Effects of Breast Cancer Genes 1 and 2 on Cardiovascular Diseases

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Abstract: Carriers of mutations of breast cancer gene 1 and/or 2 (BRCA1/2) have a higher risk of developing breast and ovarian cancers at a relatively young age. Recently, a causative role for BRCA1/2 in cardiovascular diseases has been emerging. In this review, we summarize currently available evidence obtained from studies on animal models and human BRCA1/2 mutation carriers that shows a correlation of BRCA1/2 deficiency with various cardiovascular diseases, including ischemic heart disease, atherosclerosis, and chemotherapy-linked cardiac muscle disorders. We also discuss one of the major mechanisms by which BRCA1/2 protects the heart against oxidative stress, ie mediating the activity of Nrf2 and its downstream targets that govern antioxidant signaling. More research is needed to elucidate whether the carriers of the BRCA1/2 mutations with ovarian and breast cancers have increased susceptibility to chemotherapy-induced cardiac functional impairment. (Curr Probl Cardiol 2021;46:100421.)

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Introduction

Cardiovascular diseases (CVD) and breast cancer are leading causes of morbidity and mortality in the United States. Approximately 47.8 million women are affected by CVD, and nearly 3.32 million women are affected by breast cancer.[1] older and postmenopausal women with no history of breast cancer have higher mortality linked to CVD than that linked to breast cancer.[1] On the basis of 2014 data in women provided by the Centers for Disease Control and Prevention, 1 in 3.3 deaths was associated with CVD, whereas 1 in 31.5 deaths was linked to breast cancer. However, mortality rates of breast cancer and CVD have diminished, with an average decline in the mortality of female breast cancer by 1.8% per year from 2005 to 2014 and in the mortality of CVD (both genders) by 6.7% per year from 2004 to 2014.[1]

Breast cancer gene 1 and 2 (BRCA1 and 2) encode 2 unrelated proteins with functional similarity. BRCA1 gene is located on chr17q and has 1863 amino acids,[2,3] while BRCA2 gene is localized on chr13q, has 3418 amino acids,[4] and is one of the acrocentric chromosomes in men.[2,3] Any mutations in either of these 2 genes can lead to an elevated risk of developing ovarian and breast cancers.[2,3,5,6] For example, there are approximately 12% and 1.5% risk of developing breast and ovarian cancer, respectively, during one female's life time in the Western countries, and loss-of-function mutations in BRCA1/2 genes are associated with 5%-10% of breast cancer cases in the Western world and exhibit elevated risk for ovarian cancer development.[7,8] Moreover, the cumulative breast cancer risk by age 70 years in BRCA1- and BRCA2- mutation carriers was estimated to be 65% and 45%, respectively, and the cumulative ovarian cancer risk was estimated to be 39% and 11%, respectively.[9] Therefore, BRCA1 and BRCA2 are classified as "tumor suppressor genes."[10] Mechanistically, BRCA1/2 is involved in genome integrity[11] through mediating the homologous recombination (HR) repair of double-strand DNA breaks (DSBs).[12] The HR deficiency, a functional property of BRCA1/BRCA2-deficient cancerous cells, promotes error-prone DSB repair mechanisms such as nonhomologous end joining, leading to genomic instability.[12] Although both BRCA1/2 are involved in homology-directed DNA damage repair, BRCA1 appears to be ubiquitously expressed and exhibits multiprotein interactions.[13] In contrast, BRCA2 is more involved in promoting homology-directed DNA damage repair through its direct binding to the single-stranded DNA and with RAD51 interaction.[14,15] While it has been well studied regarding the role of BRA1/2 in the pathogenesis of breast and ovarian cancers, the speculation

of the implication of BRCA1/2 in CVD is emerging due to the facts that the similar mechanisms, for example, DSBs, have been involved in both cancer development and cardiovascular

pathogenesis[16,17] and that BRCA1/2 plays an important role in DNA damage repair.[18] Indeed, some animal studies demonstrate that BRCA1/2 is implicated in cardiovascular disorders.[16,19] However, the mechanisms of BRCA1/2 protection against cardiac injury have not yet been fully elucidated. In this review, we highlight the recent findings and explore the potential role of BRCA1/2 in CVD and underlying mechanisms.

