

BRCA1, BRCA2 and primary ovarian insufficiency

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Abstract: BRCA1 and BRCA2 genes belong to the family of ataxia-telangiectasia-mutated (ATM)-mediated DNA DSB repair genes that play a critical role in the DNA double-strand break (DSB) repair. Mutations in BRCA genes significantly increase the lifetime risk of breast, ovarian, fallopian tube and primary peritoneal cancers. In addition to the increased risk for multiple malignancies, recent literature suggest that mutations in BRCA genes could lead to decreased ovarian reserve and subsequent ovarian aging. In this review, we will focus on role of BRCA1 and BRCA2 in ovarian function, particularly ovarian aging and primary ovarian insufficiency. Serum AMH values are generally lower in BRCA1 mutation carriers but not in BRCA2 mutation carriers. BRCA2 carriers were more likely to have chemotherapy-induced amenorrhea DNA not stable, linking with ovarian aging. The mechanism by which BRCA mutation in the pathogenesis of POI is the impaired function of repairing DNA breaks. Future studies investigating the knockout models to elucidate the role of the BRCA genes in ovarian development and oocyte maturation will be interesting.

1 Introduction

BRCA1 was first identified as a specific gene for early-onset breast cancer and ovarian cancer susceptibility by using positional cloning methods in 1994 (Miki et al. 1994; Futreal et al. 1994). Nevertheless, the mutation of BRCA1 may not fully explain the majority of the two kinds cancers, as only a small percentage of the cancers can detect the mutations of this gene (Miki et al. 1994; Futreal et al. 1994). At nearly the same time, the germline mutation of BRCA2 gene was identified in high-risk breast cancer families (Wooster et al. 1994; Wooster et al. 1995), indicating that the germline mutation of BRCA2 gene was also a key reason involved in the development of breast cancer. However, unlike BRCA1, germline mutation of the BRCA2 gene was not a risk factor for ovarian cancers (Wooster et al. 1995). Since then, there are extensive investigations and methods used to uncover the function of the both genes, including the knockout model in animals, the induced dysfunction of the proteins or mRNAs, the regulation of cellular signals, and so on. In the review, we use BRCA to stand for both genes when they show similar characteristics.

BRCA proteins have been proved to participate in a multitude of key cellular functions. Particularly, both genes can contribute to the repair of DNA and transcriptional regulation of down signals in response to DNA damage (Yoshida and Miki 2004). Recent studies have proved that BRCA proteins are involved in maintaining chromosomal stability, thus preventing the genome from damage (Yoshida and Miki 2004). Additionally, BRCA proteins can transcriptionally regulate several potential genes functioned in DNA

repairing, the cell cycles, the proliferation as well as the apoptosis (Yoshida and Miki 2004).

Primary ovarian insufficiency (POI) is a specific kind of ovarian dysfunction characterized by premature exhaustion of the resting pool of primordial follicles before menopause. POI affects around 1% women before the age of 40 (Coulam et al. 1986), thus accounting for one of the leading causes for female infertility. The main symptom for this disease is the irregular menstrual cycles. Patients with POI have increased serum follicle-stimulating hormone (FSH) levels and reduced serum oestradiol concentrations. The disease usually has an impact on female fertility and reproductive health in women with childbearing age. Although many studies aimed to elucidate the potential causes for POI, the real mechanism remains currently unknown. Recent studies indicated that POI might result from genetic dysfunctions, chemotherapy, radiotherapy, or surgery. An increasing number of studies show a linkage of mutation of BRCA1 and BRCA2 with breast cancer and ovarian cancer (Yoshida and Miki 2004). Since the mutation of BRCA genes can cause the inefficient repairing of damaged DNA, it may also induce the apoptosis of a single cell through this mechanism, including the oocyte in the ovary (Oktay et al. 2010; Daum et al. 2018). Because BRCA genes play critical roles in the repair of double-stranded DNA breaks, it will be plausible that germline mutations of BRCA genes can lead to accelerated oocyte apoptosis as well as depletion. Emerging evidence indicates that BRCA mutation is associated with lower ovarian response and the occurrence of POI in women (Oktay et al. 2010; Giordano et al. 2016). Additionally, mutation of BRCA1 and BRCA2 can lead into a decline in the number of primordial oocyte in women with childbearing age, which further cause the occurrence of POI. In this review, we mainly focus on role of BRCA1 and BRCA2 in POI.

