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Review

DNA repair mechanisms in mammalian germ cells

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Summary. Mammalian germ cells encounter several types of DNA damage. This damage is almost completely repaired in a short period of time to provide the maintenance of genomic integrity. The main repair mechanisms operating in mammalian germline cells are: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), DNA double strand break repair (DSBR), and post replication repair (PRR). Currently, there are relatively few publications that summarize basic information and new findings on DNA repair mechanisms used in mammalian germ cells. In the present article, we review the studies that discuss repair mechanisms operating in the female and male germ cells. We then survey some of the recent discoveries made in this field.

Key words: DNA repair, DNA damage, Germ cell, Gene expression

Introduction

Mammalian eukaryotic cells are exposed to various genotoxic agents that may cause DNA damage. This damage may lead to various biological changes including: alterations in certain gene transcriptions, reproductive failures, heritable or non-heritable mutations, cell death and life threatening diseases such as cancer (Olsen et al., 2005; Somers and Cooper, 2009). There are several defense mechanisms that function in mammalian cells. The main examples are oxyradical scavengers, apoptotic pathway, and DNA repair systems. Oxyradical scavengers, antioxidants and different types of detoxifying proteins remove some of the genotoxicant. These include oxygen-derived radicals such as hydrogen peroxide, hydroxyl ion, superoxide radical, nitric oxide (Balercia et al., 2003; Kobayashi et al., 2004). The second important defense mechanism is the apoptotic pathway (also known as programmed cell death and apoptosis) that eliminates excessively damaged cells. Thus, the apoptotic pathway prevents the extremely damaged cell from starting uncontrolled cell proliferation (Hikim et al., 1998; Tilly, 1998; Salazar et al., 2003). However, the most crucial defense mechanism operating in somatic cells, germline and preimplantation embryos is the DNA repair system which involves the repair of damaged sites in the genome (Baarends et al., 2001; Jaroudi and SenGupta, 2007).

DNA damage in an eukaryotic cell will result in one of the following: a) immediate repair of the lesion using a convenient repair mechanism; b) survival despite the damage, which may cause unexpected cell death, formation of a cancerous cell or various diseases originating from genetic mutations; or c) apoptosis (Vinson and Hales, 2002). Out of these three outcomes, sufficient repair of the DNA lesion is indispensable to maintain the integrity of the nuclear genome. Indeed, stability of the genome must be retained in male and female germ cells and early embryos for the preservation of reproductive properties. Protection of reproductive qualities of germ cells helps to assure healthy offspring. On the other hand, a limited number of mutations should be tolerated in the haploid genomes of the mammalian gametes for the purpose of evolution. Intriguingly, the DNA repair system either achieves the repair of genomic mutations formed by genotoxicant factors, or saves a limited number of mutations for evolution itself (Baarends et al., 2001; Olsen et al., 2005; Jaroudi and SenGupta, 2007). Therefore, the DNA repair sytem has to be complex to perform these important missions correctly. Up to 150 repair-related genes are cloned and sequenced, but their functions are not yet fully identified. Generally, these genes are divided into two groups according to their association, either directly or indirectly, with the DNA repair process. The first group

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consists of regulator genes involved in the organization of DNA repair (e.g. cell cycle checkpoint genes and apoptotic genes), and the second group directly participates in the repair process. Aberrations in the expression of genes from both groups may lead to increasing rates of certain morbidities such as congenital malformations, acceleration in aging, distinct types of cancers, and reproductive failures (Longley et al., 2005; Wood et al., 2005) (Fig. 1).

Main DNA repair mechanisms used by mammalian germline cells

The main repair mechanisms widely used by the mammalian somatic cells, germline cells, and preimplantation embryos can be listed as nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), DNA double strand break repair (DSBR), and post replication repair (PRR) (Baarends et al., 2001; Vinson and Hales, 2002; Wood et al., 2005). Of these, the nucleotide excision repair (NER) involves more than 25 proteins that function to replace modified nucleotides with the correct ones. DNA lesions formed by UV light, exogenous chemicals such as benzo (a) pyrene, aflotoxin B_1, and chemotherapeutic agents like cisplatin are usually repaired by the nucleotide excision repair system. NER operates through several steps involving recognition of the lesion site, incision of the damaged DNA strand, DNA synthesis, and finally ligation of the uncoupled flanks by specific ligase enzymes (Mitchell et al., 2003; Olsen et al., 2005). Three distinct NER pathways, namely, global genomic repair (GGR), transcription-coupled repair (TCR), and differentiation-associated repair (DAR) have been identified. Of these, GGR pathway functions by repairing nearly all damaged sites in the whole genome, whereas TCR is solely involved in removal of the lesions that block the transcription of the constitutively expressed genes (Tornaletti and Hanawalt, 1999). The first step of GGR is the recognition of disrupted sites by a specific protein complex known as XPC-hHR23B (xeroderma pigmentosum complementation group C-human homologue of yeast RAD23B). On the other hand, TCR requires CSA (Cockayne syndrome factor A) and CSB factors (Cockayne syndrome factor B) to detect damaged DNA regions. Indeed, CSA and CSB form a gap to provide easy access for the repair-related proteins. Then, TFIIH (general transcription factor IIH) which is composed of XPB and CPD helicases unwinds about 30 base pairs around the damaged site in both GGR and TCR pathways. The ERCC1-XPF (excision repair cross complementing group 1 protein-xeroderma pigmentosum complementation group F) and XPG (xeroderma pigmentosum complementation group G) act in cooperation, via their endonuclease activities, to cleave about 25-32 bases that contain the lesion. After that, the DNA polymerase delta and epsilon enzymes accomplish synthesis of the new DNA strand, and finally the DNA ligase I enzyme successfully ligates the free flanks. Nairn et al. (1989) suggested that the rate of the GGR subpathway largely depends on the type of the lesion revealed in the genome (Nairn et al., 1989). In contrast to TCR, the DAR system is employed in terminally differentiated cells and repairs damaged sites in the continuously transcribed or non-transcribed genes (Nouspikel and Hanawalt, 2002). Each component of the NER pathway is important in achieving successful repair of the injured sites. Functional genetic defects in the genes of NER-associated proteins are found to be related to certain diseases such as xeroderma pigmentosum, Cockayne's syndrome, Trichothiodystrophy, and various types of cancers (Lindahl, 1974; de Boer and Hoeijmakers, 2000).

