avenues for investigating hypotheses regarding mechanisms underlying the impact of pregnancy and preeclampsia on cancer development both in the offspring and in the mothers in later life. Moreover, modern high throughput technologies such as microarray and proteome analysis of the ischemic/hypoxic placenta of women with preeclampsia and in animal models of preeclampsia used in concert with hypothesis driven *in vitro* and *in vivo* mechanistic studies should provide useful insights into novel factors and pathways that may yield fruitful therapeutic strategies. Indeed, the attention paid to prevention of cancer or early treatment is promising. This is especially true considering that late stage metastatic breast cancer is largely responsible for the morbidity and mortality associated with breast cancer. With the considerable progress seen in recent years in both cancer and preeclampsia research, it appears that new lines of investigation will be forthcoming to provide fresh insights into the mechanisms underlying the relationship between preeclampsia and subsequent cancer risk.

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