Published in final edited form as:

Curr Opin Urol. 2010 November ; 20(6): 525–532. doi:10.1097/MOU.0b013e32833f1c21.

DNA MISMATCH REPAIR AND INFERTILITY

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Abstract

Purpose of the review—To highlight the significance of the abnormal DNA repair mechanism in male infertility.

Recent Findings—DNA repair defects cause a variety of spermatogenic defects in mouse models. Evidence is accumulating to demonstrate the importance of DNA repair defects in human non-obstructive azoospermia. Epigenetic changes may also play a crucial role in infertility.

Summary—The DNA in the cell needs to be constantly repaired to ensure fidelity of DNA replication, to maintain genome stability and to ensure propagation of species. The DNA repair and recombination machineries are highly conserved across the species and inactivation of these pathways may lead to replication and recombination errors. This review summarizes types of DNA lesions and DNA repair pathways, particularly focusing on highly conserved meiotic regulators the DNA mismatch repair proteins. Targeted deletions of some of these proteins results in infertility and predisposes to tumor in mutant mouse models. There is evidence for loss of some of these proteins in human male infertility. Because defective DNA repair is associated with a mutator phenotype, the risk of transmission to the offspring of these otherwise infertile men conceived using an assisted reproductive technology needs further evaluation.

Key words or phrases

DNA repair; azoospermia; infertility; mismatch repair proteins; homologous recombination

Introduction

Genetic disorders like Klinefelter syndrome and Y chromosome microdeletions are recognized genetic causes of male infertility. While investigators suspect that the majority of male infertility has an underlying genetic basis; the etiology of the majority of male infertility cases remains idiopathic [1–3]. This is particularly true for men with non-obstructive azoospermia or severe oligospermia. Defects in DNA repair during spermatogenesis is thought to underlie some types of testicular failure [4,5]. DNA repair mechanisms are important to maintain the fidelity of DNA replication during mitotic, meiotic and post-meiotic development of both male and female germ cells. Three critical DNA repair pathway exist: Nucleotide excision repair (NER), Base excision repair (BER) and DNA mismatch repair (MMR). There are two additional types of DNA repair, double-stranded break repair and recombinational repair. This review focuses on our current understanding of role of these processes in the etiology of spermatogenetic defects; however, we begin with a summary of the current knowledge of types of DNA repair to set the stage for an in depth discussion of the relevance of these pathways in gametogenesis. The potential consequences of defective DNA repair during gametogenesis and the possible consequences for children conceived by ICSI who are fathered by a severely infertile male

are also discussed. Errors in DNA mismatch repair is linked to a variety of cancers which may explain the link, in part, between infertility and testicular germ cell tumors.

DNA Mismatch repair

DNA repair is essential to the maintenance of genomic integrity and the basic processes are highly conserved throughout evolution. The mismatch repair (MMR) family of proteins was first characterized in bacteria. These proteins play a key role in repairing sequence mismatches that primarily arise during replication. Mismatches escape DNA polymerase proof-reading. The function of the mismatch repair proteins are not just restricted to mitosis, but also play critical roles for meiotic recombination and chromosome pairing.

The MMR system is comprised of MutS, MutL and MutH families, which are highly conserved across species. The homodimeric MutS protein recognizes DNA mismatches binds to the DNA and forms an active repair complex. Following mismatch recognition, the MutL protein serves to couple the MutS-DNA active repair complex to then induce exonuclease MutH activation. In humans and mice, there are several mismatch repair proteins homologous to the bacterial MutS and Mut L homologues, named MSH1-6, MLH1 and MLH3, and PMS1 and PMS2 (for post meiotic segregation)[6,7]. Heterodimers of MutS and MutL homologues form a tetraheteromeric complex that recognizes a single base pair mismatch, or a small loop of single strand DNA. Other potential downstream proteins with endo- and exonuclease activities (such as, exonuclease 1, EXO1), DNA polymerases and replication factors (replication protein A(RPA), proliferating cell nuclear antigen (PCNA), and replication factor C (RFC) are recruited to complete the repair process (Figure 1). In addition, the MutL heterodimers can also trigger apoptotic machinery to induce cell death when DNA repair fails. As described below, mutations or deletion of the *MLH1* gene in mice and humans resulted in microsatellite instability and predisposition to the early onset of certain tumors [8,9]. Germ line mutations in *MLH1* gene cause hereditary nonpolyposis colon cancer (HNPCC) [10].