The base excision repair (BER) mechanism, first

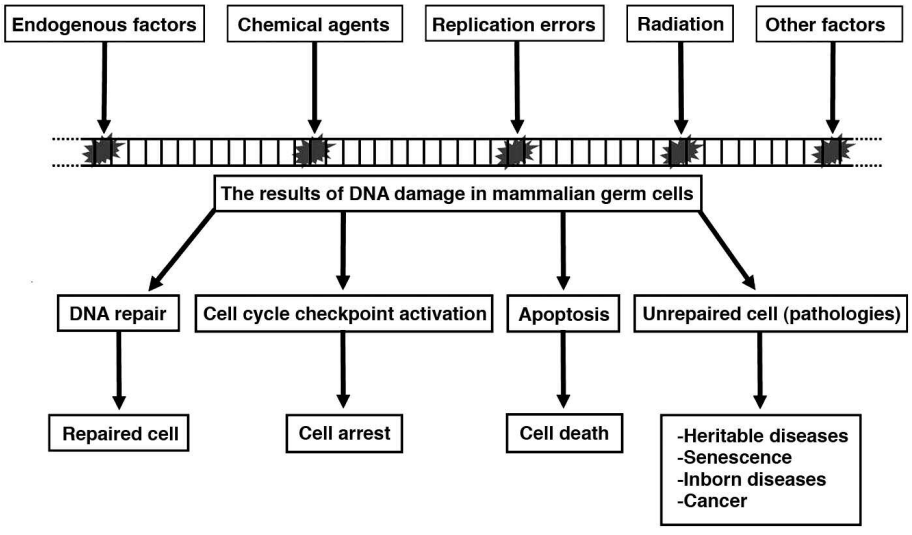

Fig. 1. Schematic diagram outlines endogenous factors, chemical agents (oxygen radicals, alkylating agents, oxidizing agents), replication errors, radiation and other factors (UV light, ionizing radiation, oxidative stress, and anti-tumour agents) that may cause DNA damage in mammalian germ cells. Thereafter, the fate of a cell with damaged DNA in the genome has been shown (Ozawa, 1995; Arnheim and Shibata, 1997; Baarends et al., 2001; Olsen et al., 2003, 2005; Aitken and Baker, 2004; Jaroudi et al., 2009; Valavanidis et al., 2009).

reported by Tomas Lindahl, is based on replacement of the modified bases via deamination, methylation, and oxidation with the correct ones (Lindahl, 1974). Modified bases are removed by specific DNA glycosylase enzymes that function in specific recognition and excision of the structurally changed bases from the genome. Apurinic/apyrimidinic sites are formed following excision of the bases, and the correct bases are rapidly synthesized by polymerase delta/epsilon. Finally, remaining free ends are faithfully ligated by ligase enzymes (Olsen et al., 2001; Jaroudi and SenGupta, 2007). Apurinic and apyrimidinic sites, also called abasic regions, frequently appear in the genome. Apurinic and apyrimidinic lesions are described as a loss of purines (adenine (A) and guanine (G)) or pyrimidins (thymine (T) and cytosine (C)) from the DNA structure, respectively (de Boer and Hoeijmakers, 2000). Generally, the BER mechanism is composed of two pathways known as the short-patch repair (SPR) and the long-patch repair (LPR). In the SPR subpathway, only one nucleotide is excised by glycosylase enzyme, and then AP endonuclease (HAP1) forms the necessary DNA ends to initiate DNA synthesis process. After that, polymerase ß accurately incorporates the correct nucleotide to the apurinic/apyrimidinic site. Finally, ligation is performed by the DNA ligase III enzyme. In the LPR mechanism, several nucleotides (2-10 nts) are excised, and then required bases are placed by polymerase δ and ε enzymes in cooperation with replication factor C (RPC) and proliferating cell nuclear antigen (PCNA). Finally, free nicks are sealed by the enzyme of DNA ligase I (Christmann et al., 2003; Jaroudi and SenGupta, 2007).

The mismatch repair (MMR) system especially functions in removing base mismatches formed by exogenous and endogenous agents that cause base deamination, oxidation, and methylation. Moreover, the MMR system plays a role in repairing the base-base mismatches derived from insertions/deletions and replication errors. Replication errors are made by DNA replication machinery that incorporates approximately one wrong nucleotide per 10^7 additions. Unfortunately, about 0.1% of mistakes generated by DNA replication machinery cannot be repaired by MMR and may lead to genetic mutations (Modrich and Lahue, 1996; Christmann et al., 2003; Kolas and Cohen, 2004). In fact, there is a discrimination during mitotic DNA replication in germline which occurs throughout life in males but only during fetal development in females (Baarends et al., 2001). MMR system comprises basic steps including recognition of the DNA lesion, strand discrimination, excision and repair. MutS and MutL proteins participate in detecting the mismatched bases in prokaryotic cells. Similar to prokaryotic cells, MutS (MSH1–6, MLH1 and MLH3) and MutL (PMS1 and PMS2) homologues are reported to be responsible for recognizing the mismatched sites, but in contrast to prokaryotic cells play roles in post-meiotic segregation in human and mouse. The heterotetrameric complex created by the interaction of two different MutS and MutL homologues proteins detect the mismatched bases and certain loop structures (Fishel et al., 1993; Umar et al., 1994; Palombo et al., 1995; Lipkin et al., 2000). Following that, the mismatched base pairs are excised by exonucleases I enzyme, and then missing nucleotides are correctly synthesized by polymerase δ enzyme (Longley et al., 1997; Genschel et al., 2002). However, the mechanism by which the MMR system distinguishes the strand that has a chemically modified base from the undamaged strand is not completely known.

