Genomic Insults and their Redressal in the Eutopic Endometrium of Women with Endometriosis

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Abstract: Endometrium, a highly dynamic tissue, is known for its remarkable ability to regenerate, differentiate, and degenerate in a non-conception cycle and transform into a specialized tissue to nurture and protect the embryo in a conception cycle. This plasticity of the endometrium endows the uterus to execute its major function, i.e., embryo implantation. However, this boon becomes a bane, when endometrium- or endometrium-like cells adhere, grow, and invade extrauterine sites, leading to endometriosis. Endometrial deposits at the extrauterine site lead to severe pelvic pain, painful menstruation, and infertility in endometriosis. Although benign, endometriotic lesions share several traits with cancerous cells, excessive proliferation, adhesion, invasion, and angiogenesis make endometriotic lesions analogous to cancer cells in certain aspects. There exists evidence to support that, akin to the cancer cell, endometriotic lesions harbor somatic mutations. These lesions are known to experience higher proliferative stress, oxidative stress, and inflammation, which may contribute to somatic mutations. However, it would be of more interest to establish whether in the eutopic endometriosis also, the mutational burden is higher or whether the DNA Damage Response (DDR) is compromised in the eutopic endometrium, in endometriosis. Such investigations may provide more insights into the pathobiology of endometriosis and may also unravel cellular events associated with the origin of the disease. This review compiles inferences from the studies conducted to assess DNA damage and DDR in endometriosis.

Keywords: endometriosis; DNA damage response; endometrium; mutations

1. Introduction

The human body is a conglomeration of approximately 30 trillion cells; several billion new cells are formed every day from preexisting cells. These new cells inherit a replica of the parental genome that ensures the preservation of the lineage, developmental and differentiation programmatic memory, and functional competence of newly formed cells. This is not an ordinary feat, considering that parental cells routinely experience several extraneous and endogenous assaults on their genomes. These assaults result in double/single-stranded DNA breaks and modifications, such as oxidation, alkylation, deamination of nitrogenous bases, DNA adduct formation, or inter- and intra-strand crosslinks. Endogenous insults result from intrinsic biochemical and molecular reactions going astray. These errors manifest as mismatches during DNA replication. DNA strands break due to aberrant topoisomerase I and topoisomerase II function, hydrolytic, and base pair lesions due to oxidative and methylation reactions, etc. Genomic integrity also gets breached due to exposure to extrinsic factors, such as environmental toxins, radiation, and various pathologies, such as cancers, infections, and also therapies in certain cases. However, these genomic assaults, which can compromise cellular survival or function if left unrepaired, are efficiently handled by healthy cells. Healthy cells are equipped with several safeguard mechanisms to detect and repair different kinds of DNA damage. These repair mechanisms include mismatch repair, base excision repair, nucleotide excision repair, homologous recombination, and non-homologous end-joining repair pathway [1].

DNA damage activates the DNA Damage Response (DDR)—a group of signaling pathways mediated by damage sensors, signal transducers, repair effectors, and arrest or death effectors [2]. DDR results in either cell cycle arrest, apoptosis, or senescence, depending upon the extent of damage, the time taken to repair DNA, the stage of the cell cycle, and also the cell context [3]. Emerging data suggest that although all cell types are equipped with evolutionarily conserved DDR and repair mechanisms, cells in different tissues respond differently to DNA damage. For example, skin cells, compared with blood cells, are known to be more resistant to radiation-induced DNA damage [4]. Irradiated human umbilical cord blood (CB) derived from fetal hematopoietic stem cells (HSCs) show a slower rate of double-stranded break repair and undergo apoptosis whereas irradiated adult mouse quiescent HSCs escape cell death [5]. Thus, fetal and adult HSCs employ different DDR pathways to the same genomic insult and have different fates. Evidence exists to suggest that some cells, despite having damaged DNA, continue to proliferate instead of undergoing cell-cycle arrest, apoptosis, or senescence [6,7]. One such example is of the estrogen receptor-positive ovarian carcinoma (BG-1) cell line treated with higher concentrations of estradiol [8]. Interestingly, DDR is also implicated in maintaining the balance between proliferation and differentiation. It was demonstrated that the DNA damage induced by oncogenic activation or UV irradiation or blockade of mitosis prompts keratinocytes to differentiate into squamous cells [9,10]. It may be added here that DDR activation in proliferating human epidermal keratinocytes is an important component of their differentiation [11]. Similarly, DNA damage influences the differentiation of stem cells [12]. Overall, it appears that DNA damage and DDR may have roles not only in disease, but also in physiology. Further, DNA damage and DDR outcomes seem to be cell-context dependent. Thus, more studies are warranted to understand the mechanisms underlying the responses of different cell types to basal DNA damage and also to DNA damage induced by extrinsic factors. Information gathered from such investigations may help us devise strategies against various cellular dysfunctions, especially those related to excessive proliferation, degeneration, differentiation, and aging.

