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REVIEW ARTICLE

A comprehensive analysis of *BRCA2* gene: focus on mechanistic aspects of its functions, spectrum of deleterious mutations, and therapeutic strategies targeting BRCA2-deficient tumors

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Abstract

*BRCA2*is the main susceptibility gene known to be involved in the pathogenesis of breast cancer. It plays an important role in maintaining the genome stability by homologous recombination through DNA double-strand breaks repairing, by interacting with various other proteins including RAD51, DSS1, RPA, MRE11, PALB2, and p53. *BRCA2*-deficient cells show the abnormalities of chromosome number. BRCA2 is also found to be involved in centrosome duplication specifically in the metaphase to anaphase transition. Inactivation or depletion of BRCA2 leads to centrosome amplification that results in unequal separation of chromosomes. BRCA2 localizes with central spindle and midbody during telophase and cytokinesis. Inactivation or depletion of BRCA2 leads to multinucleation of cell. Around 2000 mutations have been reported in *BRCA2* gene. BRCA2-deficient tumors are being taking into consideration for targeted cancer therapy by using different inhibitors like poly ADP-ribose polymerase and thymidylate synthase. The present review focusses on the role of BRCA2 in various critical cellular processes based on the mechanistic approaches. Mutations reported in the *BRCA2* gene in various ethnic groups till date have also been compiled with an insight into the functional aspects of these alterations. The therapeutic strategies for targeting BRCA2-deficient tumors have also been targeted.

Keywords Breast · Carcinoma · BRCA2 · Homologous recombination · Telophase

Abbreviations

BRCA1	Breast cancer type 1
BRCA2	Breast cancer type 2
RPA	Replication protein A
MRE11	Meiotic recombination 11
PALB2	Partner and localizer of BRCA2
SSB	Single-strand binding protein
HR	Homologous recombination
DDR	DNA damage response
ATR	Ataxia telangiectasia and Rsd3-related
ATM	Ataxia telangiectasia mutated
EMSY	BRCA2 interacting transcriptional repressor
AURKA	Serine/threonine kinase Aurora-A
UCV	Unknown classified variants
LOH	Loss of heterozygosity
NLS	Nuclear localization signal
DBD	Double-strand break domain
TS	Thymidylate synthase
PARP	Poly ADP-ribose polymerase
FU	Fluoropyrimidines

Introduction

Breast cancer is the most common diagnosed cancer in women. It has become the second leading cause of death worldwide [1]. North America and Western European populations show the high incidence rates of breast cancer, whereas African and Asian populations show the low incidence rates [2]. A total of 131,000 deaths from 464,000 cases of breast cancer were reported in Europe in 2012 and 40,000 deaths from approximately 232,000 new breast cancer cases reported in the USA in 2013 [3]. Incidence rates of breast cancer have doubled or tripled in Japan, Hong Kong, Singapore, and Korea over the past 40 years [4]. In India, age-standardized incidence rates of breast cancer range from 15 to 29 per 100,000 showing an elevated trend in the last

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2 decades. Incidence rates of breast cancer range from 14.6 to 19.6 per 100,000 women in the states of Northeast India (Aizawl district, Kamrup and Imphal) [2].

Breast cancer is a complicated heterogeneous disease. Various genetic and epigenetic alterations are responsible for malignant breast tumor progression which in turn results in activation of various hallmarks of cancer [1]. Approximately 80% of the breast cancers are sporadic [5]. The incidence of familial breast cancer is around 10%. 20–60% of these are associated with the mutations in tumor suppressor genes. The main breast cancer susceptibility genes are *BRCA1* and *BRCA2* situated on chromosome 17q21 and 13q12, respectively. These high penetrant genes are known to play an important role in fundamental processes of the cell including cell proliferation, cell development, transcription, centrosome duplication, and DNA repair mechanism [6, 7].

BRCA2 discovered in 1995, 15 months after the discovery of *BRCA1* [8], is a large gene bearing 27 exons and 3418 amino acids. There are several functional domains present in human BRCA2. It has an N-terminal domain, whose role is uncertain and has eight BRC repeats in the center, consisting of approximately 1000 amino acids that bind to the protein Bacterial RecA homolog DNA recombinase (RAD51). The C-terminal of BRCA2 has five distinct domains. The first domain is the helical domain that comprises of 190 amino acids followed by three structurally related domains named as OB1, OB2, and OB3. These three domains bear approximately 110 amino acids which exhibit structural similarity to the oligonucleotide/oligosaccharide-binding (OB) fold that exists in many eukaryotic and prokaryotic single-stranded DNA-binding proteins, such as RPA (replication protein A) and SSB (single-stranded DNA-binding protein) (Fig. 1). BRCA2 C-terminal shows high affinity toward single- and double-stranded DNA because of these three OB domains [5].

Functions of *BRCA2*

BRCA2 confers many functions as its protein maintains the genome stability by homologous recombination through DNA double-strand breaks repairing. Its elevated gene expression in response to the activity of estrogen has been related to cell cycle progression. Loss of heterozygosity at *BRCA2* locus is associated with mutations in *BRCA2* as it results in more than 50% of sporadic breast tumors. *BRCA2* gene expression is regulated by various known and unknown factors which are responsible for altered expression of *BRCA2* resulting in cancer [6].

