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The importance of DNA repair for maintaining oocyte quality in response to anti-cancer treatments, environmental toxins and maternal ageing

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BACKGROUND: Within the ovary, oocytes are stored in long-lived structures called primordial follicles, each comprising a meiotically arrested oocyte, surrounded by somatic granulosa cells. It is essential that their genetic integrity is maintained throughout life to ensure that high quality oocytes are available for ovulation. Of all the possible types of DNA damage, DNA double-strand breaks (DSBs) are considered to be the most severe. Recent studies have shown that DNA DSBs can accumulate in oocytes in primordial follicles during reproductive ageing, and are readily induced by exogenous factors such as γ-irradiation, chemotherapy and environmental toxicants. DSBs can induce oocyte death or, alternatively, activate a program of DNA repair in order to restore genetic integrity and promote survival. The

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repair of DSBs has been intensively studied in the context of meiotic recombination, and in recent years more detail is becoming available regarding the repair capabilities of primordial follicle oocytes.

OBJECTIVE AND RATIONALE: This review discusses the induction and repair of DNA DSBs in primordial follicle oocytes.

SEARCH METHODS: PubMed (Medline) and Google Scholar searches were performed using the key words: primordial follicle oocyte, DNA repair, double-strand break, DNA damage, chemotherapy, radiotherapy, ageing, environmental toxicant. The literature was restricted to papers in the English language and limited to reports in animals and humans dated from 1964 until 2017. The references within these articles were also manually searched.

OUTCOMES: Recent experiments in animal models and humans have provided compelling evidence that primordial follicle oocytes can efficiently repair DNA DSBs arising from diverse origins, but this capacity may decline with increasing age.

WIDER IMPLICATIONS: Primordial follicle oocytes are vulnerable to DNA DSBs emanating from endogenous and exogenous sources. The ability to repair this damage is essential for female fertility. In the long term, augmenting DNA repair in primordial follicle oocytes has implications for the development of novel fertility preservation agents for female cancer patients and for the management of maternal ageing. However, further work is required to fully characterize the specific proteins involved and to develop strategies to bolster their activity.

Key words: oocyte / DNA repair / primordial follicle / radiotherapy / chemotherapy / ageing

Introduction

Within the ovary, oocytes are stored in long-lived structures called primordial follicles. Each primordial follicle comprises a non-growing, meiotically arrested oocyte, surrounded by a single layer of squamous granulosa cells. Primordial follicles are the storage unit of the female germline and following their activation to enter folliculogenesis, they give rise to fully grown, mature, developmentally competent oocytes. While some studies have raised the possibility that germline stem cells may exist in the adult ovary, their contribution to ovarian function and fertility is controversial (Johnson *et al.*, 2004; Zou *et al.*, 2009). Consequently, it remains widely accepted that new primordial follicles cannot be produced after birth even if the supply becomes prematurely depleted (Kerr *et al.*, 2012a). Thus, it is essential that the genetic integrity of primordial follicle oocytes is maintained to ensure that only those of the highest quality are available for ovulation, fertilization and subsequent embryonic development.

To protect genome integrity, cells have evolved a network of pathways called the DNA damage response, which is responsible for detecting DNA damage, activating checkpoints leading to cell cycle arrest and coordinating the repair process. While there are many types of DNA lesions (Sancar *et al.*, 2004), double-strand breaks (DSBs) are considered to be the most severe form. Under most circumstances, DNA DSBs are highly detrimental to chromosome integrity, cellular function and viability. However, DNA DSBs are deliberately induced and efficiently repaired in oocytes during meiotic recombination. Indeed, meiotic DNA DSB formation, processing and repair are strictly regulated, both spatially and temporally, to ensure that apoptosis is only initiated in the event that repair does not occur (Longhese *et al.*, 2009; Baudat *et al.*, 2013; Carroll and Marangos, 2013; Collins and Jones, 2016).

In addition to the requirement for oocytes to repair physiologically induced DSBs during meiotic recombination, the oocytes maintained in primordial follicles must also have the capacity to repair breaks in their DNA during their prolonged meiotic arrest. Recent evidence suggests that DNA DSBs accumulate in primordial follicle oocytes during maternal ageing, likely as a consequence of cellular metabolism and elevated oxidative stress (Titus *et al.*, 2013). Exposure to exogenous factors, such as γ-irradiation (Kerr *et al.*, 2012c), chemotherapy (Kujjo *et al.*, 2010; Titus *et al.*, 2013) and environmental toxicants (Susiarjo *et al.*, 2007; Hunt *et al.*, 2012; Zhang *et al.*, 2012) also results in DSB formation in primordial follicle oocytes. In each of these contexts, DNA DSBs readily induce primordial follicle oocyte death (Suh *et al.*, 2006; Kerr *et al.*, 2012c; Carroll and Marangos, 2013; Hutt *et al.*, 2013; Rinaldi *et al.*, 2017; Bolcun-Filas *et al.*, 2014). The accelerated depletion of the pool of primordial follicles that ensues can lead to premature ovarian failure (POF), infertility and early menopause, the latter of which is associated with increased risk of adverse health outcomes for women such as cardiovascular disease and osteoporosis (Green *et al.*, 2009).

Alternatively, recent studies in animals and humans have provided evidence that primordial follicle oocytes are capable of executing an efficient repair program when faced with DNA DSBs, in order to protect fertility and the genomic integrity of future generations (Oktem and Oktay, 2007; Soleimani *et al.*, 2011; Kerr *et al.*, 2012c). Indeed, transcript profiling indicates that oocytes at all stages of development have the capacity to repair damaged DNA (Wang *et al.*, 2004; Zeng *et al.*, 2004; Pan *et al.*, 2005; Yoon *et al.*, 2006; Cui *et al.*, 2007; Gasca *et al.*, 2007; Menezo *et al.*, 2007; Su *et al.*, 2007; Jaroudi *et al.*, 2009; Zheng *et al.*, 2005).

For the purpose of this review, we have focused on the oocyte, but it is clear that follicles, the functional unit of the ovary, require co-operation between the oocyte and granulosa cells, as well as the ovarian stroma and vasculature, all of which could be damaged by exogenous agents, or ageing. Indeed, the factors required for oocyte DNA DSB repair also play important roles in DNA stability and DNA damage repair in somatic cells, which are beyond the scope of the current review. In this review, the main pathways that cells employ for repairing DNA DSBs will be briefly described. The DNA repair response that occurs in the context of meiosis may inform us of the potential mechanisms of DNA repair induced in the response of oocytes to exogenous damage and will therefore briefly be discussed. We will primarily focus our review on the evidence for DNA DSB repair in primordial follicle oocytes in response to exogenous genotoxic stress or ageing, touching on recent novel findings in mature oocytes, where relevant.

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Methods

Systematic PubMed (Medline) and Google Scholar searches were performed using the key words: primordial follicle oocyte, DNA repair, double-strand break, DNA damage, chemotherapy, radiotherapy, ageing, environmental toxicant. The literature was restricted to papers in the English language and includes reports dated from 1964 until 2017. The references within these articles were also manually searched.

DNA double-strand breaks

DSBs are considered to be the most toxic of the DNA damage types, as they can cause genomic rearrangements and structural changes, such as deletions, translocations and fusions in the DNA, which typically result in loss of cellular function or viability and cancer susceptibility. Indeed, even a single break is sufficient to significantly impact the cell (Jackson and Bartek, 2009). The causes of DSBs are diverse and may be of endogenous or exogenous origin. For example, the endogenous production of reactive oxygen species (ROS) is a major cause of DSBs in somatic cells (Woodbine *et al.*, 2011) and although this has not directly been confirmed in oocytes, it is firmly established that ROS accumulates in human and mouse oocytes with advanced age (Tarin, 1995; Tatone *et al.*, 2008). During the course of normal oxidative respiration, mitochondria convert a small percentage of oxygen to superoxide, which is then converted to hydroxyl radicals. These hydroxyl radicals can react with DNA to cause single-strand breaks (SSBs) that can become DSBs if multiple lesions occur close together on antiparallel strands. Exogenous stressors that cause DSBs include ionizing radiation (IR), in the form of γ-rays and X-rays. Both are part of the electromagnetic spectrum and differ only in their source of origin. X-rays are produced by an x-ray generator and gamma radiation is released by radioactive atoms as the nucleus moves from a higher (unstable) to a lower energy state (stable). Gamma rays and X-rays produce photons (packets of electromagnetic radiation) that pass though the body creating clusters of free radicals along their path (usually from water molecules), which can damage a DNA duplex and cause DSBs. Notably, ionizing radiation is also present in the environment, largely emanating from the decay of radioactive metals within the earth (Lieber, 2010). Chemotherapy drugs, such as cisplatin, cyclophosphamide and doxorubicin, are also known to induce DSBs through a variety of different mechanisms. Of relevance to this review, a number of reports have demonstrated that DNA DSBs are generated in oocytes following exposure to ionizing radiation, chemotherapeutic drugs, and environmental toxicants and more recently, studies have reported the spontaneous induction of DSBs in oocytes as a consequence of endogenous oxidative stress and maternal ageing (Kerr *et al.*, 2012b, c; Titus *et al.*, 2013).

