2 Molecular Basis of BRCA1 and BRCA2: Homologous Recombination Deficiency and Tissue-Specific Carcinogenesis

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Abstract

Mutations in BRCA1 and BRCA2 cause hereditary breast and ovarian cancer (HBOC) syndrome, and these genes play multiple critical roles in maintaining genomic stability. One particularly important function of these genes is the homologous recombination (HR) repair of DNA. HR repair is an essential error-free repair mechanism for DNA double-strand breaks that utilizes an intact sister chromatid as a template. In addition to its role in HBOC oncogenesis, HR dysfunction is a target for treatment with poly (ADP-ribose) polymerase (PARP) inhibitors. Germline mutations of *BRCA1/BRCA2* cause breast, ovarian, fallopian tube, and peritoneal cancers with high rates of genomic alterations accompanied by poor prognoses. The mechanism underlying this tissue specificity has not yet clearly been explained, but several studies have examined its possible association with estrogen signaling. In this review, we first introduced the molecular mechanisms of HR mediated by BRCA1 and BRCA2 in the context of PARP inhibitor sensitivity. We also discussed several hypotheses describing estrogen- and HR deficiency-dependent genomic instability. Understanding these mechanisms is crucial for the adequate treatment and prevention of HBOC-related cancers.

Keywords

BRCA1 · BRCA2 · PARP inhibitor · Homologous recombination · Non-homologous end-joining · Alternative end-joining · Tissue specificity · Estrogen

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2.1 Introduction

Hereditary breast and ovarian cancer (HBOC) syndrome is attributable to germline mutations in genes encoding DNA repair proteins and cell cycle checkpoints, the two most prominent being *BRCA1* and *BRCA2* [1, 2]. The primary function of the BRCA1 and BRCA2 is maintaining genomic stability. In this role, BRCA1 and BRCA2 critically control a broad range of cellular processes including DNA repair, cell cycle checkpoints, apoptosis induction, chromatin modification, and centrosome duplication. In addition to *BRCA1* and *BRCA2*, germline mutations in *PALB2* (also called *FANCN*), *RAD51C*, *ATM*, *CHEK2*, and *TP53*, which participate in homologous recombination (HR) repair and cell cycle checkpoint regulation, cause HBOC [3, 4]. This indicates that HR deficiency (HRD) is a crucial cause of HBOC. In addition to its importance in oncogenesis, HRD is also critical for the treatment of HBOC-related cancers using poly (ADP-ribose) polymerase (PARP) inhibitors, which induce synthetic lethality in cancers with HRD [5, 6].

HBOC is one of the most well-studied inheritable gene mutation-derived and tissue-specific cancers. Other inheritable tissue-specific cancers include gastrointestinal cancers and skin cancers caused by mutations in mismatch repair genes and xeroderma pigmentosum (XP) genes, respectively [7, 8]. This tissue specificity is likely the consequence of the combination of the vulnerability of specific DNA repair pathways caused by the mutations and the tissues that particularly require the pathways for their genetic stability. For example, each XP gene is required for the nucleotide excision repair of DNA adducts generated by ultraviolet light exposure [9]. However, the mechanisms underlying the tissue specificity of gastrointestinal cancers caused by mismatch repair gene mutations and breast and ovarian cancer caused by *BRCA1/BRCA 2* mutations have not been clearly explained. Oxidative stresses induced by bacterial exposure could be implicated in gastrointestinal carcinogenesis caused by mismatch repair gene mutations [10]. Concerning HBOC, several studies attempted to reveal the mechanisms underlying its tissue specificity. HRD is involved in the process, and estrogen signaling likely plays important roles.

In this review, we first introduced the fundamental mechanism of HR mediated by BRCA1 and BRCA2 to illustrate the mechanism by which PARP inhibitors induce synthetic lethality. We also discussed several hypotheses describing estrogen- and HRD-dependent genomic instability including tissue-specific DNA damage induced by estrogen receptor α (ERα)-mediated transcription, the tissue-specific paracrine effect of receptor activator of nuclear factor kappa-B ligand (RANKL) secreted from mammary glands, and tissue-specific cancer cell survival associated with the stress-responsive transcription factor NRF2 activated by estrogen.