Mice deficient in *Mlh1* gene display microsatellite instability, as well as male and female infertility (for a summary of the consequences of deletion of MLH1 and other DNA repair proteins involved in infertility in mouse models see Table 1). Failure of gametogenesis in these mice results from meiotic arrest at pachytene characterized by reduced levels of chiasmata [8,9], impaired meiotic recombination and crossing during meiosis. Similar to *Mlh1* deficient mice, *Pms2* or *Msh2* knockout mice exhibit genomic instability; however, in contrast, these mouse mutants display male infertility due to disruption of normal chromosomal synapsis [22]. *Msh2*, *Msh3* and *Msh6* null mice exhibit mismatch repair deficiency in somatic cells but their fertility is largely unaffected, although spermatogenic defects are evident in the *Msh2* deleted male mice [7,11].

Two mammalian meiosis-specific MutS homologues, MSH4 and MSH5 were identified. At the time of homologous recombination and crossing over during meiosis, the chromosomes link to stabilize, through a supramolecular proteinaceous structure comprised of complex aggregate of SYCP1, 2, 3 proteins known as Synaptonemal complex (SC). During recombination MSH4-MSH5 interacts with RAD51 interacts within the SC complex facilitating and stabilizing the interactions between homologs. Targeted inactivation of the meiosis-specific MutS homologues, *Msh4* and *Msh5* resulting in gametogenesis-specific phenotype, displaying meiotic arrest in both males and females but are not involved in DNA mismatch repair [17,25,26]. *Msh4* and *Msh5* null mice exhibit greatly diminished pairing of homologous chromosomes and failure of meiosis at zygotene stage. Decreased synapsis is most severe in *Msh5*-deficient mice. Several polymorphisms of *MSH5* are reported to be

associated with azoospermia or severe oligospermia [27], but the study groups thus far have been small and the functional significance of these variants remains undefined.

Less is known concerning the mismatch repair proteins and human male infertility. Maduro, et al., [4] presented evidence of genomic instability in both testicular cells and circulating white blood cells of men with non-obstructive azoospermia, predominantly those men with Sertoli cell only syndrome. Genomic instability was assessed by analyzing molecular features associated with microsatellite instability. Microsatellites are regions of repetitive sequences of nucleotides found in the genome, which may be mono-, di- or trinucleotide repeats, such as CAGCAGCAG. Deficient mismatch repair can result in expansions or contractions of the repetitive sequences and accordingly, microsatellite instability is used as a reliable marker of mismatch repair deficiency [28].

Immunohistochemistry revealed that a significant number of these non-obstructive azoospermic men displaying genomic instability also had defects in the mismatch repair proteins, MLH1 or MSH2. In some patients the protein was mislocalized in the cytoplasm, in other non-obstructive azoospermic men the protein was absent [4]. Importantly, the defect was not germ cell specific and affected the germ cells (if present), as well as the somatic cells. These types of deficient immunostaining are used to diagnose Lynch syndrome (a cancer syndrome described below). An example of mislocalization of MLH1 protein in a non-obstructive azoospermia (NOA) is shown in Figure 2. These studies are intriguing as they suggest that defects in mismatch repair proteins may underlie some forms of non-obstructive azoospermia. Furthermore, they suggest these men may be at risk of development of colorectal or other cancers associated with defective mismatch repair.