DNA double strand breaks, commonly seen in the germline and somatic cells, are mostly repaired by the DNA double strand break repair (DSBR) system. There are two major DSB repair pathways operating in male and female germ cells. The first one is called non-homologous end joining (NHEJ), in which broken DNA strand ends are ligated by specific ligase enzymes. Since the NHEJ pathway lacks a homologous sequence control system, it is recognized as error-prone. Deletion, inversion, and other types of abnormalities in the genome could occur as a consequence of the NHEJ repair process (Christmann et al., 2003; Leduc et al., 2008b; You et al., 2009). On the other hand, the other DSBR pathway, homologous repair (HR), operates in an error-free manner as it repairs the broken ends dependent on the homologous DNA sequence (Kanaar et al., 1998). Which of the two pathways is chosen is basically determined by whether KU (KU70 and KU80) or RAD52 binds to the damaged region. When the KU protein interacts with the damaged site, the HR pathway initiates. If the RAD52 binds to the broken ends prior to KU, the NHEJ mechanism is commenced to repair the damage (Van Dyck et al., 1999; Bassing and Alt, 2004). The most important function of the DSBR mechanism is to repair the nicks created during the crossing-over process. Meiotic crossing-over occurs between non-sister chromatids of homologous chromosomes in prophase I of meiosis I stages mammalian spermatogenesis and oogenesis. During the meiotic recombination process, the DNA strand breaks are induced by the activity of specific topoisomerase 11 (SPO11) to facilitate the exchange events. Consistent with this, SPO11 is intensively expressed by the meiotic cells and is also conserved from yeast to human (Bergerat et al., 1997; Romanienko and Camerini-Otero, 1999). After completing crossing-over, virtually all ends are normally ligated by ligase enzymes. If some of these ends remain in unligated states, they are generally repaired by the DNA double strand break repair system in germ cells (Keeney et al., 1997; Goedecke et al., 1999).

Another repair mechanism found in male and female germline is post replication repair (PRR). PRR is activated following the formation of several DNA lesions that stall the DNA replication process (Laan et al., 2005). In this regard, specialized DNA polymerase enzymes add a few nucleotides to the opposite site of the lesion so that DNA replication machinery resumes the

synthesizing activity again. PRR is different from the other repair mechanisms since it drives the DNA replication process in spite of detecting the lesion in a cell during the S phase of the cell cycle. This phenomenon may be considered an advantageous of cell fate since this repair system rescues the cell from premature termination of its DNA replication, and prevents the cell from initiating the unexpected cell death process. After DNA replication is completed, the PRR mechanism repairs the damaged sites detected previously by using other repair systems. This mechanism operates either in the error-free manner or in the error-prone one; the latter commonly leads to gene mutations (Baarends et al., 2001; Zheng et al., 2005).

Gametogenesis and DNA damage encountered in mammalian germline cells

Spermatogenesis is a long process starting once man attains sexual maturity. This process comprises three main phases defined as the spermatogonial stage, the meiotic stage, and the spermiogenesis stage. In the spermatogonial stage, spermatogonial stem cells derived from primordial germ cells proliferate throughout life, but not continuously. In the meiotic stage, daughter cells originating from spermatogonial stem cell divisions differentiate into primary spermatocytes that undergo the first meiotic division. As a result of the first meiosis I, two secondary spermatocytes are formed from each primary spermatocyte. The secondary spermatocytes proceed through second meiotic division to yield four equivalent round spermatids that contain a haploid genome. In the stage of spermiogenesis, round spermatids mature into spermatozoa, the process known as spermiogenesis. Morphological and cellular changes occurring throughout the spermiogenesis are acrosome development, condensation of the haploid genome, formation of the flagellum, and reorganization of cytoplasmic organelles. Finally, mature sperm cells are released into the lumen of seminiferous tubule, this is known as spermiation and occurs at the end of each spermatogenesis (Nayernia et al., 1996; Holstein et al., 2003).

