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Genetic Polymorphism of Vascular Endothelial Growth Factor (VEGF C936T) in the Korean Population

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Vascular endothelial growth factor (VEGF) is a potent regulator of normal and abnormal angiogenesis. Recent literature suggests that VEGF has several activities that may amplify acute inflammation reactions. Dysregulated VEGF expression has been implicated as a major contributor to the development of a number of common disease pathologies. One of common mutations in the 3'-untranslated region of the VEGF gene, a C→T exchange at nucleotide position 936, has been found to be significantly associated with VEGF expression levels in the plasma from a previous Austrian study. The frequency of this mutation could be important genetic information regarding tumor growth and angiogenesis related diseases. The aim of this study was to investigate the frequency distribution of this mutation in general Korean population. We examined the statistical data from 207 healthy Korean subjects. Observed numbers (%) of 936T were 28.5 (CT) and 3.9 (TT), respectively. The mutant allele frequency of 936T in Korean subjects was 0.18, which appeared somewhat higher than that in Austrian subjects.

Vascular endothelial growth factor (VEGF) is a major angiogenic factor and is a prime regulator of endothelial cell proliferation (La Rosa et al., 2003). VEGF is a potent and specific mitogen for vascular endothelial cells that may be involved in tumor angiogenesis (Ladoux et al., 1993; Senger et al., 2002). In addition, VEGF is able to increase capillary permeability, dilate arteries and chemotactically attract monocytes (Senger et al., 1993). A potent stimulus for VEGF expression *in vivo* and *in vitro* is hypoxia, one of the regulating elements is hypoxia-inducible factor 1, a transcription factor binding to a 28-base pair enhancer in the 5'-UTR of the VEGF gene (Mazure et al., 1996). Other regulating elements lie in the 3'-untranslated region (UTR), where binding of hypoxia-induced proteins to the VEGF mRNA could be shown, resulting in a significantly increased half-life of the mRNA. The posttranscriptional regulation affects not only the VEGF gene, but also other hypoxia-inducible genes sucuas erythropoietin or tyrosine hydroxylase (Scandurro and Beckman, 1998).

Strong expression of VEGF has been observed in a variety of tissues, including the female reproductive system, ischemic tissues, tumors and transformed cell lines (Iruela-Arispe and Dvorak, 1997). Increased VEGF expression resulting in inappropriate VEGF-induced angiogenesis is linked with tumor growth and metastasis, rheumatoid arthritis, and diabetic retinopathy (Awata et al., 2002). Mutant angiogenic VEGF may provide a genomic basis for the diversity of tumor-host response (Uthoff et al., 2002). Strong inter-individual variations of VEGF plasma levels and VEGF gene expression have been reported. Variations of the VEGF gene sequence may be responsible for these differences (Renner and Pilger, 1999).

There are three frequently observed mutations (C502T, G1612A and C936T) in the 3'-untranslated region (UTR) of the VEGF gene and one of them, a C936T exchange, may be an important determinant for VEGF plasma levels (Renner et al., 2000). Therefore, we investigated the allele frequencies and genotype distributions of the VEGF C936T polymorphism and the genotype distributions in Korean population compared with other world populations.

Materials and Methods

Human subjects

To estimate the frequency of detected mutations among the general population, DNA was collected from 207 healthy Korean subjects. These subjects had been recruited from the Health Science Center of Bundang CHA General Hospital, Korea. Their ages ranged from

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24 to 81 years (mean±SD, 45.8±16.6 years).

Polymerase chain reaction

Genomic DNA was isolated from venous blood using a Nucleospin blood kit and stored at 4°C. The primer pair was designed to amplify full sequence of the VEGF gene. PCR cycling conditions were 2 min at 94°C followed by 30 cycles with 20 sec at 94°C, 20 sec at the annealing temperature and 30 sec at 72°C.

Detection of VEGF genotypes

To analyze the frequency of detected mutations in a larger sample of subjects, we used a restriction fragment length polymorphism (RFLP) method of PCR products (Kang et al., 2000, 2003, Kim et al., 2002). The VEGF 936 C→T mutation was analyzed by amplification of segment 2 of the 3'-UTR followed by digestion with restriction endonuclease *Nla*III. The primer sequences were as follows; VEGF (C936T) primer 1 (5'-AAG GAA GAG GAG ACT CTG CGC AGA GC-3'), primer 2 (5'-TAA ATG TAT GTA TGT GGG TGG GTG TGT CTA CAG G-3'). The 936C allele remained uncut (208 bp) while the 936T allele was cut into two fragments of 122 and 86 bp.

Statistic analysis

The distribution of allele frequencies for the VEGF C936T gene was examined by χ^2 test to determine whether the observed genotype distributions conform to the Hardy-Weinberg equilibrium expectation.

Results and Discussion

Several studies have reported VEGF serum levels. Unfortunately, platelets release significant amounts of VEGF during aggregation, leading to VEGF serum levels 3- to 6-fold higher than plasma levels (Maloney et al., 1998; Webb et al., 1998). Subjects carrying VEGF 936T allele had significantly lower VEGF plasma levels than subjects carrying the VEGF 936CC genotype (Renner et al., 2000). The mechanism by which the VEGF 936T allele leads to lower VEGF plasma levels is currently unknown. One possible hypothesis is that the C936T mutation leads to a loss of a potential binding site for AP-4, which is a helix-loop-helix transcription factor enhancing expression of several viral and cellular genes by binding to specific enhancer sites (Mermod et al., 1988). It is currently unclear if the potential AP-4 binding site abolished by the C936T mutation is of any relevance to the expression of VEGF. Another possible explanation of the association between the C936T mutation and VEGF plasma levels could be a linkage disequilibrium between this mutation and another yet unknown functional mutation elsewhere in the VEGF gene sequence.

