Biochem Genet (2008) 46:356–361
DOI 10.1007/s10528-008-9150-1

NOTE

T allele for VEGF-460 Gene Polymorphism at 5′-Untranslated Region is Associated with Higher Susceptibility of Leiomyoma

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Received: 16 May 2007 / Accepted: 31 July 2007 / Published online: 7 February 2008

Abstract Vascular endothelial growth factor (VEGF) is a regulator of angiogenesis and a mediator of sex steroid-induced cell growth and differentiation. We aimed to investigate if VEGF gene 5′-UTR −460 polymorphism could be used as markers of susceptibility in leiomyoma. Women were divided into two groups: (1) leiomyoma (n=159); (2) nonleiomyoma groups (n=131). VEGF gene −460 polymorphism were detected by polymerase chain reaction and BstUI restriction enzyme analysis. Genotypes and allelic frequencies between both groups were compared. We noted that the proportions of different VEGF polymorphisms in both groups were significantly different. Proportions of cuttable (C) homozygote/heterozygote/uncuttable (T) homozygote for VEGF in both groups were: (1) 0/32/68% and (2) 0/63/37%. Higher percentage of T homozygote and T allele presented in the leiomyoma population. Proportions of C/T alleles in both groups were: (1) 16/84% and (2) 32/68%. We concluded that T homozygotes and T allele of VEGF gene −460 polymorphism are associated with higher risk of leiomyoma development.

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Heterozygotes and C allele are related with lower risk of leiomyoma formation. VEGF gene polymorphism likely contributes to the pathogenesis of leiomyoma.

Keywords Leiomyoma · Polymorphism · Vascular endothelial growth factor (VEGF)

Introduction

Leiomyoma, the most common benign uterine neoplasm, occurs in around one fourth of women during their lifetime (Cramer 1992). Numerous growth hormones (such as insulin-like growth factor, epidermal growth factor, fibroblast growth factor) that influence the growth of leiomyoma are currently being investigated (Strawn et al. 1995; Tommola et al. 1989; Ali et al. 2000). Vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen, plays a central role in normal physiological angiogenesis as well as in tumor angiogenesis (Ferrara 1999). VEGF plays a part as a mediator of sex steroid-induced cell growth and differentiation (Hyder et al. 2000a), which is required for female reproductive functions (Ferrara 2000).

Recently, a VEGF gene polymorphism with a C → T base change at the 5′-UTR (untranslated region) −460 position has been reported (Watson et al. 2000); its role in the development of leiomyoma remains unclear. In this paper, we tried to evaluate whether this VEGF gene polymorphism is a useful marker for predicting susceptibility to leiomyoma. This is the first report on this survey.

Materials and Methods

Premenopausal Taiwan Chinese women with surgically diagnosed leiomyoma and absence of leiomyoma were included. The patients were divided into a leiomyoma group ($n = 159$) and a nonleiomyoma group ($n = 131$). The study was approved by the ethical committee and institutional review board of the China Medical University Hospital. Informed consent was signed by all women who donated their blood. There were nonsignificant differences between both groups in age, weight, and height.

All women provided peripheral blood samples for genotype analyses. Genomic DNA was isolated from peripheral blood using the Genomaker DNA extractor kit (Blossom, Taiwan). About 50 ng of genomic DNA was mixed with 20 pmole of PCR primer in a total volume of 25 μl containing 10 mM Tris–HCL, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each deoxyribonucleotide triphosphate, and 1 U Amplitaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA).

For the VEGF polymorphism, a 175 bp fragment of VEGF was amplified by the polymerase chain reaction (PCR) (Watson et al., 2000). The sequences of the primers were (from 5′ to 3′ end): forward, TGTGCGTGTGGGGTTGAGCG; reverse, TACGTGCGGACAGGGCCTGA. The PCR amplification was performed in a programmable thermal cycler GenAmp PCR system 2400 (Perkin Elmer

Applied Biosystems). The PCR conditions were set as follows: one cycle at 94°C for 5 min, 35 cycles at 94°C for 20 s, 62°C for 20 s, 72°C for 20 s, and a final extension cycle at 72°C for 10 min.

The PCR product of 175 bp was mixed with 2 U *Bst*UI (New England Biolabs, Beverly, MA) and reaction buffer according to the manufacturer's instructions. The reaction mixture was incubated overnight at 60°C. After complete *Bst*UI digestion, the products included a single fragment of 175 bp for the T allele (uncuttable by the restriction enzyme) and two fragments of 155 and 20 bp for the C (cuttable) allele. Genotypes of the VEGF polymorphisms after restriction analysis were divided into T homozygote (175 bp), C homozygote (155 + 20 bp), and C/T heterozygote (175 + 155 + 20 bp).

Then 10 μl of the PCR product after *Bst*UI digestion was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. Each allele was recognized according to its size. Genotype and allelic frequencies for VEGF polymorphisms in both groups were compared. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS system with a χ^2 test was utilized for statistical analysis. A P-value < 0.05 was considered statistically significant.