2 Discovery and Function of BRCA1

BRCA1 is the first breast cancer susceptibility gene to be identified and cloned. In 1990, Hall et al. conducted a genetic chain analysis of 23 breast cancer families and found that about 40% of the families were associated with D17S74 (a marker on the 17q21 chromosome), and the average age of onset of the patient was less than 46 years old, so it was thought that early-onset family-based gland cancer was associated with a genetic mutation on 17q21 (Hall et al. 1990). In 1991, Narod and others investigated five large hereditary breast cancer/pink nest cancer families, confirmed Hall and other findings and suggested that the gene is also the susceptibility gene of nest cancer (Narod et al. 1991). In 1993 Kelsell et al. identified BRCA1 between D17S857 and D17S78 located on the long arm of chromosome 17 (Kelsell et al. 1993).

The BRCA1 protein has the following functions: (1) participation in DNA damage repair. BRCA1 is mainly manifested in its interaction with other repair proteins and related complex formation in the repair process of DNA damage. In the case of dna damage in phase S, high-disc acidification BRCA1 and RAD51/BRCA2 were transferred from the site to the DNA replication site to participate in the DNA repair response, of which RAD51 is a key protein in homologous recombination. In addition, BRCA1 can participate in DNA damage repair with RAD50-MRE11-NBS1 complex through homologous recombination (Paull et al. 2001). The zinc finger structure of BRCA1 interacts with BRAD1 to form a dipolymer, which has E3 ubiquitin connective enzyme activity. (2) Participate in transcription regulation. BRCA1 has the dual function of transcription activation and transcription suppression, the n-side zinc finger structure has DNA binding function, and the "acid group" on the C-side has the function of reverse activation. For one thing, the acidic region of the BRCA1 protein C end binds to the GAL4 DNA binding domain and acts as a transcription initiator, activating the transcription of genes such as P21, P27, MDM2 and Gadd45. For another, BRCA1 inhibits transcription of genes associated with cell proliferation, such as C-myc. In addition, BRCA1 inhibits the transcription of estrogen receptors. (3) Participate in cell dystomoment. BRCA1 may be lowered by Bcl-2 and its transcription altogether P300, or by activating c-Jun aquite end kinase/stress-activated protein kinase signal transduction pathways by p53 non-dependent, improving the expression of the Gadd45 gene, and interacting with the FAS-FAS ligand. This induces cell dysplonation. In addition, studies have shown that BRCA1's onosacidification regulates the activation of caspase3 in UV-induced insizing (Martin and Ouchi 2005). (4) Center copy. The central body replication is carried out under precise regulation, and only 1 replication occurs during normal cell cycles. This process disorder can cause the central body to increase, chromosome asymmetry separation, non-integral increase, and eventually lead to tumor formation. BRCA1 negatively regulates the replication of the central body, and its mutant models can lead to the amplification

of the central body (Hsu et al. 2001). (5) Cell cycle regulation. Under normal circumstances, the BRCA1 protein can be combined with cell cycle-dependent kinase and cell cyclekines A and D, and changes between loutur acidification and dish acidification with the temporal changes of the cell cycle. In the late G1 and S stages present a high degree of disc acidification, after the M phase to go to the building acidification. When DNA is damaged, G1/S period, BRCA1 takes zinc finger domain as the main scope, through the role of transcription factor E2F and the disc acidification of the cell cycle protein CDKS complex, inhibits the cell into the proliferation cycle, prevents cell division, induces cell dysentery (Lou et al. 2003).