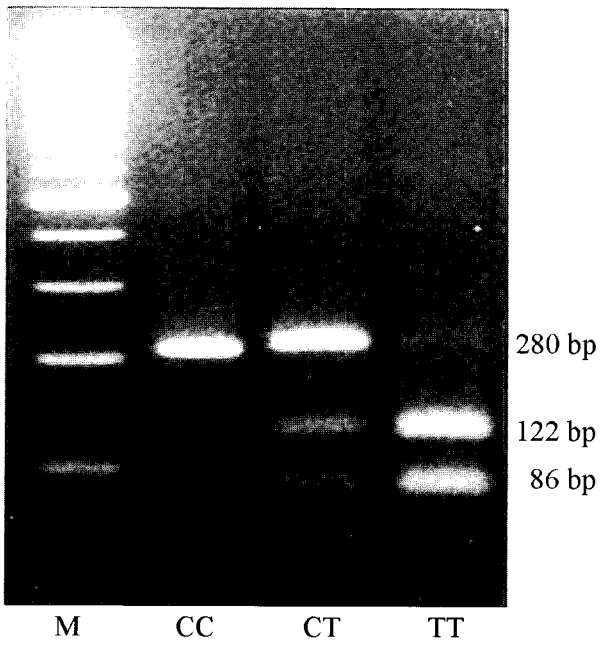

Fig. 1. RFLP analysis of the VEGF C936T mutation using enzyme *Nla*III. M: 100 bp marker DNA. CC: normal genotype, CT: heterozygous genotype, TT: homozygous mutant genotype.

This mutation is clinically important because it is associated with diabetes, angiogenesis-involved cancer and hematothrombosis (Awata et al., 2002; Faviana et al., 2002). Interestingly, it was reported that recurrent miscarriage might be linked to alteration in the expression of VEGF and VEGF receptors, due to problems in implantation (Vuorela et al., 2000). Furthermore, the genetic variations of the VEGF gene can be used to assess its possible relation to diabetic retinopathy in type 2 diabetic patients. Among the seven common polymorphisms in the promoter region, 5'-UTR and 3'-UTR of the VEGF gene, genotype distribution of the C(-634)G polymorphism differed significantly between patients with and without retinopathy, and the C allele was significantly increased in patients with retinopathy compared with those without retinopathy (Awata et al., 2002). Shahbazi et al. (2002) reported that VEGF gene polymorphisms are associated with acute renal allograft rejection. Acute rejection is a major cause of reduced survival of renal allografts.

Table 1. Genotypes and allele frequencies of VEGF C936T in healthy Korean

	CC	CT	TT	T
Observed number (%)	140 (67.6)	59 (28.5)	8 (3.9)	0.18
Expected number	139.2	61.1	6.7	

χ^2=0.33, df=1, 0.5<P<0.8, Number of individuals=207.

Table 2. Genotypes and allele frequencies of VEGF C936T in different world populations

Population	No of tested	Genotypes			Allele frequencies		References
		CC	CT	TT	C	T	
Austrian	123	85 (71.4)	30 (25.2)	4 (3.4)	0.84	0.16	Renner et al. (2000)
Korean	207	140 (67.6)	59 (28.5)	8 (3.9)	0.82	0.18	Present study

Using a screening study, six polymorphisms of the VEGF gene were identified: G(-1,877)A, T(-1,498)C, G(-1,190)A, and G(-1,154)A in the promoter region and C(-634)G and C(-7)T in the 5'-UTR (Awata et al., 2002). Additionally, there are three common mutations (the C502T, the G1612A, and the C936T) in the 3'-UTR of the VEGF gene. In this study, we have described the frequency of mutation of C→T exchange at nucleotide position 936 in the 3'-UTR of the VEGF gene in a group of healthy Korean subjects.

To analyze the frequency of the VEGF mutation at the C936T, we used RFLP typing (Fig. 1). Highest VEGF production was observed for the CC genotype (n=140, 67.6%), intermediate production was observed for the CT genotype (n=59, 28.5%) and lowest production for the TT genotype (n=8, 3.9%) (Table 1). The frequencies of alleles of VEGF 936C and 936T were 0.82 and 0.18, respectively (Table 2). These distributions of observed genotypes conformed to Hardy-Weinberg equilibrium, indicating genetic equilibrium of the different variants.

Genotype and allele frequency are summarized in Table 1. Different frequencies of VEGF C936T were compared between Korean and Austrian subjects (Table 2). The rate in Korean subjects is somewhat higher than that in Austrian subjects.

Knowledge about VEGF C936T polymorphism leading to decreased VEGF plasma levels could lead to a much better understanding of these processes, and might be of diagnostic value. We have found observed numbers (%) of 936T were 28.5 (CT) and 3.9 (TT), respectively. The mutant allele frequency of VEGF 936T was 0.18 in Korean population (Table 2). This study revealed that genotype distributions and allele frequencies of VEGF C936T polymorphism in the Korean population are not significantly different from Austrian population.

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