Homologous recombination and non-homologous end-joining

Two principal repair mechanisms are used to repair DSBs: homologous recombination (HR) and non-homologous end-joining (NHEJ) (San Filippo *et al.*, 2008; Lieber, 2010) (Fig. 1). The process of HR involves the generation of ssDNA that invades the undamaged sister-chromatid template, which allows for accurate DNA synthesis and repair. Key proteins involved in HR include, the MRE11–RAD50–NBS1 (MRN) complex, which detects and binds DSBs, recruits and activates the protein kinase ATM and then MRE11 (an endonuclease) processes the broken ends to produce single stranded DNA. RPA proteins bind to the single stranded DNA, as they do during DNA replication fork formation, however, in HR BRAC2 facilitates the replacement of RPA with RAD51 and RAD54. These Rad proteins then facilitate the invasion of the ssDNA into the undamaged sister chromatid and following the actions of polymerases, nucleases, helicases and other components, DNA ligation and substrate resolution occur (Fig. 1A).

NHEJ repair ultimately results in the two ends of the double-strand DNA break being ligated together after the removal of any single stranded DNA overhangs (Fig. 1B). The Ku70/80 proteins are involved in detecting the DSBs and activating and recruiting the protein kinase DNA-PKcs. DNA-PKcs then enlists end-processing enzymes, such as Artemis (Ahmad *et al.*, 2008), the MRN complex (Xie *et al.*, 2009) and ERCC1-XPF endonuclease (Ahmad *et al.*, 2008). Polymerases and XRCC4–ligase IV complex subsequently function to ensure the two ends of the DNA DSB are ligated together (Fig. 1B). Although NHEJ is error-prone, it can operate in any phase of the cell cycle. In contrast, HR is generally restricted to S and G2 because it uses the undamaged sister-chromatid template to mediate faithful repair.

The molecular mechanism that directs HR or NHEJ to repair a DSB remains an active area of investigation. In somatic cells, numerous studies indicate that binding of NHEJ factor Ku competes and partially blocks recruitment of HR factors, particularly in the S and G2 phases of the cell cycle (Van Dyck *et al.*, 1999; Pierce *et al.*, 2001; Sonoda *et al.*, 2006; Frank-Vaillant and Marcand, 2002). Other studies have identified PARP1 (Hochegger *et al.*, 2006) and DNA ligase IV-XRCC4 (Zhang *et al.*, 2007) as mediators of pathway activation by either blocking Ku binding and NHEJ or by suppressing the DNA end resection needed to initiate HR, respectively (Sonoda *et al.*, 2006). Cell cycle and time course analyses of checkpoint activation in human fibroblast cells demonstrated that HR and NHEJ factors were both recruited independently to the site of DNA damage, but that NHEJ recruitment preceded that of HR (Kim *et al.*, 2005). Interestingly, NHEJ factor Ku localization at the site of DNA damage was transient, whereas HR factor RAD51 persisted (Kim *et al.*, 2005). This may indicate that the two pathways may also cooperate/collaborate, with NHEJ serving as an immediate early response repair pathway (Kim *et al.*, 2005). Genetic analyses have revealed redundant and essential roles for both HR and NHEJ in different conditions and the usage of the two DSB repair pathways differs between different cell lines from the same species (Sonoda *et al.*, 2006). Combined, these studies indicate that depending on the cell and the timing and type of damage, DSB repair pathways may be differentially employed, and may compete for and collaborate on the same lesion (Sonoda *et al.*, 2006).

Components for both HR and NHEJ are expressed in mammalian oocytes, indicating that both pathways have the potential to be activated (Zheng *et al.*, 2005; Menezo *et al.*, 2007; Jaroudi *et al.*, 2009; Yukawa *et al.*, 2007; Kujjo *et al.*, 2010). Using mature *Xenopus laevis* oocytes and fertilized oocytes, it has been demonstrated that germinal vesicle stage oocytes express and utilize HR repair and are almost

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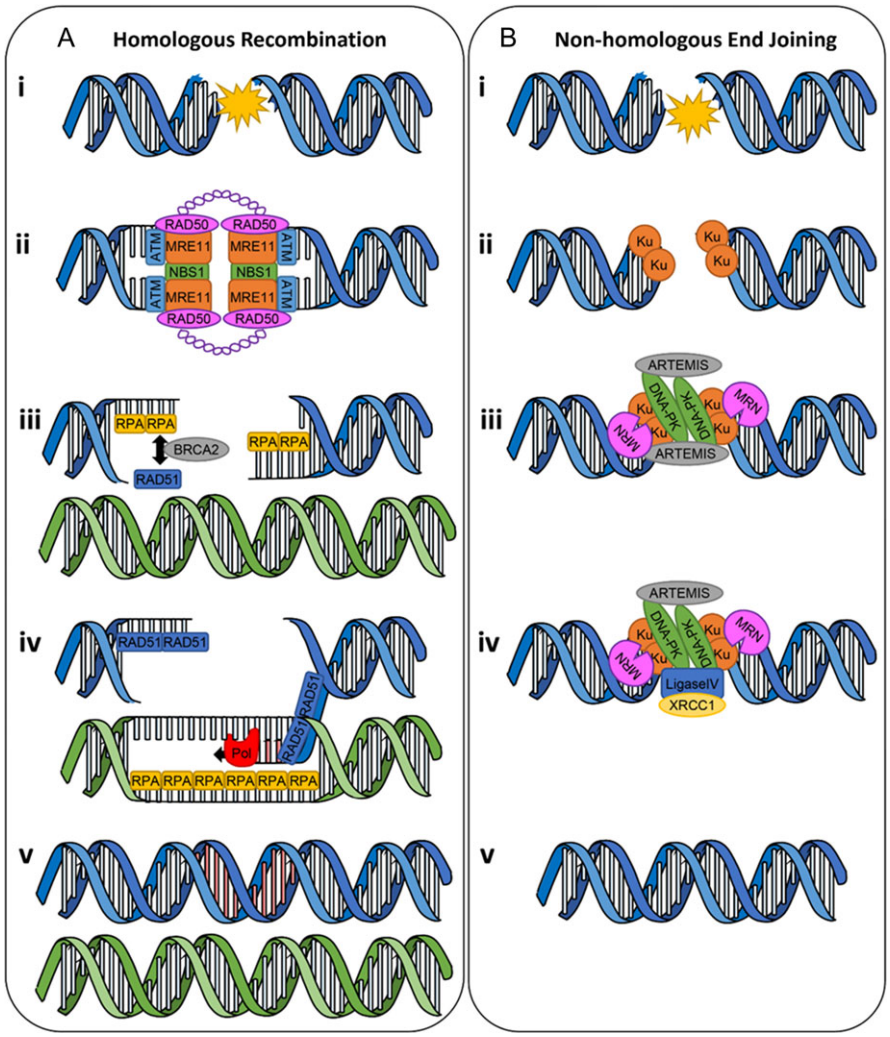