BRCA and CVD

BRCA and Ischemic Heart Disease (IHD)

Studies on human ventricular tissues from IHD patients demonstrate higher BRCA1 expression compared with control samples. Also, the study using primary cultured human fetal cardiomyocytes derived from normal hearts under the hypoxic condition exhibit increased levels of both total and phosphorylated BRCA1 compared with those cultured under the normoxic condition.[16] These observations suggest a potential implication of BRCA1 in hypoxic pathophysiology in cardiomyocytes/hearts. In addition, several studies found a correlation between single nucleotide polymorphisms (SNPs) of BRCA1/2 and CVD. For example, a study performed in Japan revealed a potential link of SNPs in the BRCA1-associated protein to the occurrence of myocardial infarction in Asian populations.[20] In addition, 2 BRCA2 SNPs localized in untranslated regions, rs11571836 and rs1799943, were correlated with a lower risk of CVD in the SHARE studies,[21] and subsequent study.[21-23] However, these findings were not reproduced in the other 2 South Asian case-control studies on the incident myocardial infarction.[22] Thus, whether SNPs of BRCA1/2 play a pivotal role in IHD in various races remains to be elucidated.

BRCA and Cardiac Remodeling

Patients with idiopathic dilated cardiomyopathy had different BRCA1 expressions in the hearts: hypertrophied cardiomyocytes had very strong BRCA1 expression but typical myopathic cardiomyocytes had weak and mosaic expression of BRCA1, while control hearts exhibited weak-to-moderate BRCA1 expression.[24] In addition, idiopathic dilated cardiomyopathy cardiomyocytes had more BRCA1 expression, whereas no

differences in BRCA1 expression were observed in the small vessels and interstitial tissues between control and diseased groups.[24] Thus, it is speculated that lower BRCA1 expression in diseased myocytes is accompanied with lower antiapoptotic and DNA damage repair activity, but with the opposite in hypertrophied myofibers.[24]

In the animal studies, BRCA1/2 has been shown to protect multireason-induced cardiac remodeling and cardiac dysfunction. For instance, the mice with cardiac loss of BRCA1 exhibits pathologic cardiac remodeling, ventricular dysfunction and increased mortality in response to ischemic or genotoxic stress.[16] It is highly likely that loss of BRCA1 in cardiomyocyte impeded DSB repair and promoted p53-mediated apoptotic signaling, therefore leading to elevated cardiomyocyte apoptosis. Correspondingly, knockout of the p53 gene in BRCA1-deficient mice prevents cardiac failure.[16] Adenoviral mediated expression of BRCA1 in spontaneously hypertensive rats is associated with reduced γH2A.X expression as an index of DSB, decreased aortic ROS generation, greater RAD51 foci, and decreased blood pressure.[19] In vitro, H_2O_2 substantially reduces BRCA1 expression coincident with an increase in ROS generation,[19] and BRCA1 overexpression reduces H_2O_2-induced ROS generation in human aortic smooth muscle cells, partially via suppression of the expression of the ROS-producing NADPH oxidase subunits Nox1 and p47phox.[19] Same as BRCA1, DNA damage causes recruitment of BRCA2 to nuclear foci.[25] Further, cells homozygous knockout for BRCA2 have similar cellular phenotypes, including chromosome instability, defects in DSB-initiated HR, and hypersensitivity to DNA damaging agents.[26] In addition to the primary role BRCA1 plays in the repair of DNA damage and thus prevents cardiomyocyte from apoptosis, BRCA1 might also regulate cardiac energy production program, which is related to cardiac dysfunction. For example, cardiomyocyte-specific deletion of BRCA1 reduces the expression of fatty acid and glucose transporters, decreases the levels of factors mediating the fatty acid and glucose oxidation, and diminished mitochondrial biogenesis, all of which results in an energy-depleted heart and eventually heart failure.[27]