2. DNA Damage and Its Repair in the Endometrium

The endometrium, the inner lining of the uterus, is a very specialized tissue with the remarkable ability to regenerate in every menstrual cycle or transform during pregnancy. In a healthy woman, the endometrium undergoes approximately 400 cycles of growth, differentiation, and shedding during her reproductive life span. While the role of hormones, especially progesterone, in endometrial differentiation, is well established, more investigations are needed to understand whether DNA damage also contributes to the endometrial differentiation process. Our recent studies demonstrated that like all other cells, human endometrial cells are equipped with DDR and DNA repair machinery. Further, the expression of factors mediating these pathways was found to differ in proliferating and differentiated human endometrium [13], indicating hormonal regulation of these pathways. There also exists data to suggest that the factors involved in various repair machinery i.e., MisMatch Repair (MMR) [14,15], Homologous Repair (HR) [16], Non-Homologous End Joining (NHEJ) [17], and Base Excision Repair (BER) [13,18] are expressed in human endometrium. Further, our unpublished data revealed the ability of endometrial epithelial and stromal cells to counteract DNA damage induced either through oxidative or toxic stress by upregulating the expression of Growth Arrest and DNA Damage Inducible (GADD45) proteins. It may be inferred from these observations that human endometrial cells have the competence to recognize and repair DNA damage. Paradoxically, emerging data demonstrates that cancer-driver somatic mutations are present even in histologically normal endometrium [19,20]. Histologically normal epithelial glands were found to harbor several somatic mutations in cancer-driver genes, such as PhosphatidylInositol-4,5-bisphosphate 3-Kinase Catalytic subunit Alpha (PIK3CA), Phosphatase, Tensin homolog (PTEN), and Kirsten Rat Sarcoma virus (KRAS) [19,21–24]. Interestingly, individual epithelial glands were found to have clones with distinct somatic mutations. This not only reflects the

genetic mosaicism of the endometrial epithelial compartment, but also hints at the permissiveness of normal endometrial epithelium to harbor cells with mutated DNA. More research, especially longitudinal studies, is needed to estimate a cost-benefit ratio of having a proportion of cells with mutated DNA in epithelial glands. It would also be of interest to know whether human endometrium evolved different adaptive mechanisms to repair DNA errors/lesions, compared with other highly proliferative tissues, such as the skin or liver. It is also not yet known how a proliferative endometrium responds in the wake of genotoxic assaults, whether its response is governed by the need to continue proliferating to generate a sufficient pool of cells for subsequent differentiation and implantation or it is dictated by the need to maintain genomic integrity for achieving functional embryo receptivity with high transcriptional fidelity. Investigations in this direction would help us gain insights into the role of basal DNA damage and DDR in endometrial functions. Further, there also exists opportunities to investigate the role of DNA damage and DDR in various endometrial pathologies, such as endometrial cancers, endometrial hyperplasia, adenomyosis, and endometriosis. This review compiles the inferences drawn from existing reports on the endometrial DNA damage response in endometriosis. For this, keywords used for the literature search on PubMed were '(DNA damage) AND (endometriosis)', '(DNA repair) AND (endometriosis)', '(Oxidative stress) AND (endometriosis)', and '(Inflammation) AND (endometriosis). Original reports were reviewed in this article.