Homologous Recombination Repair

Double-strand break (DSB) is the most deleterious type of damage to somatic cellular DNA. Meiotic recombination requires endogenously induced breaking and subsequent ligation of the DNA molecules. Unrepaired or misrepaired DSBs may lead to genomic instability resulting in genetic aberrations or cell death. During the meiotic prophase, DSB are induced in a controlled manner within a DNA molecule to initiate recombination. This action is mediated by the conserved topoisomerase, SPO11 [29]. *Spo11* knockout mice exhibit a failure to form DSBs into homologs; thus leading to faulty synapsis, recombination and ultimately meiotic failure [30–33]. SPO11 is then displaced from the DSB site by MRE11. MRE11 recruited at the DSB site, generates 3' overhangs, and in concert with a multi protein complex including RAD50, NBS1 repair and process the DNA ends for homologous recombination. RAD51 and DMC1 and associated proteins are indispensable during homology and heteroduplex formation [34]. A schematic diagram of the proteins involved in homologous recombination is shown in Figure 3. *Dmc1* null mice are infertile with gross defect in chromosome pairing [35,36]. Similarly, *Rad51* knockout mice display embryonic lethality indicating the key role of these proteins in meiosis and development [37]. A case report of a man with spermatocyte arrest showed that he had an abnormal presence of *BRCA1* with an absence of *RAD51* in early and late spermatocytes associated with a failure of XY body formation suggesting a deficiency of the processing double-strand DNA breaks at the zygotene stage of meiosis [38]. Whether this is a common finding in other cases of early maturation arrest is not known.

Cell cycle checkpoint genes involved in DNA repair

If DNA damage cannot be repaired, the activity of cell cycle checkpoint genes results in apoptosis. Pachytene arrest is sometimes observed in testicular biopsies taken from infertile men. Numerous genes are involved in the regulation of DNA damage-induced cell cycle control. Mutation in one such gene ATM causes the genetic disorder Ataxia telangiectasia.

Ataxia telangiectasia is characterized by radiosensitivity, defective cell cycle check point activation, chromosomal instability and infertility [39]. Likewise, *Atm* knockout mice are infertile due to meiotic arrest at zygotene-pachytene stage [40]. Aberrant telomere clustering and chromosomal fragmentation are also observed in *Atm* null mice [41]. ATR is an ATM-like protein, confined to the unpaired axes of meiotic chromosomes at prophase [42,43]. Mutations in *Atr* result in an embryonic lethal phenotype [44]. Similarly, *Brca1* and *Brca2* knockout mice show embryonic lethality [45], but *Brca1-p53* (tumor suppressor gene implicated in elimination of germ cells in DNA damage) double-knockout mice rescued the severe phenotypes as seen in *Brca1* and *Brca2* but the male mice were infertile due to meiotic failure [46]. Knockout mice models of *Ercc1* (excision repair cross-complementing gene 1), *Msh2*, *Tp53* representing different DNA repair pathways (like NER, MMR and cell cycle checkpoint genes) exhibit impaired germ cell function and sperm production with overlapping phenotypic features [11]. *Tp53* and *Ercc1* play a critical role in DNA damage repair during spermatogenesis [11]. *XRCC1*, *ERCC1* and *ERCC2* polymorphisms are reportedly associated with a risk of idiopathic azoospermia in the Chinese [47,48], but again whether this is true for other ethnicities or races is not known nor is the functional significance of these variants.

Other DNA repair pathways

Base excision repair (BER) focuses on the base-base mismatches or lesions induced by alkylation and oxidation. The site of damage is recognized by DNA glycosylase and the mismatched bases are removed resulting in an apurine site which is cleaved by apurine endonucleases [49]. The lesion leads to a single strand break which is further repaired by the concerted action of DNA polymerases and DNA ligases [50–53]. Human genetic diseases are not yet been directly associated with BER deficiency but occurrence of polymorphisms in genes implicated in BER pathway is evident [54].

Nucleotide excision repair (NER) pathway involves repair of large loops, bulky, helix-distorting lesions [55]. NER proteins cleave the two phosphodiester bonds leaving a gap of few nucleotides which are patched and ligated by DNA polymerases [56,57]. NER are more error prone compared to BER or MMR pathways. Severe genetic diseases like Xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy, are manifestation of inborn NER deficiencies [54].

Infertility and neoplasia

Infertile men have higher incidence of testicular germ cell tumors [58]. Of importance to the focus of this paper, errors in DNA mismatch repair such as some of those described above, are increasingly linked to the development of a variety of human cancers. DNA mismatch repair is associated with about 15% of colorectal cancer and with certain other tumors, namely retinoblastoma, melanoma, gastric, breast and ovarian cancers. Targeted inactivation of Exonuclease 1 protein in mice resulted in both infertility and cancer [24].

