Review Article

Outer Dense Fiber Proteins: Bridging between Male Infertility and Cancer

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Abstract

Background: The similarities between gametogenic and carcinogenesis processes have been noted for more than decades. Among prominent similarities between these two processes is expression of a group of antigens, namely cancer-testis antigens in both the testes and various cancer tissues. Outer dense fiber (ODF) proteins are testis-specific proteins localized to sperm tails and involved in sperm motility.

Methods: We performed a computerized search of the MEDLINE/PUBMED databases with keywords "outer dense fiber, ODF, cancer, testis, gametogenesis and infertility".

Results: The results of animal and human studies show ODF contribution to male fertility. In addition, ODFs are expressed in some cancers, including prostate adenocarcinoma, breast cancer, chronic myeloid leukemia and basal cell carcinoma.

Conclusion: ODF expression analysis in cancer may pave the way for identification of cancer biomarkers or therapeutic targets.

Keywords: Cancer, gametogenesis, infertility, outer dense fiber proteins

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Introduction

The similarities between gametogenic and carcinogenesis processes have been noted for more than decades.[1] The shared characteristics between cancer cells and those in the germ cell differentiation pathways include global hypomethylation, immune evasion, immortalization (participating in transformation), stimulation of meiosis (can result in aneuploidy), and migration (involved in metastasis).[2,3] Genetic alterations in cancer can lead to reactivation of normally silent germ line expression programs, which might confer some of the essential features of malignancy to the tumors.[4] Long before, the "trophoblastic theory of cancer" stated that cancers originate from germ cells that fail to accomplish their embryonic migration to the gonads.[5] The evidence for this theory includes, but is not limited to, the expression of α-feto protein, carcinoembryonic antigen (CEA) and human chorionic gonadotrophin in a variety of histologically different cancers, as well as the identified function of developmental genes in the process of invasion and metastases.[1] The more recent theory of "cancer stem cells" can be somehow regarded as a rephrasing of this old theory with some modifications.[6]

Some of the transiently expressed proteins in the developing germ cells are proto-oncogenes or oncogenes which have fundamental roles in spermatogenesis and reproductive processes.[7] They have been shown to be expressed in abundance only in metastatic or tumor cells but not in any other normal cells except the testes and the placenta (and the ovaries in some cases). Consequently, these antigens are known as cancer-testis (CT) antigens.[8] CT antigens are tumor-associated proteins whose expression in both tumors and testes highlights the mentioned similarities between gametogenic and carcinogenesis processes.[9] Being expressed in a wide variety of tumors,[10–16] they are potential targets for cancer immunotherapy.[17–21] However, the expression of these antigens in adult stem cells raises concerns regarding the possible side effects of cancer immunotherapy on gametogenic tissues.[22] Some CTAs such as SSX have been shown to participate in mesenchymal-to-epithelial transition (MET) by induction of matrix metalloproteinase 2 expression and changing E-cadherin levels, thus influencing cell migration and favoring metastasis and participating in self-renewal which suggest another evidence for their invovlement in cancer stem cells.[23] Recently, some CTAs have been shown to up-regulate the expression of growth, proliferation, metastasis, and stemness genes.[24] In addition to the protein coding genes, micoRNA (miRNAs) with special characteristics of cancer-testis genes are being discovered recently. Among them is mir-888 which is exclusively expressed in the testes among normal tissues whereas it is overexpressed in endometrial cancers. Furthermore, it has a potential role in male fertility.[25] In addition to miRNAs, Piwi-interacting RNAs (piRNAs) are involved in both gametogenic and carcinogenesis programs.[26] These types of small RNAs are produced from long precursor RNAs and participate in the regulation of retrotransposons.[27] Some members of this family have been shown to participate in establishing *de novo* DNA methylation of retrotransposons in fetal male germ cells.[28] On the other hand, Piwil genes expression has been associated with cancer progression in bladder cancer[29] and renal carcinoma.[30] In addition, up-regulation of piRNA pathway genes has been detected in epithelial ovarian cancer.[31] It has been suggested that aberrant PIWI function in the germline of a man might lead to retrotransposon jumping, resulting in

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progenies with higher vulnerability to cancer.[32] Besides, recent studies have shown the essential role of a family of polymerizing GTP-binding proteins named septins in numerous disorders such as leukemia, ovarian tumors, breast cancer, and male infertility.[33] These proteins participate in many cellular functions, such as membrane compartmentalization, vesicle trafficking, mitosis and cytoskeletal remodeling.[34] So, novel evidences are being found for linking gametogenic and carcinogenesis processes.