3. Endometrial DNA Damage in Endometriosis

Endometriosis is characterized by the presence of endometrium-like cells or endometriotic ectopic lesions outside the uterus. Lesion characteristics, such as size, depth, number, and the extent of adhesions to adjoining regions, are used to classify endometriosis into minimal, mild, moderate, and severe stages by the ASRM criteria [25]. Endometriotic lesions are predominantly detected on the surface of the ovary (endometrioma; OMA), peritoneum cavity wall (superficial peritoneal lesion; SUP), and also on the intestine, rectum, and the pouch of Douglas as Deep Infiltrating Endometriosis (DIE). The presence of ectopic lesions in the liver, kidney, pleural cavity, and even the brain has been reported. Some asymptomatic women with endometriosis may remain undiagnosed. However, a majority of women with the disease exhibit an array of symptoms, predominantly chronic pelvic pain, dysmenorrhea, dysuria, dyschezia, dyspareunia, and infertility. These morbidities adversely affect the quality of life of approximately 247 million women worldwide and 42 million women in India [26].

Several theories have been put forth to explain the origin of endometriosis. These include retrograde menstruation, lymphatic dissemination, stem cell induction, coelomic metaplasia, and the presence of mullerian remnants [27]. Among these, the most accepted is the retrograde menstruation theory. More support for the retrograde theory has come from recent reports indicating the clonal expansion of epithelial cells harboring cancer-driver mutations in genes, such as oncogenic PIK3CA and KRAS, in endometriotic lesions. On the other hand, these mutations remain in a subclonal state in healthy endometrium [19]. This clonal relationship, as indicated by shared somatic mutations in normal and endometriotic endometrium, supports the origin of endometriosis from the possible dissemination and growth of the menstrual phase endometrium into the peritoneal cavity. Further, endometriosis is known to be one of the risk factors for clear cell and endometrioid ovarian carcinoma [28,29]. ARID1A, PIK3CA, and KRAS genes are reported to be frequently mutated in endometriosis and ovarian carcinoma [19,21–24,30]. In 2020, Suda et al., using whole exome sequencing data from histologically normal endometrial tissues, endometriotic tissues excised from distant and adjacent sites from ovarian clear cell carcinoma (OCCC), and primary OCCC tissue, concluded that the precursors of both ovarian endometriosis and OCCC were common and present in normal endometrium. It was postulated that the accumulation of cancer-associated mutations in normal endometrium and their subsequent genomic evolution contributed to endometriosis and endometriosis-related ovarian cancer [19]. While these recent investigations on the mutational landscape

of endometrium have introduced more insights to the genetic relationship between the uterine and extrauterine endometrial samples, these reports fail to explain how a normal trait, "accumulation of somatic mutations," in eutopic endometrial epithelial cells (that are shed off every month) contributes to endometriosis in some women. Our previous study demonstrated a higher expression of DDR-associated genes in the proliferative phase in the eutopic endometrium of women with endometriosis compared with those without the disease [13]. While this may be due to higher proliferative stress in the endometrium of women with endometriosis, it remains to be established whether altered DDR in the proliferative phase modulates the endometrial differentiation program in endometriosis. Indeed, the differentiation of endometrial mesenchymal stromal cells (eMSCs) and stromal fibroblasts in women with endometriosis was found to be dysregulated [31]. It was postulated by authors that mesenchymal differentiation takes a different route in some women with endometriosis and results in a subset of senescent fibroblasts [31]. More research is needed to answer whether it is (a) the niche at extrauterine sites that is more conducive for the expansion of endometrial epithelial clones harboring mutations in cancer-driver genes or (b) the frequency of menstruation or early menarche or volume of menstrual efflux or (c) the shedding of a part of the basalis compartment along with the functionalis during menstruation in women with endometriosis.

Interestingly, 3D imaging analyses of the human endometrium have revealed the presence of rhizome-like structures in which several epithelial vertical glands are connected to horizontal glands in the basalis compartment of the endometrium [32]. Yamaguchi et al. (2022) reported the monoclonal origin of the continuum of rhizome and vertical glands [33]. Authors further proposed that after menstruation, residual glands in the basalis extend horizontally along the myometrium to form monoclonal rhizomes and each monoclonal rhizome gives rise to several vertical glands. It was further hypothesized that some rhizomes are long-lived, and during this period, some cells, including stem cell/progenitor cells in these rhizomes, are likely to acquire cancer-driver mutations while undergoing several cycles of repair and regeneration. These cells or clones with somatic mutations are likely to have a proliferative advantage and thus may contribute to endometrial regeneration by expanding the rhizome structure. However, when lodged at ectopic or other sites, these clones may contribute to pathologies, such as endometriosis. At extrauterine sites, the clones are likely to be released from anatomical constraints or these may experience paracrine influences from ectopic sites and thereby proliferate excessively. Considering the extent of heterogeneity of the genetic makeup of endometrial glands, causal events leading to the growth of endometrium at ectopic sites are likely to be stochastic and this may explain the differential susceptibility of women to endometriosis.