Role of BRCA2 in homology-directed repair

BRCA2 is necessary for the homology-directed repair mechanism. The mechanism is mediated by the formation of RAD51 nucleoprotein filament on single-stranded DNA [9]. There is elevated sensitivity toward specific DNA-damaging agents caused by BRCA2 heterozygosity and low level of RAD51 succeeding irradiation [10]. Human RAD51 is a homolog of yeast RAD51 and bacterial RecA and likewise plays a central role in homology recombination [6]. RAD51 acts on single-stranded DNA that is produced by nucleolytic abscission of a primary lesion [11]. Likewise, RAD51 uses abscised DNA double-strand breaks to form a displacement loop by pervading a homologous double-stranded DNA [14].

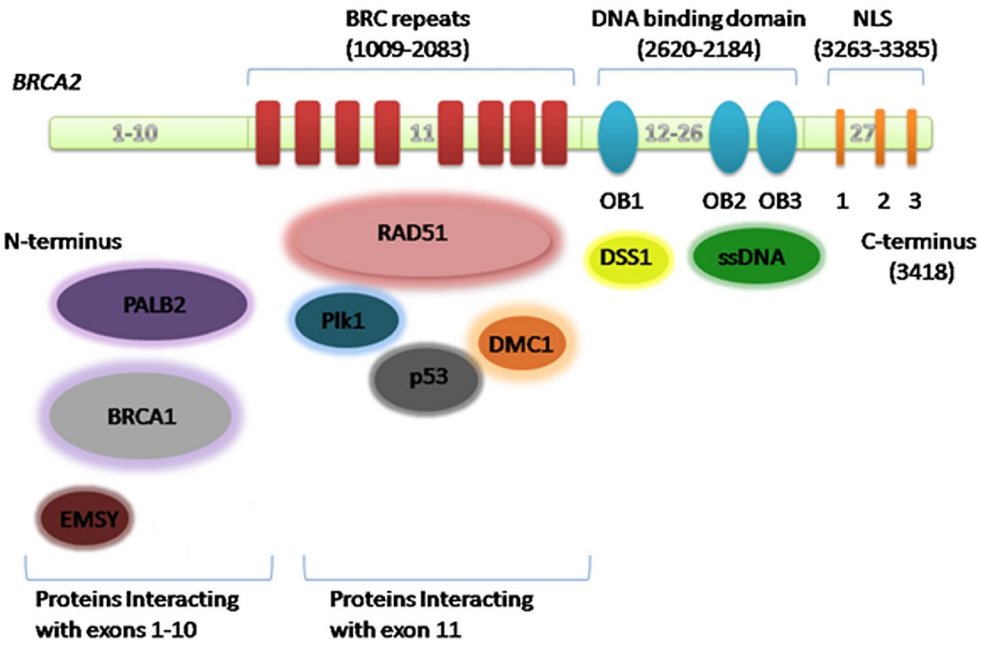

Fig. 1 Structure of *BRCA2* and its motifs for the interaction with various proteins

The BRC repeats of human BRCA2 are eight conserved RAD51 interaction motifs necessary for homology-directed repair [9]. Two types of BRC domains interact with RAD51. The first category of BRC domains forms a nucleoprotein filament by targeting RAD51 to single-stranded DNA (Fig. 1). It downregulates the RAD51 ATP hydrolysis and stabilizes this activated nucleoprotein. The other category of BRC domain has a role in the prevention of nucleation of double-stranded DNA by RAD51 [15]. Polypeptide like BRCA2 that has two BRC repeats and a DNA-binding domain has a capacity to enhance the presynaptic assembly of RAD51 on RPA-coated single-stranded DNA, leading to the suggestions that BRCA2 facilitates RPA–RAD51 exchange. RPA interaction is mediated by the BRCA2-associated deletion of SUV3 suppressor (DSS1) protein that functions as DNA mimic to facilitate RPA–RAD51 exchange on single-stranded DNA [11]. BRCA2 has direct control over RAD51. This control is applied at two levels; RAD51, which does not possess a consensus nuclear localization signal (NLS), is incapably transported to the nucleus, in some BRCA2-deficient cells. BRCA2, which has nuclear localization signal, can transport the RAD51 to the nucleus and afterward to the site of DNA damage processing [6]. A RAD51 interaction site has been identified at the C-terminal of BRCA2 apart from BRC region, bearing 200 amino acids. This region is conserved, but its sequence is different from BRC repeats [9].

The homologous recombination (HR) mechanism is conserved and error free, needed for fortunate navigation through S phase. In S phase of cell cycle, HR restores the stalled or collapsing replication forks that encountered a lesion [11]. Replication forks are endangered to secondary structures or unrepaired DNA damage. A replication fork that has the ability to restart after arrest is the stalled replication fork, whereas a collapsed replication fork results in replication dependent DNA double-strand breaks [14]. HR is necessary for the preservation of replication forks, conservation of telomere, accurate segregation of chromosomes in meiosis, and maintenance of genome integrity. Simultaneously, specious HR events in the genome in between the random repeat sequences may result in deletions and duplications. Due to such rearrangements, loss of heterozygosity can cause loss of tumor suppressor gene or translocation of proto-oncogene downward to the promoter that leads to cancer [16].