BRCA and Cardiac Damage Induced by Chemotherapy

It has been well-documented that germline mutations in BRCA gene significantly increases the risk of breast and ovarian cancer syndromes,[28,29] and these patients are usually administered with different cancer therapies including chemotherapy. Most of chemotherapeutic agents damage DNA directly or indirectly via various mechanisms.[30] Doxorubicin (DOX), also called adriamycin, belonging to anthracyclines, widely used in cancer

therapy. However, DOX-associated dose-dependent cardiotoxicity, which is mainly refractory cardiac dysfunction, has limited DOX clinical application.[31] DOX-linked cardiomyopathy usually leads to the worse outcome and currently does not have effective treatments. The mechanisms by which DOX treatment induces heart failure include the formation of DSBs, cardiomyocyte apoptosis secondary to DOX-induced DNA damage, the activation of p53, and excessive oxidative stress.[32-34] Given that DOX induces DSBs[34] and that BRCA is involved in DSBs repair,[35] the implication of BRCA1/2 in DOX-linked cardiotoxicity has been speculated. Indeed, recent findings suggest BRCA1/2 play a protective role in DOX-induced cardiotoxicity.

Studies from loss of function animal models indicate that deficient BRCA1/2 genes substantially elevate the risk of cardiac failure and mortality in mice exposed to DOX. For instance, heightened cardiac dysfunction and apoptosis are observed in cardiomyocyte-specific BRCA1 homozygous knockouts (CM-BRCA1-KO) mice compared with the wild-type (WT) littermates, which is associated with activated p53-mediated proapoptotic signalling.[16] This study also demonstrates greatly diminished RAD51-foci in the left ventricle of DOX-treated CM-BRCA1-KO mice.[16] Similar result was observed in the mice with cardiac specific deletion of BRCA2. Although mice with cardiomyocyte specific deletion of BRCA2 exhibit no discernable heart phenotypes at baseline, these mice develop more severe cardiac dysfunction and higher mortality compared to control mice.[17] DOX treatment also significantly increases apoptosis in the left ventricles of CM-BRCA2-KO mice compared with that of littermate controls.[16] Correspondingly, compared to the WT mice, microscopic examination revealed more DSBs and no RAD51 focus formation in the left ventricle of DOX-treated CM-BRCA2-KO mice.[16] Mechanistically, CM-BRCA2-KO hearts exhibit increased expression of Bax, p53 and p53-up-regulated modulator of apoptosis (PUMA) compared with WT mouse hearts, resulting in the higher Bax to Bcl-2 ratio and more cytochrome c release in the DOX-treated CM-BRCA2-KO hearts.[17] Additionally, Yao et al demonstrated that 3,3′-diindolymethane, a compound derived from the digestion of indole-*3*-carbinol and an enhancer of cell proliferation,[36] can markedly increase the BRCA1 expression in heart tissues and fibroblast, leading to the activation of the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2).[37] The Nrf2 activation in turn increases the expression of several antioxidant genes and exerts a substantial antifibrosis impact on the heart tissues in a DOX-treated animal model.[37]

Although animal studies strongly support the premise that BRAC1/2 plays an important role in cardiac phenotypes induced by DOX treatment,

whether this conclusion also applies to human patients remains not completely clear. One study with 401 female with breast cancer (232 BRCA1 and 159 BRCA2 patients; 10 with both mutations) showed the significant increase in the risk of cardiotoxicity in BRCA mutation carriers compared to general population and an overall increased risk of cardiotoxicity from anthracycline-based therapy,[38] but another study compared the risk of cardiac dysfunction of 39 women with a history of BRCA1/2 mutation-associated breast cancer treated with anthracyclines to that of 42 similarly treated women with a history of sporadic breast cancer and revealed no significantly increased cardiac risk as expected.[39] Given that BRAC1/2 plays an instrumental role in cardiac homeostasis, it is intriguing to observe the inconsistent findings between human and mice. While the exact mechanisms underlying this discrepancy are not understood, the sample size and the mutation location(s) of BRCA1/2 may play a role. These discrepant findings warrant further investigation and clarification.

