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REVIEW

Effects of DNA damage on oocyte meiotic maturation and early embryonic development

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Abstract DNA damage is one of the most common threats to meiotic cells. It has the potential to induce infertility and genetic abnormalities that may be passed to the embryo. Here, we reviewed exogenous factors which could induce DNA damage. Specially, we addressed the different effects of DNA damage on mouse oocytes and embryonic development. Complex DNA damage, double-strand breaks, represents a more difficult repair process and involves various repair pathways. Understanding the mechanisms involved in DNA damage responses may improve therapeutic strategies for ovarian cancer and fertility preservation.

Keywords DNA damage, double-strand breaks (DSBs), oocyte, embryo

1 Introduction

Increasing attention is being paid to how DNA damage influences mammalian oocytes. Women undergoing chemotherapy have an increased expectation of fertility preservation. In 19 European countries where all clinics report to the assisted reproductive technology register, a total of 350143 assisted reproductive cycles were performed in a population of 370 million, representing 947 cycles per million inhabitants [1]. Mammalian oocytes are particularly vulnerable to DNA damage, causing infertility and genetic abnormalities [2,3]. Simple forms of DNA damage such as single-site base damage or single-strand breaks (SSBs) will be repaired rapidly and easily [4]. However, complex DNA damage, DNA double-strand breaks (DSBs), represent a more difficult process with various repair pathways, which are the major focus of this review. DSBs can be induced by many exogenous or endogenous factors. During development in the fetal ovary, natural recombination can lead to DSBs at early stages of meiotic prophase I. There are two programmed DNA DSBs which occur during gene rearrangements in immunocytes or during meiotic recombination in germline cells of mammalians [5–8]. Also, DSBs occur in the cleavage stage of embryos, which does not influence the genome sequence because the DSBs can be repaired [9,10]. The DSBs are usually sensed and repaired by the DNA damage checkpoint, which can take place during three specific cell cycle stages: G_1/S, S and G_2/M [5]. During mouse oogenesis, the DNA DSBs occur mainly at the pachytene stage in oocytes of the 14–20 days post-coitum fetus [11]. The programmed DSBs of pachytene can be repaired by homologous recombination (HR) when homologous chromosomes form synaptonemal complexes (SC) [12]. However, the abnormal DNA DSBs, if not repaired immediately like the programmed DSBs, could induce chromatin remodeling [13], cell cycle arrest, cell cycle delay, apoptosis or other forms of cell death [5,14,15].

H2A histone family member X (H2AX) is one of several genes coding for histone H2A. γ-H2AX is often used as a marker for DNA DSBs damage, because H2AX becomes phosphorylated within 1–3 min after DSBs and forms foci to recruit repairing factors at break sites [16,17]. In contrast, poly ADP-ribosepolymerase 1 (PARP-1) is used as a DNA damage repair marker, a sensor of SSBs in DNA and a component of the base excision repair (BER) signaling cascade [18–20]. The typical exogenous factors which can induce DSBs include: chemical drugs, especially cancer chemotherapy, doxorubicin, etoposide [21], bleomycin (BLM) [22]; ionizing radiation (X-rays and γ-rays), neocarzinostatin [23]; physical treatment, UV-A (wavelength 315–400 nm in the solar spectrum) [22,24]. The endogenous metabolites or metabolic intermediates produced in response to these factors are reactive oxygen species and products generated as a consequence (e.g., lipid peroxides) [25].

The effect of DNA damage caused by endogenous factors resembles programmed DSBs of pachytene. It is repaired by homologous recombination (HR) when

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homologous chromosomes form synaptonemal complexes (SC) [12]. Next we focused on the DNA damage caused by exogenous factors.

2 Effects of DNA damage

2.1 Chemical drugs

Bleomycin (BLM) is an antineoplastic drug used widely in clinical applications. DNA DSBs damage will increase with elevated concentration of BLM, observed by the increasing γ-H2AX signals in mouse oocytes [22]. Germinal vesicle oocytes with DSBs damage induced by BLM can develop into metaphase II stage (MII), with lower germinal vesicle breakdown (GVBD) ratio and longer GVBD period. Also, a delayed polar body extrusion (PBE) can be observed. Once GVBD occurs, however, the PBE will not be affected. There is a higher spindle assembly checkpoint activity during the transition of metaphase I (MI) to anaphase I (AI), indicated by Ccnb1-GFP degradation. Notably, oocytes with DNA DSBs can undergo parthenogenetic activation [22].

Two widely used cancer chemotherapeutic reagents, VP-16 and BLM, combined with an *in vivo* GC-specific DNA topoisomerase II-β (TOP2) knockout mouse model are used to investigate the effects of chemotherapy-induced DNA damage on growing mouse follicles [26]. VP-16 can cause massive DSBs in the GCs of growing follicles in a time-dependent manner. This damage, related to apoptotic GC death, results in follicle atresia and ovulation failure [26]. After TOP2 activity is inhibited by a specific inhibitor ICRF-193, an effective decatenation checkpoint does not occur in fully grown oocytes, which undergo the G_2/M transition and initiation of meiosis [27]. Also, oocytes treated with ICRF-193 have serious defects in chromosome condensation and homologous chromosome segregation. Furthermore, condensed chromosomes fail to maintain their normal configuration [27].