Figure 1 Brief overview of the repair of DSBs by homologous recombination and non-homologous end joining. (**A**) Repair of DSBs by HR (Ai) DNA DSB formation. (Aii) The MRN complex (which consists of MRE11, RAD50 and NBS1) localizes to the DNA, keeping the broken ends together and initiating end resection to form single strand overhangs. ATM also localizes to break sites and is responsible for phosphorylating numerous substrates. (Aiii) Single stranded DNA is then coated by RPA until a polymerase synthesizes a complementary sequence. With the help of BRCA2, RAD51 replaces RPA and forms a nucleoprotein filament on single stranded DNA. The undamaged sister chromatids is shown in green. (Aiv) RAD51 facilitates the invasion of the ssDNA into the undamaged sister chromatid. Polymerases (Pol) then use the undamaged sequence as a template to synthesize the missing part of the broken strand. (Av) When the broken strand is restored, the junction of double stranded DNAs is resolved to form separate chromatids. (**B**) Repair of DSBs by NHEJ. Repair of DSBs (Bi) by NHEJ involves the direct ligation of the DNA ends. (Bii) The first step of NHEJ repair involves the binding of the Ku protein (a heterodimer of Ku70 and Ku80) to the broken DNA ends, which protects them from degradation and prevents unwanted interactions with other DNA. Ku also provides structural support and facilitates alignment of the DNA. (Biii) Ku then recruits DNA-PKcs (the catalytic subunit of DNA-PK), which phosphorylates DNA ligase IV and XRCC4. If a single-stranded overhang is present, terminal end processing is required to generate blunt ends before ligation can occur. This can be achieved by employing a polymerase to fill-in the gaps. Alternatively, single-stranded overhangs can be trimmed off by nucleases, possibly by the Artemis/DNA-PKcs complex. MRN may also be involved in end processing. (Biv) Following terminal end processing, the XRCC4/DNA ligase IV ligation complex joins the two blunt DNA ends together (v) generating an intact double helix.

completely devoid of the NHEJ system (Goedecke *et al.*, 1992) until metaphase II when NHEJ becomes the predominant mechanism, particularly after fertilization (Goedecke *et al.*, 1992; Hagmann *et al.*, 1996). Thus, while both HR and NHEJ repair pathways may function in oocytes, activation of each pathway may vary depending on oocyte stage.

DNA DSB repair in meiotic oocytes

While DSBs are usually pathologic, or 'accidental', there are two notable situations in which this type of lesion is actively induced: (i) DSBs are essential for T and B-cell receptor rearrangements and (ii) DSBs

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are induced in oocytes during meiosis to enable recombination. Meiotic recombination is essential for the generation of genetic diversity within a population and must be tightly coordinated and managed in order to avoid the induction of cell death (Mahadevaiah *et al.*, 2001). The repair of DSBs during meiotic recombination is well-studied and many of the proteins first identified for their role in the repair of DSBs during meiosis have been later found to be involved in the repair of pathological DSBs in somatic cells.

Meiosis

Meiosis is a cell division program in which a single round of DNA replication is followed by two consecutive sequences of chromosome segregation, resulting in the production of haploid gametes from diploid germ cells. A key feature of the first meiotic prophase is the exchange of genetic material between maternal and paternal chromosomes, referred to as recombination (Fig. 2). Recombination is crucial for creating new allele combinations and increasing the genetic diversity in the population. Furthermore, crossovers (the points where recombination occurs) are essential for ensuring that each homologue aligns on the metaphase plate and segregates properly (Hunter and Kleckner, 2001; Wang *et al.*, 2017). This is because crossovers provide the tether (visible as chiasmata) that keeps the homologous chromosomes together during the prolonged diplotene arrest. Indeed, it is essential that at least one crossover forms prior to diplotene arrest to ensure balanced chromosome segregation during the first metaphase (Hunter, 2015). A correlation between a reduced number of recombination events and increased aneuploidy has been demonstrated in mice, yeast, Drosophila and humans (Golin and Esposito, 1981; Hawley, 2003; Jeffreys *et al.*, 2003; Ottolini *et al.*, 2015). Therefore, generating and maintaining chiasmata is essential for preventing the transmission of aneuploidies to the next generation.

The induction and repair of meiotic DSBs

Similar to the situation for pathological DSBs described in the following sections of this review, failure to repair the DSBs acquired during meiotic recombination can trigger apoptosis and lead to infertility, or lead to defective embryo development and miscarriage due to gamete aneuploidies (MacLennan *et al.*, 2015) (Table I). Indeed, it is essential that meiotic recombination checkpoints detect DNA damage and activate DNA repair or apoptosis to prevent the transmission of genetic aberrations to offspring. Persistent unrepaired DNA damage evident in DMC1 and ATM deficient mice causes complete oocyte elimination prior to follicle formation (Di Giacomo *et al.*, 2005), while the primordial follicle reserve is prematurely depleted in female mice deficient in MRE11 as a consequence of failed homologous chromosome synapsis and DSB repair during meiotic prophase (Inagaki *et al.*, 2016). MCM8 is another protein involved in the recruitment of RPA and RAD51 during the repair of meiotic DSBs. *Mcm8*$^{-/-}$ mice are sterile resulting from premature depletion of the ovarian reserve and a failure in follicle development (Lutzmann *et al.*, 2012). This phenotype is likely due to a failure in the repair of meiotic DSBs. Together, these findings indicate some of the DNA damage checkpoints that exist during meiosis to prevent oocytes with DNA damage from forming follicles. Contrary to this, some evidence suggests that the checkpoints that regulate female meiosis are not as robust as in male meiosis, or in somatic cells, which is evident from the high rates of aneuploidy seen in oocytes compared with male gametes (Hunt and Hassold, 2002).

Interestingly, the repair of meiotic DSBs to crossovers in oocytes was recently shown to require a heterodimeric complex of the mismatch repair (MMR) proteins MLH1 and MLH3 (Rogacheva *et al.*, 2014). This study highlights the emerging concept that DNA repair pathways that have evolved to handle different types of DNA damage are in fact interlinked and work cooperatively to protect genomic integrity (Zhang *et al.*, 2009). While the bulk of currently available evidence focusses on traditional modes of DSB repair (i.e. NHEJ and HR), it is becoming apparent that future studies should consider the involvement of additional repair pathways, such as nucleotide excision repair (NER), MMR and cell cycle regulation, in the repair of DSBs in oocytes, whether it be during meiosis or during diplotene arrest.

DNA DSB repair in primordial follicle oocytes

After meiotic recombination is complete, oocytes undergo meiotic arrest and then assemble into primordial follicles at birth (Fig. 2). Primordial follicles are comprised of an immature oocyte surrounded by a single layer of squamous granulosa cells. The enclosed oocytes are among the most long-lived cells in the body and remain arrested part way through the first meiotic prophase at diplotene for several months in mice, or up to 50 years in humans, before being activated to resume meiosis and mature (Fig. 2). Given that the initial pool of primordial follicles is formed during fetal or perinatal life in humans and mice, respectively, and their development represents the entire supply of oocytes available to the female (Pepling and Spradling, 2001), it is essential that their genomic integrity is maintained during their long life to ensure future female fertility. Notably, primordial follicle oocytes appear to be much less tolerant of DNA damage than ovarian somatic cells or oocytes from growing follicles (Hanoux *et al.*, 2007; Kerr *et al.*, 2012a). Their increased propensity for apoptosis may be due to reduced DNA repair capacity and/or the evolution of highly sensitive apoptotic responses. Recent work described below provides compelling evidence for the latter, and suggests that primordial follicle oocytes are capable of highly efficient DNA repair.

Evidence for efficient repair of DSBs in primordial follicle oocytes

The DNA damage checkpoint which detects and eliminates oocytes with unrepaired meiotic DSBs acts around the time of meiotic arrest, although it presumably persists, given that resting primordial follicles are highly sensitive to exogenous damage. However, the precise underlying molecular mechanisms to protect genomic DNA after embryonic HR and DSB repair have not been intensively studied. If DSBs are not repaired, it is critical that those oocytes with DNA damage are eliminated through apoptosis to protect against germline mutations. Members of the p53 family, including p53, p63 and p73, have all been identified as key regulators of primordial follicle oocyte apoptosis following DNA damage (Suh *et al.*, 2006; Livera *et al.*, 2008;