Among testis-specific proteins are outer dense fiber (ODF) proteins which constitute the main cytoskeletal structure of sperm tail.[35] In human spermatozoa, the ODFs spread along more than half of the principal piece and gradually taper. There are nine *ODFs* in the mid-piece, each one associated with a microtubule doublet of the axoneme on its outer edge. In the principal piece, the number of *ODFs* is diminished to seven, and these become progressively smaller distally. The ODFs linked with the axonemal microtubules numbered 3 and 8 finish at the annulus, which shows the distal end of the middle piece (Figure 1).[36] At the anterior end of the sperm tail, they are adjacent to the paracentriolar connecting piece and stretch posteriorly into the principal piece.[37] They seem to have an indirect role in sperm motility rather than being involved in induction of active motility. They may participate in the maintenance of the elastic features of sperm tail and may provide the stretchy strength needed for protection of the sperm tail against shearing forces which occur during epididymal transport and ejaculation.[38] Other studies have indicated that some of these proteins are self-interacting proteins that make a fibrillar structure with the microtubule network.[39] The ODFs include at least 14 polypeptides,[39] four of which have been well studied in human as well as animal studies. In humans, they are encoded as *ODF1* (NM_024410, OMIM no. 182878), *ODF2* (NM_002540.3, OMIM no. 602015), *ODF3* (NM_053280.3, OMIM no. 608356), and *ODF4* (NM_153007.3, OMIN no. 610097). Disorganization of external microtubule doublets and ODFs have been shown to be associated with impaired sperm motility and male infertility in animal models.[40] More recently, *ODF* genes have been shown to be expressed in a variety of tumors which implies that they can be included in the CT family. In the present study, we summarize the recent data regarding the role of ODF proteins in sperm structure and function as well as male fertility and cancer.

ODF proteins discovery and function

ODF1

ODF1 mRNA has been detected in round spermatids in rat. Its translation is significantly increased in the maturation phase of spermiogenesis during which a marked increase occurs in the diameter of ODFs.[41] It has a putative leucine zipper dimerization motif in the N-terminus and the PCX repeats in the C-terminus. Self-interaction of this protein is in part mediated by a leucine zipper domain in the rat.[42] The human gene has been shown to be localized to band q22 of chromosome 8. An antiserum raised against a synthetic peptide derived from the N-terminus of the encoded sequence has spotted the 32 kDa protein in an extract of human sperm flagella.[43]

ODF2

ODF2 has been discovered through its functional interaction with *ODF1*.[44] *ODF2* is a major *ODF* protein with the ability for self-interaction and two leucine zipper motifs, one of which facilitates its binding with *ODF1*.[45] It can also bind cdk5, which after activation can phosphorylate *ODF1*.[46] It also has mutual interactions with Tssk4. Tssk4 can alter the phosphorylation state of *ODF2*, while *ODF2* can induce the autophosphorylation activity of Tssk4 at Ser-197.[47] *ODF2* proteins are made up of about 590 amino acids with an estimated molecular mass of 70 kDa.[39] Two leucine zipper motifs located in their C-terminal region are involved in the interaction with the leucine zippers of *ODF1*.[48] *ODF2* expression is not limited to male germ cells as its variant named Cennexin1 is a prevalent scaffold piece of the centrosome, the microtubule (MT)-organizing center of the cell and is associated with mother centrioles in a cell cycle-dependent pattern.[49] Centrosomes control the polarized organization of MTs in interphase cells and participate in the mitotic spindle structure which is necessary in separating chromosomes in mitotic cells.[50] However, the cell cycle of *ODF2* (-/-) cells has not been affected. In *ODF2* (-/-) cells, distal/subdistal appendages vanish from mother centrioles, making it hard to discriminate mother from daughter centrioles. In *ODF2* (-/-) cells, the primary cilia are completely disrupted which can be rescued by exogenous *ODF2* expression. Consequently, *ODF2* is crucial for generation of distal/subdistal appendages and formation of primary cilia, but not for other cell-cycle-related centriolar roles.[51] Its human gene is localized to 9q34.11. Depletion of Cenexin-1 from HeLa cells delocalized centrosomal PLK1, decreased gamma-tubulin recruitment to centrosomes, and changed localization of some centrosomal proteins needed for microtubule nucleation and function in addition to severe mitotic defects resulting in multipolar chromosomes and apoptotic cell death in some cells.[52]