In some cases, DNA mismatch repairs are due to inherited mutations, as in Lynch Syndrome. Lynch syndrome is caused by inactivating mutations of genes that encode mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2). Generally, the Lynch syndrome patients are born with a germ line mutation in a mismatch repair gene and then develop an inactivation of the second wild-type allele in their tumors [28]. This is consistent with Knudson's two hit hypothesis for carcinogenesis resulting from inactivation of tumor suppressor genes [28]. Apart from harboring a distinct type of colorectal cancer, these families have higher incidence of other malignancies, including those associated with multiple extracolonic cancers such as endometrial cancers, stomach, ovarian, small bowel, pancreatic, hepatobiliary tract, brain, upper uroepithelial tract and possibly prostate [59].

Upper urinary tract urothelial carcinomas have also been reported in the context of Lynch Syndrome, but incidence of testicular germ cell tumors are very rare [60]. Other possible potential mechanisms between infertility and testicular germ cell tumor includes testicular dysgenesis syndrome (if this exists), the Hiwi protein, chromosome 12 aneuploidy, and Y-chromosome instability [58].

Epigenetic mechanisms of Mismatch Repair Defects in Malignancy and Infertility

Another important mechanism controlling DNA mismatch repair is methylation of the promoter regions of MMR genes. Somatic hypermethylation of *MLH1*-gene promoter causes inhibition of gene transcription and functionally mimics an inactivating gene mutation [28]. If both *MLH1* alleles become hypermethylated, *MLH1* will be deficient and microsatellite instability will occur. This route to colorectal cancer development occurs with aging in patients who do not have Lynch syndrome or mutations in the mismatch repair genes, yet develop malignancies with mismatch repair defects and microsatellite instability [61,62]. Less commonly, constitutional epimutations of *MSH2* were identified in some families with early-onset colorectal and endometrial tumors [61], while the majority of those cases with constitutional *MLH1* epimutation (while rare) do not have a strong family history of colorectal cancers. Although promoter hypermethylation of MMR genes in the context of infertility is yet to be explored, promoter hypermethylation and silencing of several infertility associated genes like *VASA* and *MTHFR* [63,64] was recently reported. Several other epigenetic mechanisms, like histone modification [65] and RNA interference, may also play a part. Although epimutations are reversed during the process of spermatogenesis (potentially correcting a spontaneous epigenetic error in the father), there could be transmission of the epimutation if there is a genetic factor that influences this occurrence [61].

Another potential epigenetic mechanism is the presence of SNPs (single nucleotide polymorphisms). A SNP within an important regulatory element may lead to a reduction in transcriptional output and increased propensity to hypermethylation. SNPs are more frequent in those shown to have meiotic arrest upon testicular biopsy suggesting compared to those with normal spermatogenesis who are obstructed. These mutations were confined to testicular biopsies, whereas, no polymorphisms were observed in blood from either group. Our unpublished data also show several SNPs in *MLH1*, *MSH2* and *MSH6* genes associated with non obstructive azoospermia. This observation suggests that these are de novo mutations, which arise in the germ cells during spermatogenesis.

Consequence for assisted reproductive technology

With the advent of assisted reproductive technologies, such as *in vitro* fertilization with intracytoplasmic sperm injection, it is now possible to bypass the natural barriers that exclude fertilization by a defective sperm. Embryos with severe DNA repair defects usually undergo spontaneous abortion. Whether incidence of congenital abnormalities following ICSI is different or not from the general population is controversial with investigators having conflicting views [66–69]. In a meta-analysis of just 8 published studies by Woldringh, et al., [66], 55 out of 1973 children born after ICSI showed abnormal karyotypes, but there was no statistical significance or major congenital defects reported. In another study, major malformations that result in functional impairment or requiring surgical correction were observed in four children conceived by ICSI [70]. In both of the above studies, the groups were small and heterogenic. In contrast, others reported that prenatal karyotype abnormalities increase with decreasing sperm count and an increased incidence of birth defects in children conceived by ICSI [71–74]. Meta-analyses results from 25 studies

suggest a statistical significance of about 30–40% increased risk of birth defects associated with ART [75]. Importantly, the incidence of birth defects represents just one measure of the safety of these procedures and it is clear that highly controlled, prospective studies of birth defects, such as those published from the Bounduelle group, and systemic defects in children conceived by ICSI are needed. Increased genetic polymorphisms seen in infertile men, raises the potential for these variants to be inherited. These polymorphisms may confer increased risk of infertility and possible increased risks of somatic defects later in life. Longer term follow up of children born through ICSI is required to understand the genetic consequences in these offspring.