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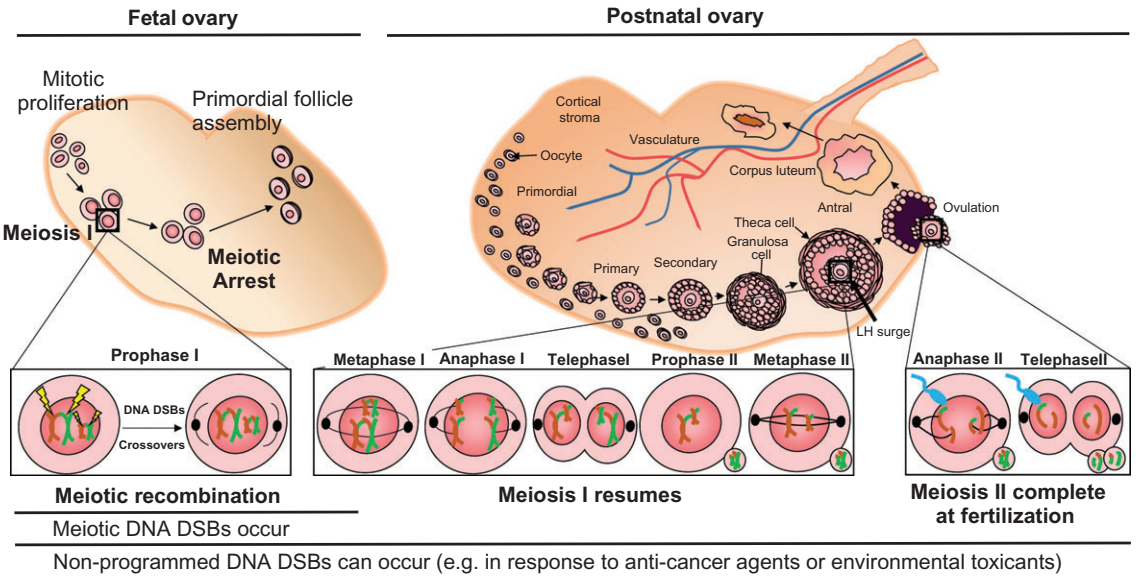

Figure 2 Timeline of human ovarian development, meiosis and folliculogenesis. During fetal ovarian development, physiological and programmed induction and repair of DNA DSBs occurs in precursor oocytes during prophase I, to facilitate homologous recombination. These oocytes are then arrested at diplotene of meiotic prophase I. By birth, all oocytes are assembled into primordial follicles, the functional unit of the ovary which comprise the oocyte, surrounded by somatic granulosa cells. A finite supply of primordial follicle oocytes form the ovarian reserve. These primordial follicles may be activated to undergo folliculogenesis, a process in which the oocytes and follicles develop through primary, secondary, and antral stages, before one dominant follicle is ovulated. Upon the LH surge (beginning at puberty), meiosis resumes in the dominant follicle just prior to ovulation, before being arrested at metaphase II. Meiosis is only completed upon fertilization of the ovulated oocyte. The ovulated oocyte leaves behind remnants to form the corpus luteum, required to hormonally support a potential conceptus. The remaining growing follicles undergo atresia (not shown). The mechanisms of repair of meiotic DSBs in fetal oocytes have been well-characterized and may inform us of the repair pathways employed by oocytes to repair subsequent non-programmed DNA damage sustained throughout life. In addition to meiotic DSBs, oocyte DNA damage may be sustained at any time throughout life and may accumulate in any follicle stage. Oocyte DNA DSBs can occur in response to exogenous factors, such as anti-cancer treatments and environmental toxins, or endogenous production of reactive oxygen species, which are enhanced with age.

Kim *et al.*, 2013; Bolcun-Filas *et al.*, 2014; Adhikari *et al.*, 2016). In particular, the TAp63 isoform is expressed at high levels in primordial follicle oocytes and is an essential mediator of DNA damage-induced oocyte death through transcriptional activation of the pro-apoptotic Bcl-2 family members PUMA and NOXA (Kerr *et al.*, 2012c). Interestingly, TAp63 expression is down regulated as oocytes leave the resting pool (Kim and Suh, 2014), which may partially explain why growing oocytes are more resistant to DNA damage-induced apoptosis than primordial follicle oocytes. When oocyte apoptosis was prevented in *Puma*$^{-/-}$ and *Puma*$^{-/-}$ *Noxa*$^{-/-}$ mice, γ-H2AX DSB foci induced by γ-irradiation, were completely resolved within 5 days. Levels of phosphorylated ATM, which were elevated within 3 h of γ-irradiation treatment, were also restored and primordial follicle apoptosis was limited after 5 days. Interestingly, healthy offspring were produced, demonstrating that the DNA repair was sufficient to maintain genomic stability in primordial follicle oocytes (Kerr *et al.*, 2012c). Similarly, elimination of checkpoint kinase 2 (Chk2) in mice prevents the loss of primordial follicles following irradiation and the surviving primordial follicles give rise to healthy offspring (Bolcun-Filas *et al.*, 2014). These studies establish the molecular mechanisms of DNA-damage induced apoptosis, namely regulation by Chk2 (Bolcun-Filas *et al.*, 2014), TAp63 and PUMA (Kerr *et al.*, 2012c), and strongly suggests that oocytes are capable of highly efficient repair, but specific repair pathways were not investigated.

Of the two mechanisms responsible for repairing DSBs, HR is likely to be the pathway of choice for primordial follicle oocytes because sister chromatids are present, which can serve as a template for accurate, error-free repair. Kujjo *et al.* (2010) were the first to localize a HR DNA repair factor, namely RAD51, within primordial follicle oocytes in mice. Breast cancer type 1 and 2 susceptibility genes (BRCA1 and BRCA2) belong to the family of ATM-mediated HR DNA DSB repair factors, vital for protecting chromosomal integrity (Rosen, 2013). Subsequently, Titus *et al.* (2013) demonstrated a crucial functional role for BRCA1 in DSB repair during both meiosis and in primordial follicle oocytes post-natally. *BRCA1* and *BRCA2* homozygous deletion in mice result in embryonic lethality (Hakem *et al.*, 1998). However, heterozygous deletion of *BRCA1*, but not *BRCA2* in mice results in impaired reproductive capacity, characterized by low primordial follicle counts and an age-related increase in the accumulation of DNA DSBs in surviving follicles relative to wild-type mice. Notably, DNA DSBs were absent in the granulosa cells of BRCA1-null mice (Titus *et al.*, 2013). Additionally, serum AMH concentrations, often used to estimate primordial follicle reserve and predict menopausal age (Pankhurst, 2017), were prospectively compared in

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Table I DSB repair deficient mouse models.

Repair pathway	Protein	Role	Mouse phenotype	Ovarian phenotype	Role in meiosis	Role in post-natal repair	Reference
NHEJ	KU70	Forms heterodimer with KU80 to act as a regulatory component of DNA-PK	Defective B, not T cell maturation, lymphoma development, sensitivity to severe combined immunodeficiency disease and IR	Unknown	Unknown	Unknown	Li *et al.* (1998)
	KU80	KU80 protein is only stable when co-expressed with KU70	Early ageing independent of tumor formation	Unknown	Unknown	Unknown	Difilippantonio *et al.* (2000), Li *et al.* (2007)
	DNA-PKcs	Serine/threonine kinase activated by CHK2 and ATM to repair DSBs. Inactivated once repair is complete	Sensitivity to severe combined immunodeficiency disease and IR	Unknown	Unknown	Unknown	Kirchgessner *et al.* (1995)
	DNA ligase IV	A nuclear enzyme that joins breaks in the phosphor-diester backbone of DNA	Lymphopoiesis is blocked and V(D)J joining does not occur. Fibroblasts show sensitivity to IR. Mutation leads to late embryonic lethality	Unknown	Unknown	Unknown	Frank *et al.* (1998)
	XRCC4	Forms a complex with DNA ligase IV that re-joins two DNA ends in the last step of V(D)J recombination and NHEJ to repair DSBs	Late embryonic lethality, defective lymphogenesis and neurogenesis manifested by extensive apoptotic death of newly generated post-mitotic neuronal cells	Unknown	Unknown	Unknown	Gao *et al.* (1998)
HR	RPA	ssDNA overhangs are bound by the heterotrimeric replication protein A (RPA) complex with high affinity, a prerequisite for the loading of the strand-exchange proteins; RAD51 and DMC1	Early embryonic lethality exhibited in homozygous mutants. Defects in DNA DSB repair, precipitated chromosomal breaks and aneuploidy evident in heterozygous mutant mouse embryonic fibroblasts	Unknown	Unknown	Unknown	Wang *et al.* (2005)
	MCMDC2	Promotes formation and/or stabilization of strand invasion intermediates that permit alignment of homologues	Both male and female mice deficient in MCMDC2 are infertile, although mice exhibit no obvious somatic cell defects	Oogenesis is blocked, with a complete loss of oocytes soon after birth	✓	✗	Finsterbusch *et al.* (2016)
	MCM8	Facilitates DNA elongation	Both male and female mice are sterile. Embryonic fibroblasts show growth defects and chromosomal damage and cannot overcome transient inhibition of replication fork progression	Females are infertile and have only arrested primary follicles	✓	✗	Lutzmann *et al.* (2012)
	DMC1	Mediates the search for homologous sequences	Males and females are sterile with arrest of gametogenesis in the first meiotic prophase	Oocytes eliminated before follicle formation, suggesting a DNA damage-dependent response	✓	Unknown	Di Giacomo *et al.* (2005), Qin *et al.* (2007)
	BRCA1	Functions synergistically with RAD51 to perform a key role in HR during DNA repair and may also regulate mitotic progression in concert with CHK2	Homozygous mutation results in embryonic lethality and developing embryos show signs of a cellular proliferation defect associated with activation of the p53 pathway	Heterozygous loss of BRCA1 is associated with reduced ovarian reserve, POF, accumulation of DSBs in oocytes and ovarian ageing	✓	✓	Hakem *et al.* (1998), Titus *et al.* (2013)