ODF3

This protein has been shown to be expressed in the flagella of the elongated spermatids and along the entire length of the tail in mature sperm.[53] In addition to testis and epididymis, it has been shown to be expressed in rat brain, suggesting a possible role in the cytoskeletal structure. Secondary structure predictions showed that *ODF3* is a coiled-coil protein.[54] The human gene is located on 11p15.5. In humans, its amino acid sequence has 6 Pro-Gly-Pro repeats, which are also seen in the mouse and *Drosophila melanogaster* orthologs.[53]

ODF4

It was first isolated from a subtracted complementary DNA (cDNA) library of mouse testis. Its mRNA expression has been shown from late pachytene stage-spermatocytes to elongated spermatids in the seminiferous tubules.[55] Subsequently, the human ortholog of *ODF4* was cloned and shown to be expressed solely in the testes. The 30 kDa protein encoded by the mRNA was identified in the flagellae of ejaculated sperm, and its gene was mapped to chromosome 17.[56]

ODF splicing variants

Cenexin1

ODF2 has a splicing variant named Cenexin1 with an additional 167-amino acid (aa) distinctive C-terminal extension (molecular mass of 93 kDa). These two variants have diverse functions in male germ cells and somatic cells.[52,57] While ODF2 is principally expressed in the pachytene spermatocytes,[58] Cenexin1 high expression has been detected in somatic tissues. Cenexin1 but not *ODF2* has a distinctive role in ciliogenesis and cell cycle

progression at the centrosome both in the early cell cycle stages, as well as late G2 and M phases.[57]

Other variants

Cloning of *ODF3* and *ODF4* cDNA from the testis of a fertile patient suffering from prostate adenocarcinoma resulted in identification of two alternative splice variants of these genes. The first variant corresponds to *ODF3* and lacks 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). The second one represents a novel splicing variant for *ODF4* and lacks some parts of exon 1 (nucleotides 292 to 636 of the mRNA sequence). 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 have been suggested as the underlying mechanisms for such in-frame deletions.[59]

ODF proteins roles in fertility

Sperm is produced through a sequential process which includes proliferation of spermatogonia, meiotic prophase of spermatocytes, and radical morphological alterations of spermatids. In the last step, known as spermiogenesis, formation of the tail is a critical event. Sperm tail is produced via a complex process including organization of the axoneme, transport of periaxonemal proteins from the cytoplasm to the tail, and gathering of the *ODFs* and fibrous sheath.[53] Although *ODFs* have been shown to be indispensable elements of sperm tail, their contribution to human male fertility has been assessed in only few human studies so far. Axonemal and *ODF* defects have been identified in asthenozoospermia patients as a consequence of ciliapathies, such as primary ciliary dyskinesia and Kartagener's syndrome.[60] In addition, a previous study has shown that developmental defect of *ODFs* results in severe sperm tail abnormalities and infertility in humans.[61] Another study has shown a significant decrease in *ODF1* mRNA and protein expression in the ejaculated spermatozoa of asthenozoospermic men, which might be responsible for low sperm motility.[62] A recent study has shown lower expression and less compact localization of *ODF1* and *ODF2* proteins in sperm samples of asthenozoospermic male patients compared to normospermic males which has been attributed to disruption in *ODF* protein expression during maturation process in the epididymis of these patients.[63] A recent case report of an infertile man with severe asthenozoospermia showed lack of axoneme and *ODFs* in the principal piece.[64] Although the underlying defect in spermatids has not been detected, as the defects were seen in more than 95% of sperms, the condition can be attributed to a genetic defect.

The results of these human studies are in accordance with those of animal studies. For instance, it has been demonstrated that the RO072 ES cell derived homozygote knock out of *ODF2* leads to embryonic lethality,[65] while XL169 ES cell derived heterozygote knock out results in severe defects in sperm tail development and male infertility in mice with a high percentage chimerism.[45,66] However, mice of low-medium percentage chimerism were fertile. More than half of the epididymal sperms had bent tails, with one or more complete *ODFs* absence and the deficiency of one or more axonemal microtubule doublets.[45] Another study has shown that disruption of odf2/cennexin in mice results in primary cilia dyskinesia in addition to male infertility.[67]