Etoposide is also a TOP2 inhibitor, which can cause DSBs damage in oocytes even in a low concentration ($5\ \mu g \cdot mL^{-1}$) as in somatic sells [28]. Ataxia telangiectasia mutated (ATM) is a master regulator of the DNA damage response pathway. Mouse oocytes can enter the meiosis phase in the presence of low concentration of etoposide. However, $> 80\%$ of oocytes will arrest at G_2/M with higher levels ($50–100\ \mu g \cdot mL^{-1}$) of etoposide, because the ATM-dependent DNA damage checkpoint is activated [21]. Cell division cycle 25 (Cdc25) is essential for meiotic resumption in mouse oocytes. It is the inhibition of Cdc25B, and not degradation of Cdc25A, which is responsible for the activation of oocyte DNA damage checkpoint [21].

A novel anticancer agent MLN4924, as a NEDD8-activating enzyme E1 (NAE) inhibitor, was identified by high throughput screening. MLN4924 can efficiently inhibit cullin neddylation, which inactivated CRL/SCF E3 ligase to cause substrate accumulation. As a result, a DNA damage response is triggered, cellular apoptosis is induced, and remarkable anticancer effects are observed both *in vitro* and *in vivo* [29–31]. MLN4924 activates DNA damage responses and the apoptosis pathways, as shown by the accumulations of pH2AX, pCHK1, pCHK2, cleaved caspase-3, and the cyclin-dependent kinase inhibitors p27 and p21 [32].

2.2 Ionizing radiation

Oocyte sensitivity to ionizing radiation (IR) varies widely according to the follicle/oocyte stage and the species. The effects of genetic changes depend on the types of initial DNA damage, such as base damage, base loss, SSBs and DSBs. Also, exposure to IR may result in structural chromosome anomalies, including chromosome-type aberrations and chromatid-type aberrations [4,33].

Neocarzinostatin (NCS) is often used as an ionizing radiation mimetic to induce DSBs [34,35]. A lot of γ-H2AX foci are associated with chromatin in oocytes followed by NCS treatment. Lower PBE indicates that NCS can inhibit oocyte maturation in a dose dependent manner [23]. This is similar to the somatic cells, in which NCS treatment can inhibit cellular proliferation by G_2 cell cycle arrest and induce apoptosis [36].

IR can induce apoptosis of the oocyte in primordial follicles as in somatic cells [37]. Exposure to IR during oogenesis and the diplotene stages of ovarian development can induce the loss of primordial follicles in the postnatal ovary, whereas half of the follicular reserve remains present after irradiation during the zygotene/pachytene stages. This different sensitivity is related to the level of caspase-2 expression [37]. By western blotting and caspase activity analysis, caspase-2 is activated 2 h after irradiation. Inhibition of caspase-2 activity can prevent cleavage of caspase-9 and partially prevent loss of oocytes in response to irradiation. Therefore, caspase-2-dependent activation of the mitochondrial apoptotic pathway is one mechanism involved in depletion of the primordial follicle pool [4,37].

2.3 Physical damage

Laser micro-beam dissection (LMD) can cut the nuclei precisely and effectively, so it is regarded as a physical treatment to induce DNA damage [38,39]. UV-A has been used as a LMD to induce DSBs in germinal vesicle oocytes and zygotes [22,24], as in various kinds of somatic cells [40–42]. LMD can induce DSBs to inhibit or delay G_2/M transition in oocytes [22]. Chromosome fragmentation cannot affect spindle organization, chromosome segregation and PBE, once GVBD occurs [22]. Also, early embryonic cleavage and development can be disturbed by LMD-induced DSBs damage in the female pronucleus

[24]. In addition, DNA-damaged blastomeres of 4- or 8-cell fail to divide and form compact morulae [24]. Oct4 (a marker of the inner cell mass) and Cdx2 (a marker of trophoblast cells) are expressed normally in the intact blastomere, but not in the DNA damaged blastomere of 2-cell embryos. It is possible that the embryos have their own mechanisms to delete DNA-damaged blastomeres, resulting in failure to develop further.

3 DNA damage repair

There is much evidence for mammalian oocytes repairing various kinds of DNA damage. The repair occurs either spontaneously or as a consequence of exposure to exogenous factors. Of course, the efficiency of DNA repair varies with different oocyte stages.

Usually, cells respond to DNA damage caused by exogenous and endogenous factors by arresting the cell cycle to allow time for the damage to be repaired. The DNA damage is usually sensed and repaired by the DNA damage checkpoint, which can take place during three specific cell cycle stages: G_1/S, S and G_2/M [5]. For oocytes that have completed DNA synthesis, the checkpoint operates mainly at meiosis (not strictly regarded as G_2/M). Depending on the nature of the DNA damage, it is repaired by a number of different mechanisms during the checkpoint mediated arrest. Simpler forms of DNA damage, such as single sites of base damage or SSBs, will be repaired rapidly and easily by three main pathways, namely base-excision repair, nucleotide excision repair, and mismatch repair [43].