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Table I *Continued*

Repair pathway	Protein	Role	Mouse phenotype	Ovarian phenotype	Role in meiosis	Role in post-natal repair	Reference
	BRCA2	Binds ssDNA and interacts with the recombinase RAD51 to stimulate strand invasion, a vital step of HR	Mutation results in embryonic lethality and developing embryos show signs of a cellular proliferation defect associated with activation of the p53 pathway	Mice are fertile and there are no overt meiotic or fertility defects.	✗	✗	Hakem *et al.* (1998), Titus *et al.* (2013)
	RAD51	Strand exchange protein	Oogenesis defects	Oocytes progress to metaphase I after superovulation but display precocious separation of sister chromatids, aneuploidy, and broken chromosomes at metaphase II	✓	✓	Kuznetsov *et al.* (2007)
Both	MRE11	Forms a complex with the RAD50 required for NHEJ and possesses increased ssDNA endonuclease and 3′ to 5′ exonuclease activities. In conjunction with a DNA ligase, MRE11 promotes joining of non-complementary ends using short homologies near the ends of the DNA fragments	Mitotic cells exhibit cell cycle checkpoint defects, genome instability, and hypersensitivity to IR. Males exhibit increased meiotic recombination	Females with a mutation in MRE11 (ATLD1/ATLD1) exhibit premature oocyte elimination attributable to defects in homologous chromosome pairing and double-strand break repair during meiotic prophase	✓	Unknown	Inagaki *et al.* (2016).
	RAD50	Component of the MRE11 complex, essential for cell proliferation.	Required for viability of proliferating bone marrow, but not hepatocytes or cerebral Purkinje cells	Unknown	Unknown	Unknown	Adelman *et al.* (2009)
	NBS1	Component of MRE11 complex. Activated by ATM and required to sense DNA DSBs and activate CHK2	Mice are viable, growth retarded, hypersensitive to IR and display normal spermatogenesis	Ovaries are degenerated with lack of follicles or oocytes—oogenesis is abolished.	✓	✗	Kang *et al.* (2002)

women with and without BRCA1 mutations. The data revealed that women with BRCA1 mutations exhibited significantly reduced serum AMH levels, despite a similar mean age between groups (Titus *et al.*, 2013). This indicates that women carrying BRCA1 mutations have diminished ovarian reserve and is supported by reports of impaired response to ovarian stimulation (Oktay *et al.*, 2010) and premature menopause (Rzepka-Gorska *et al.*, 2006) in women with BRCA1 mutations. Clearly further investigations are required to uncover the key players that mediate primordial follicle oocyte DNA repair in response to exogenous DNA damage and ageing.

Ionizing radiation

Radiotherapy is well-known to cause ovarian damage, permanent infertility and early menopause (Baker, 1971; Wallace *et al.*, 2003). In humans, the effective sterilizing dose (ESD) of fractionated radiotherapy (causes POF immediately after treatment in 97.5% of patients) is predicted to decrease with increasing patient age. This is due in part to the natural decline of oocyte number with age, thus at birth the predicted ESD is 20.3 Gy; at 10 years, 18.4 Gy; at 20 years, 16.5 Gy; and at 30 years, 14.3 Gy (Wallace *et al.*, 2005). Doses below these levels are expected to still cause DNA damage, but do not result in infertility, thus have the potential to cause genetic disorders in offspring. Numerous epidemiological studies have investigated the clinical or sub-clinical pregnancy outcomes of people exposed to medical, occupational or accidental radiation exposure. Interestingly, these studies provide little evidence of adverse pregnancy outcomes after exposure (Adriaens *et al.*, 2009). This may indicate that sufficient DNA repair occurs in oocytes after radiation exposure to prevent inheritance of genetic errors or that damaged oocytes were efficiently eliminated from the ovary via apoptosis.

More precise analysis of the effects of radiation can be performed on animal models as accurate counts of follicles can be performed and DNA damage measured. A low dose (0.1 Gy) of IR is sufficient to induce 90% loss of oocytes and infertility in adult wild-type female mice (Morita *et al.*, 2000). Interestingly sensitivity to radiation is dependent on the age of the mouse and the stage of the oocyte (Peters and Levy, 1964; Selby *et al.*, 1991), with oocytes surviving

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doses of IR at post-natal day (PN)0 that result in almost complete oocyte destruction 7–21 days after birth, when oocytes were diplotene arrested (Peters and Levy, 1964; Kim and Suh, 2014). This difference in survivability was thought to be due to differences in chromosome arrangement and meiotic stage of the oocytes (Peters and Levy, 1964). An alternative hypothesis suggests that mechanisms blocking TAp63 activation in new born oocytes, possibly carried over to prevent apoptosis in response to meiotic DSB at E18.5, may explain early primordial resistance to IR (Kim and Suh, 2014). TAp63 is highly expressed in primordial follicles and phosphorylation and activation of TAp63 has been correlated with oocyte apoptosis in response to DNA DSB (Suh *et al.*, 2006). Interestingly, at birth phosphorylation and activation of TAp63 is extremely low in primordial oocytes, even after IR, which indicates the presence of mechanisms that may block TAp63 activation during meiotic DNA recombination (Kim and Suh, 2014). Significantly, blocking ATM in older mice completely inhibited TAp63 phosphorylation and significantly reduced oocyte loss, whereas blocking DNA-PKcs did not (Kim and Suh, 2014). Moreover, blocking calyculin A (CA)-sensitive Ser/Thr phosphatase, which functions to downregulate TAp63 phosphorylation, in new born mice resulted in an increase in TAp63 phosphorylation (Kim and Suh, 2014). Therefore, activation of TAp63 in primordial oocytes is likely to be regulated by calyculin A (CA)-sensitive Ser/Thr phosphatase and ATM in response to meiotic or IR-induced DNA DSBs. Similarly, the resistance of growing oocytes to radiation is assumed to be due to the reduction in TAp63 expression and the accumulation of repair factors after oocyte activation.

Chemotherapy

Different classes of chemotherapy drugs have been demonstrated to exert different degrees of off-target damage to the ovary and have been reviewed extensively (Morgan *et al.*, 2012; Bedoschi *et al.*, 2016) (Fig. 3). Briefly, chemotherapeutic agents can be broadly divided into five classes including alkylating agents, anti-tumor antibiotics, platinum based drugs, antimetabolites and taxanes. These can further be classified based on their ability to be cell cycle or non-cell cycle specific. Cell cycle specific chemotherapeutics exert their primary action only during specific phases of the cell cycle. For example, the plant alkaloid, vinblastine has been shown to bind to tubulin in mouse secondary oocytes, causing microtubule crystallization and metaphase I arrest (Russo and Pacchierotti, 1988). In contrast, non-cell cycle specific chemotherapeutics, including platinum based agents such as cisplatin, as well as alkylating agents like cyclophosphamide can target cells at any stage of the cell cycle, including G_0, and can thus damage both quiescent and proliferating cells (Torino *et al.*, 2014). These compounds are of particular relevance for this review because they have the capacity to damage the DNA of diplotene arrested oocytes. Additionally, these agents are reported to exert the greatest immediate damage to the ovary in clinical (Meirow, 2000) and biological studies (Yucebilgin *et al.*, 2004; Desmeules and Devine, 2006; Oktem and Oktay, 2007; Nozaki *et al.*, 2009; Petrillo *et al.*, 2011; Yuksel *et al.*, 2015), leading to depletion of the ovarian reserve, infertility and premature menopause.

