Review Article

Proteins as the Molecular Markers of Male Fertility

Eswari Beeram, Bukke Suman, Bysani Divya

Department of Biochemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

Abstract

Proteins play a key role in many functions such as metabolic activity, differentiation, as cargos, and cell fate regulators. It is necessary to know about the proteins involved in male fertility to develop remedies for the treatment of male infertility. However, the role of the proteins is not limited to particular aspect in the biological systems. Some of the proteins act as ion channels such as catsper, and protein such as Nanos is a translational repressor in germ cells and expressed in prenatal period whose role in male fertility is not clearly understood. Rbm5 is a pre-mRNA splicing factor necessary for sperm differentiation whose loss results in deficit in sperm production. DEFB114 is a beta-defensin family protein necessary for sperm motility in lipopolysaccharide-challenged mice. TEX101 is a plasma membrane specific germ cell protein whose function is not clearly identified. Gpr56 is an another adhesion protein whose null mutation leads to arrest of production of pupps. Amyloid precursor protein in Alzheimer's disease plays a role in male fertility whose function is uncertain which has to be considered while targeting them. The study on amyloid precursor protein in male fertility is a novel thing, but requires further study in correlation to Alzheimer's disease.

Keywords: *Amyloid precursor protein, DYNLT1, Nanos2, RBM5*

Introduction

Nanos is a highly conserved gene for the reproduction in *Drosophila melanogaster* as RNA-binding protein.[1-7] It plays several roles in the fly drosophila, but the role of it in fertility of humans has yet to be studied. There are three types of Nanos gene: Nanos 1, Nanos 2, and Nanos 3[8] in which Nanos 3 involves in the migration of primordial germ cells to the gonads[7] and its presence in spermatogonia is necessary for differentiation during spermiogenesis.

Some of the proteins such as ser/thr protein phosphatases and kinases are well known for regulating enzymes by phosphorylation and dephosphorylation events, but their role in male fertility was not well known. However, the protein phosphatase 4 is one of the ser/thr phosphatase of well-studied and found to be necessary for preventing errors in the genetic exchange from mother to child. It plays a key role in the maintenance of synaptonemal complex and generation of programmed ds breaks which is necessary for crossover events.[9] ser/thr protein kinase causes phosphorylation of H1, H2, H2AX, and H3,[10] which is required for the chromatin remodeling in mitosis and meiosis and known to play a role in DNA compaction also.

Ion channels such as catsper which is an anion transporter known for maintaining the ion fluxes and membrane potential and ion balance inside the cell. It is also involved in male fertility through regulating the sperm motility. Slc26 is an anion transporter necessary for transport of monovalent and divalent anions such as chloride (Cl^-), sulfate ($SO42^-$), iodide (I^-), and bicarbonate ($HCO3^-$)[11] and involved in differentiation process in mouse. Catsper is the ion channel required for maintaining sperm motility through ca^{2+} ion fluxes.

Proteins that counteract the oxidative stress found in seminal plasma such as SOD, catalase, and GPx and nonenzyme antioxidants such as α-tocopherol, urate,[12-15]

Address for correspondence: Dr. Eswari Beeram, Department of Biochemistry, Sri Venkateswara University, Tirupati - 517 502, Andhra Pradesh, India.
E-mail: eswaribeeram@gmail.com

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naphthoquinone, and HCO_3^- were previously known for the oxidative stress prevention, but now these are known to be involved in sperm functional parameters also.

Another protein GPR 56 was one of the adhesion G-protein-coupled receptors that function in cell adhesion through G-protein-coupled signaling.[16] The first report of its involvement in male fertility through testis cord remodeling was found by Chen *et al*.

DYNLT1 is a 14 Kda protein occupying the L1 inner arm of cytoplasmic and flagellar dynein components.[17,18] It is also present in oocytes, sperm tails,[19,20] and Golgi complexes.[21] It also functions in dynein-independent manner and cell fate regulator in neural progenitor cells.[22]

There are many proteins that play a role in male fertility, but is mainly focused on key molecules involved in male fertility.