2.2 Structure and Binding Partners of BRCA1/BRCA2 Proteins

BRCA1 comprises functional domains including an N-terminal RING finger, exon 11, a coiled-coil domain, and C-terminal tandem BRCT repeats, whereas BRCA2

possesses BRC repeats and single-stranded DNA (ssDNA) binding domains (Fig. 2.1). BRCA1 constitutes a RING heterodimer ubiquitin E3 ligase with another structurally similar RING finger protein BARD1 [11–13] that participates in heterochromatin formation by ubiquitinating histone H2A [14–16]. The BRCT repeats of BRCA1 interact with the phosphorylated forms of CtIP, FANCJ (also called BRIP1 or BACH1), or Abraxas (also called ABRA1) [17–23]. FANCJ is a DNA helicase that is critical for the repair of DNA damage including DNA crosslinking, and homozygous mutation of this gene causes Fanconi anemia [24]. Abraxas is an adopter protein connecting BRCA1 with RAP80, which interacts with ubiquitin chains generated at DNA double-strand breaks (DSBs) [21–23]. Whereas BRCA1 complexed with CtIP and FANCJ performs HR through DNA end resection, BRCA1 complexed with Abraxas antagonizes and fine-tunes HR [25–27]. The coiled-coil domain of BRCA1 recruits BRCA2 to sites of DNA damage. This domain interacts with PALB2, which bridges BRCA1 and BRCA2 [28, 29]. BRCA2 interacts with ssDNA through the ssDNA binding domain in its C-terminus, and it comprises eight repeats of the BRC domain, each of which is capable of interacting with one RAD51 molecule [30–33]. *BRCA1*, *BRCA2*, *PALB2*, and the *RAD51* homolog *RAD51C* are also known as the Fanconi anemia genes *FANCS*,

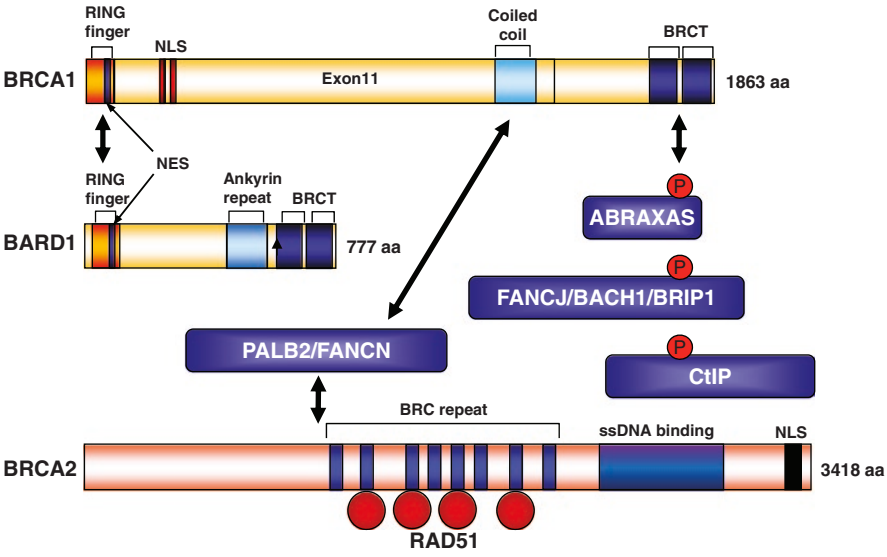

Fig. 2.1 Molecular structures of BRCA1, BRCA2, and their binding partners. BRCA1 comprises an N-terminal RING finger domain, exon 11, a coiled-coil domain, and C-terminal tandem BRCT domains. BRCA2 comprises eight repeats of the BRC domain and a single-stranded DNA binding domain. The RING domain interacts with the structurally similar protein BARD1, whereas BRCT repeats interact with the phosphorylated forms of CtIP, FANCJ, or Abraxas. The coiled-coil domain interacts with BRCA2 via PALB2. The BRC domains in BRCA2 interact with RAD51. The nuclear localization signal (NLS) and nuclear export signal (NES) are also presented. *P* phosphorylation

FANCD1, *FANCN*, and *FANCR*, respectively, and homozygous mutations in these genes cause Fanconi anemia [34–38].

