Expression of vascular endothelial growth factor and its receptor in the epididymal sperm of adolescent male rats*****★

Ai Qing-yan, Zhao Yu-feng, Wang Yan-mei, Miao Nai-zhou, Ma Li, Yang Jia-zhou

Abstract

BACKGROUND: Vascular endothelial growth factor (VEGF) and its receptor fms-like tyrosine kinase-1 (Flt-1) have a very important position in the field of male reproduction. However, it is still unclear about their expression meaning and regulatory mechanism in the reproductive system.

OBJECTIVE: To study the expression and location of VEGF and Flt-1 in the epididymal sperm of adolescent male rats.

METHODS: The expression of VEGF and Flt-1 was detected in 10 adolescent SD rats. The concentration of the sperm was (30–40)×10^9/L. Immunofluorescent staining was used for VEGF and Flt-1 expression and location in the sperm.

RESULTS AND CONCLUSION: Immunofluorescent staining showed that VEGF and Flt-1 were both localized in the acrosome of sperm head, as well as in the neck, middle and principal segment of sperm tail. Specific expression patterns of VEGF and Flt-1 in the sperm of rats display that they may participate in spermiotelcosis, relevant to movement, capacitation and acrosome reaction of the sperm.

Department of Human Histology and Embryology, Medical School of Yan'an University, Yan'an 716000, Shaanxi Province, China

Ai Qing-yan★, Master, Lecturer, Department of Human Histology and Embryology, Medical School of Yan'an University, Yan'an 716000, Shaanxi Province, China
aqy_2001@163.com

Correspondence to: Yang Jia-zhou, Associate professor, Department of Human Histology and Embryology, Medical School of Yan'an University, Yan'an 716000, Shaanxi Province, China
jiazhouy@163.com

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INTRODUCTION

Vascular endothelial growth factor (VEGF) is also named as vascular permeability factor (VPF) or blood vessel opsonin. It is either the specific mitogen of vascular endothelial cells (VEC) or the superactive angiogenesis inducer. It is widely distributed in the soma. And it brings into full play by the means of mediated receptor[1]. VEGF receptor belongs to the family of tyrosine protein kinase c-fms. It forms dimeride when it is stimulated by VEGF. The residue of its protein tyrosine produces a phosphorylation reaction. The signals are transferred. As a result, phosphatase Cγ can be activated, the hydrolysisof phosphatidylinositol 4, 5-bisphosphate occurs to generate 1, 4, 5- inositol triphosphate and diacylglycerol. It stimulates the rise of Ca^{2+} content and activates the opening of protease C[2]. According to the reports of the scholars abroad, VEGF and its receptor are expressed in the testis, epididymis, glandula seminalis and prostatic gland. Besides, high-concentration VEGF protein is also detected from the semen[3].The experiment observed the expression and location of VEGF and fms-like tyrosine kinase-1 (Flt-1) in the epididymal sperm of normal rats.

MATERIALS AND METHODS

Design

Experiment based on observation.

Time and setting

The experiment was completed in the Medical College of Yan'an University in February 2010.

Materials

Animals

Ten adolescent male SD rats, clear and healthy, approximately 7–8 weeks old, weighing 180–200 g, were involved in the experiment. All were purchased from the Experimental Animal Center of the Medical College, Xi'an Jiaotong University.

Reagents and instruments are listed as follows:

Reagent and instrument	Resource
Anti-rat VEGF, Flt-1 monoclonal antibody	Newmarker Company, America
Goat serum, FITC signed Anti-rabbit IgG	Beijing Zhong Shan Jin Qiao Biotechnology Limited Company, China
Triton-X 100, aminoacetic acid	Beijing Chemicals Factory, China
Fluorescent digital microscope	Olympus, Japan

Methods

Sample collection and dealing

Bilateral epididymises of rats were extracted. Then the adipose tissues were separated and the fresh epididymises was cut and put into 0.01 mol/L phosphate buffered saline (PBS) (pH 7.4). The supernatant after the precipitation was removed followed by 2 200×*g* centrifugation, 15 minutes. The sample was fixed with 40 g/L paraform for 30 minutes, then centrifuged at 2 200×*g* for 15 minutes. After washed twice in PBS containing 50 mmol/L glycine, 2 200×*g* centrifugation for 15 minutes was performed again. The sample was adjusted with PBS to the sperm concentration of (30–40)×10^9/L; smearing and drying in the open-air was done for immunofluorescence staining.