Various intracellular and intercellular signaling events and enzyme activities caused by the induction of DNA damage are collectively termed as DNA damage response (DDR) that finally leads to regulation of DNA replication, cell cycle arrest, and repair of DNA damage. DDR also has an impact on downstream cell fate decisions including cell death or senescence [17]. There are two mechanisms by which DNA double-strand breaks are repaired in mammalian cells: One is non-homologous end joining in which broken double-strand breaks are ligated together simply without homology and other is homology-directed repair in which double-strand breaks are organized into exposed single strands that can either couple with single-stranded DNA homologue or initiate homologous recombination by disrupting double-stranded DNA [6]. Homologous recombination repair pathway depends upon the availability of damaged free sister chromatid DNA, whereas non-homologous end-joining pathways are independent of the presence of replicated DNA but are less accurate [17].

DSB repair requires end processing for which implication of nuclease meiotic recombination 11 (MRE11) is needed. As MRE11 is a nuclease, it promotes 5′–3′ polymerization critical for homology-directed repair and also 3′–5′ exonuclease activity which trim the DNA ends for repair. BRCA2 has a protective role during replication fork stalling, and specifically it prevents nascent strands at stalled forks from degradation by MRE11 [9].

DSS1 is used as a biomarker for various cancers and is also the candidate gene for split foot/hand syndrome. It is a small and highly acidic protein, consisting of 70 residues. Like BRCA2, DSS1 is also critical for DNA double-strand breaks and replication fork repair. It has an important role to play in proteasomal assembly and different aspects of RNA metabolism. It has been reported that for proteasomal assembly in *S. Cerevisiae*, Dss1 acts as a chaperone and in *S. pombe* it works as an ubiquitin-binding subunit. DSS1 is coupled with a helical region present adjacent to OB1 of BRCA2 (Fig. 1). It has many acidic residues present in its solvent exposed loop by which it targets RPA and acts as a DNA mimic to facilitate the assembly of the RAD51 [11].

DNA damage results in single-stranded breaks and double-stranded breaks in DNA. Ss DNA breaks are sensed by RPA leads to the activation of transducer ATR, whereas ds DNA breaks are sensed by H2AX that leads to the activation of transducer ATM. ATM/ATR results in activation of BRCA1, BRCA2, and p53 genes. *BRCA2* binds to RAD51 and DSS1 resulting in RAD51–BRCA2–DSS1 complex that forms displacement loop. PALB2 interacts with BRCA1 and facilitates BRCA2 for the stable intranuclear localization so that BRCA2 can accomplish homologous recombination successfully. p53 interacts with RAD51 and activates apoptosis or cell cycle arrest.

BRCA2 is important for the interaction of RAD51 to form D loop. Ataxia telangiectasia mutated/ataxia telangiectasia and Rad3-related (ATM/ATR) and histone variant (H2AX) are damage sensors that lead to the formation of BRCA2–RAD51–DSS1 complex (Fig. 2).

Another protein p53 (Cellular tumor antigen) also interacts with RAD51 in the region between 125 amino acids to 220 amino acids important for suppression of homologous recombination. It can activate apoptosis or cell cycle arrest