2.3 BRCA1/BRCA2 Functions in HR and the Synthetic Lethality of PARP Inhibitors

2.3.1 HR Mediated by BRCA1/BRCA2 and a Backup Pathway Mediated by PARP1/PARP2

DNA damage can be broadly classified as two types: single-strand breaks and more cytotoxic DSBs. There are at least four mechanisms of DSB repair: non-homologous end-joining (NHEJ), single-strand annealing (SSA), alternative end-joining (Alt-EJ, also called Alt-NHEJ or microhomology-mediated EJ), and HR (Fig. 2.2). Most DSBs are repaired by NHEJ. NHEJ is available throughout all cell cycle phases, but it is most important during G1 phase when HR is not available [39]. NHEJ simply joins the blunt ends of DSBs via a process mediated by the heterodimer Ku70/Ku80 complexed with DNA-dependent protein kinase catalytic subunit (DNA-PKcs), XRCC4, and DNA ligase 4 (Lig4) (Fig. 2.2a) [40–41]. NHEJ is therefore relatively error-prone, and despite the deletion of residues at the broken ends in some instances, such deletions in most genetic regions do not affect cellular viability.

Contrarily, HR is an error-free process that is ideal for genetic stability, but it is only available during S and G2 phases when sister chromatids are accessible as

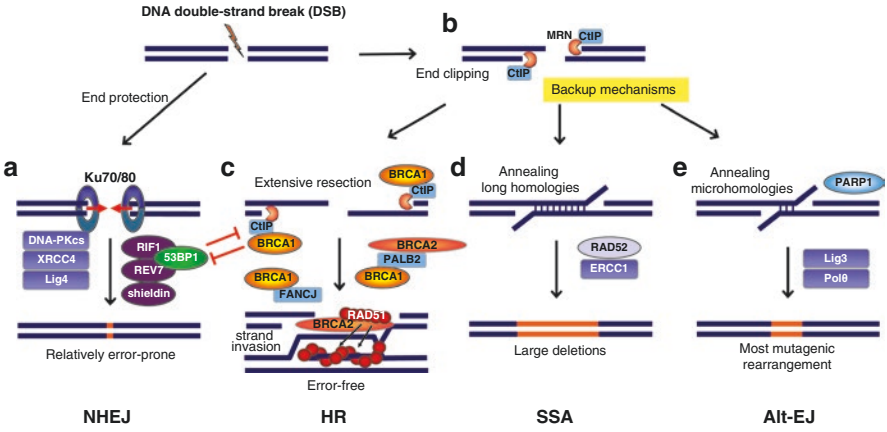

Fig. 2.2 Double-strand break (DSB) repair machineries. Four distinct mechanisms of DSB repair, namely, (**a**) non-homologous end-joining (NHEJ), (**c**) homologous recombination (HR), (**d**) single-strand annealing (SSA), and (**e**) alternative end-joining (Alt-EJ), and their representative repair proteins are presented. End-clipping by CtIP-MRN (**b**) is also presented. NHEJ simply joins the blunt ends of DSBs in a relatively error-prone process, whereas HR is an error-free pathway mediated by BRCA1, BRCA2, and RAD51. SSA and Alt-EJ are backup pathways for end-clipped DSBs in cells with HR deficiency. SSA and Alt-EJ lead to significant genetic alterations. Poly (ADP-ribose) polymerase 1/2 (PARP1/PARP2) is required for Alt-EJ

templates for recombination [39]. Directed by CtIP, a protein capable of interacting with the C-terminal BRCT domain of BRCA1, DSBs are first processed by the MRN complex consisting of MRE11, RAD50, and NBS1 for endonucleolytic clipping, in which the nuclease activity of MRE11 removes small amounts (~20 bp) of DNA at the broken ends (Fig. 2.2b) [42–44]. Once the DNA end is clipped, DNA is no longer a substrate for NHEJ, and it should be repaired by HR. One of most critical functions of BRCA1 in HR is extending DNA resection by supporting CtIP, which is phosphorylated in S phase and is therefore capable of binding to BRCA1, to generate ssDNA of sufficient length for strand invasion of the sister chromatid (Fig. 2.2c) [45, 46]. Another critical role of BRCA1 is the recruitment of BRCA2 to DSB sites via PALB2 [28, 29, 47]. While BRCA2 directed to DSBs interacts with ssDNA, multiple RAD51 molecules on BRCA2 are transferred onto ssDNA to create ssDNA-RAD51 filaments that invade the sister chromatid and execute homology searching via the recombinase activity of RAD51 [30–33].