Although the ovotoxicity of cell cycle specific chemotherapeutics have been less well characterized, increasing evidence suggests that the anthracycline anti-tumor antibiotic, doxorubicin, can also considerably impact the ovarian reserve (Ben-Aharon *et al.*, 2010; Soleimani *et al.*, 2011; Roti Roti *et al.*, 2012; Xiao *et al.*, 2017). Thus, the assumption that chemotherapeutics predominantly target rapidly dividing cell types remains to be unequivocally proven in the ovary. Together, the body of evidence described below suggests that alkylating agents like cyclophosphamide, platinum-based drugs such as cisplatin and also those in the topoisomerase inhibitor category, such as doxorubicin, cause direct damage to primordial follicle oocytes (Fig. 3). In the surviving follicles, it is likely that DNA repair pathways are activated and to date, this hypothesis has been experimentally supported with regard to RAD51 activation in response to doxorubicin treatment (Kujjo *et al.*, 2010) (Fig. 3), as discussed below.

Furthermore, in mice, loss of protein phosphatase 6 (*PP6c*) (a factor recruited to DSB sites by DNA-PK and required for NHEJ-mediated repair of DSBs) caused persistent γH2AX foci and defective DNA repair in primordial follicle oocytes (Hu *et al.*, 2016). These mice are also more sensitive to DNA damage induced in response to treatment with the anti-tumor antibiotic, zeocin. This was evident from increased γH2AX foci, as well as accelerated oocyte depletion compared with wild-type mice. Together, these data emphasize that not all women are likely to respond uniformly to various anti-cancer treatments, but rather, underlying defects in DNA repair capacity could dictate the individual response.

Cisplatin acts by promoting the formation of inter-strand DNA cross-links and intra-strand DNA adducts, which disrupt transcription and cell division. Cisplatin has been shown to induce DSBs and activate the DNA damage response in mouse primordial follicle oocytes, demonstrated by γH2AX staining within 8 h of treatment (20 μM) *in vitro* in PN5 mouse ovarian tissue (Gonfloni *et al.*, 2009) and within 12 h of treatment (20 μM) in cultured PN8 mouse ovaries (Rossi *et al.*, 2017). Cleaved caspase-3 positive primordial follicle oocytes can be detected within 24 h (Gonfloni *et al.*, 2009) and TUNEL by 36 h (Rossi *et al.*, 2017), indicating that cisplatin treatment causes oocyte DNA damage and apoptosis. This was substantiated by reduced primordial follicle counts in the PN5 cultured tissues (Gonfloni *et al.*, 2009). In agreement, primordial, but not secondary, follicles were reduced *in vivo* in mice at PN9 following a single dose of 5 mg/kg cisplatin. In the same report, breeding studies demonstrated reduced fertile lifespan after only a single dose of cisplatin (Gonfloni *et al.*, 2009). These findings were later supported by Kerr *et al.* (2012b), who also showed that cisplatin mediated primordial follicle apoptosis in a direct manner and led to reduced fertile lifespan in multiple strains of mice. Importantly, while *in-vivo* exposure to cisplatin appeared to damage all primordial follicle oocytes in these studies, those that survive give rise to apparently normal offspring, providing evidence that cisplatin-induced DNA lesions can be repaired (Gonfloni *et al.*, 2009; Kerr *et al.*, 2012b). This is consistent with studies of fertility in female cancer survivors showing that maternal cisplatin treatment is not associated with adverse pregnancy or health problems in their children (Chow *et al.*, 2016).

Cyclophosphamide is a widely used chemotherapeutic and immunesuppressive alkylating agent with potent ovarian toxic effects, attributed to its ability to cause DNA cross-links and target metabolically active cells within the ovary, such as granulosa cells. While studies investigating the impact of cyclophosphamide on human primordial follicles are limited, there is strong evidence for the induction of DNA damage, including DSBs, and oocyte apoptosis (Oktem and Oktay, 2007). For example, when 24-week fetal human ovarian tissue comprising

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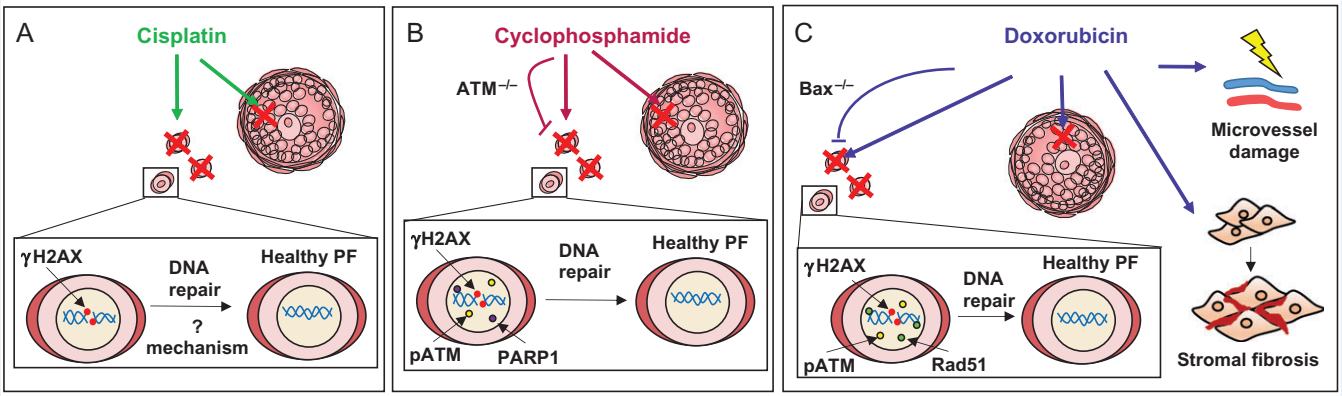

Figure 3 Cellular targets in the ovary and mechanisms of DNA damage and repair in response to chemotherapy. Different classes of chemotherapeutics exert different effects on the ovary. Each drug class may cause different a different type of DNA damage response, and thus a different DNA repair response. The cellular targets and mechanisms of DNA damage and repair in the ovary in response to cisplatin, cyclophosphamide and doxorubicin have been most well-characterized and are depicted. (**A**) Cisplatin induces nuclear γH2AX and apoptosis (red crosses) in primordial follicles (PF) and granulosa cells of growing follicles. The mechanisms of DNA repair in surviving PF remain unknown in response to cisplatin. (**B**) Cyclophosphamide induces apoptosis (red crosses) in PF and granulosa cells of growing follicles. PF apoptosis is induced in an ATM-dependant manner and nuclear γH2AX, pATM and PARP1 are thought to mediate PF DNA repair. (**C**) Doxorubicin induces apoptosis (red crosses) in PF and granulosa cells of growing follicles. PF apoptosis is induced in a BAX-dependant manner and nuclear γH2AX and pATM localize to PF, while RAD51 has been experimentally proven to facilitate oocyte DNA repair. Doxorubicin is also known to damage vessels and cause stromal fibrosis which may indirectly damage primordial follicles.

meiotically arrested primordial follicle oocytes was xenografted into mice, a single dose of cyclophosphamide resulted in significant primordial follicle death by apoptosis (Oktem and Oktay, 2007). Follicle loss was evident as early as 12 h post-cyclophosphamide treatment, with a 12 and 53% reduction in primordial follicle counts after 12 and 24 h, respectively, and TUNEL-positive oocytes were observed.

Direct effects of cyclophosphamide on the quiescent primordial follicle pool have also been demonstrated in mouse models. Cyclophosphamide was shown to reduce the primordial follicle reserve by approximately half in mice 7 days following a single dose of either 50 or 75 mg/kg (Meirow *et al.*, 2004). Likewise, exposure of mouse ovarian tissue to cyclophosphamide metabolites (e.g. phosphoramide mustard) induced γH2AX-positive DNA DSBs predominantly in oocytes, not granulosa cells, within 18 h and depleted primordial follicle numbers within 24 h (Petrillo *et al.*, 2011). Interestingly, blockade of the kinases that phosphorylate H2AX significantly increased phosphoramide mustard-mediated primordial follicle loss, suggesting that the alternative (i.e. increasing DNA repair signaling) could exert ovoprotective effects (Petrillo *et al.*, 2011). DNA damage response factors, including ATM and PARP1 were activated within 12 h of phosphoramide mustard treatment in PN4 rat ovaries, while treatment with the ATM inhibitor, KU55933, prevented phosphoramide mustard-mediated follicle loss at all stages, highlighting an ATM-dependant DNA damage mechanism (Ganesan and Keating, 2016). Some evidence suggests that DNA repair occurs in primordial follicle oocytes in mice treated with cyclophosphamide. The rate of viable conceptuses was dramatically reduced one week following a single dose of cyclophosphamide (75 mg/kg), indicating immediate depletion of mature follicles (Meirow *et al.*, 2001). Ten-fold rates of fetal malformations were recorded in surviving offspring from mating females 1–3 weeks post-cyclophosphamide treatment, implying that cyclophosphamide induced DNA damage in these oocytes (Meirow *et al.*, 2001). Interestingly, reduced malformation rates were observed from 4 weeks onwards and by 12 weeks post-cyclophosphamide treatment, this rate dropped to 3%, suggesting that DNA repair occurred in surviving primordial follicles and was sufficient to give rise to healthy offspring (Meirow *et al.*, 2001).