Spermatogonial stem cells are susceptible to genotoxic agents coming from endogenous metabolites or environmental factors that may lead to various types of DNA damage (Figure 1). A great number of divisions in the spermatogonial stem cells occur at the beginning of spermatogenesis. This high level of mitotic activity may cause an increase in the formation of damaged sites in the nuclear genome originating from DNA replication machinery errors. Other spermatogenic cells, including secondary spermatocytes, spermatids, and spermatozoa do not undergo DNA synthesis. Formation of new DNA damage derived from the DNA replication errors is therefore unlikely. However, high levels of DNA damage generated by the genotoxic agents and cellular metabolites have been detected in the nuclear genome of certain male germ cells, especially spermatozoa (Evenson et al., 2002). There are two main reasons that elevated sensivity of male germ cells to DNA damage-inducing agents may arise. First, there may be alterations in transcriptional/translational activities of DNA repair genes, e.g. elongating/elongated spermatids, and sperm cells inevitably down-regulate repair-related transcripts during spermiogenesis stage. The reason for this reduction is that nuclear genomes of these cells are strictly condensed by replacement of 85% of the total basic histones with protamins. This exchange will prevent unfavorable effects of the DNA-damaging agents. However, defects in the protamination process may lead to an increased occurrence of certain DNA damage, including single and double strand breaks, formation abasic sites, the appearance of modified bases and interstrand DNA-DNA and DNA-protein cross-links (Bennetts et al., 2008). Deficiencies in the protamination process might originate from paternal aging, a decrease in the levels of the follicle stimulating hormone or leutinizing hormone, genetic mutations in protamine 1 and 2, and chemotherapeutic agents (etoposide, cisplatin and bleomycin) commonly used for the treatment of testicular cancers (Spermon et al., 2006; Plastira et al., 2007; Aleem et al., 2008). The other main reason for elevated sensitivity of male germ cells is the loss of some crucial cellular events, such as apoptosis, during spermatogenesis. Male germ cells usually use the apoptotic pathway at the beginning of the spermatogenesis to remove cells with excessive damage (Lewis and Aitken, 2005). Lack of stimulation of the apoptotic pathway may increase the number of sperm cells that have high levels of DNA damage in their genomes (Sawyer et al., 2003). A spermatozoon that possesses high levels of DNA damage in the nuclear genome can lead to unsuccessful fertilization of a mature oocyte in metaphase II phase of meiosis II. Thus, the accumulated DNA damage in the sperm is also seen in the zygotic genome after fertilization. This damage is usually repaired successfully during fertilization by maternal DNA repair enzymes transcribed from maternal stored mRNAs (Brandriff and Pedersen, 1981; Aitken and Krausz, 2001). If the damage is not repaired, the pre-implantation embryo derived from the unrepaired zygote may not be able to develop properly and may even remain at an arrested state in the zygotic stage. Occasionally, these kinds of embryos can pass the zygotic arrest and then certain developmental abnormalities including abortions, congenital anomalies, and an increase in the incidence of childhood cancers may occur (Ji et al., 1997; Brinkworth, 2000; Lee et al., 2009).

Many genotoxicants leading to DNA damage in male germline have been detected in recently-performed studies. One of these genotoxicants is 8-oxoG (8-oxoguanine) which is largely produced by environmental agents such as exhaust from the cars Damage caused by 8-oxoG originates from misincorporation of the oxidized dGTP or oxidized guanine as a result of the oxidative metabolisms and is commonly repaired by BER

mechanism (Shen et al., 1997). In addition, oxidized pyrimidins (thymine glycols (TG), 5-hydroxycytosine (5-OHC) and purines formed by oxygen radicals have been indicated to cause misincorporations in rodent and human male germ cells (Olsen et al., 2003). Other risk factors capable of inducing DNA damage in male germ cells are paternal aging and smoking. Increasing paternal age may correlate with high levels of DNA fragmentation and decondensation in human sperm DNA (Singh et al., 2003; Belloc et al., 2009). Potts et al. (1999) have reported that there were significantly higher numbers of DNA strand breaks in heavy smokers' spermatozoa than the control group (Potts et al., 1999). Furthermore, smoking mediates increases in the levels of oxidative DNA lesions such as 7,8-dihydro-8-oxoguanine (8-oxoG), decrease conception rates, and increases the incidence of childhood cancers (Ji et al., 1997; Zitzmann et al., 2003). Aside from paternal aging, excessive caffeine consumption may be responsible for increasing the rate of DSB formation in human sperm (Leduc et al., 2008b). Also, oxidative stress adversely affects the integrity of the sperm DNA. There are two major adducts defined as 8-hydroxydeoxyguanosine (8OHdG) and ethenonucleosides seen in human sperm DNA. 8OHdG is formed following the oxidative attack on sperm DNA; ethenonucleosides (1,N6-ethenoadenosine and 1,N6-ethenoguanosine) are indirectly produced as a consequence of lipid peroxidations (Badouard et al., 2008). These DNA adducts weaken the DNA backbone via making glycosyl bonds attached to the base-ribose unit. As a result of this effect, abasic sites and strand breaks are formed on the DNA backbone (Lindahl and Andersson, 1972). The major sources of oxidative stress in the male reproductive system are derived from large numbers of leukocytes in testicular tissues, radiations, redox reactions, mitochondrial reactive oxygen species (ROS), and impaired sperm metabolisms (Aitken et al., 1989, 2005; Aitken and Baker, 1995; Koppers et al., 2008).

In females, primordial germ cells undergo a few mitotic divisions and then differentiate into oogonia. Throughout fetal development, these oogonia mature into primary oocytes that enter into first meiotic division and become arrested in the diplotene stage of the prophase I of meiosis I. Impressively, these primary oocytes maintain a dormant state in primordial follicles until puberty. At puberty, the primary oocyte completes meiosis I and turns into a secondary oocyte that also remains at metaphase II stage of meiosis II up to fertilization. One secondary oocyte is released from the ovary at the end of each ovarian cycle in humans. While the released oocyte is transported in the tuba uterina, it can be fertilized by a competent spermatozoon. Male and female pronuclei formed after the normal fertilization process fuse faithfully to generate the zygote nucleus. Then, the zygote starts a series of rapid mitotic cell divisions called segmentation to produce 2-cell, 4-cell, 8-cell, morula (approximately 16-cell), and blastocyte during early embryonic development. The blastocyte includes an inner cell mass (ICM) localized on one side of the blastocoel cavity and an outer cell mass (OCM) at the periphery of the blastocyte (Fleming et al., 2001; Ozturk and Demir, 2009).

