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Expression of vascular endothelial growth factor (VEGF) transcript and protein in the testis of several vertebrates, including endangered species

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Abstract

Vascular endothelial growth factor (VEGF) is known to influence the testis function. To establish the role of VEGF in the testis of a variety of species, we analyzed the expression of VEGF transcript using human gene-specific primers by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis in the testes of 18 vertebrates, including a few endangered species. An amplicon of 566 bp representing $VEGF_{165}$ was identified in testis of all species in this study. Sequence analysis of these amplicons revealed 84 to 96% homology to available human VEGF sequence and to the VEGF sequences of other species in GenBank. Immunohistochemical analysis revealed expression of VEGF protein, primarily in Sertoli and Leydig cells and occasionally in the germ cells of the testis sections. It can be concluded from this study that expression of VEGF transcript is conserved in the testis of several vertebrates and may have a role in the process of spermatogenesis.

Keywords: VEGF; Testis; Spermatogenesis; Vertebrates; Endangered species

1. Introduction

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF) or vasculotropin, induces proliferation of endothelial cells and increases permeability of the vessel wall [1,2]. The VEGF gene is located on chromosome 6p21.3 in humans. A group of related growth factors includes vascular endothelial growth factor-A (VEGF-A), VEGF-B, VEGF-C, VEGF-D and placenta-derived growth factor (PIGF) [1,2]. VEGF-A is produced by different cell types, including vascular smooth muscle cells (VSMC), macrophages, fibroblasts, and tumor and endothelial cells [2–9]. The expression of several known VEGF-A isoforms is induced by hypoxia, growth factors, such as TGFb, bFGF, or PDGF, and cytokines, such as IL-1β and IL-6 [4–9].

VEGF-A is a hypoxia-inducible peptide essential for angiogenesis and targets nonvascular cells in a variety of tissues and cell types. VEGF-A is generated in several isoforms, resulting from alternative splicing of VEGF pre-mRNA [2]. The most common isoforms are $VEGF_{121}$ and $VEGF_{165}$, which consist of 121 and 165 amino acids, respectively [2]. The longer $VEGF_{189}$ and $VEGF_{206}$ isoforms are also synthesized [2]. Owing to the presence of an N-terminal signal sequence,

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VEGF is secreted from the cells. The shortest isoform, $VEGF_{121}$ does not bind to extracellular matrix, $VEGF_{165}$ is partially free and partially bound, and $VEGF_{189}$ and $VEGF_{206}$ are largely bound to heparan sulfate [2].

Normal physiological role for VEGF in angiogenesis during embryo development and postnatal growth has already been established [10,11]. In the adult female, VEGF has been shown to be involved in active angiogenesis that occurs during ovarian follicular and luteal phases of estrous cycle in rodents [12] and the menstrual cycle in primates [13]. Simultaneously, enhancement of angiogenesis and vascular permeability has also been observed in the uterine endometrium [14]. Unlike the adult female, there is no active angiogenesis in the testis of the adult male even though an enormous amount of VEGF is produced by the testis [15]. VEGF-A isoforms stimulate endothelial and hematopoietic stem cells through two high affinity membrane-bound receptor tyrosine kinases, FLT1 (also known as VEGFR1) and KDR (also known as VEGFR2 and FLK1) [16]. Following the binding of VEGF-A, each receptor elicits a distinct cellular response: FLT1 regulates cell-cell interactions [17,18], chemotaxis [19], and cell survival [20], while KDR is responsible for the majority of signaling leading to proliferation, survival, and differentiation processes [17,18]. Data also suggest potential functional roles of VEGF-A in the postnatal testis, as KDR and FLT1 are expressed in testicular germ cells in humans [21], rats [22], mice [23], and cattle [24], while somatic Sertoli and Leydig cells produce VEGF-A [25]. Two studies have shown that overexpression of $VEGF_{121}$ and $VEGF_{165}$ leads to detrimental effects on male fertility in transgenic mice [26,27]. Microarray analysis identified VEGF-A as a candidate gene important for germ cell function in normal and grafted bovine testis in situ [28], and a study suggests that VEGF-A may regulate germ cell proliferation in the bovine testis [29]. VEGF have been implicated in germ cell homeostasis [30], and several studies have demonstrated the importance of VEGF for male fertility [26,27,31]. VEGF acts to stimulate functional mechanisms in undifferentiated germ cells during postnatal bovine testis development to support the proliferation and/or survival of these cells [24]. It is, therefore, evident that VEGF exerts a differential effect of proliferation or differentiation during the process of spermatogenesis.

To date, no report exists that describes expression of the VEGF gene in the testis of wild and endangered species, including lower vertebrates. The objective of the present study was to analyze the expression of VEGF transcript and protein in the testis of several vertebrates, including endangered species and lower vertebrates, such as reptile and fish.

2. Materials and methods

2.1. Collection of the testes

Testes samples from adult domestic species were collected from local slaughter house. Testes samples from wild animals of various age groups were collected from Nehru Zoological Park, Hyderabad, India following the death of the animals. Testes samples from stray dogs and squirrels were collected from animals that died in road accidents. Testes samples from cat fish was collected from local fish seller. A small piece of testis tissue was submerged immediately after collection in RNAlater (Ambion, Inc; http://www.ambion.com) following manufacturer's instructions and stored at −20 °C until isolation of RNA. For histochemical analysis, testes tissues were immediately fixed in Bouin's fixative following collection and processed, embedded, sectioned, and stored until further analysis. Only tissue sections that were preserved optimally and, which showed fewer degenerative changes were chosen for immunohistological analysis. The list of animals and their respective sample size is given in Table 1.

2.2. Isolation of RNA and reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was prepared from testes tissue of 18 vertebrates. For positive control, RNA was prepared from human embryonic kidney (HEK) cells. The stored testes tissue pieces were removed from RNALater and processed for RNA isolation using Trizol reagent (Invitrogen; http://www.invitrogen.com) according to the manufacturer's instructions. Extracted RNAs were diluted with DEPC-water and incubated with 10 U of RNase free-DNase (Roche, http://www.roche.com) for 30 min at room temperature. Incubating the samples at 70 °C for 15 min inhibited DNase activity and samples were stored on ice. Random Primers and RNase OUT (both from Invitrogen) were added to the RNA solution, incubated for 5 min at 65 °C and set on ice. For reverse transcription, MMLV high performance reverse transcriptase (Epicentre Biotechnologies, http://www.epibio.com) was added to the RNA solution and incubated for 10 min at 25 °C, for 60 min at 37 °C and for 5 min at 90 °C (RT +). At the same time, the reactions without the addition of reverse transcriptase