Proteins Involved in Male Fertility through Phosphorylation and Dephosphorylation Events

Serine/threonine protein phosphatase PP4 homolog PPH 4 was normally found in budding yeast, and its presence in *Caenorhabditis elegans* and *D. melanogaster* showed that this protein is necessary for the two main events DNA ds break formation initiation and crossover formation along with synapsis-independent pairing and prevention of nonhomologus pairing in autosomes during the synapsis. However, it does not prevent the synapsis-dependent pairing of homologous chromosomes. The homology of these proteins is about 92% at amino acid level in humans than that of mouse. The protein is necessary for formation of chiasma, thus recombination without effecting the loading of recombinant proteins Rad 51. Ds break inducing property of phosphatase is age dependent and the enzyme alsoregulates meiosis.[9]

PP4 is necessary for the conversion of foci in to crossovers through COSA-1 in the yeast and dephosphorylates SUN protein which is required for synapsis-independent pairing.[9]

SSTK is a small protein kinase found on chromosome 8 and distributed with high similarity in mammals, and it is expressed in almost all the tissues. Phylogenetic analysis showed moderate similarity of SSTK to the testis-specific ser/thr kinases TSSK1, TSSK2, and TSSK3, MAP kinase/microtubule affinity-regulating kinases MARK and MARK4, and the ELKL motif kinase EMK1.[10]

This protein consists of N and C lobes of a protein kinase domain containing catalytic and ATP-binding domains, in which catalytic residues are K41, E60, D135, N140, D154, and T170, a glycine-rich motif in phosphate-binding loop and the conserved sequence DFG in the active site. SSTK consists of tyr phosphorylating domains which are similar to phosphorylation inhibitory domains of cyclin-dependent kinases Cdc2 and Cdk2 and TTY sequence similar to T–X-Y phosphorylation motif found in the activation loop of MAP kinases.[10]

SSTK even though associate with HSP90-1, HSP70, and HSP70-1 does not phosphorylates the above but required for proper maintainance of structure of the sperm head, sperm motility, and DNA condensation in sperm head as it phosphorylate H1, H2A, H2AX, and H3 but not the H2B, H4, and TP1.[10]

Another protein TSSK found to have kinase activity is necessary for male fertility as they are localized in the spermatids, which is HSP90 dependent. It was found that this kinase physically associates with HSP90 and showed reduced expression after the incubation with HSP90 inhibitors and kinases. TSSK-1, 2, 4, 6 phosphorylates H2A whereas TSSK3 does not show any kinase activity against H2A and where as TSSK-1,2,6 show decreased expression when treated with HSP 90 inhibitors, indicating that HSP90 is for maintainance of their half- life and catalytic activity also. TSSK 2 and 6 undergoes ubiquitination directly when HSP90 was inhibited and undergoes proteasomal degradation without any change in their mRNA levels.[23]

Ion Channels and Transporters as Male Fertility Factors

Another protein catsper is present in sperm tail and necessary for sperm motility, calcium influx, and fertilization in mice. Catsper 1 was found to be one of the four proteins of calcium channels necessary for male fertility in mice[24] and humans also. In case of sperm motility, ca^{2+} influx is necessary for hyperactivation, and in female genital tract, the ca2 + influx is necessary for capacitation and high motility penetration of sperm into oocyte.

Slc26A8 is a testis anion transporter expressed in sperm and necessary for sperm motility and fertilization potential. It is an anion transporter and not involved in the maturation of gonads and normal appearance of the mice. Slc26A8 is found to be localized in the annulus that connects the midpeice to that of principal piece of the flagella. It was shown that the ability of consumption of ATP was reduced due to defects in mitochondrial sheath even though the motor protein expression is normal and was found that the normal maturation of

sperm and capacitation is compromised in the mice and humans with null mutation of the protein.[25]

Is Oxidative Stress Preventing Enzymes and Structural Maintenance Proteins Necessary for Sperm Function

In relation to oxidative stress, superoxide dismutase, catalase, and glutathione peroxidase play an important role in protection against ROS but their role in male fertility was yet to be determined. SOD changes were associated with changes in sperm count whereas catalase with sperm morphology and GPx was found to be not associated with any of the sperm parameters.[26]

There is no contributional research to nuclear matrix proteins up to now. Hence, studies about nuclear matrix proteins of sperm were proven to be useful as these are also involved in male fertility. Proteins identified in the sperm head were mostly chaperons, cytoskeletal proteins, peroxiredoxins, isomerases, and other enzymes.[27]

Other Proteins Involved in Male Fertility

Amyloid precursor protein is normally known for its activity in Alzheimer's disease, but its role in male fertility was not known. This protein was first identified in testis and studied for its interaction with testis. It is now known to interact with RANB9 protein and 37 proteins, in which COPS5 has highest correlation coefficient whereas CD81 and CD99 with $C = 0.029$ and 0.064, respectively.[28]

GPr56 is an adhesion protein necessary for male fertility. It was found to be expressed in testis cords and PM cells and localized in Sertoli cells and germ cells, but found to be absent in interstitial cells. GPr56 is highly expressed in Sertoli cells and spermatogonial cells with reduced expression in PM cells. Production of progeny with defective testis is seen in GPr56 null mice as the spermatic cords are disrupted and scattered instead of forming tubular structures. There is basement membrane disruption in the testis with no alterations in follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, probably showing no effect on these hormones.[29]