It has been shown that co-administration of bleomycin, etoposide, and cisplatinum (BEP) to male rats results in abnormal number of *ODFs* (either too many or too few), hemilateral absence of the axoneme and the *ODFs* as well as malformed mitochondrial sheath. However, the treated rats were fertile.[68] In addition, selenium-deficiency in rats leads to production of abnormal sperm with deficiency of one or more axonemal microtubule doublets of the axoneme as well as the corresponding *ODF*.[69] Consequently, the degree of *ODF* defects and their structure are determinants of fertility state. An *in vivo* functional study has shown no significant difference between Cenexin1 wild type (WT) and *ODF2* over-expressing transgenic mice with an Odf2+/- background. Both mouse lines were viable and fertile which shows the functional similarities between these two variants. Yet, Cenexin1 S796A mutant expressing mice showed a defect in fertility in a way that the number of their progeny was less than half when compared with Cenexin1 WT/ *ODF2* transgenic mice. A defect has been found in the differentiation from round spermatids to elongated spermatids in this transgenic mouse. Besides, the total germ cell number and structure of the seminiferous tubule were relatively different from those of normal mice. It is possible that this type of mutant Cenexin1 fails to localize at the appropriate site in the sperm tail which leads to a defect in the differentiation of the sperm tail.[66]

ODF expression analysis in cancer

Patient samples

ODF1, *ODF2* and *ODF3* have been shown to be expressed in a fraction of basal cell carcinoma (BCC) samples but not in normal skin biopsies. In addition, *ODF2* expression has been correlated with infiltrative subtype of BCC, whereas *ODF1* expression was associated with tumor location. The differential expression of *ODF2* in distinct histopathologic subtypes of BCC has been suggested as a clue to discriminate pathological features of BCCs based on their gene expression profile in future.[11] Furthermore, *ODF1* and *ODF2* expression has been shown in prostate adenocarcinoma patients but not in benign prostate hyperplasia which implies their role in differentiation of these two disorders. Furthermore, mean serum prostate specific antigen (PSA) level was considerably higher in prostate cancer patients expressing *ODF2* compared to patients who did not express this gene.[12] As PSA levels have been shown to be associated with higher cell proliferation and lower cell apoptosis index,[70] a link might exist between *ODF2* expression and cell proliferation rate in prostate cancer patients. Another study aimed at expression analysis of CT antigens in breast cancer patients failed to detect *ODF3* expression in any of patients' samples.[10] The expression of *ODF4* has been shown to be upregulated in breast cancer tissues compared with their normal adjacent tissues. In addition, its expression has been correlated with HER2/neu overexpression but no other clinicopathological characteristics of patients. Consequently, it has been proposed as a putative breast cancer biomarker at the transcript level.[71] Moreover, *ODF4* expression has been detected in about one third of chronic myeloid leukemia patients but none of healthy donors.[13]

Cell line studies

ODF2 has been shown to be expressed in a variety of cancer cell line including LNCaP (prostate cancer), MCF-7 and WHIM12 (breast cancer), HCC4017, H460 and H1299 (non-small cell lung cancer), Saos-2 (sarcoma), SK-MEL-2 and SK-MEL-37

