

LEIOMYOMA AND VASCULAR ENDOTHELIAL GROWTH FACTOR GENE POLYMORPHISMS: A SYSTEMATIC REVIEW

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SUMMARY

Leiomyomas (myoma or fibroid) are the most common gynecologic tumors that occur in women of reproductive age, but their molecular pathogenesis is still unknown. Since the growth of leiomyomas involve numerous vascular factors, an association between the leiomyoma and growth factors is suspected. Vascular endothelial growth factor (VEGF) is one of the most important angiogenic growth factors. VEGF regulates angiogenesis and mediates sex steroid-induced cell growth and differentiation. VEGF-mediated activities seem to contribute to the pathogenesis of leiomyoma. Genetic variations, including polymorphisms, in VEGF might also be associated with the complex pathogenesis of leiomyomas. Here, we performed a systematic review of the roles of VEGF and its polymorphisms in the pathogenesis of leiomyoma. [*Taiwan J Obstet Gynecol* 2010;49(3):247-253]

Key Words: leiomyoma, polymorphism, vascular endothelial growth factor

Introduction

Uterine fibroids (leiomyoma or myoma) are the most common gynecologic tumors in women of reproductive age. The prevalence of leiomyoma reportedly ranges from 20% to 50% of women over the age of 30 years [1]. However, very little is known about its molecular pathogenesis. Similarly, the regulation of its growth is poorly understood. It is believed that angiogenesis, the process by which new capillaries develop from pre-existing blood vessels, may be involved. In fact, leiomyoma is related to complex autocrine and paracrine interactions, and the effects of sex-steroid hormones on uterine cells [2]. Genesis and growth-promoting factors are responsible for their development.

Growth factors are multifunctional cytokines that regulate many biological functions, ranging from growth and differentiation to cellular apoptosis [3]. Growth factors are also associated with cellular proliferation and differentiation, angiogenesis, extracellular matrix modification and immunomodulation [3]. Hyperexpressed growth factors and increased production of growth factor peptides may elicit autocrine effects or act as cocarcinogens [4]. Growth factors function as autocrine or paracrine mediators of estrogen to maintain, differentiate and enhance tumor growth [2].

Growth factors are involved in the pathogenesis of leiomyomas and regulation of angiogenesis, which is necessary for the growth of leiomyomas [5]. Growth factors could preferentially promote the growth of leiomyoma cells compared with myometrial cells [6]. Thus, growth factors and their receptors may play important roles in the pathogenesis of leiomyomas [7]. The presence of growth hormone receptor mRNA suggests that the human uterus is a target tissue for growth hormone action [8]. Uterine leiomyosarcomata has been shown to oversecrete growth hormone [7].



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The mRNA expression of growth factors is regulated by ovarian hormones [9]. Estrogen may exert its mitogenic effects on leiomyomas *via* estrogen-dependent growth factors [10]. Hypoestrogenism caused by a gonadotrophin-releasing hormone analog is also associated with decreased levels of growth factors [11]. Growth factors are also responsible for uterine myogenesis [10]. Changes in growth factor binding to the myometrium may play a role in the pathogenesis of leiomyomata [12].

Among the growth factor family, vascular endothelial growth factor (VEGF) might be one of the most important angiogenic growth factors in terms of angiogenesis regulation [13]. VEGF is a potent proangiogenic factor, and is an essential growth factor for vascular endothelial cells. The development of leiomyoma is associated with exposure to ovarian sex steroids and an increased requirement for vascular supply for their growth. These observations suggest that VEGF and other proangiogenic factors might be involved in the development of leiomyomas [14].

VEGF Expression

The contribution of VEGF to tumor angiogenesis is well understood. VEGF is up-regulated in many tumors [15] and VEGF protein was detected in the culture media from a range of tumor cell lines [16]. VEGF mRNA was also detected in numerous tumors and metastases, with immunoreactivity for VEGF localized on tumor cells and in the stromal matrix. VEGF might be released into the surrounding stromal matrix, which might contribute to tumor growth and metastasis in a paracrine manner through angiogenesis and increased vascular permeability. Some investigators have reported no correlation between serum VEGF levels and tumor vascular density. These findings suggested VEGF may promote tumor growth by direct pro-survival effects in tumor cells [17].