3 Discovery and Function of BRCA2

The BRCA2 gene was discovered by American scientist Wooster in 1994 and positioned at 13q12-13. The gene is about 70kb long, of which the coding area contains 10987bp, rich in AT (about 64%). There are 27 coded exons, of which the 11th exon is very large, with 4932bp. The mRNA of BRCA2 is about 10kb long and the encoded BRCA2 protein contains 3418 amino acid residues. The 11th exon of the BRCA2 gene encodes 8 BRC structures containing 30-40 amino acid residues, which runs through the middle of the BRCA2 protein and is highly conservative, and the domain is the binding site of BRCA2 protein and RAD51 protein (Wooster et al. 1994; Wooster et al. 1995).

BRCA2 protein is mainly involved in DNA double-stranded damage repair (Arnold et al. 2006; Tutt et al. 2001). In the homologous recombination of double-stranded DNA damage repair mediated by the RAD51 protein, the DNA binding activity in BRCA2 protein DBD plays a direct role in the binding activity of RAD51 in the BRC structural domain. When a double-stranded DNA is damaged, a SIGNAL of DNA damage is sent, Signals are further activated such as ATM, ATR and other kinase activity, catalytic BRCA2-RAD51 complex, phosphorylation occurs, make it activated, and then BRCA2 protein carries RAD51 protein to transport to the double-stranded DNA damage site, participate in the recombinant repair of double-stranded DNA damage (Venkitaraman 2001).

4 BRCA1 and POI

Recent literatures indicates that in addition to the increased risk for several malignancies in women with germline mutations of BRCA genes, these women may also have diminished ovarian reserve.

It has been proved recently that healthy women with child-bearing age and with BRCA mutations showed diminished primordial follicles and increased oocyte DNA damage (Lin et al. 2017). By using histology and immunohistochemical analysis, the study directly provided strong evidence of reduced ovarian reserve and accelerated primordial follicle loss as accompanied with increased oocyte DNA damage in women with BRCA

mutations on paraffin-embedded ovarian sections (Lin et al. 2017). AMH is a type of hormone that regulate the growth differentiation and formation of follicle. Serum AMH level is usually considered as a biomarker of ovarian reserve as it is mainly derived from granulosa cells in the ovary. It can also be used to detect the age of menopause as the number of granulosa cells falls over age which cause ovarian aging. The normal menopause time takes place between the age of 40 to 51 gradually and the value of AMH falls as the time goes on. The low serum AMH value indicates a signal of ovarian aging in women. Based on the ability to reflect the number of antral and pre-antral follicles in the ovaries, a wide range of clinical applications of circulating AMH have been suggested, including assessment of ovarian reserve in women. Additionally, BRCA1 mutation carriers showed lower serum AMH levels than women without BRCA1 mutations (Wang et al. 2014). All these studies indicated that BRCA1 mutations were involved in the pathogenesis of premature ovarian aging, thus causing POI. Consistent with these studies, reproductive reserve was reduced, primordial follicle numbers were lower, and DSBs were significantly increased in remaining follicles in BRCA1-deficient mice compared with age matched wild-type mice. Furthermore, oocyte-specific knockdown of *Brc1* gene significantly increased DSBs and reduced the survival rate of oocyte, whereas *Brc1* overexpression showed the converse effects (Titus et al. 2013). In contrast to these studies, a recent study indicated that BRCA1 mutation carriers had AMH values that were similar to non-carrier women, and the reason for this may be attributed to races in the population investigated (Johnson et al. 2017).

5 BRCA2 and POI

In addition to the BRCA1, abnormal of BRCA2 may also be involved in the pathogenesis of POI.