BRCA and Atherosclerosis

Atherosclerosis is the major culprit for stroke, acute coronary syndromes, and other vascular diseases,[40] and is believed to be a response to injury.[41-43] Atherosclerosis is related to the flow disturbances that damage the endothelium,[44] followed by platelets' adhesion, macrophages' penetration into the subendothelium, excessive oxidative stress and inflammation, low density lipid (LDL) oxidation, and proliferation of smooth muscle cells.[45] Risk factors for atherosclerosis identified in the Framingham Heart Study include hypertension, smoking, elevated LDL, diabetes, and left ventricular hypertrophy.[46] Dysfunctional endothelium, which can be caused by oxidative damage associated to overproduced ROSs, significantly contributes to the initiation and progression of atherosclerosis.[47] Also, DNA damage and repair is involved in the endothelial dysfunction and contributes to the progression of atherosclerosis.[48] The speculation for BRCA genes to be implicated in atherosclerosis comes from the facts that (1) both BRCA1 and 2 are expressed in endothelial cells,[49-51] and (2) as mentioned above, BRCA genes play a pivotal role in the repair of DNA damage. Indeed, studies have shown that cells null for BRCA1/2 gene are more sensitive to oxidative stress.[52,53] Correspondingly, overexpression or silencing of BRCA1 protects or exaggerates inflammation- and DOX-induced endothelial cell apoptosis, respectively.[40,41] Mechanistically, BRCA1 limits apoptosis of endothelial cells and thus improves endothelial function. In addition, overexpression of BRCA1 greatly attenuates ROS generation, upregulates endothelial NO

synthase, phosphorylates Akt, and promotes the expression of vascular endothelial growth factor. Also, overexpression of BCRA1 in mice ameliorated capillary density and restored blood flow better in the ischemic hindlimb compared with the control mice.[54] In addition, BRCA1-overexpression protects ApoE($^{-/-}$) mice fed a Western diet against development of severe aortic plaque lesions.[54] On the contrary, BRCA1 levels are decreased in the plaque samples of human atherosclerotic carotid artery compared with the adjacent normal tissues.[54]

Insulin resistance (IR) is a valuable predictor for the pathogenesis of diabetes, a disease with characteristics of heightened endothelial dysfunction, excessive inflammatory responses, and atherosclerosis.[55] BRCA1/2 mutation carriers are found to have IR-reduced levels of insulin-like growth factor 1 (IGF-1), while these carriers with breast cancer were reported to have elevated levels of IGF-1.[56,57] Since extremely high and low serum IGF-1 concentrations are linked to an elevated risk of IR,[58] BRCA1/2 mutation carriers exhibit doubled risk of developing diabetes within the 15-year from the breast cancer diagnosis compared to the healthy carriers, and the risk is particularly high for females who have a body mass index $>$ 25.0 kg/m2.[59]

Asselbergs and colleagues have shown a potent correlation between 2 BRCA2 SNPs and low-density lipoprotein levels in a large-scale, gene-centric meta-analysis,[60] and Ortega et al. reported that BRCA1 expression is substantially increased in both omental and subcutaneous adipose tissues obtained from subjects with obesity.[61] In line with its role in suppressing fatty acid biosynthesis, the expression of BRCA1 is upregulated in preadipocytes, and downregulated during adipogenesis, whereas P-acetyl-CoA carboxylase decreases during the human adipocyte differentiation allowing lipid biosynthesis.[61] In agreement with the above observations, deletion of BRCA1 gene induces a marked increase in the fatty acid synthesis by preventing dephosphorylation of acetyl coenzyme A carboxylase alpha.[62]

BRCA and Nrf2

Nrf2 is a key transcription factor that mediates the activity of a wide array of genes involving antioxidation and detoxification in response to oxidative and xenobiotic stress.[63] Under physiological conditions, Nrf2 is in association with Kelch like-ECH-associated protein 1 (Keap1), a stress sensor, and Cullin 3, a ubiquitination E3 ligase, and is localized in the cytoplasm.[64] Nrf2 is a ubiquitination target for Cullin 3, and Keap1 acts as a substrate bridge, which bring in Nrf2 and promotes its ubiquitination by Cullin 3.[65] Therefore, Nrf2 has a short half-life of only 20 minutes under

