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Medical Hypothesis

Aberrant alteration of vascular endothelial growth factor-family signaling in human tubal ectopic pregnancy: what is known and unknown?

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Abstract: More than 98% of ectopic pregnancies occur in the Fallopian tube. Because many facets of tubal ectopic pregnancy remain unclear, prediction, prevention and treatment of tubal ectopic pregnancy are still a major clinical challenge. Compelling evidence suggests that angiogenic growth factors are involved in normal and abnormal implantation. While acknowledging the importance of an intrauterine pregnancy requires the development of a local blood supply and angiogenesis, we hypothesize that the hypoxic- and estrogen-dependent regulation of vascular endothelial growth factor/placental growth factor expression, secretion, and signaling pathways that are possibly involved in the pathophysiology of tubal ectopic pregnancy. Our hypothesis may also lead to a new therapeutic strategy for women with tubal ectopic pregnancy.

Keywords: VEGF, VEGF receptor, fallopian tube, ectopic pregnancy

Introduction

Human ectopic pregnancy (EP) complicates up to 2% of all pregnancies in the western world [1] and it shows a significant number of maternal deaths in the first trimester [2, 3]. More than 98% of EPs occur in the Fallopian tube [1]. It is well known that the primary function of Fallopian tubes is to provide the proper microenvironment for fertilization and to transport the embryo to the uterus for implantation [4]. Because the Fallopian tubes are not accommodated to hold a growing embryo, the implantation and growth of the embryo will cause the Fallopian tube to rupture if the EP is not surgically or medically treated [2]. The etiology of tubal EP is still unknown. A major limitation to understanding the pathophysiology of tubal EPs is that no existing mouse models can establish causative roles for factors implicated in the pathogenesis of tubal EP [1, 5]. Although several risk factors are associated with tubal EP in women [1, 6, 7], no experimental studies have firmly established causative roles for any of the factors implicated in the pathogenesis of tubal EP. Furthermore, clinical evidence points out other factors may be involved in the pathogenesis of tubal EP [1]. In this article and the accompanying figure, we hypothesize that the hypoxic- and estrogen-dependent regulation of vascular endothelial growth factor (VEGF) / placental growth factor (PIGF) expression, secretion, and signaling pathways that are possibly involved in the pathophysiology of tubal ectopic pregnancy.

The expression of VEGF/PIGF and their receptors during the intrauterine and ectopic pregnancies

VEGF is a key regulator of physiological and pathological angiogenesis [8]. Its family consists of VEGF-A (generally called VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and PIGF [9]. Sequence comparisons indicate that PIGF has 42% amino acid sequence identity with VEGF-A

[10]. VEGF-A forms homodimer with itself and/or heterodimers with PlGF, and acts by binding to its two cell surface tyrosine kinase receptors, VEGFR1 (FLT-1) and VEGF2 (KDR/FLK-1). PlGF binds only to VEGFR1 [9]. Compelling evidence suggests that these potent angiogenic growth factors are involved in normal and abnormal implantation [9]. For example, they participate in the regulation of early placental angiogenesis, maternal stromal changes and spiral artery remodeling [9]. In the human Fallopian tube, VEGF is localized in the epithelial cells, smooth muscle cells, and blood vessel cells in a region-specific manner [11, 12], and significantly higher levels of tubal VEGF and VEGF receptor mRNAs are detected in women with a hydrosalpinx [13], which is defined as tubal dilation and abnormal fluid accumulation [7]. Moreover, in vitro studies have shown an increase in VEGF and soluble VEGF receptor secretion in human tubal epithelial cells and stromal fibroblasts in response to hypoxic stimulation [14]. It has been shown that VEGF-A and VEGF receptor mRNAs are significantly increased in the implantation site compared to non-implantation sites of human Fallopian tubes [15], and these are associated with trophoblastic invasion into the tubal wall in vivo [16]. These results suggest that the development of tubal EP could contribute to the elevation of circulating VEGF-A levels. In fact, circulating levels of VEGF-A have been shown to change in women with EP [17-23]. Moreover, ligation of the Fallopian tube, which mimics the tubal occlusion that likely induces an EP in women, increases VEGF protein expression in rat Fallopian tubes [24]. Furthermore, in vitro experiments have shown that insulin-like growth factor-1 and interleukin-1β directly regulate VEGF and VEGFR expression in human tubal epithelial cells and stromal fibroblasts [25, 26]. Collectively, these observations show that different molecules contribute to the regulation of VEGF synthesis and secretion, but their precise roles in tubal EP are not clearly understood.