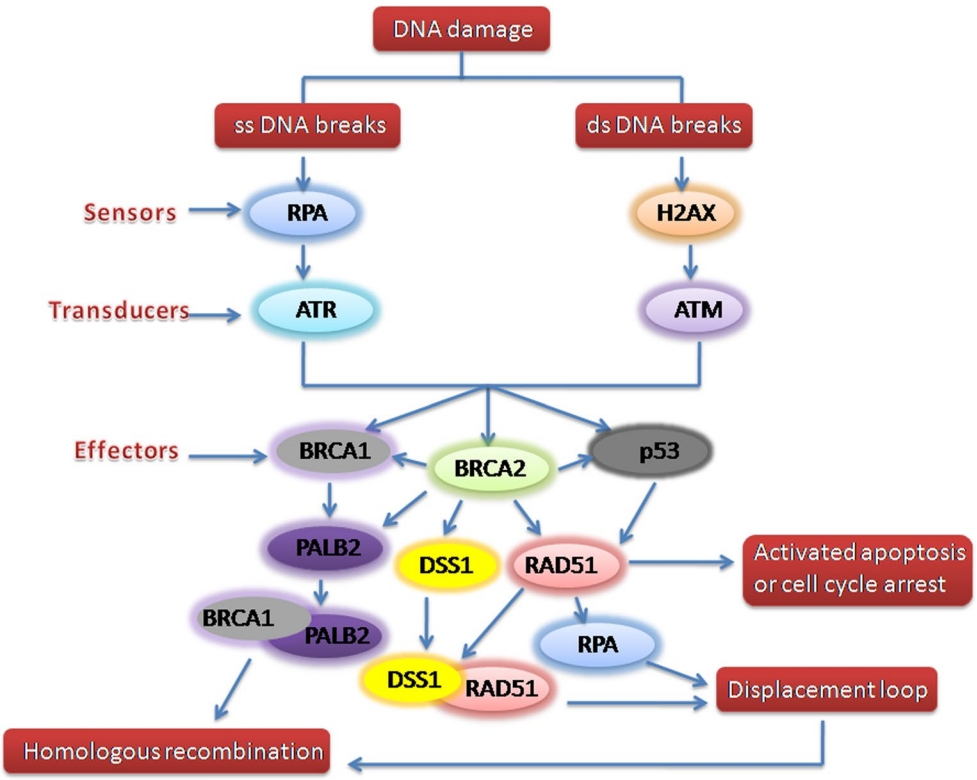

Fig. 2 Role of BRCA2 in DNA DSB repair by homology recombination; DNA damage results in single-stranded breaks and double-stranded breaks in DNA. Ss DNA breaks are sensed by RPA leading to the activation of transducer ATR, whereas ds DNA breaks are sensed by H2AX that leads to the activation of transducer ATM. ATM/ATR results in activation of BRCA1, BRCA2, and p53 genes. *BRCA2* binds to RAD51 and DSS1 resulting in RAD51–BRCA2–DSS1 complex that forms displacement loop. PALB2 interacts with BRCA1 and facilitates BRCA2 for the stable intranuclear localization so that BRCA2 can accomplish homologous recombination successfully. p53 interacts with RAD51 and activates apoptosis or cell cycle arrest

depending on severity of damage. p53 hinders RAD51-mediated strand exchange and prevents oligomerization. p53 also interact with BRC domain of BRCA2 via its DNA-binding domain (Fig. 1). BRCA2 downregulates the transcriptional activity of p53 [16].

DNA DSB happens during the S phase of the cell cycle following endogenous or exogenous damage resulting in single-stranded DNA strands. BRC repeats of *BRCA2* bind to RAD51 (DNA recombinase) and DSS1 at exon 11 resulting in RAD51–BRCA2–DSS1 complex. This complex acts together and repairs the damage by homologous recombination using sister chromatids as template [8]. c-myc is one of the most common amplified oncogene in different types of human cancers, facilitating transformation of thousands of genes through transcriptional regulation of c-myc leading to DNA double-strand breaks and disruption of DNA damage repair mechanism. c-myc upregulates the miRNAs specifically miR-1245 that are associated with tumorigenesis, resulting in suppression of BRCA2 expression via binding to its 3′UTR transcript [18]. A novel protein BRCA2 interacting transcriptional repressor (EMSY) is located at 11q13.5 that is found to be amplified in 13% of breast cancers. It bears 1322 amino acids and binds to BRCA2 at exon 3 (Fig. 1). It inhibits activation of reporter raised by a fusion protein named as BRCA2-GAL4. It has a role in DNA damage response as it colocalizes with phosphohistone 2AX (DNA damage marker) and presumably with BRCA2. It has been reported that BRCA1/2 inactivation leads to EMSY amplification and, thereby, affects various cellular processes [19]. It induces genomic instability and also has the ability to invalidate the G1 arrest persuaded by DNA damage.

BRCA2 acts as a scaffold protein that facilitates the formation of high-order complexes of proteins of biological importance. It interacts with copious protein partners including BRCA1, RAD51, DSS1, PALB2, Plk1, p53, and oncoprotein EMSY.

Recent studies have identified that N-terminal of BRCA2 is associated with partner and localizer of BRCA2 (PALB2) and C-terminal is essential for the interaction with RAD51 [12]. Approximately, 50% of PALB2 is associated with BRCA2 and more than 50% forms complexes with it (Fig. 1). PALB2 facilitates BRCA2 for the stable intranuclear localization so that BRCA2 can accomplish homology recombination successfully [20].

In an endogenous complex of proteins BRCA2 coexist with BRCA1 and this interaction is mediated by PALB2. At DNA damage sites, PALB2 colocalizes with both BRCA2 and BRCA1. BRCA1 might work as an upstream regulator of BRCA2 and PALB2. Interaction between PALB2 and BRCA1 is critical for homology recombination [13]. N-terminal coiled-coil domain of PALB2 interacts with BRCA1, and C-terminal WD40 domain interacts with BRCA2. PALB2 self-interact via its coiled-coil domain [14]. The interaction of BRCA2 with various proteins during recombination repair and their functions are summed up in Fig. 2.

Role of BRCA2 in cell cycle regulation

Genomic instability is classified into two types; one is microsatellite instability which is related to a mutator phenotype, and other is chromosome instability which is related to gross chromosomal abnormalities. Centrosome plays an important role in maintaining the chromosome stability by facilitating the formation of the bipolar mitotic spindle during cell division. Various chromosome abnormalities which cause unequal distribution of chromosomes leading aneuploidy or polyploidy of daughter cells are usually due to the multipolar mitotic spindle. Recent studies have revealed that apart from DNA repair, BRCA2 is also a participant in cytokinesis. *BRCA2*-deficient cells show the abnormalities of chromosome number on account of BRCA2 dysfunction [8].

