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*Short Communication*

# Expression of splice variants of cancer-testis genes *ODF3* and *ODF4* in the testis of a prostate cancer patient

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**ABSTRACT.** The outer dense fiber (*ODF*) genes encode proteins that co-assemble along the axoneme of the sperm tail. Recently, it was demonstrated that some *ODF* genes are aberrantly expressed in tumors, including prostate adenocarcinoma, basal cell carcinoma, and chronic myeloid lymphoma. We cloned *ODF3* and *ODF4* cDNA from the testis of a patient suffering from prostate adenocarcinoma and found two alternative splice variants of these genes.

**Key words:** ODF3; ODF4; Cloning; Alternative splicing; Cancer-testis gene

 

## INTRODUCTION

Recent studies have identified the expression of two groups of keratins during mammalian spermatogenesis: the classical epidermis-type keratins expressed in spermatogonia, spermatocytes, and spermatids and the outer dense fiber (Odf) proteins expressed predominantly during mammalian spermiogenesis (Kierszenbaum, 2002). The Odf family of proteins co-assembles along the axoneme during growth of the sperm tail (Kierszenbaum, 2002). In humans, they are encoded by *ODF1* (NM_024410), *ODF2* (NM_002540.3), *ODF3* (NM_053280.3), and *ODF4* (NM_153007.3) (Kierszenbaum, 2002; Miyamoto et al., 2004). Odf proteins maintain the passive elastic structures and elastic recoil of the sperm tail (Fawcett, 1975) and protect the sperm tail from shear forces during epididymal transport (Baltz et al., 1990).

*ODF3* is a testis-specific gene expressed in haploid germ cells. The human gene is located on 11p15.5. Its rat ortholog is expressed in epididymides and brain, suggesting a possible role in the cytoskeletal structure (Petersen et al., 2002). In humans, its mRNA consists of 1331 nucleotides with 7 exons (http://www.ncbi.nlm.nih.gov/nuccore/NM_053280.3), encoding 254 amino acids (http://www.ncbi.nlm.nih.gov/protein/NP_444510.2). Its amino acid sequence has 6 Pro-Gly-Pro repeats, which are also seen in the mouse ortholog protein as well as in 2 other reported proteins of *Drosophila melanogaster* (Egydio de Carvalho et al., 2002). The protein is expressed in the flagella of the elongated spermatids and along the entire length of the tail in mature sperm (Egydio de Carvalho et al., 2002).

*ODF4* is a testis-specific gene that is located on 17p13.1 and is expressed in the outer dense fibers (ODFs) of the tails of mature sperm. It was first isolated from a subtracted cDNA library of mouse testis (Nakamura et al., 2002). Later, a human ortholog was cloned, and its mRNA was found to be expressed exclusively in the testis. The 30-kDa protein encoded by the mRNA was detected in the flagellae of ejaculated sperm, and its gene was mapped to chromosome 17 (Kitamura et al., 2003). Then, the full-length cDNA of it was isolated using 5'-random amplification of cDNA ends (RACE) and 3'-RACE (Miyamoto et al., 2004). Its mRNA consists of 1149 nucleotides with 3 exons (http://www.ncbi.nlm.nih.gov/nuccore/NM_153007.4; NCBI, 2012) and encodes for 257 amino acids (http://www.ncbi.nlm.nih.gov/protein/NP_694552.2; NCBI, 2012).

Previously, we analyzed the expression of *ODF3* and *ODF4* genes along with two other members of this group, *ODF1* and *ODF2*, in patients with prostate cancer and benign prostate hyperplasia using reverse transcriptase (RT)-polymerase chain reaction (PCR). Although *ODF1* and *ODF2* were expressed in 10% of prostate cancer samples, *ODF3* and *ODF4* were not expressed (Ghafouri-Fard et al., 2010a). In addition, we analyzed *ODF4* expression in patients with chronic myeloid leukemia and found that it was expressed in 30% of patients (Ghafouri-Fard et al., 2012). *ODF3* was expressed in 2.6% of basal cell carcinoma samples, but *ODF4* was not expressed in these samples (Ghafouri-Fard et al., 2010b). Here, we examined *ODF3* and *ODF4* transcripts in a testis sample from a patient with prostate adenocarcinoma.

## MATERIAL AND METHODS

### Patient and sample

Testis tissue was obtained from a normal fertile male patient undergoing orchidectomy as a part of his treatment for prostate cancer. The participant provided informed consent.

Study approval was obtained from the Investigation Review Board at Tehran University of Medical Sciences. This study was also approved by the Ethics and Clinical Studies Committee of Tehran University of Medical Sciences.

## RNA extraction, cDNA synthesis, and RT-PCR

Total RNA was isolated from testis tissue using the Tripure isolation reagent (Roche, Mannheim, Germany) according to manufacturer instructions with minor modifications. The mRNA expression of *ODF4* was evaluated using RT-PCR. RT was carried out in each sample using random hexamer primers (Pharmacia, Sweden) and MMLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) on 1 mg total RNA. PCRs were performed using specific primers for *ODF3*, *ODF4*, and *HPRT1* for checking cDNA quality. *ODF3* and *ODF4* primers were designed to cover all reading frame of the corresponding gene.