Conclusion

Mismatch repair (MMR) genes are well studied in hereditary non-polyposis colorectal cancer because MMR mutation carriers are at high risk for several extracolonic malignancies. The proteins of the DNA repair pathway play a crucial role in gametogenesis. Knockout mouse models and targeted deletions of these genes involved in different repair mechanisms exhibit male and female infertility. Studies in human subjects, especially in NOA men with respect to genomic instability have progressed. Defective MMR results in a “mutator” phenotype with increased rate of spontaneous mutations and microsatellite instability. The challenges to the development of epigenetic therapies for infertility should be investigated. Further studies to elucidate the molecular mechanisms of mammalian recombination and the role of these MMR proteins in infertility are needed to provide a better insight. These characteristics may have health consequences for the men with non-obstructive azoospermia and may present a risk to their children conceived by ICSI. Thus, with the increasing knowledge of the underlying genetic cause of male infertility, patients should be encouraged for counseling before ICSI.

Acknowledgments

Supported in part by 2P01 HD 36289 and the NIH Cooperative Centers Program in Reproductive Research (U54 HD07495) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health to DJL.

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Figure 1. Overview of mismatch repair in eukaryotes

The MutS α heterodimer (MSH2-MSH6/3) binds the mismatch and recruits MutL α heterodimer (MLH1-PMS2). A conformational change upon the exchange of ADP with ATP allows sliding of the complex away from the mismatch. Latent endonuclease activity within the PMS2 subunit of MutL α introduces nicks into the discontinuous strand (indicated by pink arrows) generating 5' entry sites for nuclease EXO1. EXO1 degrades the nicked strand and the remaining single stranded DNA is protected by RPA. POL δ and its cofactors PCNA and RFC fill in the gap with DNA ligase I completing the repair by sealing the final nick.

Figure 2. Subcellular localization of MLH1 protein

Infertile control men, MLH1 displayed diffuse cytoplasmic staining with a strong perinuclear accentuation, one of the NOA patient showed aberrant localization of MLH1

Figure 3. Meiotic Recombination pathway

Homologous unpaired acrocentric chromosomes are depicted above (A and B). Topoisomerase, SPO11, initiates recombination by inserting double-strand breaks (DSBs) into homologous sister chromatids. SPO11 is then displaced from the DSB site by MRE11, which in turn generates 3' overhangs as well as recruiting RAD50 and NBS1 to repair and process the DNA ends in preparation for homologous recombination. The resulting leads to the canonical Szostak model with double Holliday junctions and crossover products.

MMR genes phenotypes in mouse knockouts

Table 1

Gene	Phenotype	Cancer	Expression	Ref
<i>Msh2</i>	Fertility Fertile Some germ cell loss and SCO tubules (diameters <200 μm)	Lymphoma, GI and skin tumors	Highly expressed in mouse spermatogonia and leptotene/zygotene spermatocytes.	11,12,13,14,15
<i>Msh3</i>	Fertile	Late stage GI tumors	-	15,16
<i>Msh4</i>	Infertile Failure of spermatogonial maturation beyond zygonema.		Accumulation within nuclear matrix during leptotene of prophase I. Formation of MSH4 foci on zygotene unpaired chromosomes with the number of foci decreasing until mid-pachynema.	17
<i>Msh5</i>	Infertile Incomplete and nonhomologous chromosome pairing	Lymphoma and GI tumors	Expression elevated during spermatogenesis Possible role during prophase I stages	18
<i>Msh6</i>	Fertile Infertile	Lymphoma and GI tumors	Elevated expression observed in testis	19,20
<i>Mlh1</i>	Failure of crossing over and premature desynapsis of homologous chromosomes	Lymphoma, GI and skin tumors	Localised in discrete foci along the synaptonemal complex during pachynema in spermatocytes	8,9,21
<i>Pms1</i>	Fertile	-	-	21
<i>Pms2</i>	Male Infertile	Lymphoma	High levels in leptotene/zygotene spermatocytes	15,21,22
<i>Mlh3</i>	Infertile	-	High levels in pachytene spermatocytes	23
<i>Exo1</i>	Infertile	Lymphoma	Elevated expression observed in testis	24