It has been proved in *Drosophila* that BRCA2 has a dual role in the repair of both mitotic and meiotic DNA breaks and the efficient activation of the meiotic recombination checkpoint (Klovstad et al. 2008). Significantly lower AMH levels in BRCA2 mutations carriers was observed compared with control women (Johnson et al. 2017). Additionally, the same study also identified 15 signals involving various DNA repair mechanisms. At least one of these genes, RAD51, is also important in the BRCA2 pathway. It is possible that the lower AMH levels in BRCA2 carriers occurred due to variants in other DNA repair genes associated with the BRCA2 pathway. The loss of BRCA2 function led to reduced recruitment of RAD51 to double-stranded breaks in DNA. The recruitment of RAD 51 to double-stranded breaks in DNA is necessary both for the repair of breaks that occur during recombination in meiosis and for the repair of DNA in mitotic cells (Weinberg-Shukron et al. 2018). However, a recent study showed that mean serum AMH level not significantly reduced in women with BRCA2 mutation status (Phillips et al. 2016). Consistently, a recent study also suggested that no

reduction in AMH level was found in the mutations carriers (van Tilborg et al. 2016). This can be explained that BRCA2 can also repair DNA breaks, decreased BRCA2 gene expression in mutated model typically occurs at the end of reproductive window, and the proportion of BRCA2 gene expression among all DNA genes is small (Son et al. 2019). Additionally, compared with BRCA1 mutations, where decline in the function of gene expression occurs at an earlier reproductive age and exhibit accelerated ovarian aging, women with BRCA2 mutations exhibit more latter ovarian aging (Oktay et al. 2015). Many studies demonstrate that a series of cancer treatments like radiation therapy and chemotherapy will cause the damage in ovarian reserve, leading into reduced AMH value and ovarian aging. This will effect those women whose with child-bearing age and willing to have children. Women with breast cancer for chemotherapy are at risk of ovarian failure because of the accelerated depletion of ovarian follicles (Valentini et al. 2013). The same study also suggested that the proportion of women experiencing amenorrhea with BRCA1 mutations was higher than those with BRCA1 mutations. However, probability of amenorrhea in women who carry BRCA mutations after chemotherapy are not at particularly high risk of menopause (Valentini et al. 2013). All these results suggested that ovarian aging in women after chemotherapy was mainly induced by the drugs.

6 Potential mechanisms for BRCA mutations in POI

BRCA1 and BRCA2 are previously identified as tumor suppressor genes, which enables the integrity of the whole genome through the repair of DNA double stranded breaks. The loss function of the two BRCA genes are unable to synthesize proteins to repair the DNA DSBs and causing apoptosis of cells, including the oocyte. The potential mechanism of POI is premature depletion of the primordial pool due to impaired repair of double stranded DNA breaks. The ovarian aging can be caused by an inefficient DNA repair and increased accumulation of DNA breaks, which can further cause mutagenesis and even cell death, leading to POI (Lin et al. 2017).

There is strong association between DNA damage and repair and ovarian aging, which can be mediated by cell death and senescence (van Tilborg et al. 2016; White and Vijg 2016). Because of the masking of most severe symptoms by prophylactic oophorectomy or cancer in BRCA mutations, it is less likely that an impact of BRCA mutations on fertility until can be easily found in early reproductive age (Titus et al. 2013). BRCA1 is needed for meiotic spindle assembly as well as cohesion function between the chromatids. And therefore the impact of BRCA1 mutations on reproductive function can be more visible. The absence of an influence of BRCA2 gene mutations on ovarian reserve in female mice and women may indicate that the reduce of the function in BRCA2 gene mutated women occurs at the

vary end of reproductive life (Titus et al. 2013). Additionally, this mutation model may have functions that are very different from women with BRCA1 gene mutation.

7 Conclusion

Serum AMH values are generally lower in BRCA1 mutation carriers but not in BRCA2 mutation carriers. BRCA2 carriers were more likely to have chemotherapy-induced amenorrhea DNA not stable, linking with ovarian aging. The mechanism by which BRCA mutations in the pathogenesis of POI is impaired repair of DNA breaks.

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