The end-clipped DSBs in cells with HRD caused by BRCA1/BRCA2 dysfunction cannot be repaired by NHEJ, and they are cytotoxic if they are left unprocessed. Therefore, such lesions are repaired by the alternative backup pathways SSA and Alt-EJ (Fig. 2.2d and e). The majority of such DNA lesions are repaired by Alt-EJ, the most mutagenic repair pathway that anneals the broken ends with minimal homologous sequences (called microhomology), whereas SSA anneals DNA regions with longer homologous repeated sequences [48, 49]. Hence, SSA and Alt-EJ are beneficial for cancer cell survival but disadvantageous for individuals because they lead to genetic alterations and cancer. The genetic alteration created in this process can be detected as signature 3 genetic scars by next-generation sequencers, which are often used to assess HRD in cancer specimens for therapeutic purposes [50]. Importantly, PARP1/PARP2 is required for Alt-EJ in addition to DNA single-strand break repair (SSBR) [48, 51]. Therefore, PARP inhibitors inhibit the backup pathway required for the survival of cells with HRD.

HR and NHEJ are antagonistically regulated by BRCA1 and 53BP1 in response to DSBs. Whereas 53BP1 protects the DSB ends from CtIP-MRN-mediated end-clipping and consequently directs DSB repair toward the NHEJ pathway, BRCA1 blocks the action of 53BP1 by protecting CtIP [52, 53]. Interestingly, suppression of 53BP1 or its functional partners RIF1, Rev7, and Shieldin dramatically restores HR in BRCA1-/BRCA2-deficient cells and therefore causes PARP inhibitor resistance [54–62]. As described previously, the two major functions of BRCA1, namely, ssDNA elongation and BRCA2-RAD51 recruitment, are mediated through the CtIP-MRN complex and PALB2, which interact with BRCT and the coiled-coil region of BRCA1, respectively. In addition, a recent analysis revealed that exon 11 of BRCA1 is required for the suppression of 53BP1 [63]. Deletion of this exon coupled with *TP53* knockout leads to breast cancer in mice [64]. Interestingly, whereas homozygous deletion of the coiled-coil domain ($BRCA1^{CC/CC}$) or exon 11 ($BRCA1^{\Delta 11/\Delta 11}$) induces a Fanconi anemia-like phenotype with a low birth frequency or embryonic lethality, respectively, compound heterozygous mice possessing a combination of each deletion ($BRCA1^{CC/\Delta 11}$) were born at Mendelian frequencies indistinguishable from those of wild-type mice [63]. Hence, the 53BP1 counteraction

and BRCA2 recruitment, namely, DNA end resection and RAD51 loading, are individually essential functions for BRCA1.

2.3.2 Essential Role of BRCA1/BRCA2 in DNA Replication with PARP Trapping

DSBs are generated by exogenous insults, such as ionizing irradiation and topoisomerase II inhibitors, and endogenous insults accompanied by DNA replication (Fig. 2.3). DNA adducts represent a common cause of stalled replication. DNA adducts are generally removed by base excision repair followed by SSBR performed by PARP1 and to a lesser extent by PARP2 (Fig. 2.3a) [65–67]. When this pathway fails to function, for example, in the presence of PARP inhibitors, replication is maintained by either translesion synthesis (Fig. 2.3b), template switching (Fig. 2.3c), or HR (Fig. 2.3d). There are at least four pathways to continue replication, and therefore, HRD does not greatly affect cell viability in this situation. However, the situation differs if fork stalling is prolonged. In cases of prolonged stalling, forks are cleaved by the MUS81-EME1 nuclease complex, generating one-ended DSBs that absolutely require HR to restart break-induced replication (Fig. 2.3e) [68–70]. Prolonged fork stalling is caused by DNA secondary structures such as R-loops and G-quadruplexes, as well as PARP trapping, a phenomenon in which PARP