Cytokinesis starts with the obstruction of a membrane-bound actinomycin ring and ends up by membrane fusion resulting in two daughter cells. The formation of cleavage furrow depends on spindle midzone that comprises a microtubule-based structure formed at anaphase modulated by several regulators of cytokinesis. Interestingly, accumulation of BRCA2 has been reported at the spindle midzone [21]. Normally cytokinesis is instigated by the actinomycin contractile ring. Accumulation of myosin II does not occur within the furrow, in BRCA2-depleted cells [8].

According to some studies, BRCA2 is found to be involved in centrosome duplication specifically in the metaphase to anaphase transition. Inactivation or depletion of BRCA2 leads to centrosome amplification which in turn results in unequal separation of chromosomes. It has been observed that during telophase and cytokinesis BRCA2 localizes on central spindle and midbody and inactivation or depletion of BRCA2 leads to multinucleation of the cell. In G2-M transition, BRCA2 is found to interact with HMG20b which is a kinesin-like coiled protein, and any disruption in this interaction leads to defects in the completion of the cell division [22]. Suppression of BRCA2 in cell shows a delay in G2-M transition, a period when a mitotic kinase Plk1 phosphorylates BRCA2 (Fig. 1) [23]. In M phase, Plk1 hyperphosphorylates BRCA2 and also dephosphorylates the protein when cell escapes M phase, suggesting a role of BRCA2 in regulating M phase progression [10]. During spermatogenesis, the expression of *BRCA2* is high, and at the time of synaptonemal complexes formation, the protein is confined to meiotic chromosomes suggesting a role of *BRCA2* in meiotic recombination as well [24]. HMG20b is a member of (HMG) high-mobility group of DNA-binding proteins that are not sequence specific and also have two coiled-coil regions at C-terminal that are kinesin like. HMG20b directly interacts with human BRCA2, and its depletion has been reported to delay mitosis resulting in anomalies in cell division and generation of binucleate daughters [25]. There is a critical role of serine/threonine kinase Aurora-A (*AURKA*) in the maintenance of genetic stability. It regulates bipolar spindle assembly, centrosome separation, and chromosome segregation. A number of centrosomes and multipolar spindles increase with the amplification of Aurora-A. Although the mechanism is still unclear, it is suggested that Aurora-A and BRCA2 work synergistically and regulate genomic instability [26]. Aurora-B and BRCA2 proteins are involved in the formation of midbody. Aurora-B is also controlled by another protein named BARD1, which is a BRCA1-associated ring domain protein 1 suggesting a link between Aurora-B, BARD1, and BRCA2 [27]. Dosage suppressor of Mck1 Homolog (DMC1), a paralogue of RAD51 particularly for meiosis needed for meiotic recombination, has been found to interact with BRC repeats of *BRCA2*. Like RAD51, it forms octameric rings that bind to DNA and catalyze strange exchange by establishing helical nucleoprotein filaments. RAD51 and DMC1 both are found to colocalize at the time of meiotic recombination to nuclear foci. BRCA2 is found to coordinate the activities of both RAD51 and DMC1 as reduced expression of BRCA2 results in reduced number of DMC1 and RAD51 foci [28].

Previous studies have demonstrated that BRCA2 is required for BubR1 acetylation which is needed for proper chromosome segregation in mitosis. BRCA2 forms a complex with BubR1 at prometaphase. The absence of BRCA2 abolishes the acetylation of BubR1 leading to reduction in its levels in mitosis. Reduced level of BubR1 weakens the spindle assembly checkpoint, hence resulting in aneuploidy [29].

BRCA2 interacts with HMG20b and its depletion leads to delay in mitosis, anomalies in cell division, and generation of binucleate daughters. Aurora-A and BRCA2 works synergistically and regulates chromosome segregation. Aurora-B and BRCA2 proteins are involved in the formation of midbody, and knockdown of Aurora-B results in anomalies in cell division. BRCA2 also coordinates the activity of DMC1 which is important for meiotic recombination and chromosome segregation. BubR1 regulates bipolar spindle assembly and centrosome separation by interacting with BRCA2 (Fig. 3).

Involvement of BRCA2 and its interaction with various proteins including HMG20b, DMC1, BubR1, Aurora-A, and Aurora-B along with their effects in cytokinesis process are depicted in Fig. 3. Aurora-B is a cytokinetic protein that colocalize with BRCA2. Knockdown of Aurora-B results in abnormal cell division and polyploidy. It is now clear that whenever there is disruption in BRCA2 function, cytokinesis is also impaired and there is elevated incidence of binucleated cells [8].