Similar to the male germline, the female germ cells encounter diverse types of DNA damaging-agents during the production of mature oocytes. Besides these genotoxicants, DNA replication errors occurring during the S phase of the cell cycle in oogonia may lead to certain gene mutations. Indeed, unlike premeiotic male germ cells, a primary oocyte is generally formed after approximately 20 mitotic divisions during fetal development (Baarends et al., 2001). However, premeiotic male germ cells divide more than 1000 times prior to entering into meiosis in a 50-year-old male. Many more mitotic divisions in spermatogenesis than oogenesis are significant since spermatogenesis may play a major role in the evolutionary process (Baker et al., 1996; Hurst and Ellegren, 1998). It is generally accepted that most of the damage appearing in mature oocyte may originate from the long delay in prophase I of the meiosis I until puberty (Drost and Lee, 1995; Huttley et al., 2000). Encountered genotoxicants such as radiation and other environmental agents are able to affect the genome of female germ cells during oogenesis. In fact, radiosensitivity of an oocyte largely depends on the follicular stage (Adriaens et al., 2009). Studies performed on animals demonstrate that the stimulation of DNA damage by ionizing radiation in male and female germ cells results in low birth weight, congenital anomalies, and miscarriages (Jacquet, 2004).

DNA repair mechanisms in mammalian male germ cells

DNA damage occurring in human spermatozoa is found to be associated with changes in the fundamental reproductive events, including a decrease in conception rates, impairment of embryonic development, and an increase in the number of abortions. Operating repair mechanisms throughout spermatogenesis mend the DNA damage to prevent these kinds of unfavorable outcomes. There are a number of studies associated with the DNA repair mechanisms being used during spermatogenesis (Beumer et al., 1997; Hamer et al., 2003; Ahmed et al., 2007, 2010a,b). Information related to DNA repair mechanisms in male germline usually arise from different applications, such as unscheduled DNA synthesis (UDS), alkaline elution, the comet assay, and various immunochemical techniques. Although these methods could detect the existence of DNA damage, they are unable to give precise knowledge about the nature of the injuries (Aitken et al., 2009).

The main modifications in the bases, such as methylation, deamination, abasic sites and oxidations, are generally repaired by the BER mechanism in the male germline. Indeed, the BER repair system efficiently functions during mammalian spermatogenesis from primordial germ cells to elongated spermatids. The

expression of BER-associated genes such as Mpg and Apex are demonstrated in elongated spermatids (Aguilar-Mahecha et al., 2001). Methylpurine and uracil DNA glycosylases that participate in the BER system are also detected in male mice germ cells (Grippo et al., 1982; Engelward et al., 1993). Furthermore, high levels of ligase I, Ape (mouse homologue of human Hap1), Xrcc1, Polß, and ligase III have been documented in mouse spermatogenic cell nuclear extracts when compared to the somatic cells (Intano et al., 2001) (Fig. 2). Knock out studies have been performed to understand more precisely the functional features of certain BER genes. When Polß and Apex (Ref-1) genes were separately knocked out in mice models, embryonic lethality was determined (Gu et al., 1994). Furthermore, disruption of the Polß/Pms2 genes in mouse causes embryonic lethality (Friedberg and Meira, 2006). Embryonic lethality at E15.5–16.5 dpc was observed in mice whose DNA ligase I gene was ablated (Bentley et al., 1996). On the other hand, when some of the BER genes such as Nth1, Apng, Udg and Mnth1 were knocked out, the mice were viable and exhibited no anomalies (Nilsen et al., 2000a; Ocampo et al., 2002; Takao et al., 2002).

NER actions in spermatogenic cells are lower than somatic cells. High levels of NER activity are not required in male germ cells, because most of the damaged cells can be eliminated by programmed cell death (Xu et al., 2005). Gene expression studies related to NER showed that Ercc1 is transcribed during spermatogenesis as the primordial germ cell (PGC) progresses to the elongated spermatid (Hsia et al., 2003). In addition, the Rpa gene is detected in primary spermatocytes and round spermatids at low levels. Other genes such as Pcna (in primary spermatocytes and elongated spermatids), and Xpd (Ercc2) (in round spermatids) are synthesized in certain cells throughout spermatogenesis (Shannon et al., 1999) (Fig. 2). However, Xpf is only produced by spermatogonia, primary spermatocytes, secondary spermatocytes, and round spermatids (Jansen et al., 2001).

Spermatogenic cells in early stages of spermatogenesis are capable of repairing single-strand breaks (SSB) faithfully in humans, hamsters, and rats (Cleaver, 1989; Van Loon et al., 1991; Olsen et al., 2003). Rad51 and Dmc1, considered to be necessary for HR subpathway in DSBR, are expressed in primary spermatocytes; therefore the HR pathway seems to be important during the meiotic process. Rad51 potentials in binding to the single strand-DNA and possesses DNA-dependent ATPase activity thereby inducing strand exchanges. Additionally, Dmc1 has been shown to be associated with carrying out meiotic division properly in mice (Pittman et al., 1998; Shinohara and Ogawa, 1999; Tarsounas et al., 1999; Ronen and Glickman, 2001). Tarsounas et al. (1999) observed that Rad51 and Dmc1 proteins interact with each other and colocalize in the chromosome cores in spermatocytes at early prophase I phase of meiosis (Tarsounas et al., 1999). Therefore, the knockout of the Rad51 gene results in embryonic lethality in mice (Thacker, 1999). Indeed, there is no operating homolog recombination in round, elongating and elongated spermatids, since they do not have a sister chromatid. Thereby, these cells use the non-homologous end joining pathway of the DSBR mechanism, which has been identified as error-prone. Goedecke et al. (1999) have shown that the Ku70 gene required for NHEJ can be expressed in spermatids of mice (Goedecke et al., 1999). In addition, Leduc et al. (2008) observed that elongating spermatids express topoisomerase II beta (Top2b), tyrosyl-DNA phosphodiesterase 1 (Tdp1), gamma-H2AX (also known as H2AFX). Tdp1 is an enzyme that functions in repairing of topoisomerase-mediated DNA damage. Also, detection of H2AX protein in elongating spermatids shows that there is a response to DNA damage (Leduc et al., 2008a).