physiological conditions.[66] However, under oxidative stress, Keap1 is oxidized on the important cysteine residues, thereby disrupting the Keap1-Cul3 ubiquitination system. The un-ubiquitinated Nrf2 accumulates and translocates into the nucleus, where it binds a small protein called Maf to form a heterodimer.[67] The formed heterodimer then binds the antioxidant response elements (AREs) in the upstream promoter region of target genes and starts the transcription of several antioxidant genes, including superoxide dismutases, catalase, glutathione-S-transferase, NAD(P)H dehydrogenase (quinone 1) (NQO1), heme oxygenase-1 (HO-1), γ-glutamylcysteine synthase, and glutathione peroxidases.[68,69] Hence, Nrf2 can augment a wide spectrum of cell defense processes to detoxify potentially harmful molecules through inducing the expression of these antioxidant enzymes.

As mentioned above, several critical free radical scavenging enzymes, which contain AREs in their gene promoters, are transcriptionally mediated by Nrf2. Given that ROS plays a critical role in the pathogenesis of CVDs, it is understandable that as an antioxidative stress mediator, Nrf2 is associated with the protection of the heart against functional/histologic damage caused by excessive ROS production. Indeed, Nrf2 activation was protective for the heart against I/R-induced cardiac injury in both in vivo and vitro. The direct evidence for the involvement of Nrf2 in mediating cardiac function comes from a study using Nrf2 knockout (Nrf2-KO) mice, which showed that Nrf2-KO mice developed cardiac diastolic dysfunction and cardiac hypertrophy.[70] In addition, activation of Nrf2 signaling by different approaches prior to cardiac I/R significantly decreased infarct size and facilitated cardiac functional recovery.[71,72] A more recent study revealed the implication of Nrf2 in sulforaphane-mediated cardiac protection from diabetic cardiomyopathy, as evidenced by the loss-of-function for Nrf2 exacerbated the cardiac dysfunction in mouse diabetic models compared with the WT mice.[73] Mechanistically, the Nrf2-KO mouse cardiomyocytes had decreased activity of SERCA2,[70] an important regulator of intracellular calcium concentration and a therapeutic target for treatment of heart failure.[74] Also, in H9c2 cardiogenic cells, increased Nrf2 expression plays an important role in suppressing the increase in the intracellular ROS levels induced by simulated I/R in vitro.[75] Another important mechanism by which Nrf2 protects the heart against insults is that Nrf2 improved the function of mitochondria in response to ROS insult,[76] and mitochondrial dysfunction is knowingly involved in a variety of human diseases, including CVDs. Collectively, the above findings point to an important beneficial role for Nrf2 in the development of CVD. Although the protective role of Nrf2 in cardiovascular system has been well explored in animal models, its potential

involvement in human CVDs and its activation as a potential therapeutics have not been well examined. Some studies found that the Nrf2 expression was downregulated in the left ventricles of patients with diabetic cardiomyopathy and in aging human hearts,[77,78] and exercise was able to partially recover the Nrf2 levels in the heart.[78] Thus, the approaches, including molecules, that can activate Nrf2 signaling may have potential clinical applications in the future and are under active exploration. For example, a number of small molecules that are derived from natural products are able to induce the Nrf2 expression through abolishing the Keap1-induced Nrf2 degradation.[79] Also, *dimethyl fumarate, a molecule that possesses anti-inflammatory and cyto-protective activity, potentiates Nrf2 function.*[80] Interested readers are referred to some comprehensive reviews for more details.[79,81]