Involvement of *BRCA2* in various other cancers

Apart from breast cancer, BRCA2 is known to be mutated in other cancers including male breast cancer, ovarian cancer,

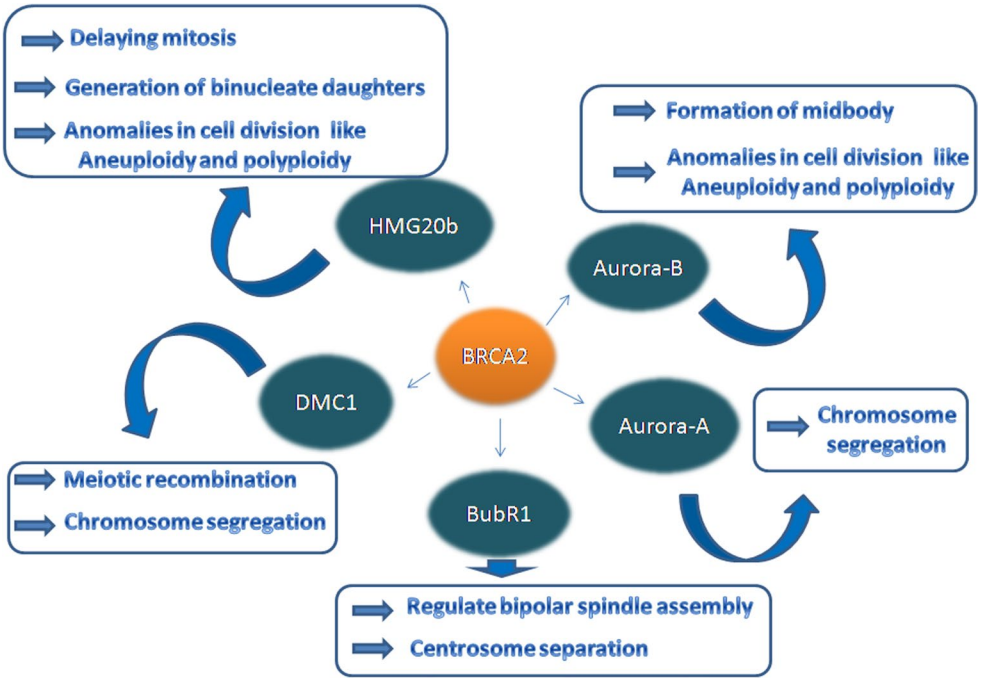

Fig. 3 BRCA2 interacts with various proteins involved in cell cycle regulation. BRCA2 interacts with HMG20b, and its depletion leads to delay in mitosis, anomalies in cell division, and generation of binucleate daughters. Aurora-A and BRCA2 work synergistically and regulate chromosome segregation. Aurora-B and BRCA2 proteins are involved in the formation of midbody and knockdown of Aurora-B results in anomalies in cell division. BRCA2 also coordinates the activity of DMC1 which is important for meiotic recombination and chromosome segregation. BubR1 regulates bipolar spindle assembly and centrosome separation by interacting with BRCA2

prostate cancer, and pancreatic cancer. In addition to breast cancers, the carriers of BRCA2 mutations have greater risk of getting ovarian, pancreatic, prostatic, bile duct, gall bladder, and stomach cancers [30].

Male breast cancer is a heterogeneous disease and has many molecular differences in contrast to female breast cancer. Approximately, 4–14% of incidence of male breast cancer is due to mutations in *BRCA2* [31]. *BRCA2* has higher mutation rate than *BRCA1* in male breast cancer, in comparison with female breast cancer [32]. 5–10% men with mutations in *BRCA2* gene will eventually result in male breast cancer [33]. Higher prevalence rates of BRCA2 mutations are observed in Sweden, Hungary, Iceland, and Ashkenazi Jewish populations [34].

In heredity ovarian cancer, *BRCA* genes have a central role to play. However, the mutations of *BRCA2* are less frequent than *BRCA1* [35]. Approximately, 30–40% of sporadic ovarian cancers are caused due to the loss of heterozygosity (LOH) occurring at *BRCA2* locus [36].

The mutations in *BRCA2* gene have also been implicated in the pathogenesis of prostate cancer. Specifically, 999del5 single *BRCA2* mutation has been observed in Iceland. Approximately, 2.7% of patients with prostate cancer carried this mutation [37].

Pancreatic carcinoma is the fifth common cause of death in the USA. One of the main causes of pancreatic cancer is a deletion at the *BRCA2* locus. 6174delT mutation has been found in approximately 0.8% pancreatic cancer patients in USA [38]. Also, *BRCA2* mutations along with *BRCA1* have been reported to be responsible for approximately 2% of colon cancer cases in Jewish population. According to a study, 6174delT BRCA2 mutation is responsible for increased risk of stomach, esophageal, and hematopoietic carcinomas. Apart from carcinomas, BRCA2 is also related to cutaneous and ocular melanomas [34].

Variants of *BRCA2*

More than 2000 mutations have been reported in *BRCA2* gene including many single nucleotide substitutions, deletions, and insertions located in coding or noncoding sequences. Usually, the site of mutations is exon 10 and exon 11 which commonly include insertions or deletions that elevate the premature stop codon ending and missense alterations in gene, resulting in functional disturbance in BRCA2 protein [39]. Unknown classified variants (UCVs) are the pathological consequence of numerous missense mutations accounting for over 50% of all announced mutations in *BRCA2* [5]. It has been reported that usually the segments that are separated out of *BRCA2* contain Rad-51-binding motif, nuclear localization signal (NLS) and double-strand break domain (DBD) that are critical for its function and alterations in these regions might result in the malfunctions of the protein [39].