Doxorubicin is an anthracycline anti-tumor antibiotic and one of the most commonly used cell cycle specific anti-cancer chemotherapeutics. Its mechanism of action remains inconclusive, although it has been reported to inhibit DNA resealing by forming the doxorubicin–DNA–topoisomerase II complex and activation of p53 mediated apoptosis in cancer cells (Minotti *et al.*, 2004). In mice, doxorubicin induces γH2AX staining in the nucleus of primordial follicle oocytes and dramatically reduces primordial follicle numbers, with no change in growing follicle numbers (Jurisicova *et al.*, 2006; Roti Roti *et al.*, 2012), suggesting that doxorubicin rapidly induces direct primordial follicle oocyte DNA damage and apoptosis. Interestingly, Kujjo *et al.* (2010) assessed DNA repair in mice and found that resistance of oocytes to doxorubicin-mediated apoptosis was strain dependent and specifically correlated with the presence of RAD51. Furthermore, *in-vitro* microinjection of a RAD51 blocking antibody sensitized mature MII oocytes to doxorubicin-mediated apoptosis in ICR mice, while microinjection of recombinant RAD51 increased survival (Kujjo *et al.*, 2010). When apoptosis was prevented in BAX deficient-mice, doxorubicin-mediated DNA damage was resolved (Kujjo *et al.*, 2010), confirming that the degree of efficiency of DNA DSB repair is an important factor influencing the chemo-sensitivity of oocytes.

Similar results have been reported for human primordial follicles. Soleimani *et al.* (2011) treated SCID mice xenografted with human ovarian cortical tissue with a single dose of 10 or 100 mg/kg

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Table II DNA repair factor mutations associated with premature ovarian ageing in women.

Gene	Online Mendelian inheritance in man (OMIM) database number	Reference
HFM1	615 684	Wang *et al.* (2014)
STAG3	608 489	Caburet *et al.* (2014)
MCM8	608 187	AlAsiri *et al.* (2015)
MCM9	610 098	Wood-Trageser *et al.* (2014)
CSB-PGBD3	609 413	Qin *et al.* (2015)
MSH5	617 442	Guo *et al.* (2017)

doxorubicin, then recovered the tissues after 24 h. Doxorubicin treatment induced γH2AX-positive DNA DSBs and apoptosis in primordial follicle oocytes, but no staining in the surrounding granulosa cells. These data indicate that doxorubicin chemotherapy directly damages the DNA of human oocytes, resulting in primordial follicle depletion. In the same study, GV oocytes and small pre-antral follicles were collected from the ovaries of doxorubicin or vehicle treated mice and examined for γH2AX lesions or AC-3 positive staining. Of the γH2AX-positive oocytes, ~66% were apoptotic. The DNA damage sustained in the doxorubicin-treated oocytes also triggered the activation and nuclear translocation of the DNA repair mediator, ATM. The remaining proportion of oocytes and granulosa cells were found to be non-apoptotic, strongly suggesting that DNA repair enabled their survival (Soleimani *et al.*, 2011).

Overall, evidence for oocyte DNA repair in response to various chemotherapeutics discussed above is primarily based on findings in mouse models. The relevance of the chemotherapy dose used in such studies may be a limiting factor when extrapolating findings from animal models to humans. Therefore, caution should be used when interpreting the clinical relevance of such findings. Regardless, many female cancer patients remain fertile after cisplatin chemotherapy treatment (Chow *et al.*, 2016), supporting findings in mice and strongly suggesting that human primordial follicle oocytes are capable of DNA repair in the face of genotoxic exposure. Indeed, one recent study highlighted a maintenance of primordial follicle density in human ovarian biopsies in female Hodgkin lymphoma patients treated with a commonly administered chemotherapy regime comprising adriamycin, bleomycin, vinblastine and dacarbazine (McLaughlin *et al.*, 2017). Although DNA damage or repair factors were not investigated in this study, this finding also suggests that DNA repair occurs in human primordial follicles in response to this chemotherapy regimen.

Reproductive ageing

One of the major challenges in women's reproductive health is the age-related decline in reproductive capacity. Reproductive ageing is characterized by the progressive decline in oocyte number and quality, leading to loss of fertility and ovarian function, cycle irregularity and eventually menopause (Templeton *et al.*, 1996; Baird *et al.*, 2005; Tatone *et al.*, 2008; Balasch and Gratacos, 2012). To date, most efforts to understand female reproductive ageing have focussed on the mechanisms of aneuploidy and have primarily analysed growing/fully mature oocytes (Angell, 1994; Hassold *et al.*, 2007; Fragouli *et al.*, 2011; Handyside, 2012). By contrast, the earliest stages of oocyte development and the underlying mechanisms that bring about the overall reduction in oocyte quality, or contribute to depletion of the ovarian reserve, have not been studied in detail. Importantly, however, oocytes exist as primordial follicles for most of their lifetime (up to fifty years in women) and problems arising during their prolonged arrest could contribute to the age effect.

It is now well established that somatic cell ageing is associated with an accumulation of DNA DSBs and the inability to repair this damage appropriately (Lieber and Karanjawala, 2004; Gorbunova *et al.*, 2007). With this concept in mind, it has been hypothesized that DNA damage and loss of DNA repair capacity contributes to diminished oocyte number and quality during reproductive ageing. In support of this hypothesis, DNA DSBs have been shown to accumulate with age in the oocytes of mouse primordial follicles and human germinal vesicle stage oocytes, with concomitant downregulation of the key DNA repair genes BRCA1, MRE11, RAD51 and ATM (Rzepka-Gorska *et al.*, 2006; Oktay *et al.*, 2010; Titus *et al.*, 2013). A similar decrease in BRCA1, RAD51 as well as γH2AX expression was also observed in aged female rat and buffalo primordial follicle stage oocytes (Govindaraj *et al.*, 2015, 2017). In addition to genes involved in the repair of DSBs, Govindaraj *et al.* (2015) reported a decrease in the expression of ERCC2 (excision repair cross-complementing group 2, also called xeroderma pigmentosum group D-XPD) in aged rats. The protein encoded by ERCC2(XPD) is a vital member of basal transcription factor BTF2/TFIIH complex, which is an essential element in the repair of both damaged bases and SSBs (Drapkin and Reinberg, 1994; Iyer *et al.*, 1996). Together, these results clearly support the hypotheses that diminished DNA repair capacity contributes to oocyte ageing and DNA repair is critical for maintenance of oocyte quality. Another consideration is that if DNA repair capacity declines with age, then oocytes from older women may be more sensitive to exogenous DNA damaging agents, such as radio- or chemotherapy. This intrinsic characteristic may contribute to older women experiencing a much higher incidence of acute ovarian failure during or immediately following cancer treatment (Petrek *et al.*, 2006; Letourneau *et al.*, 2012). This phenomenon has previously been solely attributed to the fact that older women have a smaller primordial follicle reserve at the commencement of treatment, compared with younger women (Meirow and Nugent, 2001), and thus loss of follicles from an already reduced follicle pool is more likely to induce POF by the end of treatment (Meirow *et al.*, 2010).

Indeed the intrinsic ability to repair DNA damage may, in part, account for the natural variation in age at menopause in women. Stolk *et al.* (2012) performed a meta-analysis of 22 genome-wide association studies in 38 968 women of European descent and identified several DNA repair genes to be associated with age at natural menopause. MCM8 and DMC1 were the highest significant genes associated with the age of natural menopause. MCM8 is expressed at low levels in the somatic tissues and gonads, and *Mcm8*$^{-/-}$ mice are infertile (Lutzmann *et al.*, 2012). As mentioned earlier, DMC1 encodes for a protein that is essential for meiotic HR, and mutations in this gene cause POF (Qin *et al.*, 2007). How these genes and DNA repair pathways contribute to menopause remains unclear, but the data above indicates a critical role for DNA DSB repair in human reproductive ageing.