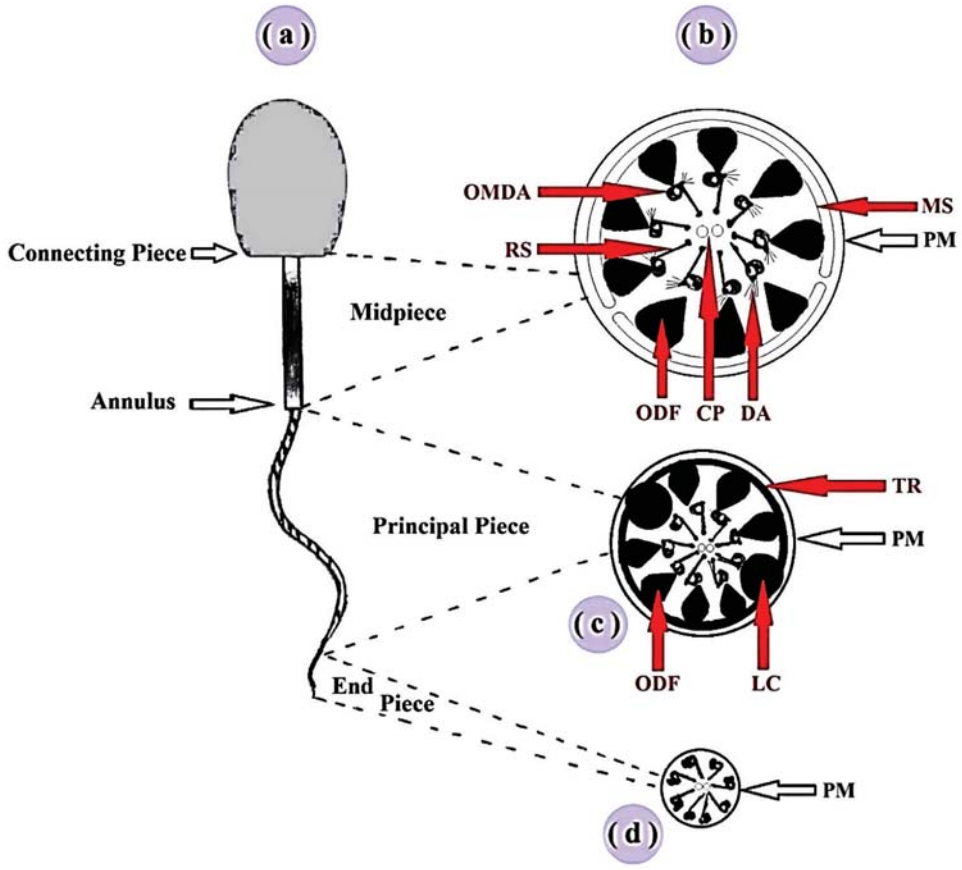

Figure 1. Structure of sperm tail; **a)** Main structural parts of mammalian spermatozoa; **b)** A cross-sectional segment of the midpiece showing outer dense fiber (ODF), plasma membrane (PM), mitochondrial sheath (MS), dynein arms (DA), radial spokes (RS), central pairs of microtubule doublets (CP) and the microtubule doublets of the axoneme (OMDA); **c)** A cross-sectional segment of the principal piece showing ODF, longitudinal columns of the fibrous sheath (LC), transverse ribs (TR) and PM; **d)** A cross-sectional segment of the end piece (Modified and redrawn).[74]

(melanoma) as well as SK-OV-6 and SK-OV-3 (ovarian cancer). However, *ODF1* expression has been detected in a selected number of these cell lines including some melanoma and sarcoma derived cell lines and WHIM12 triple negative breast cancer cell line.[72] *ODF4* has been shown to be expressed in both MDA-MB-231 and MCF-7 breast cancer cell lines with higher expression in the former. Considering the more aggressive behavior of MDA-MB-231 compared to MCF-7 cells, this higher expression implies participation of *ODF4* in malignant phenotype.[71] Another study has demonstrated the down-regulation of *ODF4* expression in MDA-MB-231 cells following treatment with anticancer materials derived from *Lactobacilli*.[73] On the other hand, *ODF3* expression has not been detected in any of the above mentioned cell lines.[10,72]

Discussion

Considering the similarities in the two fundamental processes of gametogenesis and carcinogenesis, evaluation of the observed links may facilitate the discovery of new markers as well as therapeutic targets in both fields. ODF proteins are among proteins whose function in male fertility has been clarified for more than a decade at least in animal models but their contribution to cancer development is being analyzed just recently. Considering the exclusive testis-specific expression of most *ODF* proteins, their aberrant expression in cancer has important clinical implications. Most importantly, they fulfill the first and most central feature of tumor biomarkers which is specific expression in tumor tissues and not in the normal counterpart. There are still many questions regarding the role of these proteins in the process of tumorigenesis. However, considering the indirect role of the encoded proteins in sperm motility, aberrant expression of these antigens in cancer cells may be involved in cell migration and metastasis. Moreover, the principal role of *ODF2* in centriole formation implies that its aberrant expression in cancer cells may be implicated in abnormal mitosis and aneuploidies seen in cancer cells.

Although the expression of *ODF* genes has been assessed in a few types of cancer samples, there is little known regarding their association with patients' clinicopathological characteristics or patients' survival which is at least partly due to the relatively small sample size of the studies. Nevertheless, a comprehensive functional characterization of CT antigens has shown an association between *ODF2* expression and survival in some oncogenic backgrounds.[72]

Although animal studies have shown the crucial role of ODF proteins in sperm tail structure, there are few human studies

aimed at identification of their participation in male fertility. The recent case report of absence of ODFs in the majority of sperms in a patient[64] suggests that mutational analysis of *ODF* genes may show the underlying genetic defects in a fraction of infertile men. At the end, it is worth emphasizing the fact that the expression of ODFs is not limited to male cancers. These genes are encoded by autosomes and possibly silenced through epigenetic mechanisms in normal tissues other than the testes. But their aberrant expressions have been detected in both male and female patients suffering from cancer, so they are putative cancer biomarkers and therapeutic targets for both genders.

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