Immunofluorescence staining

The specimen was hatched with 3% H_2O_2 for 30 minutes to wipe out endogenous peroxidase activity, then immersed with 1% Triton X-100 about 15 minutes to increase the penetrability of organization, and closed with 5% goat serum for 25 minutes at the room

temperature. Anti-rat VEGF, Flt-1 monoclonal antibody (1∶50) was added at 4 ℃ overnight; in addition, FITC labeled anti-rabbit IgG (1∶100) was added at 37 ℃ hatching for 1 hour. The specimen was rinsed twice with 0.01 mol/L PBS (pH 7.2–7.4) for each step, each time for 5 minutes. Glycerol-sodium bicarbonate was used as wet sealant. The specimen in the negative control group was inoculated in 0.1 mol/L PBS (pH 7.2–7.4) instead of primary antibody. The rest steps were similar as above mentioned. The whole process was observed with the fluorescent digital microscope. Yellow-green fluorescent sperm was considered as positive.

Main outcome measures

The expression and distribution of VEGF and Flt-1 in the male rats.

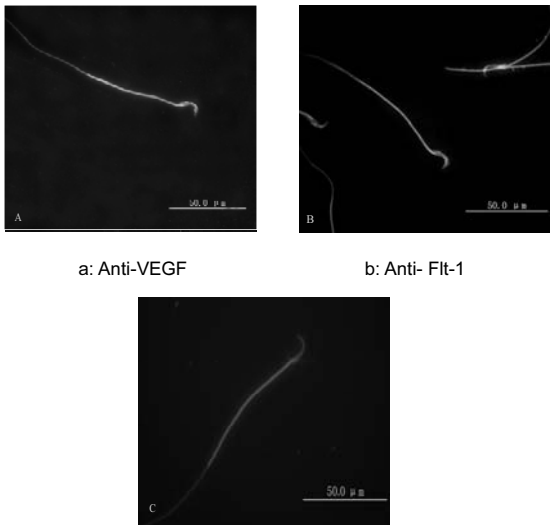
RESULTS

Quantitative analysis of experimental animals

Ten SD rats were involved in the result analysis.

Expression of VEGF and Flt-1 in the sperm of adolescent male rats

Under the fluorescent digital microscope, both VEGF and Flt-1 positive proteins were allocated in the acrosome of sperm head, as well as in the neck, middle and principal segment of sperm tail. No positive staining was shown at either the sperm nucleus or at the end piece of sperm tail (Figure 1).



a: Anti-VEGF b: Anti- Flt-1

c: Negative control

Figure 1 Immunofluorescent expressions of vascular endothelial growth factor (VEGF) and its receptor fms-like tyrosine kinase-1 (Flt-1) proteins in the adolescent rat epididymal sperm (Bars: 50 μm)

DISCUSSION

VEGF is a kind of angiogenesis factor with dissolubility and endothelial cell specific mitogen factor, mediating many endodermis and non-endodermis effects, such as promoting cell caryocinesia and chemotaxis, inhibiting cell apoptosis, inducing angiogenesis, elevating vasopermeability, mediating Ca^{2+} inflow and improving extracellular matrix.