During normal pregnancy, VEGF isoforms are involved in building the placenta [9]. VEGF-A is expressed in the human placenta throughout gestation [27], and placenta-specific PlGF has a similar role as VEGF during normal intrauterine pregnancy [9]. We have recently shown that serum PlGF levels are significantly increased in the middle and late stages of intrauterine pregnancy compared to the early stage of intrauterine pregnancy. Although some previous studies have indicated that serum PIFG levels are elevated in tubal EP compared to intrauterine pregnancy [22, 28], we are unable to find a significant difference in serum PlGF levels between women with tubal EP and gestational age-matched women with intrauterine pregnancy.

Multiple factors regulate the VEGF/PlGF signaling pathways during the onset of tubal EP

Cellular responses to reduced oxygen availability (hypoxia) are recognized as critical in normal development and physiology, as well as are implicated in pathological processes [29]. Hypoxia-inducible factor (HIF), a critical hypoxia sensor, is a heterodimeric complex composed of three alpha subunits (HIF-1α, HIF-2α, and HIF-3α) and a stable beta subunit (HIF-1β, also known as aryl hydrocarbon receptor nuclear translocator (ARNT)) [30]. Hypoxic HIF activity is controlled primarily through post-translational modification and stabilization of the HIF-1α and HIF-2α subunits, and HIF-1β expression levels constitute important determinants of hypoxia responsiveness [30]. It is well known that heterodimeric complexes of HIF-1α/β translocate to the nucleus and activate several hypoxia-associated genes, including VEGF [9]. VEGFs are synthesized, secreted and activated by a variety of tissues/cells in vivo [9]. Based on the results from our and other laboratories, we hypothesize a working model for regulation and activation of VEGF isoforms and their receptor signaling pathways during tubal implantation. Disruption of the local environment such as a low oxygen level, possibly due to embryo implantation in the Fallopian tube, induces elevated levels of HIFs [30]. We propose that implantation in the Fallopian tube leads to the coordinated activation of a transcriptional cascade in response to the presence of excessive hypoxia (**Figure 1**). As a result, tubal VEGF-A levels are increased and this activates the VEGF signaling cascades in an autocrine manner. On the other hand, increased levels of tubal VEGF causes them to be released into the circulation, and this leads to activation of the VEGF signaling cascades in a paracrine manner. Both autocrine and paracrine regulation may result in tubal fluid secretion, vascular defects, angiogenic dysfunction, and tubal wall damage.