VEGF and VEGF receptors (VEGFR) are expressed by numerous endothelial and non-endothelial tumor cells. VEGF exerts its biological effects by binding to one of two tyrosine kinase receptors (VEGFR-1 and VEGFR-2) [13]. VEGF plays a major role in leukemia and lymphoma [18]. Furthermore, VEGF is highly expressed in a variety of solid tumors [19]. VEGF expression is also correlated with malignant disease progression [20]. VEGF overexpression in tumors is associated with increased angiogenesis, proliferation, and metastasis [21]. Phosphorylated VEGFR was also observed in numerous solid tumors, including lung cancers, breast cancers, lymphoma, and melanoma [22]. Other than

endothelial cells, many peripheral cell types have stained positive for phosphorylated VEGFR, including macrophages, fibroblasts, and myofibrils.

VEGF expression is potentiated by a variety of hormones and cytokines. VEGF mediates angiogenesis in a variety of estrogen target tissues [23]. VEGF expression was significantly correlated with estrogen receptor status and inversely correlated with tumor grade [24]. Meanwhile, estrogens increase the expression of VEGF mRNA in the uterus [14]. VEGF is a stimulator and prognostic factor for breast and uterine tumors [14]. VEGF is regulated by estradiol and tamoxifen in the uterus and by estradiol in breast cancer cells [14]. Furthermore, nitric oxide was reported to mediate VEGF signaling [25]. The mitogenic effects of VEGF are inhibited by antagonists of nitric oxide synthase [25]. VEGF expression is also affected by p53 status [26].

VEGF and its receptors have been identified in several reproductive tissues, including the endometrium [27], placenta [28], fallopian tube, ovary [29], corpus luteum, ovarian follicles, endometrial vessels, embryonic implantation sites [30], trophoblast cells, and yolk sac [31]. VEGF contributes to luteal angiogenesis, corpus luteum development, and progesterone production during mid-pregnancy [32]. VEGF can diffuse into the extracellular environment and might be present in biological fluids, including peritoneal fluid and blood [13].

VEGF expression is significantly higher in deeply infiltrating endometriosis as well as during vascularization [33]. VEGF exists in several isoforms, all of which are potent stimulators of angiogenesis [34]. The differential expression of VEGF in leiomyomas compared with the adjacent myometrium indicates that local angiogenesis may be important in the development and growth of leiomyoma [35]. High levels of VEGF in serum and peritoneal fluid has been reported to be associated with increased mitotic activity in endometriotic lesions [13].

VEGF gene expression

The *VEGF* gene is an important determinant of VEGF plasma levels [36]. Endothelial cell proliferation, tubule formation, and tumor growth may be diminished by down-regulation of endogenous VEGF [37]. VEGF is subject to multilevel regulation at the transcriptional, posttranscriptional, translational, and posttranslational levels during embryogenesis and in adulthood [38]. VEGF is produced by various cells, including vascular smooth muscle, endothelial and inflammatory cells, and has direct effects on vascular endothelial and smooth muscle cells through the activity of receptor tyrosine kinases [39]. VEGF has been shown to increase the

ability of endothelial cells to produce nitric oxide, which may improve endothelial function [40].

VEGF gene expression is modulated by a variety of effectors, including cytokines [41,42], lipopolysaccharide [43], hormones [41], and hypoxia [42]. Dysregulated *VEGF* expression is implicated in the pathogenesis of a number of diseases. Increased *VEGF* expression resulting in inappropriate *VEGF*-induced angiogenesis is associated with tumor growth and metastasis [44], rheumatoid arthritis [45], and diabetic retinopathy [46]. The ability to produce *VEGF* in response to hypoxia is associated with the development of collateral vessels and protection against myocardial disease [47].

The identification of the related genes is essential for genetic diagnosis and gene therapy for genetic diseases. Genetic studies of multifactorial diseases such as leiomyoma are difficult to perform because of uncertainties of a polygenic trait. Biallelic expression of the gene might lead to overexpression of growth factors and increased mitogenic activity [7]. In our previous reports, we found no associations between leiomyoma and polymorphisms in estrogen and androgen receptors, or in interleukin (IL)-1 (IL-1 β -511 promoter, IL-1 β exon 5), IL-4, tumor necrosis factor, p53 or p21 [48–52]. We have also noted that the insulin-like growth factor II *Apal* polymorphism is associated with leiomyoma susceptibility (Hsieh et al, unpublished data).

Regulation of the human *VEGF* gene, which is located at chromosome 6p12-21, is extremely complex [52]. *VEGF* is a determinant of the rate and extent of angiogenesis, playing a major role in endothelial mitogenesis. Upregulation of *VEGF* may be related to metastases in cancer, and may also play a role in cardiovascular pathophysiology by initiating and propagating angiogenesis, and the development of collateral vessels [42]. *VEGF* is a potent stimulus of vascular permeability that plays a major role in embryonic vasculogenesis and adult vascular remodeling [41]. *VEGF* is also required for cyclic blood vessel proliferation in the female reproductive tract [41]. *VEGF* induces endothelial cell proliferation, promotes cell migration, and inhibits apoptosis. Dysregulation of *VEGF* expression contributes to the development of solid tumors by promoting tumor angiogenesis [42].