TEX101, a testicular germ cell-specific protein, was found to be located in the plasma membrane of germ cells. It does not affect the mating, but secreted as one of the glycosylphosphatidylinositol-anchored proteins by TACE into the seminal fluid. However, the mechanism of action was not clearly understood.[30]

DEFB114 is the β-defensin that is highly expressed in the caput and corpus regions of epididymis. It shares some of the cysteine-conserved regions with β-defensin members along with cysteine pairing regions like Cys10–Cys24, Cys3–Cys31, and Cys14–Cys32. It is involved in maintenance of male fertility through preserving sperm motility in lipopolysaccharide (LPS)-challenged mice by neutralizing it in a dose-dependent manner.[31]

Rbm5 is the pre-mRNA splicing factor present in the nucleus and cytoplasm of spermatocytes and round spermatids. R263 was found to be the highly conserved region in the Rbm5, and loss of function allele mutation in Rbm5 does not produce pupps which proves that allele is necessary for binding of RNA in the region of RNA recognition motif and also found to be necessary for change in structure of β-strand which is required for binding of RNA. This mutation does not effect the fertility in females. The protein is necessary for sperm differentiation and the mutation leads to loss in sperm production due to arrest at stage 8 of haploid sperm development, which is independent of hormones FSH, LH, and testosterone. Round spermatids stage is necessary for the conversion of histones to protamines. Its absence leads to testis atrophy as it is highly expressed in testis compared to other tissues.[32]

St5 as it is involved in MAP kinase pathway necessary for cell growth, Post testicular maturation and fertilization. Splicing mediated by Rbm5 results in 126kDa fragment and regulates MAP kinase pathway through phosphorylation of ERK1/2. Regulation in sperm differentiation in mutant than in wild type indicates the regulation in tumor growth.[33]

Nanos 2 is the protein encoded by the gene Nanos that acts as translational repressor in germ cells and expressed in the cytoplasm of the germ cells of seminiferous tubules of the testis in prenatal period. The molecular weight was found to be more than 15 Kda. It is highly expressed in the peritubular cells, and localization is different for prenatal and adult tubules. H68Q and H109H are the mutations localized in the zinc finger domain commonly found in Nanos 2. It form complexes with the proteins (DAZ and BOL, PUMILIO2, NANOS1), but its role in male fertility is not clearly understood.[33]

DYNLT1 is a gene that is found to be associated with t-complex of testis. It is localized in sperm head, mid piece, and sperm tail, and in infertile men, the localization was restricted to head and midpiece only. It is known to cause male sterility in its absence and restoration of the fertility with BAC construct of the protein in not only mice and fly but also in humans. Its molecular weight was found to be 14 Kda. It is a component of microtubular network, and overexpression of HSP 90

along with DYNLT1 leads to phosphorylation of Thr 94 which plays a role in cell division. DYNLT1 is found to be involved in sperm division and differentiation.[34]

Discussion

Proteins play a key role in the biological systems such as enzymes, transcription factors, antibodies, and cytokines, so instead of focusing on the sperm morphology, and semen parameters focussing on molecules involved in maintaining integrity of genome proves to be helpful in understanding disorders related to male fertility. Some of the proteins are ion channels maintaining ca2 + influx during motility and capacitation. Some of them are useful for DNA compaction as they are involved in phosphorylation of histones in sperm. The absence of some proteins leads to loss of fertility as they are responsible for sperm division and differentiation like DYNLT1 and found to be involved in cargo binding, lymphocyte division, vesicular transport, and human embryo implantation.

Most of the proteins which are discussed here are responsible for sperm motility and male fertility, but not focused in the female. Amyloid precursor protein in patients with Alzheimer's disease should be studied for interactions related to fertility. Some of the proteins act as enzymes preventing oxidative stress and play a role in sperm count and morphology whereas defensin DEFB114 found to preserve fertility in LPS mice which seems to be its involvement in immune reactions may be as a pattern recognition molecule.

Some of the proteins are known to interact with other proteins and involved in prevention of over cell division in germ cells like Rbm5 and also act as splicing factor of pre mRNAs of apoptotic proteins such as caspase, FAS receptor, and C-FLIP. It indicates its control on cell division preventing the tumorigenic growth in tissues apart from male fertility.

Hence studies on these aspects by various scientists explain us to focuss on functional proteins involved in male fertility instead of high cost invitro aspects.

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Conflicts of interest

There are no conflicts of interest.

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