The primer sequences are listed below:

*ODF3* forward primer for cloning: 5'-cgggatccatgacggaggaggtatgg-3'

*ODF3* reverse primer for cloning: 5'-gggatccttattccacatcaaccagcag-3'

*ODF4* forward primer for cloning: 5'-ggaattcatggatgcagagtactctgg-3'

*ODF4* reverse primer for cloning: 5'-ggaattcttacacatgtgtatccttctgc-3'

*HPRT1* forward primer: 5'-gcattgttttgccagtgtcaa-3'

*HPRT1* reverse primer: 5'-attgtaatgaccagtcaacaggg-3'

*HPRT* primers spanned different exons of this gene in order to prevent false-positive results caused by genomic DNA contamination of the RNA preparation.

PCR was performed using 30 cycles in a thermal cycler at an annealing temperature of 60°C for *HPRT1*, and 56°C for *ODF3* and *ODF4*. Each set of PCRs contained a negative control containing all components of the PCR mixture except for cDNA, which was substituted with water. The integrity of the RNA was checked in each PCR by amplification of the *HPRT1* gene segment. PCR products were subjected to electrophoresis and run on a 1.2% agarose gel containing ethidium bromide, which was then photographed under ultraviolet light.

## Cloning

The open reading frames (ORFs) of the *ODF3* and *ODF4* genes were amplified using specific primers with *Bam*HI restriction sites for *ODF3* cloning and *Eco*RI restriction sites for *ODF4* cloning. PCR was performed on cDNA of the testis sample using specific primers to amplify the entire ORFs of *ODF3* and *ODF4*. PCR products were then digested by the appropriate restriction enzymes and were inserted into the restriction sites of the Pmal-C2X vector. Competent XL1-Blue *Escherichia coli* cells were transformed with the recombinant vectors. Positive clones were selected by PCR using a primer specific for the desired gene and a specific primer for the vector (M13_PUC_F: CCCAGTCACGACGTTGTAAAACG). Recombinant vector sequences were then confirmed by sequencing.

## Sequencing

The specificity of the primers was also confirmed by sequencing mini-preparations of

the selected clones with MBP_F_ primer (GATGAAGCCCTGAAAGACGCGCAG) using an ABI Prism3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

## RESULTS

RT-PCR of cDNA from the testis sample showed two differently sized products for *ODF3* and one for *ODF4* (Figures 1 and 2). Cloning and sequencing of all products confirmed the specificity of the primers (Figures 3 and 4). The longer *ODF3* product represented the previously identified variant, whereas the sequence of the shorter product represented a variant lacking exon 4 and some parts of exons 3 (nucleotides 492 to 703 of the mRNA sequence) and 5 (nucleotides 826 to 967 of the mRNA sequence). Sequencing of the *ODF4* product showed that it lacked some parts of exon 1 (nucleotides 292 to 636 of the mRNA sequence). Schematic alternative splice sites for *ODF3* and *ODF4* exons are shown in Figures 5 and 6, respectively. Alternative splicing sites in exons 3 and 5 of the *ODF3* variant as well as an alternative splicing site in exon 1 of the *ODF4* variant led to in-frame deletions. The newly identified *ODF3* and *ODF4* variants were submitted to GenBank, and their accession Nos. are FJ436414-1 and FJ23552601, respectively.

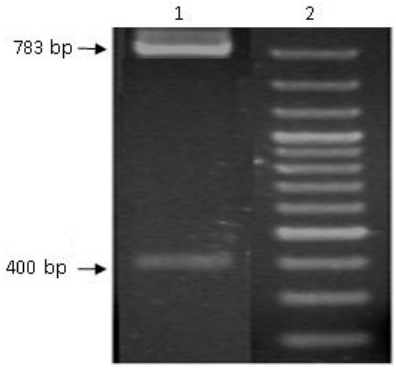


**Figure 1.** RT-PCR result for amplification of open reading frame of *ODF3*: *lane 1* = results for testis sample; *lane 2* = 100-bp DNA ladder.

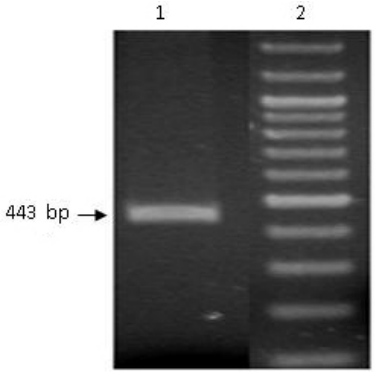


**Figure 2.** RT-PCR result for amplification of open reading frame of *ODF4*: *lane 1* = results for testis sample; *lane 2* = 100-bp DNA ladder.