MMR is active during spermatogesis, but its activity dramatically declines after completing meiosis and is not detected in elongated spermatids. However some genes like Msh2 that have roles in MMR mechanism are expressed in most of the male germ cells including PGCs, gonocytes, spermatogonia, and primary and secondary spermatocytes (Richardson et al., 2000; Zheng et al., 2005). Knockout mice models focusing on the Msh2, Msh3 and Msh6 genes have been separately performed. Although these mice have a deficiency in the MMR repair system in somatic cells, no failures associated with fertility have been observed (Arnheim and Shibata, 1997; de Wind et al., 1999). On the other hand, if either of Msh4 and Msh5 is knocked out, meiotic arrest and impairment in homologous

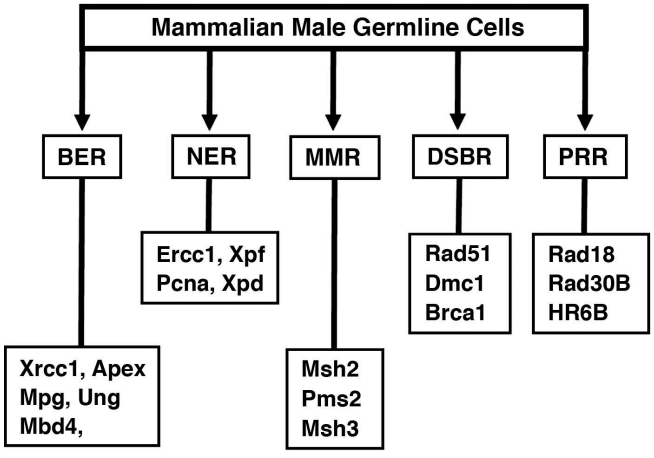

Fig. 2. Schematic diagram of genes expressed in main repair mechanisms frequently used in mammalian male germline cells during spermatogenesis. BER: base excision repair, NER: nucleotide excision repair, MMR: mismatch repair, DSBR: double-strand break repair, PRR: post replication repair (Shannon et al., 1999; Baarends et al., 2001; Intano et al., 2001; Christmann et al., 2003; Laan et al., 2005; Zheng et al., 2005; Leduc et al., 2008b).

chromosome pairing in male and female mice occurs (de Vries et al., 1999; Kneitz et al., 2000). Pms2 ablated mice models show genomic instability and male-restricted infertility. Also, there are defects related to pairing of homologous chromosomes in spermatocytes, but no complete meiotic arrest is seen in male mice. Interestingly, the oocytes faithfully carry out chromosomal pairing without requiring the Pms2 gene (Baker et al., 1995). These studies suggest that MMR is not only needed for mitotic divisions but also that it plays crucial roles during meiosis, including proper establishment of homologous recombination and inhibition of recombination between mismatched sites (Baarends et al., 2001). Another repair system taking place during spermatogenesis is post replication repair (PRR). Several proteins (Rad18, HR6B and Rad30b) of PPR are detected in meiotic prophase and in the post-meiotic development of spermatids. Rad18 and Rad30b (mammalian homologue of Rad30) involved in the PRR in human and mouse are found in high levels in primary spermatocytes and round spermatids, respectively. The specific reason for the late expression pattern of these genes in spermatogenic cells has not been investigated (McDonald et al., 1999; Tateishi et al., 2000). HR6B, another conserved PPR protein, may be involved in histone to protamin transition, repair of exogenous factor-induced lesions in spermatids, and chromatin remodeling during meiotic prophase. The conditions of impaired spermatogenesis, increased programmed cell death of pachytene spermatocytes, and male-restricted infertility are observed in HR6B gene knockout mice models (Roest et al., 1996; Baarends et al., 1999).

DNA repair mechanisms in mammalian female germ cells

The BER mechanism functions in removing damaged bases commonly stimulated by reactive oxygen species during oogenesis. The Mpg gene, a member of BER is extensively expressed by the unfertilized oocytes and cumulus cells in mice (Hendrey et al., 1995). Other genes of BER such as Mbd4, Ogg1, Ung, Polß, Xrcc1 and Apex are found to be expressed at high levels in human oocytes, germinal vesicle (GV) and stages after fertilization (Nilsen et al., 2000b; Zheng et al., 2005; Menezo et al., 2007; Jaroudi et al., 2009) (Fig. 3). On the other hand, NER proteins accumulate in synapsis and recombination sites in female germ cells that enter meiotic division. Furthermore, Xpc and Ercc2 proteins are weakly translated in rhesus monkey oocytes (Jaroudi and SenGupta, 2007).