International Agency for Research on Cancer (IARC) has given a standardized method five-tier system (classes 1–5) to classify variants. According to this system, 1 is considered non-pathogenic and 5 is considered definitely pathogenic. By using this system, Biswas et al. classified 8 variants including G25R, W31C, W31R, F2406L, I2944F, S2695L, E3002K, and N3124I. These variants are located at the N-terminal of PALB2 and C-terminal of DNA-binding

domain that necessary for its interaction with BRCA2. Variants W31C, W31R, E3002K, and N3124I lead to disruptive protein structure, whereas I2944F has no effect on protein structure. Another variant Phe2406 of BRCA2 is necessary for its interaction with DMC1 [40].

Global distribution of *BRCA2* mutations

Various polymorphisms have been reported in BRCA2 gene around the globe. These are broadly classified into four types: silent, non-synonymous, unclassified whose impact on BRCA2 gene is unclear, and harmful. In Czech population, c.475G>A and c.7007G>A are the deleterious variants of *BRCA2*. Likewise, associated variants that have been identified are S1832P, T2766I, N2781I, and K2860T. It was predicted that these variants are non-synonymous having negative effect on BRCA2 function in Danish population. Another variant, c.9023A/C, causing replacement of proline with histidine, leads to establishment of a turn in BRCA2 protein structure. Further, c.72A>T, c.68−80insT, c.793+34T>G, and 4088insA mutations have been identified in a Finnish population. According to Miramar et al., K3083E or 9475A>G are non-pathogenic polymorphisms that introduce lysine-to-glutamine exchange resulting in truncated and non-functional protein. 6174delT is the founder mutation in *BRCA2* reported in Ashkenazi Jewish population [39]. Wordwide most frequent polymorphisms in *BRCA2* gene were reported to be c.475G>A, c.7007G>A, c.476−2>G; c.7007G>A; c.8755−1G>A; c.9117+2T>A and c.9118−2A>G,1342A>C, S1832P, T2766I, N2781I and K2860T, c.72A>T, K3083E or 9475A>G [39].

The mutations identified in *BRCA2* gene reported in European and American populations are summed up in Table 1. It has been reported that N372H *BRCA2* variants were associated with elevated breast cancer risk in women from UK. According to some recent studies AluSx/Sx (dup 9700) and AluSx/Sp (del2352ins12) rearrangements have been observed in English, Dutch, and German origin populations. In Swedish population, 4486delG is the frequently occurring founder mutation located at exon 11 of *BRCA2*. In Italian population, variants c.7963C>T, c.2950G>T, and c.289G>T causing truncated BRCA2 protein by producing premature stop codon have been reported. Moreover, two new mutations 6174delT and 7525_7526insT were identified in exon 15 of *BRCA2* gene. Both mutations are premature stop codon insertion/deletion. These mutations cause disruption of nuclear localization signals that will interfere *BRCA2* functions, including DNA repair and nuclear penetration.

Table 1 *BRCA2* gene mutations in European and American populations

Country	BRCA2 mutations	Cancer	References
Canada/USA	185delAG, 5382insC, 6174delT	Breast	[41]
Iceland	999del5, 6174delT, 995delG	Male and female breast	[41]
USA, France	982del4	Male and female breast, ovarian	[43]
Sweden	4486delG	Male and female breast	[43]
Netherland	6503delTT, 8295T4A, 9900insA, 5579insA, 5573insA, 7647delTG	Breast, ovarian	[43]
France and Spain	9254del5	Male and female breast, ovarian	[43]
Britain, Belgium, Netherland and Sweden	6503delTT, 9303ins31	Male and female breast, ovarian	[43]
Germany, Canada, USA, Spain, France, Switzerland, Italy and Belgium	3034del4, 5910C3G, 6676insTA, 2041insA, c.289G>T	Male and female breast, ovarian	[43]
France (French-Canadian)	2816insA	Breast, colon, lever, lung, bladder, stomach, skin	[44]
	G6085T	Male and female breast, prostrate, colon, skin	[44]
	6503delTT	Breast, brain, colon, lymphoma	[44]
	8765delAG	Male and female breast, ovarian, bone, colon, fallopian tube, prostrate, stomach, lung, pancreas, kidney, liver, mouth, throat, uterine, brain	[44]
Canada, France, Hungary, Sweden, USA and UK	6174delTT	Male and female breast, ovarian	[43]
Hungary, Sweden and UK	9326insA	Male and female breast	[43]
Spain	185delA, 3374delA, 3492insT, 4082delA, 6076del4, 6857delAA, 6857delAA, 3034del4, 8297insTT, 9254del5, E3096X	Male and female breast, ovarian	[45]

This alteration has an important role in PARP inhibitors resistance. The founder mutation c.156−157insAlu, found in the exon 3 of *BRCA2*, in Portuguese population is a targeted insertion instead of random insertion of 350 base pairs from the subfamily of AluYa5 resulting in its transactivation domain loss [39].