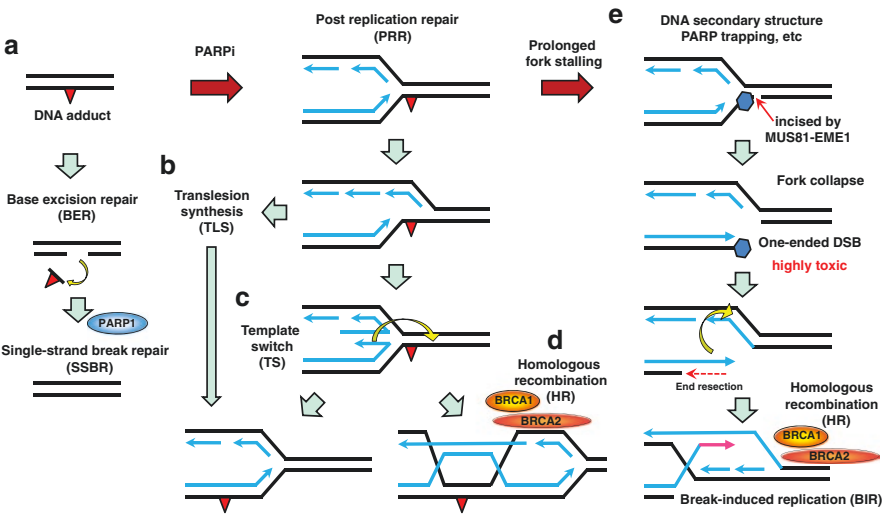

Fig. 2.3 BRCA1/BRCA2 function in DNA replication. DNA adducts are generally removed by base excision repair (BER) followed by single-strand break repair (SSBR) conducted by poly (ADP-ribose) polymerase 1/2 (PARP1/PARP2) (**a**). Translesion synthesis (**b**), template switching (**c**), and homologous recombination (HR) (**d**) function as backup mechanisms for BER. Prolonged stalled forks caused by DNA secondary structures or PARP trapping are cleaved by the MUS81-EME1 nuclease complex, resulting in one-ended double-strand breaks, which are highly toxic and require HR for cell survival (**e**)

persistently interacts with damaged DNA sites while its enzymatic activity is suppressed by PARP inhibitors [71]. Because one-ended DSBs are highly toxic, the ability of PARP inhibitors to induce PARP trapping is important for the induction of synthetic lethality in *BRCA1*- or *BRCA2*-mutated cancers with HRD. In addition to HR, BRCA1 and BRCA2 play additional essential roles in this process. BRCA1 and BRCA2 protect stalled replication forks against nucleolytic degradation by nucleases including MRE11, EXO1, and DNA2, thereby preventing fork collapse [72–78]. BRCA1 and BRCA2 are also required for preventing DNA damage driven by R-loops [79, 80], which are DNA-RNA hybrids that often accumulate at sites of DNA secondary structures including G-quadruplexes [81]. Collisions between the DNA replication machinery and R-loops result in fork collapse and subsequent DSBs. BRCA1 and BRCA2 associate with the DNA-RNA hybrid helicase SETX and mRNA export factor TREX-2, respectively, to resolve R-loops [79, 80]. G-quadruplexes are stacked structures built in guanine-rich DNA regions, such as rDNA, telomeres, and promoter sequences, with DNA motifs containing four stretches of three or more consecutive guanines [82–85]. Importantly, G-quadruplex-interacting compounds that stabilize G-quadruplex formation and therefore cause stalled replication forks sensitize cells to PARP inhibitors in PARP inhibitor-resistant BRCA1-/BRCA2-deficient tumors [81, 86, 87]. The G-quadruplex stabilizer CX-5461 is currently in a clinical trial of patients with BRCA1-/BRCA2-deficient tumors [87].

2.4 Hypotheses for Tissue-Specific Carcinogenesis

Germline mutation of BRCA1 or BRCA2 causes breast and ovarian cancers including ovarian cancer-related fallopian tube and peritoneal cancers. Although such mutations also cause other cancers such as prostate and pancreatic cancers, the incidence of cancer is much higher in the breasts and ovaries. The mechanism by which this tissue specificity occurs is not completely understood at present. However, accumulated evidence indicates that estrogen signaling is an important factor contributing to tissue specificity.

Clinically, the incidence of *BRCA1/BRCA2* mutation-derived breast cancer is significantly reduced by the suppression of estrogen signaling by treatment with the anti-estrogen tamoxifen or risk-reducing salpingo-oophorectomy [88–90]. *BRCA1* mutation-derived breast cancer was also prevented by oophorectomy in a mouse model [91]. Interestingly, complementation of estrogen, but not progesterone, in these mice resulted in breast cancer development. These estrogen-dependent phenotypes support the hypothesis that the tissue specificity of HBOC is ascribed to its estrogen dependency. However, the mechanism by which HRD or other functional deficiencies attributed to germline mutation of *BRCA1/BRCA2* contribute to estrogen-dependent carcinogenesis is not completely understood. HBOC carriers possess heterozygous germline mutations of *BRCA1/BRCA2*, and a second hit in the intact allele, such as loss of heterozygosity, triggers carcinogenesis [92–94]. Because the total loss of function caused by homozygous *BRCA1/BRCA2* mutation

