

Study of VEGF-A Gene Polymorphism in the Patients with Nasopharyngeal Angiofibroma

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Abstract

Background: The vascular endothelial growth factor A (VEGF-A) play a crucial role in development and regulation of activity both of the blood and lymphatic vessels.

Material and Methods: For definition of the genetic markers C963T, G405C, -1154A of VEGF-A gene we used systems of the genetic analysis PyroMark Q24.

Results: The analysis of the data has shown that the patients, at the analysis of a genetic marker C963T, have a genotype CC, whereas the given genotype was not found out in the practically healthy people. At genotyping of a marker G405C of VEGF-A gene in group of the patients the increase in frequency of prevalence of a genotype CC (40,0 %) was noted, in comparison with control group (15,0 %). In case of research of G-1154A polymorphism the decrease (10,0 %) in prevalence of a genotype AAi, increase in quantity of detection of a genotype GG (50,0 %) in group of the patients with angiofibroma was observed, in comparison with the control (30,0 % and 10,0 %, respectively).

Conclusion: Identified VEGF-A gene polymorphism suggests that single nucleotide substitutions in the promoter region of genes are unique and have a definite impact on features of functioning proteins and gene expression.

Introduction

The vascular endothelial growth factor A (VEGF-A) is a member of the family of structurally closed proteins, which together with receptors (VEGFR) play a crucial role in development and regulation of activity both of the blood and lymphatic vessels. VEGF-A effects on the development of new blood vessels (angiogenesis) and on the survival of the immature blood vessels (vascular support), linking with two closed structurally membrane tyrosine kinase receptors (VEGFR-1 and VEGFR-2) and activating them. Besides, the data of

last years testify that VEGF-A is not only the main stimulator of angiogenesis, but also a lymphagenous factor [1, 2, 3, 4, 5].

In spite of the fact that the identity of the human genome is extremely high, at a level of sequences of genes the differences between two individuals make about 0,1 % [2]. The point mutations, that is replacements of single nucleotides (SNP-single-nucleotidepolymorphism), appeared to be the most frequent reason of distinctions in the structure of genes. There have been revealed two classes of high-affined VEGF-A-linking sites on the

Table 1. Distribution of the Frequencies of VEGF-A Gene Genotypes in the Patients with Nasopharyngeal Angiofibroma

Groups	VEGF-A Genotype, % (abs)									VEGF-A Alleles, % (abs)					
	C963T			G405C			-1154A			C963T		G405C		-1154A	
	C/T	T/T	C/C	G/C	G/G	C/C	A/G	A/A	G/G	C	T	G	C		
Patients, n =20	(6)	(11)	(3)	(7)	(5)	(8)	(8)	(2)	(10)	(28)	(12)	(10)	(30)	(28)	(12)
	30,0	55,0	15,0	35,0	25,0	40,0	40,0	10,0	50,0	70,0	30,0	25,0	75,0	70,0	30,0
	±7,2	±7,9	±5,7*	±7,5	±6,9	±7,8*	±7,8	±4,8*	±7,9*	±7,2	±7,2	±6,8*	±6,8*	±7,2*	±7,2*
Controls, n=20	(10)	(10)	(0)	(10)	(7)	(3)	(10)	(6)	(4)	(30)	(10)	(28)	(12)	(30)	(10)
	50,0	50,0	-	50,0	35,0	15,0	50,0	30,0	20,0	75,0	25,0	70,0	30,0	30,0	70,0
	±7,9	±7,9		±7,9	±7,5	±5,7	±7,9	±7,3	±6,3	±6,8	±6,8	±7,2	±7,3	±7,2	±7,2

*-p<0,05

cells and it is suggested, that such sites are required for monocytes in VEGF-dependent chemotaxis. It is shown, that similar low molecular (120-130 kDa) receptors exist on the cells of a tumor and link VEGF-A165, but not VEGF-A121. Thus, the special type of a tumor and endothelial cells express low affinity proteins which selectively link coded sequences [1].

The purpose of the present study was evaluation of the prevalence of the most typical polymorphic markers of the VEGF-A factor: C963T, G405C, -1154, in the patients with nasopharyngeal angiofibroma.

Material and Methods

For definition of the genetic markers C963T, G405C, -1154A of VEGF-A gene there was used method of pyrosequencing. The technique of detection of genetic polymorphisms of VEGF-A genes included the following stages:.

1. DNA isolation from the clinical material. There were used standard methods with the set of reagents "DNA-sorb-B" manufactured by FGUN " Central Scientific Research Institute of epidemiology" of Rospotrebnadzor.

2. PCR-amplification of a fragment containing polymorphic genetic locus. At amplification of the DNA fragment one of the pair of primers was linked at the 5'-end with biotin; the DNA chain, which will serve as a matrix for pyrosequencing, is amplified with biotinylated primer.

3. Preparation of the samples of PCR-product. This procedure included amplicon incubation with particles of sefarose, covered with streptavidin, amplicon denaturation and series of consecutive washings resulting in one-chained PCR- product, fixing on the particles of sefarose.

4. Immobilization of PCR-product on the solid surface and annealing of sequencing primer in the area of analyzing genetic locus. These processes resulted in duplex between DNA-matrix and sequencing primer, necessary for performance of reaction of pyrosequencing synthesis.