Obermair *et al* [4] reported for the first time that the concentration of VEGF in the seminal plasma has a great impact on the reproduction of human beings. If putting VEGF and its receptor on the surface of the sperm, the expression of Flt-1 is very dramatic in the acrosome of the sperm head. This experiment involved the healthy adolescent male rats as objects to detect the expression of VEGF and Flt-1 protein in the sperm of the pididymis by means of immumofluorescence staining. VEGF and Flt-1 protein were expressed in the sperm of rats. In addition, the immunofluorescence staining showed comparatively strong results at the apical body of the head of the sperm as well as the cervical segments, middle piece and principal piece of the tail. It illustrates that the sperm cell is one of the target cells of VEGF[5]. Therefore, it can be estimated that the combination of VEGF and Flt-1 not only participates in the spermiotelcosis, but also plays an important role in movement, capacitation and acrosome reaction of the sperm.

The most important change for the sperm in the maturation process of the epididymis is the physico-chemical property change of the membrane structure and its physico-chemical propertyis change, including the changes of the permeability and flowability of the membrane, membrane lipid, membrane protein, membrane receptor. The expression of VEGF and Flt-1 protein at the apical body and tail of the epididymis shows that they can affect different ion channels in an autocrine and paracrine manner and participate in the spermiotelcosis. The combination of VEGF and its receptor may participate in the spermiotelcosis of the perforatorium by redecorating the membrane structure. They may also form or activate the Ca^{2+} pathway and cause internal flow of the Ca^{2+} at the tail of spermatozoon. In addition, they may activate the information transfer in cells and promote the sperm to take on the activation movement so as to obtain the motor capacity[6]. Among the ions which influence the physiological functions of the sperm, Ca^{2+} in the intracytoplasm of the sperm may have the dominant position in controlling the azoospermia[7-8]. As the second messenger in cells, Ca^{2+} plays a very crucial role in various physiological stages of the sperm, including the movement of the sperm, capacitation, acrosomal reaction and sperm-egg binding[9]. Ca^{2+} signal realizes its control over the movement of the sperm by the adjustment of Ca^{2+} concentration in cells which is adjusted by the relevant Ca^{2+} ion channels on the cytolemma of the sperm[10]. It is found that many proteins which have connection with the axial filament microbules at the tail of the sperm are substrates of protein kinase C. They are all indispensible for the priming and the maintenance of the motion of the sperm[11]. The protein phosphorylation is made by the protein kinase C. Furthermore, the motion of the sperm is adjusted, and sperm activity and contents are closely connected with the motion of the sperm.

Numerous studies have shown that Ca^{2+} also depends on capacitation[12]. Ca^{2+}/Calmodulin can activate adenyl cyclase to increase the composition of the cAMP. It can also realize the degradation of the cAMP by cAMP cylic nucleotides. Ca^{2+} plays both the positive and negative effects on capacitation and relevant signals[12]. In the process of the sperm capacitation, the protein tyrosine phosphorylation increases. It is estimated that the combination of VEGF and Flt-1 might promote or adjust the capacitation of the sperm.

Acrosomal reaction is a process of Ca^{2+}-depended exocytosis. The ingression of Ca^{2+} to the cell is an indispensible condition for the acrosomal reaction. The sperm plasma membrane depolarizes and the ion channel of Ca^{2+} opens. Ca^{2+} that entered induces the acrosome reaction of Ca^{2+} dependence. What is more, the alteration of Ca^{2+} in the process of the acrosome reaction is also very important[13]. The perforatorium of mammals includes inositol triphosphate receptor of high-density 1, 4, 5. And the combination of inositol triphosphate 1, 4, 5 and ER specificity receptor activate calcium channel and release Ca^{2+}. Inositol triphosphate 1, 4, 5 stimulates the Ca^{2+} concentration to heighten. Hence, the sensitivity of the receptor to Ca^{2+} enhances. As a result, it stimulates the calcium channel to open and Ca^{2+} to release[14]. There are 95 000 PTK receptors in the sperm of both men and mice. They are on the cytolemma which is at the lateral apical body of the head. Surprisingly, they can be combined with the transparent glucoprotein ZP3. Moreover, they are widely known as the ZP3 receptor. When the sperm is combined with the pellucid zone, PTK realizes the phosphorylation of the residue of the tyrosine in the inner segment of the cell. Besides, the residue of the tyrosine in the inner segment of the cell is recognized and combined by the target protein with the structure of SH2 and then induces sperm acrosome reaction through transmembrane signal transduction[15]. The combination of VEGF and its receptor on the surface of the sperm plays an important role in inducing the acrosomal reaction. Furthermore, Ca^{2+} also plays a crucial role in the producing of the zygote.