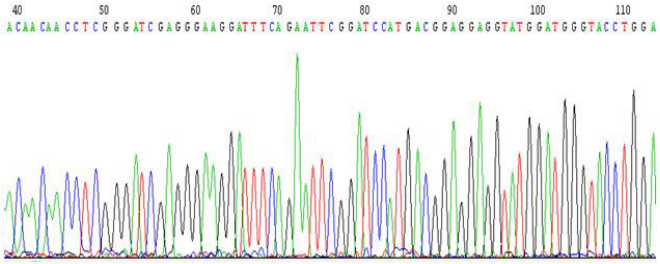

**Figure 3.** Sequencing results of *ODF3* clone using the MBP primer.

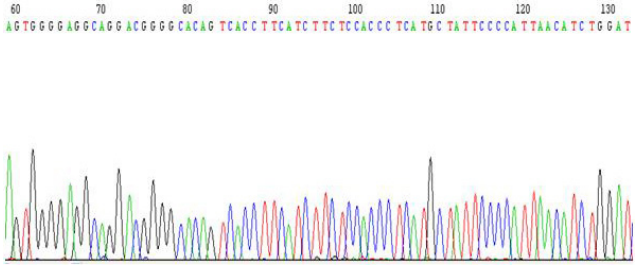

**Figure 4.** Sequencing results of *ODF4* clone using the MBP primer.

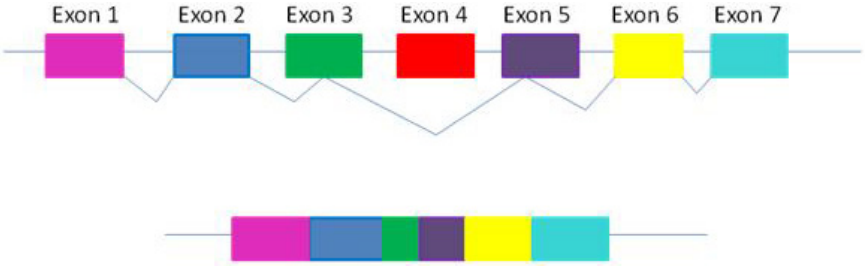


**Figure 5.** Schematic alternative splicing of *ODF3* exons.

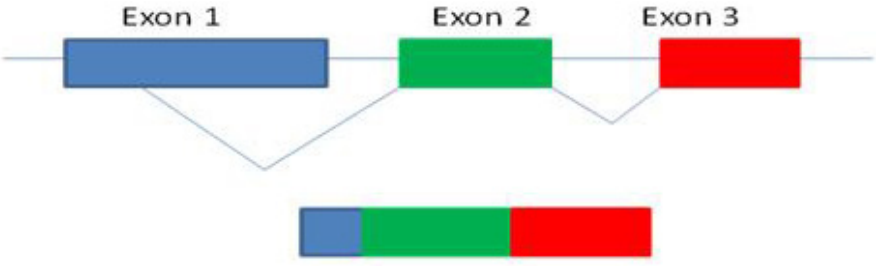


**Figure 6.** Schematic alternative splicing of *ODF4* exons.

## DISCUSSION

In higher organisms, a central mechanism for increasing complexity and information capacity from the genome to the transcriptome is alternative splicing (Chen and Zheng, 2008). Recent advances in the field of bioinformatics and the application of expressed sequence tags and exon-exon junction microarrays have revealed that approximately two-thirds of human genes are alternatively spliced (Kan et al., 2001; Johnson et al., 2003). Earlier studies had estimated that cassette exons compose up to 60% of alternative splicing cases in the majority of species (Mironov et al., 1999; Stamm et al., 2000). Identification of splice variants of human genes is important in many biological fields, including cancer research. Splice variants can also serve as cancer biomarkers (Brinkman, 2004). Alternative splicing is a mechanism involved in the regulation of many cancer-associated genes. During the process of tumor formation, the splicing process can be altered and controlled to switch to specific alternative splice forms, which could play a key role in carcinogenesis (Brinkman, 2004). Here, two cancer-associated genes *ODF3* and *ODF4*, with aberrant expression in some tumors, were shown to have splice variants in the testis tissue of a fertile man suffering from prostate adenocarcinoma. Prostate tissue responds to paracrine hormone stimulation from the testes. Although Odfs have not been shown to be involved in hormone synthesis, future studies should investigate whether splice variants of these two genes affect the crosstalk between the testis and prostate. Aberrant expression of some *ODF* genes (*ODF1* and *ODF2*) in prostate cancer samples raises the possibility that these genes are involved in tumorigenesis. This hypothesis should be examined in the future. In addition, a lack of expression of the common variant of *ODF4* in this sample and normal fertility of the patient implies that the small variant might be functional in spermatogenesis. Nevertheless, the relationship between this variant and interaction between the testis and prostate must be investigated further.

## ACKNOWLEDGMENTS

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