Few strides have been made to investigate whether endometriosis is associated with higher endometrial DNA damage. Lymphocytes isolated from women with endometriosis, following exposure to bleomycin, were found to have a higher number of chromatid breaks compared with those from women without the disease [34]. This shows that the blood cells of women with endometriosis are more susceptible to DNA damage. The eutopic endometrium in women with endometriosis also shows higher DNA damage. The uutopic endometrial epithelial as well as stromal cells show a higher number of foci of the γH2AX—a DNA damage marker in women with endometriosis, compared with their counterparts of control women [13,16]. In both investigations, women with endometrioma (stage III–IV) were included as study participants. These observations were in contrast to the conclusion drawn by Hapangama et al. [35].

This discordance in results may be due to the investigations of endometrial samples from different subtypes of endometriosis. In addition to genomic DNA, mitochondrial DNA damage has been investigated for its association with endometriosis. A 4 kbp deletion was found in the mitochondrial genome of eutopic endometrium from women with endometriosis. The frequency of this deletion was found to be higher in the tissues from chocolate cysts (endometrioma) compared with myoma, adenomyoma, and normal endometrium [36]. In the mitochondrial genome, 1.2 and 3.7 Kb deletions are reported to have the potential to serve as

a promising biomarker of endometriosis [37]. Mutations in the mitochondrial DNA of eutopic and ectopic endometrium from women with endometriosis have also been reported [38]. Seventeen somatic mutations identified in mitochondrial DNA were predicted to cause defects in the oxidative phosphorylation system. Evidence also suggests that mitochondrial energy metabolism is reduced in the ectopic and eutopic endometrium from non-human primates (*Macaca fascicularis* and *Macaca mulatta*) with endometriosis [39]. Altogether, these reports suggest higher DNA damage in the eutopic endometrium of women with endometriosis.

4. Potential Causes of DNA Damage

Although the data on endometrial DNA damage and mechanisms that counteract DNA damage in the context of endometriosis are limited, it is apparent that the extent of DNA damage in the endometrium in women with endometriosis is higher compared with those without the disease. The higher endometrial DNA damage seen in endometriosis could be because of replicative stress, oxidative stress, inflammation, or environmental toxicants, as there exists a significant amount of data to suggest that the eutopic as well as ectopic endometrium in women with endometriosis experience replicative stress, an imbalance in oxidant and antioxidant factors, and also inflammation.

4.1. Proliferative Stress

There exists sufficient data to suggest that the eutopic endometrium in women with endometriosis shows a higher proliferative index [40,41]. In addition, a higher expression of telomerase was reported in the epithelial cells of the proliferative and secretory phases and the stromal cells of the secretory phase eutopic endometrium in women with peritoneal endometriosis [35]. Other reports have also demonstrated an increase in telomerase [42] and telomerase-associated gene [43] expression and activity [42] in the secretory phase eutopic endometrium in women with endometriosis. A recent study showed higher levels of DNA replication of ATP-dependent helicase/nuclease 2 (DNA2) in the eutopic and ectopic endometrium of women with endometriosis [44]. Similar results were reported for eMSCs isolated from the eutopic and ectopic endometrium of women with endometriosis [44]. Expression of Ki67, a proliferation marker, was found to be higher in the proliferative phase eutopic endometrium in women with endometriosis, compared with women with uterine polyps [45]. In addition, the secretory phase endometrium in women with peritoneal endometriosis was found to have a higher expression of proliferating cell nuclear antigen (PCNA) [35]. Nucleolin expression was also found to be higher in proliferative and secretory phase eutopic endometrium in women with endometriosis [35]. Investigations from our laboratory and other groups also indicate a higher proliferative index in the eutopic endometrium of women with endometriosis as revealed by a higher number of stromal cells per unit area and a significantly higher PCNA expression in the proliferative phase endometrium in women with endometriosis, compared with the phase-matched endometrial samples from control women [13].