MMR is known to be activated after DNA replication is completed (G2/M checkpoint) in female germ cells. The main MMR genes such as Mlh1, Pms1, Pms2, Msh2, Msh3, and Msh6 are reported in human MII oocytes and blastocytes (Fig. 3). Although Mlh1, Msh2, Pms1, TfIIh, Cdk7, Csb, Rpa1 and Msh6 are highly transcribed, Msh3 is shown to be expressed in low level in human MII oocyte. The expressions of the genes (Csb, Gtf2h1, and Mms19l) related to the TCR subpathway of the MMR system have been demonstrated in human MII oocytes (Jaroudi et al., 2009). In addition, TCR genes are also observed in human GV oocytes, but their translational activity gradually decreases through the embryonic genome activation which occurs at 4-8 cell stages (Braude et al., 1988; Telford et al., 1990). Impressively, the Mlh1 protein of the MMR repair system has also been observed in the meiotic synaptonemal complex formed in the pachytene spermatocytes and oocytes (Baker et al., 1996). Both Baker et al. (1996) and Edelman et al. (1996) have shown that the Mlh1-ablated mouse models are infertile due to pachytene arrest and impairment in meiotic replication process (Baker et al., 1996; Edelmann et al., 1996).

The HR subpathway of the DSBR system is more commonly used than NHEJ in human metaphase stage oocytes and blastocytes. Therefore, Rad52 and Rad51

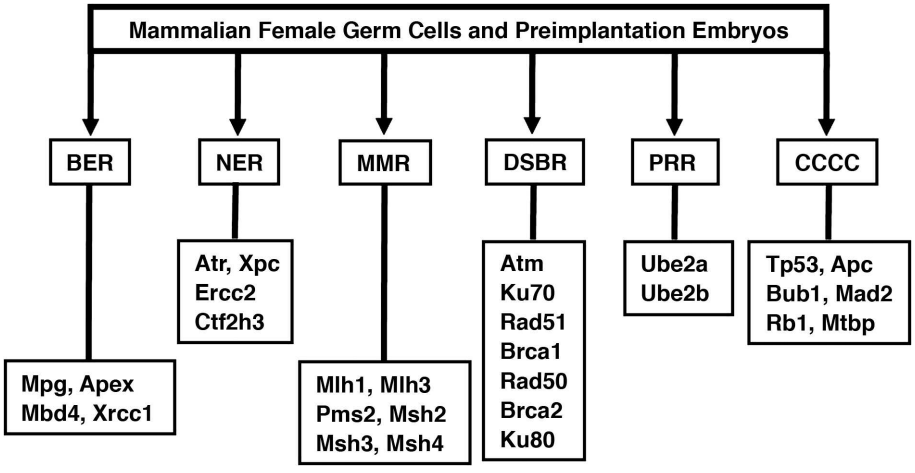

Fig. 3. Schematic diagram of genes expressed in the main repair mechanisms frequently used in mammalian female germ cells and preimplantation embryos (Jurisicova et al., 1998; Vinson and Hales, 2001; Zheng et al., 2005; Jaroudi and SenGupta, 2007; Jaroudi et al., 2009). BER: base excision repair, NER: nucleotide excision repair, MMR: mismatch repair, DSBR: double-strand break repair, PRR: post replication repair, CCCC: cell cycle checkpoint control.

genes which are crucial in the HR mechanism are highly transcribed in human MII oocytes and blastocytes. Brca2, known to play a role in activating the HR pathway through its interaction with Rad51, is also detected in human GV and MII oocytes (Menezo et al., 2007; Jaroudi et al., 2009). Additionally, Brca1 is required to trigger the HR mechanism in human oocytes. Although Jaroudi et al. (2009) could not find Brca1 mRNA in MII oocytes, Wells et al. (2005) and Menezo et al. (2007) demonstrated the presence of Brca1 expression in human MII and GV oocytes, respectively (Wells et al., 2005; Menezo et al., 2007; Jaroudi et al., 2009). In addition to synthesis of HR-related transcripts, NHEJ genes, including Ku70 (Xrcc5) and Ku80 (Xrcc6), are also expressed at medium levels in human MII oocytes (Jaroudi et al., 2009) (Fig. 3).

PARP-1 (poly (ADP-ribose) polymerase-1) is also found to contribute significantly to the DNA repair mechanism in male and female germ cells (Cherney et al., 1987). Functional features of the PARP-1 protein in the DNA repair process are broadly reviewed by Christmann et al (2003) (Christmann et al., 2003). They found three functions. 1) Direct interaction with Xrcc1 and polymerase ß proteins, which are critical molecules in the base excision repair system. 2) Participation in remodeling of chromatin structure after occurrence of DNA damage. PARP-1 is capable of binding to single strand breaks created by alkylating agents and ionizing radiations to stimulate strand displacement and DNA synthesis (Menissier-de Murcia et al., 1989). Furthermore, automodified PARP-1 can bind to 20S proteasome to induce the proteolytic activity of this complex (Mayer-Kuckuk et al., 1999). The induced 20S proteasome complex achieves degradation of histone proteins which are damaged by oxidative stress. Thus, DNA repair proteins easily enter into DNA strands to repair the damaged sites. 3) Interaction with several proteins including, telomerase, p21, check point proteins, DNA ligase III, NF-κß, iNOS (inducible nitric oxide) and p53, which have critical roles in cellular physiology (Pleschke et al., 2000). Jaroudi et al. (2009) showed the expression of PARP1 mRNA in human MII oocyte and blastocyte. Although repair functions of PARP during spermatogesis have been explained up to a certain level, its biological and clinical features have not been definitively clarified in female germ cells (Agarwal et al., 2009; Jaroudi et al., 2009).