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Furthermore, mutations in key DNA DSB repair factors are correlated with premature ovarian ageing in women (Table II), suggesting that a fully functional DNA repair response is vital for normal fertile lifespan. While the underlying cause of premature ovarian ageing in these studies is unclear, it is possible that failure to repair DSBs generated during meiosis reduces the primordial follicle endowment. Alternatively, such mutations could also reduce the capacity of diplotene arrested oocytes to repair and survive DNA damage incurred post-natally. Indeed, defects in repair have the potential to result in loss of oocyte number if apoptosis is induced and loss of oocyte quality if oocytes evade cell death activation, both of which could contribute to reproductive ageing.

Environmental toxicants

There are many environmental toxicants that affect female fertility, such as endocrine disruptors (e.g. some pesticides, phthalates and phytoestrogens) which mimic, minimize or block cellular response to endocrine signals in organs such as the adrenal gland, thyroid and ovary. Importantly, some of these endocrine disruptors, such as Bisphenol A, have been shown to induce DNA damage in somatic cells (Iso *et al.*, 2006; Izzotti *et al.*, 2009) and sperm (Meeker *et al.*, 2010; Dobrzyńska and Radzikowska, 2013) of mice and humans. Similarly, some commonly used pesticides have also been shown to induce DNA damage in human cells *in vitro* (Ahmed *et al.*, 1977; Ündeğer and Başaran, 2005) and *in vivo* (Garaj-Vrhovac and Zeljezic, 2000). While few studies have examined the direct DNA damaging effects of these compounds on oocytes, some have provided evidence of interference in meiosis and DNA recombination.

Bisphenol A (BPA) is a high-production estrogenic endocrine disrupting chemical, used in the synthesis of plastics. In rodents and primates, BPA has been linked to the inhibition of meiosis initiation, resulting in reduced germ cell cyst breakdown and abnormalities with meiotic recombination and chromosome segregation (Susiarjo *et al.*, 2007; Hunt *et al.*, 2012; Zhang *et al.*, 2012). Significantly, in human fetal oocytes, BPA has been associated with a decrease in oocyte survival (Brieno-Enriquez *et al.*, 2011b) and an increase in the expression of DNA DSBs and repair genes (Brieno-Enriquez *et al.*, 2011a). Moreover, BPA causes DNA damage in cultured cells in an estrogen receptor (ER) dependant manner, as demonstrated by comet assays and the presence of γH2AX foci (Iso *et al.*, 2006). As ER is expressed in mature oocytes (Wu *et al.*, 1992), it remains possible that environmental toxicants such as BPA could damage oocyte DNA and activate DNA repair or apoptosis.

Benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon and is one of the most prevalent environmental carcinogens. BaP is prevalent in cigarette smoke, various oils, coal tar, automobile exhaust fumes, margarine, butter, fats, fruits, vegetables, cereals and in charbroiled steaks. BaP exposure leads to the formation of N2-benzo(a) pyrene DNA adducts that need to be removed by NER (Wani *et al.*, 2000). Significantly, in mice and humans, BaP exposure results in a significant increase in chromosome abnormalities and DNA damage in oocytes (Basler and Rohrborn, 1976; Einaudi *et al.*, 2013; Shamsuddin and Gan, 1988). Notably, elevated levels of DNA breaks were observed, which may have resulted from adduct excision and repair and/or through direct genotoxicity from increased ROS. Interestingly, after *in-vivo* exposure of female mice to BaP, there was significant DNA damage in super-ovulated MII oocytes and supporting cumulus cells collected 4 and 6 days after gavage but not 2, 15 or 22 days after gavage (Einaudi *et al.*, 2013). While the authors suggested that oocytes at different stages of folliculogenesis were more or less sensitive to BaP (Einaudi *et al.*, 2013), an alternative hypothesis is that oocytes at different stages were more or less able to repair the damage. Oocytes in antral follicles (exposed 2 days before induced ovulation) would have all the repair factors similar to GV/MII oocytes and may have rapidly repaired DNA damage before ovulation. Primary follicles (exposed 15 days before induced ovulation) and primordial follicles (exposed 22 days before induced ovulation) may have had sufficient time to repair damage or more severely affected oocytes may have undergone apoptosis. Interestingly, oocytes in early antral and large pre-antral follicles (4 and 6 days before induced ovulation) did not repair DNA, or undergo apoptosis, possibly supporting the authors' conclusion that these oocytes were more susceptible to BaP. However, more in-depth *in-vivo* analysis is required to determine if BaP induced DNA damage is repairable in oocytes. Similarly, analysis of other DNA damaging environmental compounds could be used to investigate the activation of different DNA repair pathways in oocytes.

Evidence for DNA damage response in mature oocytes

In response to the LH surge and GV breakdown, the oocyte progresses through meiosis I, and arrests at metaphase of meiosis II, where it remains until fertilization (Fig. 2). The DNA damage response in growing follicles has previously been reviewed (Carroll and Marangos, 2013; Collins and Jones, 2016). However, of note, recent evidence suggests that fully grown oocytes are not capable of establishing a robust prophase checkpoint in response to genotoxic exposure (Marangos and Carroll, 2012). Instead, an oocyte specific DNA-damage checkpoint has recently been discovered, whereby oocytes respond to DSBs by establishing metaphase I arrest, independent of ATM or ATR (Lane *et al.*, 2017), but dependant on the spindle-assembly checkpoint (Collins *et al.*, 2015; Marangos *et al.*, 2015). These findings are consistent with mouse oocytes in response to etoposide (Marangos *et al.*, 2015), IR, UVB and bleomycin (Collins *et al.*, 2015). This DNA damage checkpoint provides a potential mechanism to circumvent the production of embryos with chromosomal abnormalities and genomic instability. Furthermore, the DNA damage-induced metaphase I arrest is compromised in oocytes from mice of advanced age (Marangos *et al.*, 2015), highlighting DNA damage as a potential cause for age-related infertility. Although these studies highlight a new mechanism of DNA damage response, they do not provide insight into whether DNA repair occurs in these oocytes, or the potential mechanisms of repair.

Future directions: augmenting DNA repair to protect oocytes during ageing or cancer treatment

Recent evidence suggests that targeting oocyte DNA repair could be a useful strategy for preventing oocyte death in response to cancer

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treatment or prolonging reproductive lifespan. A notable example is the study reported by Kujjo *et al.* (2010) showing that supplementation of oocytes from ageing mice with recombinant RAD51 decreased the occurrence of spontaneous DNA DSBs, suppressed apoptosis, and restored the developmental competence of embryos. Additionally, manipulation of mediators of oocyte apoptosis in mouse models prevents depletion of the ovarian reserve and confers resistance to ageing and anti-cancer treatment. For example, a large number of oocytes survive radiation exposure and produce healthy offspring in apoptosis deficient PUMA (Kerr *et al.*, 2012c) and CHK2-null mice (Rinaldi *et al.*, 2017). Chk2 is a checkpoint kinase which acts downstream of ATM in order coordinate the elimination of oocytes with unrepaired DNA. Importantly, in addition to genetic studies in mice, the transient use of a CHK2 inhibitor was also shown to prevent irradiation mediated primordial follicle depletion, and furthermore, apparently healthy offspring were produced (Rinaldi *et al.*, 2017). Indeed, there has been a surge in studies aimed at pharmacological prevention of chemotherapy-mediated ovarian damage and primordial follicle loss (Roness *et al.*, 2014). Many of these strategies involve preventing damaged oocytes from dying and thus fertility and offspring health rely on the ability of oocytes to faithfully and efficiently repair themselves. Because enhancing DNA repair globally could impair the efficacy of cancer treatment, a targeted approach would be required to exploit the DNA repair capacity of oocytes for the purpose of fertility preservation during ageing and cancer treatment and such an approach has not yet been developed.

Conclusions

During meiosis, oocytes efficiently repair hundreds of intentionally induced DNA DSBs. Accumulating evidence now indicates that, despite a predilection for the induction of apoptosis, mitotically arrested primordial follicle oocytes can also repair DSBs in their DNA arising as a consequence of the ageing process or following exposure to genotoxins. Further work is required in order to identify the full suite of proteins involved and to determine whether primordial follicle oocytes only utilize HR or whether NHEJ is employed under certain circumstances. A deeper appreciation of the DNA repair capacity of oocytes is important for understanding the factors responsible for safeguarding the quality of ovarian reserve during reproductive life. In the future, this information may be exploited for the design of new therapies to inhibit oocyte death, restore oocyte quality and/or preserve fertility during ageing and anti-cancer treatments.

Authors' roles

All authors researched data for the article and provided substantial contributions to discussions of the content. All authors contributed to writing the article and to the review and/or editing of the article before submission.

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Conflicts of interest

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