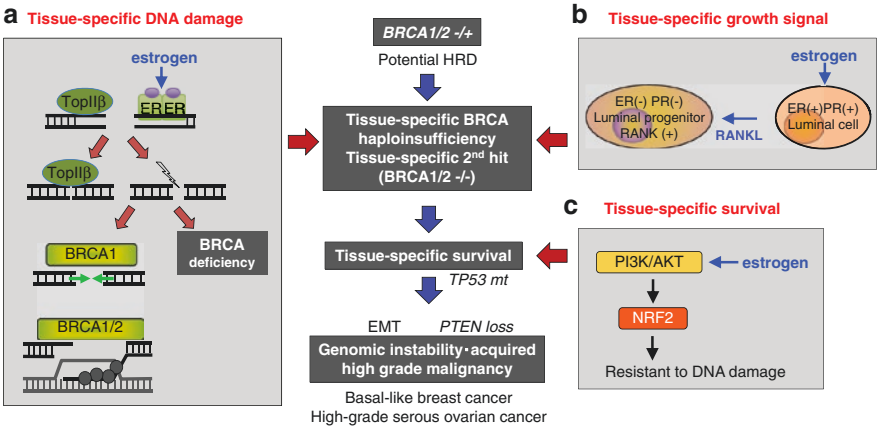

Fig. 2.4 Models of tissue-specific carcinogenesis induced by *BRCA1/BRCA2* mutation. Tissue-specific carcinogenesis caused by estrogen receptor α (ER)- and topoisomerase IIβ (TopIIβ)-dependent DNA damage (**a**), estrogen-dependent growth escalation induced by paracrine receptor activator of nuclear factor kappa-B ligand (RANKL) (**b**), and estrogen-dependent cell survival induced by NRF2 (**c**) are presented

leads to apoptosis induced by p53 activation, inactivation of the apoptosis pathway via the simultaneous mutation of *TP53* or by other conditions is required for cells to survive and develop into cancer [64, 95, 96]. Hence, tissue-specific carcinogenesis may be caused by a tissue-specific second hit, tissue-specific survival, or both (Fig. 2.4). In the next sections, representative evidence supporting these mechanisms induced by estrogen signaling is introduced.

2.4.1 Tissue-Specific DNA Damage Induced by ERα-Mediated Transcription

Estrogen-bound ERα translocates to the nucleus and functions as a transcription factor. The ERα-conducted transcription process requires topoisomerase IIβ (TopIIβ)-mediated transient truncation and rejoining of double-stranded DNA (dsDNA) to relax dsDNA distortion [97]. ERα and androgen receptor (AR) both control this process. In the case of AR, DSBs are generated via incomplete rejoining of the truncated ends of dsDNA, leading to the fusion gene *TMPRSS2-ERG*, the most common driver mutation of prostate cancer [98, 99]. Importantly, HRD, including that induced by BRCA1 depletion, accelerates the production of *TMPRSS2-ERG* [98]. This strongly suggests that AR-mediated transcription causes prostate cancer in *BRCA* mutation carriers. It is possible that a similar mechanism underlies the development of estrogen- and HRD-generated breast and ovarian cancers (Fig. 2.4a). In addition, it has been reported that TopIIβ frequently fails to rejoin truncated dsDNA ends in the presence of estrogen and remains attached to the 5′ ends of DNA [100]. BRCA1 complements this process by removing TopIIβ

adducts from the DSB ends and completing the rejoining. BRCA depletion leads to the remarkable accumulation of TopIIβ-DNA cleavage complex intermediates upon estrogen treatment. These findings suggest that BRCA1 dysfunction or HRD specifically exacerbates genomic instability in tissues expressing ERα or AR, thereby promoting carcinogenesis.