DNA repair mechanisms and repair related gene expression patterns in preimplantation embryos in mammalians, including humans, rhesus monkeys, and mice, have been reviewed to a large extent by Jaroudi et al. (2009) (Jaroudi et al., 2009). Apex, Mbd4 and Xrcc1 members of the BER repair system are extensively expressed from zygotic stage to blastocyte in the rhesus monkey (Vinson and Hales, 2001). Atr, Xpc and Ercc2 genes that belong to the NER repair system are transcribed in rhesus monkey embryos from zygote to blastocyte stage (Jurisicova et al., 1998; Zheng et al., 2005). On the other hand, Ctf2h3 gene expression occurs throughout preimplantation embryo development in mice (Yoshikawa et al., 2006). The MMR genes in preimplantation embryos of rhesus monkeys, involving Mlh1, Mlh3, Pms2, Msh2, Msh3 and Msh4 genes are translated during early embryonic development (Vinson and Hales, 2001; Hirano and Noda, 2004; Zheng et al., 2005) (Fig. 3). Atm, Brca1, Brca2 gene expressions are also demonstrated in human preimplantation embryos (Wells et al., 2005). Interestingly, Atm levels gradually increase from fertilized oocytes to blastocyte stages. Brca1 has a weak expression in the oocyte, morula and blastocyte stages, but its level is increased at the 4- to 8-cell stages in humans (Xin et al., 2000). Ube2a, a post-replication repair gene, is abundant among the stored maternal mRNAs, and its level gradually rises at 8-cell stage onwards (Zheng et al., 2005).

Cell cycle checkpoint genes such as Tp53, Apc, Bub1, Mad2, Rb1, and Mtbp are expressed in rhesus monkeys, mice and humans throughout early embryonic development (Horne et al., 1996; Jurisicova et al., 1998; Wells et al., 2005; Zheng et al., 2005). Chek1, a DNA damage-response gene, is produced by 1-cell, 2-cell and 4-cell preimplantation embryos in mice. Chek1 expression is also observed in the oocytes and during preimplantation embryo development up to the blastocyte stage in the rhesus monkey (Zeng et al., 2004; Zheng et al., 2005). Many repair genes required for recognition of damaged sites are also transcribed in mammalian post-implantation embryos (Vinson and Hales, 2001).

DNA damage-induced cell cycle checkpoint control system in germ cells

Like somatic cells, germ cells are capable of controlling the integrity of the genome throughout their cell cycle. When a damaged site appears in the DNA structure, the cell cycle arrest system is activated to block cell division until the damaged region is completely repaired by one of the appropriate DNA repair mechanisms explained above. In fact, cell cycle arrest that occurs at G1/S and G2/M checkpoints takes place before DNA synthesis and mitosis, respectively, in somatic cells (Franca et al., 1998; Spiller et al., 2009). The observations from fluorescent in situ hybridization (FISH) analysis of cleavage stage human embryos shows that these embryos do not have any checkpoint control system. If the repair system fails, stimulated cell cycle checkpoint genes trigger the initiation of apoptotic processes to completely remove the blastomeres or the blastocyte cells (Aquilina et al., 1999; Harrison et al., 2000). Pro-apoptotic genes Bax and Bak are found to be expressed in MII oocytes and blastocytes. Bad, another pro-apoptotic gene, is expressed only in human blastocytes and not in MII oocytes. On the other hand, the expression of anti-apoptotic Bcl-2, Bcl-w genes is detected at low levels in both human MII oocytes and blastocytes, but Bcl-xl is absent in both groups (Jaroudi et al., 2009). Thus, the apoptotic process in MII oocytes

and blastocytes is balanced by expression levels of pro- and anti-apoptotic genes. As in early embryos, there is no cell cycle checkpoint control in pachytene spermatocytes. However, these cells are capable of checking their genome integrity and can be eliminated by programmed cell death pathway if it is required (Baarends et al., 2001).

Atm (ataxia telangiectasia) and Atr (Atm and Rad3-related) play major roles in the damage-induced cell cycle control system in germ cells. Atm and Atr have protein kinase activities and couple with proteins in detection of DNA damage and cell cycle arrest (Wells et al., 2005). Studies show that the Atm expressed by spermatocytes and oocytes is also involved in regulation of meiotic cell cycle progression. Consistent with this, meiotic arrest is observed at the pachytene stage of zygotes in Atm knockout mice models, and these mice are also infertile (Keegan et al., 1996; Xu et al., 1996). However, Atr is an Atm-like protein and binds to unpaired regions of meiotic prophase chromosomes (Moens et al., 1999; Brown and Baltimore, 2000). Therefore, certain mutations in the Atr gene may commonly cause lethality in the embryos (Brown and Baltimore, 2000). Furthermore, both Atm and Atr proteins, which are frequently included in phosphorylation of certain proteins, participate in complexes formed at damaged DNA sites (Cortez et al., 1999). One of the Atm substrates is Brca1, which contributes to the process of NER and DSBR repair (Cortez et al., 1999; Welcsh et al., 2000).

Conclusion

Mammalian germline and early embryos commonly encounter various factors that may cause damage in the nuclear and mitochondrial genomes. Metabolites derived from the cellular metabolisms, chemical agents, radiation and replication errors are found among these factors. The damaged sites in the nuclear or mitochondrial DNA are faithfully repaired by the main repair mechanisms called BER, NER, DSBR, MMR and PRR. In fact, these repair systems should be efficiently operating during the lifespan of the cells to maintain cellular events and to generate healthy gametes and preimplantation embryos.

A limited number of studies has reported on the expression pattern of different DNA repair genes in male and female germ cells. New studies are required to completely understand the roles of DNA repair genes during gametogenesis and preimplantation embryo development. Further determination of these functions may introduce significant approaches associated with reproductive failures in in vitro fertilization (IVF) applications. Superovulation protocols and in vitro culture conditions (mediums, temperature and oxygen/carbon dioxide levels) also need to be investigated in order to observe the causal effects on the expression of DNA repair genes. In addition, since the oocyte brings numerous mitochondria to the zygote, repair systems operating in the mitochondria should be investigated in detail to clarify their effects on the fertilization process and early embryo development.

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