4.2. Oxidative Stress

A relationship between oxidative stress and endometriosis has been extensively studied. Immunolocalization of an oxidized derivative of Guanosine (a DNA base)-8-hydroxy-2′-deoxyguanosine (8-OHdG), an oxidative stress marker, was found to increase with the severity of endometriosis [18]. In addition, significantly higher levels of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were found in women with moderate and severe endometriosis, compared with mild and minimal endometriosis [46]. Higher levels of thiols, advanced oxidation protein products (AOPP), protein carbonyl, and nitrates/nitrites were found in the peritoneal fluid of women with deep infiltrating endometriosis [47]. Eutopic and ectopic endometrial stromal and epithelial cells in women with endometriosis were found to produce more oxygen anion (O^{2-}) and demonstrated higher proliferative capacity in response to oxidative stress [48]. Higher levels of lipid peroxides were also found in the peritoneal fluid of women with endometriosis [49]. Total

oxidant capacity and lipid peroxide levels were higher in the serum and follicular fluid samples from women with endometriosis [50,51]. Follicular fluid from infertile women with endometriosis also had higher nitrate/nitrite levels, compared with infertile women [52]. Higher levels of vitamin E, an antioxidant, were found in women with endometriosis [53]. Vitamin C levels were lower in the follicular fluid of women with endometriosis [51]. Levels of antioxidants, such as glutathione peroxidase and superoxide dismutase, were found to be reduced in the peritoneal fluid of women with endometriosis [49,51]. Total antioxidant levels were also lower in the peritoneal fluid of women with endometriosis [49].

Higher oxidative stress in endometriosis has been attributed to dysregulated iron metabolism. Iron released from hemorrhage or hemolysis was found to be accumulated in endometriotic lesions/peritoneal fluid [54]. A higher menstrual influx via retrograde menstruation can contribute to iron overload. Higher levels of iron, ferritin, and hemoglobin have been found in the peritoneal fluid of women with endometriosis [54–58]. Further, lower levels of hemopexin (a scavenger of heme) are reported in the peritoneal fluid of women with endometriosis [59]. Lesions were also found to have higher iron deposits in endometriosis [60]. Iron conglomerates were detected in endometriotic lesions contributing to oxidative stress [61]. Interestingly, endometriotic epithelial cell proliferation was found to be higher in response to human menstrual debris supplemented with erythrocytes injected in nude mice [62]. This proliferation subsided in response to desferrioxamine, an iron chelator.

4.3. Inflammation

There is ample evidence to implicate endometriosis as an inflammatory disease. Inflammation is reported to be linked with the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which contribute to DNA adducts, including 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OxodG) and 8-nitroguanidine [63]. NO forms peroxynitrite (ONOO⁻) upon reaction with superoxide (O^{2-}). NO and O^{2-} are also generated by neutrophils and macrophages in the inflammatory microenvironment. These DNA adducts lead to the formation of apurinic sites [64]. Furthermore, inflammatory cytokines, such as Tumor Necrosis Factor (TNF), released by macrophages, generate O^{2-} and contribute to genomic instability [65]. Higher levels of TNF have been detected in the peritoneal fluid [66] and serum [67] of women with endometriosis.

Neutrophil numbers were found to be higher in the peritoneal fluid of women with endometriosis [68,69]. Higher levels of IL-8, a chemoattractant of neutrophils, were detected in the plasma [70] and peritoneal fluid [69–71] of women with endometriosis. Neutrophils showed decreased apoptosis when incubated with peritoneal fluid from women with endometriosis [72]. The levels of human neutrophil peptides (HNP) released by activated neutrophils, α-defensins, are also reported to be elevated in the peritoneal fluid of women with endometriosis and their levels correlated with the severity of the disease [70]. α-defensin acts as a chemoattractant for T cells, dendritic cells, and monocytes/macrophages. Macrophage numbers were found to be higher in all phases [72], including the proliferative phase [73] of the menstrual cycle in the eutopic endometrium of women with endometriosis. In another study, the endometrial macrophage population was found to be lower throughout the menstrual cycle in endometriosis [74]. The frequency of CD163⁺ macrophages/M2 macrophages (anti-inflammatory macrophages) was lower, whereas that of CD68⁺ macrophages/M1 macrophages (pro-inflammatory) was higher in the eutopic endometrium of women with endometriosis [73,75]. Another macrophage chemoattractant, monocyte chemotactic protein-1 (MCP-1), was found to be elevated in the eutopic endometrium of women with endometriosis [76]. Monocytes in-vitro differentiate into macrophages, rather than into dendritic cells, in response to the peritoneal fluid from women with advanced stage endometriosis [77]. In addition, peritoneal macrophages demonstrate a reduced phagocytotic potential [78] and release pro-inflammatory cytokines TNF, IL-6, and IL-1β in endometriosis [79]. A higher proliferation and invasion of the stromal cells of the endometriotic lesions were observed, when primed with macrophages from healthy