Mutations identified in *BRCA2* gene in Asian populations are listed in Table 2. Pakistan has more incidence of breast cancer as compared to other Asian populations although BRCA1 and BRCA2 mutations only contribute 12% of all breast cancer cases. Missense 3337C>T mutation in *BRCA2* is the founder mutation of this population. In Punjab itself, the incidence of *BRCA2* mutation is 33% against multiracial women. Surprisingly, in Indian population, the 185delAG deletion has the high incidence of 16.3% nearly similar to 18% in Ashkenazi Jews [39, 42].

Therapeutic strategies for targeting BRCA2-deficient tumors

Thymidylate synthase (TS) is a mediator of DNA replication and genomic integrity in the cell. This enzyme is a well-established target for many anticancer drugs like fluoropyrimidines (5-FU) and pemetrexed (folate analogs). Thymidylate synthase can be used for the downregulation of BRCA2 to induce complementary lethality [53]. Poly ADP-ribose polymerase (PARP), a member of nuclear protein family with scaffolding properties, has the ability to recruit DNA repair proteins. The most important member of this family is PARP1 which is incorporated in the base excision repair mechanism. PARP1 binds to the catalytic subunit of the DNA protein kinase and assemble ATM (ataxia telangiectasia mutated), topoisomerase1, and MRE11, all required in DSB repair. Thus, PARP inhibitors are used for the treatment of BRCA1/2-deficient tumors. Iniparib with gemcitabine/carboplatin are used for triple-negative breast cancer [54]. BRCA-deficient cells have defects in DSB repair, and therefore, they are more dependent on PARP1 for the maintenance of genomic integrity [55]. BRCA2-deficient cells are sensitive to PARP1 inhibitors because of the incapability to repair the collapsed replication forks [56]. Olaparib is another PARP1 inhibitor which is in phase III trial of treatment for BRCA-deficient tumors [57]. Recent studies have identified that BRCA mutated tumors also exhibit sensitivity toward DNA-damaging drugs that are platinum based. Cisplatin a platinum-based drug alone or in combination with PARP inhibitor (AZD2281) can be used against BRCA-deficient tumors [58].

Single-stranded DNA can form secondary structures under physiological conditions in vitro. These structures are known as G4s. It has been observed that telomere G-rich DNA also adopts G4 configuration in vivo. PDS is a G4 interacting compound that stabilizes G4 configuration as it has higher binding specificity for G4s in comparison with duplex DNA. Therefore, PDS can be used against BRCA-deficient cells as it decreases their viability [59].

Conclusion and future perspectives

BRCA2 is the main susceptibility gene for breast cancer. It encodes for large size protein that forms high-order complexes with other proteins of biological importance. It interacts with copious protein partners including BRCA1, RAD51, DSS1, PALB2, Plk1, P53, and oncoprotein EMSY. Moreover, this large protein has functions in many important pathways including homology-directed repair, genome instability, centriole duplication, and cytokinesis. It has become the focus of intense attention as its functions provide important insight into the molecular mechanisms underlying the tumorigenic process. In addition, BRCA2 is a promising target in therapeutic strategies. PARP inhibitors are used for the treatment of BRCA1/2-deficient tumors, while iniparib with gemcitabine/carboplatin is used for triple-negative breast cancer. We are now in a position to drill very deep into the mechanisms of the regulation of DNA repair and the cell cycle control by *BRCA2* gene and its protein,

Table 2 *BRCA2* gene mutations in Asian populations

Country	*BRCA2* mutations	Cancer	References
Russia	695insT, 1528del4, 9318del4, S1099X	Breast	[39]
Japan	5802delAATT, 8732C>A, c.2835C>A	Breast, ovarian	[46]
China	7883delTTAA, 2811delACAA, 7805del370, 9098insA, 9118insA, 5804del4, 2822del4, 9143delT, 9325delA, T711G, G8415T, G8415T, C7280G, 5028delC	Breast, ovarian	[47, 48]
Pakistan	5057delTG, 6679insAA, 5869delAAAT, 3337C4T	Male and female breast, Ovarian	[49, 50]
India	185delAG	Breast	[39]
Africa	c.2826 2829del, c.6447 6448dup, c.5771 5774del, 5999del4	Breast	[39]
Korea	c.2259delT, c.865A>C, c.1114A>C, c.2350A>G, c.2971A>G, c.4334A>C, c.5785A>G, c.6091A>G, c.10234A>G	Breast	[51]
Iran	c.6033_6034 insGT, c.9317G>A (Trp3106Ter)	Male and female breast, liver	[32, 52]

but still many more details are left to be discovered. The next decade will yield many new advances as the genetic tools that have emerged, such as RNAi, give us the ability to perform more gene discovery in these mechanisms and detailed analysis of protein functions, especially the roles of the many kinase substrates are being identified. BRCA2 also serves the target in therapeutic strategies for many drugs like PARP inhibitors. The mechanism of PARP inhibition may serve to sensitize endocrine sensitive and/or HER2-positive breast tumors to DNA-damaging chemotherapy. Each of the aforementioned challenges are the areas that deserves further study and are the subject of ongoing clinical investigation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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