The expression of VEGF in the fallopian tube of the ox which is in the oestrous cycle is relatively steady. And the expressions of its receptor show cyclical variation. Especially, the transcripts of Flt-1 increase dramatically before the ovulation. VEGF is mainly located on the surface of uterine tubal epitheliu. The protein concentration of the douche of the fallopian tube is obviously higher than the other period of the oestrous cycle [1, 16]. The periodic changes of the fallopian tube VEGF and its receptor expressions provide fertilization with a good microenvironment. Hence, it might prove that the combination of VEGF in the tube with the VEGF in the sperm will directly influence the fertilizability of the sperm[17]. The age of the male and the concentration of the VEGF in the ejaculate can be regarded as an index of the external fertilization[4]. Studies have shown that concentration of VEGF in the ejaculate affects ART significantly. Such as IVF and pregnancy rate in ICSI. The higher the concentration of VEGF in the ejaculate, the higher the pregnancy rate. It even increases more than six times in such a circumstance[18], VEGF not only adjusts the male reproduction by affecting the corpuscular receptor of genital duct, but also influences the pregnancy rate by directly adjusting the sperm cells.

The different expressions of VEGF and Flt-1at the head and tail of the sperm show that they might affect different ion channels by means of autocrine or paracrine. They not only participate in the spermiotelcosis, but also play an important role in harvesting the motor capacity and fertilizability of the sperm. All in all, they play an important role in the male reproduction. However, there is still a long way to study its expression and regulatory mechanism in the genital system.

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血管内皮生长因子及其受体在大鼠附睾内精子上的表达*****★

艾庆燕，赵豫凤，王艳梅，苗乃周，马　莉，杨加周(延安大学医学院组织胚胎学教研室，陕西省延安市　716000)

艾庆燕★，女，1975 年生，陕西省延安市人，汉族，2008 年延安大学毕业，硕士，讲师，主要从事生殖医学研究。
通讯作者：杨加周，副教授，延安大学医学院组织胚胎学教研室，陕西省延安市 716000

摘要

背景：血管内皮生长因子及其受体在男性生殖领域中占有重要地位，但其在生殖系统中表达的意义和调节机制仍不十分清楚。

目的：观察血管内皮生长因子及其受体类 fms 酪氨酸激酶在青春期大鼠附睾内精子上的表达定位情况。

方法：取 10 只青春期雄性 SD 大鼠双侧附睾，分离得到浓度为(30~40)×10^9 L^{-1} 的精子，涂片，免疫荧光法检测精子上血管内皮生长因子及其受体类 fms 酪氨酸激酶的表达定位情况。

结果与结论：免疫荧光结果显示，血管内皮生长因子及其受体类 fms 酪氨酸激酶阳性蛋白均定位于大鼠附睾精子头部的顶体、尾部的颈段、中段和主段，尾部末段和精子核未见阳性染色。提示，血管内皮生长因子及其受体类 fms 酪氨酸激酶可能参与了精子的成熟过程，与精子的运动能力、获能和顶体反应有关。

关键词：血管内皮生长因子；受体；类 fms 酪氨酸激酶；精子；免疫荧光
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本文创新性：

提供证据：检索 CNKI 数据库最近 10 年的相关资料。检索关键词为：“血管内皮生长因子、受体、类 fms 酪氨酸激酶、精子”。未见与文章密切相关文献。

创新点说明：目前，对于血管内皮生长因子在男性生殖道中的作用知之甚少；理解血管内皮生长因子在生殖道中的作用，有利于揭示引发不育的原因及改善生殖功能的方法。