women [80]. Macrophages from women with endometriosis led to the increased clonogenicity and self-renewal capacity of stromal and epithelial cells [81]. Collectively, these reports suggest that macrophages in women with endometriosis create an inflammatory microenvironment.

Alarmins or damage-associated molecular patterns (DAMPs), such as high-mobility group box 1 (HMGB1), are reported to be highly abundant in the menstrual fluid of women with endometriosis compared with control women with other gynecological conditions [82]. Endometriotic stromal cells stimulated with HMGB1 expressed higher levels of vascular endothelial growth factor (VGEF) [82]. In addition, HMGB1-treated eutopic endometrial stromal cells from women with endometriosis showed higher proliferation [83]. IL-33, another alarmin, was found to have increased expression in women with endometriosis [84,85]. Intraperitoneal injections of IL-33 stimulated the growth and vascularization of the lesion in mice [85].

4.4. Environmental Toxicants

DNA in the eutopic and ectopic endometrium may be more susceptible to damage in women with endometriosis because of higher replicative stress, oxidative stress, and inflammation. It is also likely that endometrial DNA gets exposed to more environmental toxicants in women with endometriosis. Studies have shown a correlation between dioxin exposure and peritoneal endometriosis [86] or stage III/IV endometriosis [87]. Evidence also exists to suggest a relationship between exposure to 2,3,7,8-Tetrachlorodibenzo—p-dioxin (TCDD) and endometriosis development in rhesus monkeys [88]. Polychlorinated biphenyl (PCB) and 1,1-dichloro-2,2,-bis (4-chlorophenyl)-ethene (DDE) [88] and phthalate [89–92] have been associated with risk of endometriosis. However, other studies could not establish an association between TCDD [93], Dioxin [94], PCBs [94] exposure, and endometriosis. These discrepant results can be due to the different ethnicities of the populations investigated and the small cohort sizes [94].

4.5. Estrogenic Toxicity

Endometriotic lesions are capable of synthesizing estrogen in-situ. Elevated levels of estrogen have been attributed to the higher expression of aromatase in the ectopic endometrium of women with endometriosis [93–95]. Estradiol stimulates the production of prostaglandins, specifically prostaglandin E2, which further stimulate the activity of aromatase [96]. Estrogen signaling is mediated by nuclear estrogen receptors. Higher expression of the estrogen receptor β (ERβ) is reported in women with endometriosis [95–98]. ERα is reported to be of significance in initiating lesion development mediated by estrogen. Further, IL-6 was found to be attenuated in ERα knockdown mice [99], demonstrating estrogen/ERα/IL-6-mediated cross-talk in endometriosis development. Another study showed the importance of ERβ in cell survival via higher IL-1β production [100].

The eutopic endometrium in women with endometriosis is also reported to have higher levels of estradiol [101]. Higher levels of estradiol cause genotoxicity through adduct formation. Estrogen hydroxylation at C4 produces metabolites, such as 4-hydroxy-estrone (4-OHE$_1$). 4-OHE$_1$ forms quinones catalyzed by peroxidases/CYP450. Quinones interact with DNA and form adducts, such as 4-OHE1(E2)-1-N3Ade and 4-OHE1(E2)-1-N7Gua. Higher levels of estrogen metabolite 4-OHE$_1$ are reported in the eutopic endometrium in women with endometriosis [102].