One possible contradiction for this scenario is that ovaries produce, but are not affected by, estrogen, and the ovarian epithelium does not express ERα. However, it has been suggested that the origin of *BRCA1/BRCA2* mutation-derived ovarian cancer could be the fallopian tubes opposed to the ovaries. The typical subtype of ovarian cancers attributed to *BRCA1/BRCA2* mutations is high-grade serous ovarian cancer (HGSC), which is the most aggressive phenotype of ovarian cancers and is normally detected in its advanced stage, making it impossible to distinguish its origin. However, as risk-reducing salpingo-oophorectomy has become widely performed in *BRCA1/BRCA2* mutation carriers, cancers are more likely to be detected in the early stage. Interestingly, fallopian tube cancer is more common than ovarian cancer in such early-stage cancers [101–104], suggesting that the majority of advanced cancers that were previously recognized as ovarian cancer could actually have been fallopian tube cancer. In addition ovarian cancer detected in the early stage could have originated in the fallopian tube because the fimbria cells of the fallopian tube physiologically move to the ovaries during ovulation. In a mouse model, Cre-mediated conditional inactivation of *BRCA1* or *BRCA2*, together with *TP53* and *PTEN*, which are frequently altered in ovarian cancers, in the fallopian tube led to the development of HGSC and peritoneal metastases, in addition to serous tubal intraepithelial carcinomas [105]. Importantly, inactivation of these genes in the ovarian epithelium did not promote the development of such cancers. Thus, the fimbriae of the fallopian tubes have been recognized as principal sites for HGSC in the pelvis. Because the fimbriae strongly express ERα, the DNA damage induced by ERα-mediated transcription is compatible for the tissue specificity of *BRCA1/BRCA2* mutation-induced carcinogenesis.

2.4.2 Tissue-Specific Growth Signaling by Estrogen in BRCA1-Defective Progenitor Cells

Breast cancers caused by germline *BRCA1* mutation are commonly the triple-negative subtype, which lacks ERα, progesterone receptor (PR), and human epidermal growth factor receptor 2 expression, and most of them are classified as basal-like cancer, which exhibits a similar gene expression profile as mammary basal stem cells. However, basal-like breast cancer caused by germline *BRCA1* mutation originates in ERα- and PR-negative luminal progenitor cells but not in basal stem cells. *BRCA1* inactivation in basal stem cells leads to adenomyoepithelioma, but not to basal-like breast cancer, whereas that in the luminal progenitor cells leads to basal-like breast cancer [106, 107]. Interestingly, BRCA1-defective luminal progenitor cells are hypersensitive to estrogen and progesterone despite being negative for ERα and PR. The mechanism underlying the contradiction is the paracrine effect of

neighboring luminal cells. ERα- and PR-positive mature luminal cells secrete RANKL in response to estrogen signaling, thereby promoting the growth of BRCA1-defective and ERα- and PR-negative luminal progenitor cells and resulting in the development of basal-like cancer [108–111] (Fig. 2.4b). Of note, it has been reported that the RANK inhibitor denosumab prevented *BRCA1* mutation-derived breast cancer in a mouse model [111].

2.4.3 Tissue-Specific Survival Mediated by Estrogen

In addition to a tissue-specific second hit, it has been proposed that estrogen-mediated survival in BRCA1-deficient cells may be the basis for tissue-specific carcinogenesis [112]. In support of this hypothesis, estrogen-induced NRF2 reactivation in BRCA1-defective cells has been revealed to generate tissue specificity (Fig. 2.4c). NRF2 is a master transcription factor of antioxidant pathways that protects cells against oxidative stress-induced DNA damage [113, 114]. BRCA1 physically interacts with NRF2 and promotes its stability and activation by blocking its interaction with the ubiquitin ligase KEAP1 [115]. Thus, BRCA1 deficiency suppresses NRF2 responses and leads to cell death with reactive oxygen species accumulation. Notably, estrogen increases NRF2 protein expression and activity through activation of the PI3K-AKT-mTOR pathway [116]. In vivo, the survival defect of BRCA1-deficient mammary epithelial cells is rescued by pregnancy, and estrogen administration stimulates the growth of BRCA1-deficient mammary tumors in male mice.

2.5 Perspectives

The mechanisms underlying the biology of carcinogenesis induced by *BRCA1/BRCA2* deficiency have intensively been studied for more than two decades. Although the initial questions have not been entirely clarified, tremendous progress has been achieved in the basic understanding of the responsible mechanisms, leading to many benefits in the treatment and prevention of HBOC. This includes synthetic lethality mediated by PARP inhibitors. Future works may focus on the mechanisms of acquired resistance and strategies to overcome this resistance. In addition, surgical and medical prevention strategies should also be improved on the basis of the mechanisms revealed by those efforts.

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