However, DSBs represents a more difficult repair process involving various different repair pathways. DSBs repair is executed by two major mechanisms, non-homologous end joining (NHEJ) in somatic cells and HR in germline cells [10,44,45]. Notably, both NHEJ and HR are functional in zygotes [10]. DSBs are mainly sensed by ATM and ataxia telangiectasia and Rad3-related (ATR) [46,47]. The ATM phosphorylates directly or indirectly more than 30 substrates such as the checkpoint kinase 2 (CHK2), DNA-PK and Mre11-complex [48,49]. The activated CHK2 pathway can arrest the cell cycle, followed by the DNA-PK and/or Mre11-complex pathway, which is activated to repair the damaged DNA [49]. After DSBs are repaired, the checkpoint proteins become inactivated to allow the cell cycle progression resume [50].

The damage response is so tightly coordinated that cell cycle resumes as soon as the damage is repaired. However, if the extent of DNA damage could not be repaired completely, programmed mechanisms of cell death become active in order to remove the cells, usually by apoptosis [51,52].

Oocytes contain half of the homologous chromosomes and a minimum amount of cytoplasm after the first meiotic division. Then oocytes enter the second meiotic division without DNA replication and an inter phase. At this stage oocytes are ovulated and fertilization finished. Egg activation triggers the completion of the second meiotic division and initiation of the first embryonic cell cycle. Since the second meiosis is short and without DNA synthesis, there should be one DNA damage repair in meiotic metaphase I before the DNA damage reaches the developing embryo. However, little is known about the possible meiosis DNA damage repair in meiotic metaphase I. It is not known how sensitive the metaphase I is and which factors are involved. Notably, interstrand crosslinks (ICLs) damage caused by mitomycin (MMC) could not inhibit or delay meiotic division in either metaphase I or metaphase II, although the quality and development of preimplantation embryos are influenced [23]. So it is possible that only DSBs damage could establish a meiotic metaphase arrest checkpoint.

The arrest of meiosis caused by DNA DSBs damage is often compared with the arrest at G_2/M transition in somatic cells. So it is essential to distinguish the molecular mechanisms that govern resumption of meiosis and checkpoint resumption from the G_2-arrest induced by DNA damage [53,54]. There are many differences between prophase I-arrest in oocytes and G_2-arrest in somatic cells. Plk1 is the principle target for Cdc14B-APC/Cdh1 in somatic cells [55], whereas CCNB1 is the primary target in mouse oocytes [56,57]. Plk1 is essential for G_2-checkpoint recovery [58], but dispensable for resumption of meiosis, even enhancing the process [53]. DSBs, prior to entry into mitosis, activate a G_2-checkpoint that delays initiation of mitosis in the presence of damaged DNA. ATM is also required for the DNA lesions in oocytes, including natural DSBs at early stages of prophase I during the fetal ovary development, and unnatural DSBs damage caused by exogenous factors, such as UV and drugs. Female mice with absence of ATM are completely infertile due to meiotic problems at prophase I as a response to damaged DSBs [59,60]. Meiotic DSBs are initiated by Spo11via a transesterification reaction. Oocytes from Spo11-deficient mice exhibit prophase I arrest and die during postnatal development [60]. These findings suggest that normal oocyte maturation and reproduction are closely connected with DSBs and DNA repair [61]. Both oocytes arrested at prophase I and somatic cells arrested at G_2 must resume the cell cycle at an appropriate time for future development.

Somatic cells arrested at G_2 depend on the action of Cdc14B and APC-Cdh1, both of which are also essential for the prophase I arrest of mouse oocytes [56,57]. Notably, Cdc14B is sequestered in the nucleus in normal somatic cells not suffering DNA damage, and leaves the nucleus following DNA damage [55]. In oocytes arrested in prophase I, Cdc14B does not localize in the nucleus, which suggests that it may be active [57]. After DNA damage, the resumption of somatic cell cycle requires Plk1 and Cdc25B [62], both of which are required for normal mitotic entry [62,63], whereas resumption of meiosis only

requires Cdc25B [62]. In addition, protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A) are also involved in resumption of meiosis and G_2-checkpoint recovery. Okadaic acid (OA), a PP1 and PP2A inhibitor, can induce resumption of meiosis of oocytes cultured with a PDE inhibitor [64–66]. Also, OA can override the G_2-checkpoint and induce premature mitotic entry in human cancer cells [67] and in HeLa cells independent of Cdk1 activity [68].

Oocyte maturation and embryonic development is sensitive to DNA damage. Substantial evidence now exists to concerning the DNA damage checkpoint by which the mammalian oocyte responds to DNA damage. However, unlike mitosis, cell cycle arrest is absent in M-phase or in the early embryos when DNA damage occurs. So the DNA damage in meiosis is very different. Why is the DNA damage checkpoint not activated in fully grown oocytes? What mechanisms are recruited in meiosis to repair DNA damage? Thus there are still many puzzles involved in the DNA damage and repair of mammalian oocytes.

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