5. DNA Repair in Women with Endometriosis

Attempts have been made to investigate whether genetic variations in DNA repair genes make women more or less susceptible to endometriosis. X-ray repair cross-complementing 4 (XRCC4), a core player involved in the NHEJ pathway, was found to have c.1394G > T polymorphism in Iranian and Taiwanese women with endometriosis [103,104]. This polymorphism in the promoter region might affect the expression of the XRCC4 protein and consequently, DNA repair. Incidentally, studies have demonstrated a reduced expression of XRCC4 in the eutopic endometrium of women with endometriosis, com-

pared with healthy women [17]. XRCC3 polymorphism at the p.Met241Thr genotype in Turkish women has been reported to be associated with endometriosis [105]. PPARγ c.161T > C and PPARγ p.Pro12ALA polymorphisms were found to be associated with endometriosis in Japanese and German populations, respectively [106,107]. p.Pro72Arg polymorphism in the p53 gene was found to be associated with the risk of endometriosis in Italian women [108,109], but not in Mexican [110], Chinese [111], Taiwanese [112], Iranian [113], and Pakistani [114] populations. This polymorphism might influence mRNA splicing and influence p53 gene expression and DNA-protein interaction. In addition, women with XRCC1 codon p.Trp194Arg, p.Gln399Arg, and XRCC3 codon p.Met241Thr polymorphism were found to have higher damage in the DNA of their lymphocytes treated with bleomycin [115]. Another study showed an association between XRCC1 polymorphism p.Arg399Gln with the severity of endometriosis in the Taiwanese population [116]. Polymorphisms in NER proteins, such as ERCC1 (rs11615 TT), ERCC2 (rs1799793 AA), and ERCC6 (rs2228528 AA), were also associated with a risk of endometriosis [117].

Investigations have been undertaken to assess the expression of various DNA repair genes in the endometrium in women with endometriosis. BRCA1, BRCA2, RAD51, and ATM mRNA levels were found to be reduced in the eutopic endometrium in women with stage III-IV endometriosis, compared with women with neoplasm [16]. However, it remains to be ascertained whether the reduced levels of these DNA repair proteins contribute to unrepaired double-stranded DNA breaks or whether the endometrium adopts other mechanisms to ensure genomic integrity. On the other hand, expression of MSH2, a mismatch repair protein, was found to be higher in the eutopic stromal cells and endometriotic lesions of women with endometriosis, compared with the eutopic endometrium in women with leiomyoma [14]. In contrast, Fuseya et al. reported reduced levels of MSH1 and MSH2 in eutopic endometrium in women with ovarian endometriosis [15]. MMR protein levels (MSH1 and MSH2) were lower in the ectopic endometrium compared with the paired eutopic endometrium. Endometriotic lesions were found to have a hypermethylation of the MLH1 promoter. The methylation pattern correlated with low expression of MLH1 protein expression [118]. Matta et al. reported an increased DNA repair capacity in the lymphocytes of women with endometriosis, compared with women without endometriosis. However, in this study, the control group included women with breast cancer [119]. Our studies demonstrated a reduced endometrial expression of XRCC4, a core protein involved in the NHEJ repair pathway, in women with endometriosis, compared with women without endometriosis [17]. Expression of OGG1, another oxidative stress response gene, was found to be reduced in the ectopic endometrium of women with endometriosis [19].

Poli-Neto et al. analyzed five eutopic endometrial gene expression datasets, namely, GSE4888 [120], GSE6364 [121], GSE7305 [122], GSE7307 (GEO repository), and GSE51981 [123] to compare endometrial transcriptomes in women with and without endometriosis. The meta-analysis revealed downregulation of DNA repair genes (ATRX, BRIP1, EXO1, FANCI, FANCL, FEN1, MSH2, MSH6, NEIL3, PARPBP, PACIP1, PCNA, PDS5B, POLA1, POLE2, POL1, PRKDC, RPA1, SMC6, USP1) in the proliferative phase eutopic endometrium of women with endometriosis compared with control women [124]. However, RNA Seq analysis carried out by our group revealed a trend towards upregulation in the endometrial expression of DNA damage repair genes in women with endometriosis during their proliferative phase, compared with control women without endometriosis. This aligned with our observations indicating a higher number of γH2FX loci in the eutopic endometrium of women with endometriosis. Endometrial expression of DNA repair genes is upregulated probably to counteract higher DNA damage in endometriosis. We further observed that higher DNA damage in the endometrium persists in the mid-secretory phase of the menstrual cycle in women with endometriosis. However, a trend towards downregulation in the endometrial expression of DNA repair genes was apparent during the mid-secretory phase in women with endometriosis, compared with phase-matched samples from healthy women [13]. Several factors reported to be involved in DDR in proliferating cells are downregulated in differentiated cells. This may render differentiated cells resistant to genotoxic stimuli. In the context of eutopic endometrium

in women with endometriosis, it is likely that persistent DNA damage due to oxidative stress or inflammation may lead to accumulation of unrepaired DNA lesions; eutopic endometrium with damaged DNA is likely to form lesions at conducive ectopic sites.