5. Sequencing of PCR-product – performance of the reaction of pyrosequencing and analysis of the results obtained.

For realization of reaction of pyrosequencing we used systems of the genetic analysis PyroMark Q24. As the object of research was characterized polymorphic locuses in the human genome, and position of the single-nucleotide polymorphism was known there was used opportunity for automatic processing of the result with the software of the devices used. On the basis of relative height of peaks on the program there was determined homo- or heterozygous state by polymorphic locus. The analyzed sequence for analysis of genetic markers C963T, G405C, -1154A of a gene VEGF-A was ACCA/GTGCTGGGT, C/GGACAGGGGCAAAGTG, GAA/GGGGCTGAGGC, accordingly for each marker.

Table 2. Differences Between Expected and Noted Frequencies of Heterozygosis of Gene VEGF-A Genetic Markers

Groups	C963T			G405C			-1154A		
	Noted heterozygosis	Expected heterozygosis	D	Noted heterozygosis	Expected heterozygosis	D	Noted heterozygosis	Expected heterozygosis	D
Patients n=20	0.3	0.42	0.4	0.4	0.37	-0.07	0.4	0.42	0.05
Control n=20	0.50	0.37	-0.26	0.50	0.42	-0.16	0.50	0.42	-0.16

Results

The results of VEGF-A genotyping in the patients with nasopharyngeal angiofibroma are presented in table 1. The analysis of the data has shown that the patients, at the analysis of a genetic marker C963T, have a genotype CC, whereas the given genotype was not found out in the practically healthy people. At genotyping of a marker G405C of VEGF-A gene in group of the patients the increase in frequency of prevalence of a genotype CC (40,0 %) was noted, in comparison with control group (15,0 %). In case of research of G-1154A polymorphism the decrease (10,0 %) in prevalence of a genotype AAi, increase in quantity of detection of a genotype GG (50,0 %) in group of the patients with angiofibroma was observed, in comparison with the control (30,0 % and 10,0 %, respectively).

The expected frequency of distribution of genotypes on balance of Hardy-Waiberg (BHW) in group of the patients at the analysis of a genetic marker C963T of a gene VEGF-A has made: C/C =0.49; C/T =0.42; T/T =0.09; in group of the control: C/C =0.56; C/T =0.37; T/T =0.06. The observable frequency of distribution of genotypes on BHW in group of the patients was: C/C =0.55; C/T =0.30; T/T =0.15 ($\chi^2=1.6$; P=0.2); in group of the healthy donors: C/C =0.50; C/T =0.50; T/T =0.0 ($\chi^2=0.1$; P=2.2).

At the analysis of a genetic marker G405C of a gene VEGF-A the expected frequency of distribution of genotypes by BHW in group of the patients was: G/G =0.06; G/C =0.37; C/C=0.56; in group of the control: G/G =0.49; G/C =0.42; C/C =0.09. The observable frequency of distribution of genotypes in group of the patients was: G/G =0.05; G/C =0.40; C/C =0.55 ($\chi^2=0.1$; P=0.8); in group of the healthy donors: G/G=0.45; G/C=0.50; C/C=0.05 ($\chi^2=0.7$; P=0.4).

At the analysis of a genetic marker G1154A of gene VEGF-A the expected frequency of distribution of genotypes by BHW in group of the patients was: G/G =0.49; G/A=0.42; A/A=0.09; in group of the control: G/G=0.09; G/A=0.42; A/A=0.49. The observable frequency of distribution of genotypes in group of the patients was: G/G =0.50; G/A =0.40; A/A =0.1 ($\chi^2=0.05$; P=0.8); in group of the healthy

donors: G/G =0.05; G/A =0.50; A/A =0.45 ($\chi^2=0.7$; P=0.4).

In table 2 the results of definition of distinctions between expected and observable frequencies of heterozygosis of genetic markers of gene VEGF-A are given. The reliable distinctions in definition of heterozygotes among the investigated groups were not revealed: both in skilled, and in control groups the quantity of heterozygotes varied within the limits of 40-50 %, the least number of heterozygotes was observed at the analysis of a genetic marker C963T in the patients with angiofibroma.

Discussion

Expression of VEGF is stimulated by set of the proangiogenic factors, including epidermal growth factor, main fibroblast growth factor, thrombocytary growth factor, interleukin 1 β , and factors of external environment, such as pH, pressure and concentration of oxygen. The similar influence consists in mediated through VEGF stimulation of important for angiogenesis and lymphangiogenesis factors, including antiapoptotic proteins, molecules of cellular adhesion and metalloproteinase. However, the fact is conclusive, that production of VEGF in reply to standard stimuli varies between the people, and in a population there are met stable low-producing and high-producing phenotypes, at the constant structure of synthesized protein [6].

The researches performed for revealing of the frequency of polymorphism prevalence of the VEGF-A gene allowed to establish, that in the patients with nasopharyngeal angiofibroma there was determined genotype CC of the genetic marker C963T, increase of CC genotype frequency up to 40,0 % at the analysis of a marker G405C, decrease of frequency of genotype AA up to 10,0 %, and increase of frequency of genotype GG up to 50,0 % at the analysis of a marker G-1154A. In the investigated groups of the patients and control at

the analysis of genetic marker C963T there was observed distribution of frequencies of genotypes of this polymorphism without deviation from BHW, i.e., it was corresponded to expected ($\chi^2=0.1$; $P=2.2$).

Conclusions

Revealed polymorphism of gene VEGF-A allows to assume, that single-nucleotide replacements in the promoter region are individualized and have special effect on the features of the protein functioning and on the gene expression.

In the patients with nasopharyngeal angiofibroma there was observed genotype CC of genetic marker C963T, increase in frequency of CC genotype to 40,0 % at the analysis of marker G405C, decrease in frequency of AA genotype up to 10,0 % and increase in frequency of GG genotype to 50,0 % at the analysis marker G-1154A.

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