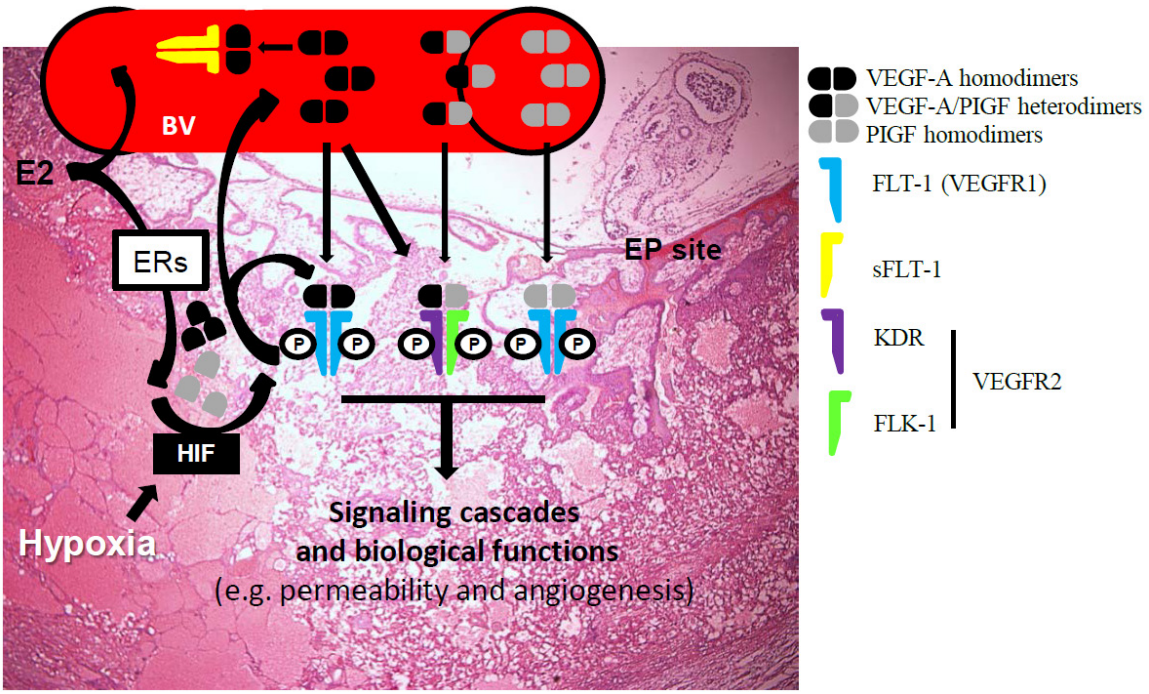

Figure 1. Hypothesis for the hypoxic- and estrogen-dependent regulation of VEGF/PIGF expression, secretion, and signaling pathways that are possibly involved in the pathophysiology of tubal EP. Histopathologic photomicrograph of the Fallopian tube (ampulla) affected by an EP. The tubal wall is extensively infiltrated by chorionic villi and trophoblastic cells (H&E staining). Implantation in the Fallopian tube leads to the coordinated activation of a transcriptional cascade in response to the presence of excessive hypoxia. As a result, tubal VEGF-A levels are increased and this activates the VEGF signaling cascades in an autocrine manner. On the other hand, increased levels of tubal VEGF causes them to be released into the circulation, and this leads to activation of the VEGF signaling cascades in a paracrine manner. Both autocrine and paracrine regulation increase the permeability of the tubal vasculature and induce angiogenesis during Fallopian tubal implantation. Soluble FLT-1 binds VEGF and is the most potent regulator of VEGF activity in vivo. Moreover, E2-dependent regulation of VEGF expression and VEGF secretion may occur indirectly through HIF isoforms during implantation in the Fallopian tube. FLT-1, fms-like tyrosine kinase 1; sFLT-1, soluble FLT-1; KDR, kinase insert domain-containing receptor; FLK-1, fetal liver kinase 1; ERs, estrogen receptors; HIF, hypoxia-inducible factor. BV, blood vessel.

The Fallopian tube is a dynamic, steroid hormone–responsive tissue [4]. 17β-estradiol (E2) is a steroid hormone and contributes to a diverse array of Fallopian tubal functions in vivo [31]. The physiological actions of E2 are mediated by its interaction with estrogen receptor (ER), which exists as two different subtypes, ERα and ERβ [31]. Both ER subtypes are expressed in normal human Fallopian tubes [31-34]. Moreover, ERα is frequently lost in the implantation and non-implantation site (our unpublished data) of the Fallopian tube in women who have suffered from EP [32, 35]. Although the VEGF gene promoter harbors the estrogen response element [35], whether E2 is able to directly regulate VEGF-A expression via the activation of ER signaling in human Fallopian tubes is not fully understood. On the other hand, animal studies suggest that E2 and hypoxia can cooperate to regulate the same target in the Fallopian tube. For example, the expression of erythropoietin, a potent anti-inflammatory cytokine, is increased by both E2 and hypoxia in mouse Fallopian tubes both in vivo and in vitro [36]. Treatment with E2 followed by hypoxic stimulation significantly reduces VEGF-A protein synthesis and release in human endometrial tissues in vitro [37]. Because the C-terminal domain of HIF-1β, a potent coactivator of ER-dependent transcription, is essential for the enhancement of ER transcription [38], E2-dependent regulation of VEGF expression and secretion may be indirect through HIF isoforms during the Fallopian tubal implantation (**Figure 1**).

Conclusions and future directions

Successful reproduction is critically dependent upon normal angiogenesis. However, the pic-

ture of VEGF-family signaling in the Fallopian tube is still unclear with many gaps in our understanding the molecular details of VEGF/PlGF and their receptor interaction under pathophysiological conditions. While acknowledging the importance of an intrauterine pregnancy requires the development of a local blood supply and angiogenesis, our hypothesis emphasizes the importance of the crosstalk between hypoxia and estrogen in the regulation of VEGF-family signaling pathways during the onset of tubal EP. From a disease mechanism perspective, it was necessary to expand upon clinical research on circulating VEGF isoforms to understand the mechanisms by which their synthesis is regulated and to understand their biological functions during tubal implantation. Such research would allow for a better understanding of the actual diagnostic values of VEGF-A and PlGF. Although medical treatment of unruptured tubal EP using methotrexate has been established [2, 3], development of more potent and safer medical treatment is needed due to limited indications and side effects of methotrexate. Further studies assessing the levels of VEGFR activation and inhibition in human Fallopian tube in situ need to be carried out. When the specific VEGF signaling pathways that contribute to the tubal implantation are identified, anti-VEGF therapy may be of great benefit for women who have developed a tubal EP.

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Conflict of interest statement

The authors report no conflict of interest.

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