6. Conclusions

A large body of data links DNA damage, impaired DNA damage response (DDR), and aberrant DNA repair capacity with various pathologies, such as cancers, neurodegenerative diseases, premature aging, and cardiovascular diseases. Additionally, the role of DNA damage, DDR, and DNA repair in various physiological settings, such as the generation of immunoglobulin and T cell receptor diversity, telomere homeostasis, cell proliferation, and differentiation, the protection against pathogens is unequivocally established. However, DNA damage, DDR, and DNA repair capacity of human endometrium in health and disease, especially in endometriosis, have not received much attention. This review compiles the major inferences drawn from the investigations undertaken in this direction (Figure 1). A majority of these reports have focused on the expression/level of various components of DDR pathways and their dysregulation in the eutopic or ectopic endometrium of women with endometriosis. Some strides have also been made to assess whether genetic variations in DDR or DNA repair genes are associated with endometriosis risk. Collectively, these investigations highlight aberrations in the expression or levels of some DDR factors in the eutopic as well as ectopic endometrium of women with endometriosis, compared with women without endometriosis. More investigations are needed to resolve queries, including (I) whether these aberrations in DDR and DNA repair gene expression play a causal role in endometriosis or if these derangements appear after the onset of endometriosis; (II) how the activities of various DDR proteins are controlled in different phases of the menstrual cycle; (III) whether ectopic tissues preferentially employ a specific DDR pathway. Further, in the wake of emerging reports indicating an association between DDR and metabolic reprogramming, it would be of interest to investigate the potential effects of modulated DDR on endometrial metabolism and other functions in endometriosis. More knowledge about the DDR and DNA repair mechanisms that operate in the endometrium may open novel avenues for treating or managing endometriosis in the future.
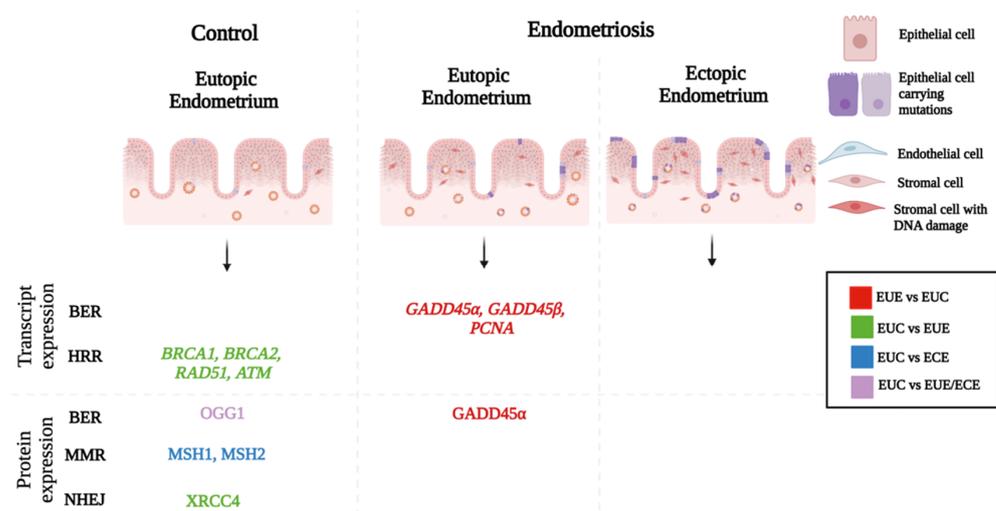
Figure 1. Endometrial expression of genes/proteins involved in DNA damage response (DDR) and DNA repair in endometriosis. The figure highlights some of these factors displaying higher expression in EUE (eutopic endometrium in women with endometriosis) or EUC (eutopic endometrium from women without endometriosis) or ECE (ectopic endometrium in women with endometriosis), compared with their counterparts at eutopic or ectopic sites. The box indicates the categories that were compared for the relative expression of DDR or DNA repair factors. This illustration also reflects a higher frequency of epithelial cells with mutations in ectopic lesions. (Created with BioRender.com).

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