Systematic Review

Numerous gene polymorphisms have been reported to be associated with the pathogenesis of leiomyoma, including cytochrome *P450c17 α* gene**A2* allele [53], estrogen receptor*12 or 13 thymine-adenine dinucleotide repeat [54], *insulin-like growth factor II* gene and

the *small nuclear ribonucleoprotein polypeptide N* gene [55]. However, some researchers found no association between *VEGF* genetic presentation and leiomyoma. Furthermore, there was a non-significant difference between *VEGF* mRNA levels in the proliferative and secretory phases of the cycle [56].

Reviewing the MEDLINE database, we found some reports describing associations between *VEGF* polymorphisms and individual disorders (Table). However, few researchers have reported associations between *VEGF* gene polymorphisms and leiomyoma. We recently reported that the 5'-UTR-460 polymorphism in the *VEGF* gene might contribute to the pathogenesis of leiomyoma [57]. We also observed that *VEGF*T homozygotes and T allele are associated with higher susceptibility of leiomyoma development. These findings strongly suggest a molecular correlation between *VEGF* and leiomyoma, and that *VEGF* is involved in the pathogenesis of leiomyoma.

Genomic imprinting is defined as a gamete-specific modification that causes differential expression of the two alleles of a gene in somatic cells [58]. Alternation or loss of imprinting (LOI) of growth factors is related to oncogenesis, either through inactivation of a tumor suppressor gene or activation of a growth-promoting gene [59]. Biallelic expression of *VEGF* may be an early event in tumorigenesis. When both alleles of the growth factor are expressed in the tumor tissue, it is likely that more growth factor mRNA and protein will be produced [60]. LOI may contribute to the growth of these tumors through an autocrine or paracrine mechanism. With LOI, the growth factor is overexpressed, contributing to the growth or development of the neoplasm [60].

Summary

In summary, we suggest that growth factors are associated with the pathogenesis of leiomyomas. Polymorphisms in growth factor genes might play a role in the complex pathogenesis of leiomyoma. If so, polymorphisms in the *VEGF* gene may be a useful marker to predict susceptibility to leiomyoma. However, the actual role of *VEGF* polymorphisms in the development of leiomyoma warrants further studies. The long-term aim was to find and develop useful markers for early detection of these diseases. Our understanding of the underlying biophysical and biochemical mechanisms will help us to develop new diagnostic criteria beyond histologic evaluation, and permit the identification and validation of molecular targets for future drug discovery.

Table. Correlations between *VEGF* gene polymorphisms and individual diseases

SNP locations	Correlation	No correlation
-2578C/A	Thyroid cancer [61], breast cancer [62], hepatic cancer [63], gastric cancer [64]	Colon cancer [65], inflammatory bowel disease [66], diabetes [66], breast cancer [67]
-1498 C/T	Breast cancer [62], hepatic cancer [63], diabetic retinopathy [68]	-
-1154A/G	Recurrent pregnancy loss [69], laryngeal squamous cell carcinoma [70]	Inflammatory bowel disease [66], diabetes [71]
-634C/G	Colorectal cancer [72], Kawasaki disease [73], diabetic retinopathy [74], osteonecrosis of the femoral head [72], breast cancer [95], ventricular septal defect [76]	Diabetic retinopathy [77], thyroid cancer [61], inflammatory bowel disease [66], diabetes [71]
-509C/T	-	Pterygium [78]
-460T/C	Leiomyoma [57], hydrocele [79], prostate cancer [80], vesicoureteral reflux [81], gastric cancer [82]	Lung cancer [83,84], pterygium [78], gastric cancer [85], ovarian cancer, cervical cancer, endometrial cancer [86], ovarian cancer [87]
+405G/C	Lung cancer [83], endometriosis [88], preterm birth [89]	Lung cancer [84], ovarian cancer [87]
+813C/T	Sarcoidosis [90,91]	-
+936C/T	Oral squamous cell carcinoma [92], lung cancer [83], breast cancer [93], colorectal cancer [72], leukemia [72], endometriosis [94], preeclampsia [95], gastric cancer [85], oral cancer [96]	Diabetic retinopathy [77], familial mediterranean fever [97], lung cancer [84], thyroid cancer [61], ovarian cancer, cervical cancer, endometrial cancer [86], endometriosis [72], ovarian cancer [87]

VEGF=vascular endothelial growth factor; *SNP*=single-nucleotide polymorphism.

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