Results

Genotype proportions of different VEGF-460 gene polymorphisms in both groups were significantly different ($P = 0.001$, Table 1). A higher percentage of the T homozygote was present in the leiomyoma population. There was no individual with the C homozygote in either group. Proportions of C homozygote, heterozygote, and T homozygote for VEGF were 0/32/68% in the leiomyoma group and 0/63/37% in the nonleiomyoma group.

Allele frequencies for VEGF-460 gene polymorphism between both groups were also significantly different (Table 2). There was a dominant presentation of the T allele in the leiomyoma population as compared with the normal population

Table 1 Distribution of VEGF-460 gene polymorphism in women with and without leiomyoma

Genotype	Leiomyoma $n = 159$ (%)	Nonleiomyoma $n = 131$ (%)	P-value[a]
Cuttable homozygote (CC)	0	0	0.001
Heterozygote (C/T)	51 (32.1)	83 (63.4)	
Uncuttable homozygote (TT)	108 (67.9)	48 (36.6)	

[a] Comparison of two genotypes (C/T, TT); P-value calculated by χ^2 test

Table 2 Allelic frequency for VEGF-460 gene polymorphism in women with and without leiomyoma

Allele	Leiomyoma $n = 318$ (%)	Nonleiomyoma $n = 262$ (%)	P-value[a]
C	51 (16)	83 (31.7)	0.001
T	267 (84)	179 (68.3)	

[a] P-value calculated by χ^2 test

($P = 0.001$). Proportions of C/T alleles in both groups were 16/84 and 32/68%, respectively (Table 2).

Discussion

Leiomyoma is the most common tumor in women, but its etiology remains unclear. Myoma growth may be derived from growth and proliferation of a single smooth muscle cell (Townsend et al. 1970). Numerous hormones and cytokines are associated with leiomyoma formation, including estrogen, progesterone (Hyder et al. 2000b), insulin-like growth factor (Strawn et al. 1995), epidermal growth factor (Tommola et al. 1989), fibroblast growth factor (Ali et al. 2000), etc. Uterine tissue contains numerous growth factors and their receptors (Boehm et al. 1990). Several growth factors, including VEGF, insulin-like growth factor, fibroblast growth factor, and platelet-derived growth factors, are present in the uterine endometrium (Ferriani et al. 1993). Furthermore, leiomyoma contains more growth factor mRNA than does myometrium, which may be related to the genesis and progression of these tumors (Boehm et al. 1990).

Overexpression of VEGF may be related to numerous tumors, including leiomyoma (Hyder et al. 2000a), endometrial cancer (Stoner et al. 2000), breast cancer (Adams et al. 2000), placental rejection (Huminiecki et al. 2001), male fertility (Huminiecki et al. 2001), hepatocellular carcinoma (Zheng et al. 1998), colorectal cancer (Akagi et al. 2000), esophageal cancer (Uchida et al. 1998), chronic hypoxic pulmonary hypertension (Chen et al. 1998), Kawasaki disease (Hamamichi et al. 2001), leukocytoclastic vasculitis (Viac et al. 1999), pyogenic granulomas (Bragado et al. 1999), astrocytic gliomas (Oehring et al. 1999), and polymyalgia rheumatica (Meliconi et al. 2000). VEGF is related to the growth of hepatocellular carcinoma (Zheng et al. 1998). It is a useful indicator for the metastasis and survival of hepatocellular carcinoma (Zheng et al. 1998) and oral squamous cell carcinoma (Maeda et al. 1998). The antibody against VEGF may be a potentially effective antimetastasis agent for the treatment of lung metastasis (Wang et al. 1997). Abnormally high or low VEGF in human semen is correlated with a lower pregnancy rate following IVF (Huminiecki et al. 2001).

In contrast, several investigators disagree about these associations. VEGF polymorphism is not associated with susceptibility to several illnesses, including nephrotic syndrome (Postlethwaite and Brenchley 1999), mountain sickness (Maloney et al. 2000). In this study, we observed that the genotype distribution for VEGF in individuals with leiomyoma was significantly different from that in the normal population. The VEGF T homozygote and T allele are related to a higher risk of leiomyoma development. The heterozygote and the C allele are related to a lower risk of leiomyoma development. We also note that there are few individuals with the C homozygote (0%) and the C allele (31.7%) in the general population, which was inconsistent with the previous reports. The fluctuation of the VEGF*C allele in Caucasians ranged around 50–55% (Watson et al. 2000; Holt et al. 2003). In contrast, the C allele was at the level of 31.7% in the normal controls. The discrepancy might be due mainly to the ethnic differences between Asians and Caucasians.

In conclusion, an association exists between leiomyoma and the VEGF gene 5′-UTR −460 polymorphism. VEGF-460*T homozygote and T allele are associated with higher risk of leiomyoma development. The heterozygote and the C allele are related to a lower risk of leiomyoma formation. VEGF polymorphism might become a useful marker for predicting leiomyoma susceptibility. It likely contributes to the pathogenesis of endometriosis. This could provide the database for a further survey of VEGF polymorphisms. The real role of the VEGF polymorphism in the development of leiomyoma, however, remains to be clarified. Furthermore, the effect of other growth factor polymorphisms on leiomyoma development merits further surveys.

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