The primary mammary epithelial cells collected from BRCA1-KO mice have low expression of Nrf2-targeted antioxidant enzymes and high ROS accumulation, which compromises cell survival in vivo.[82] Correspondingly, increased Nrf2 expression decreases ROS levels in and rescues survival of BRCA1-KO cells.[82] A recent study shows that deletion of BRCA1 gene reduces Nrf2 expression since the recruitment of BRCA1 to the promoter/enhancer sequences of Nrf2 is required for Nrf2 expression and thereby Nrf2-dependent antioxidant signaling.[83] Oxidative stress upregulates BRCA1 expression, which causes Nrf2 accumulation.[82] Hence, as aforementioned, the identified functional interaction between BRCA1 and Nrf2 are mainly based on the findings that (1) Nrf2 expression is downregulated in BRCA1-KO cells; (2) increased BRCA1 expression by oxidative stress stabilizes Nrf2, and (3) overexpression of Nrf2 regains antioxidative function in BRCA1-KO cells. However, few studies have been performed to probe the functional interaction between BRCA1 and Nrf2 in cardiovascular settings, although the protection of both BRCA1/BRCA2 and Nrf2 for cardiovascular system has been previously explored.[84] Recently, Yao et al. demonstrated that 3,3′-diindolymethane, a compound derived from the digestion of indole-*3*-carbinol and an enhancer of cell proliferation,[36] attenuated DOX-linked cardiac fibrosis through increasing the BRCA1 expression in heart tissues and fibroblast, which was associated with the Nrf2 activation,[37] thereby showing a functional cooperation between Nrf2 and BRCA1 in a drug-induced cardiac disease model. While BRCA2 is widely assumed to act like BRCA1 to mediate Nrf2 function, the direct evidence underlying this assumption is still missing. More studies that will be carried out to further confirm the existence of a functional link between BRCA1/BRCA2 and Nrf2 in the cardiovascular setting are expected in the future.

Mechanistically, BRCA1 interferes with Keap1-mediated Nrf2 degradation.[83] Nrf2 levels are tightly governed by Keap1 through direct interaction on 2 domains, ETGE and DLG.[85] ETGE is a stronger interacting domain between Nrf2 and Keap1 compared with the DLG motif.[86] BRCA1 was found to compete with Nrf2 for binding to ETGE domain, thus dissociating Nrf2 from its interaction with Keap1 and promoting its stability, as evidenced by the observation that the presence of exogenous BRCA1 abolished the ubiquitination of Nrf2 by Keap1 in 293T cells.[82] Thus, BRCA1 offers cell protection at least in part through mediating the Nrf2-dependent antioxidative stress activity. However, several questions remain to be answered. For instance, does BRCA2 have the similar function as BRCA1 to mediate the Nrf2 activity through direct physical interaction with Nrf2? Also, the direct binding between BRCA1 and Nrf2 was uncovered in cancerous cells. Does this hold true in the cardiovascular system at baseline and/or in a diseased setting?

BRCA1 and BRCA2 influence the Nrf2 activity also through functional interaction with partner and localizer of the BRCA2 (PALB2), a major BRCA2 binding partner. Different regions on PALB2 regulate physical interactions with BRCA1 and BRCA2.[87-91] For example, coiled-coil motifs present on PALB2 (amino acids 9-44) and BRCA1 (1393-1424) dictate the physical interaction between PALB2 and BRCA1, and this interaction potentiates DSB-initiated HR and resistance to mitomycin C,[88-90] indicative of that BRCA1, BRCA2, and PALB2 act in concert in the event of DNA repair.[92] PLAB2 interacts with the N-terminal domain of BRCA2, and controls the subnuclear localization, stability, recombination repair, and DNA damage checkpoint functions of BRCA2.[92] In addition, the functional redundancy between BRCA2 and PALB2 is also noted.[93] Interestingly, PALB2 contains an ETGE-type Keap1 binding motif and can effectively compete against Nrf2 for Keap1 binding,[94] thus promoting the stability of Nrf2 and nuclear localization. Functionally, PALB2 retains Nrf2 in the nucleus and reduces the cellular ROS level.[94] PALB2 also mediates the rate of Nrf2 export from the nucleus to cytosol upon induction.[94] The structure of BRCA1, BRCA2, PALB2, KEAP1, and Nrf2, and their interaction is illustrated in Figures 1 and 2. While it is clear that (1) BRCA1, BRCA2, and PALB2 participate in the repair of DNA cooperatively in cells in response to DNA damage signals, (2) PALB2 mediates Nrf2 activity, and (3) BRCA1 regulates Nrf2 function, whether and how BRCA1/BRCA2 affects the activity of Nrf2 through PALB2 or vice versa, particularly in the cardiovascular (patho) physiology await further exploration.

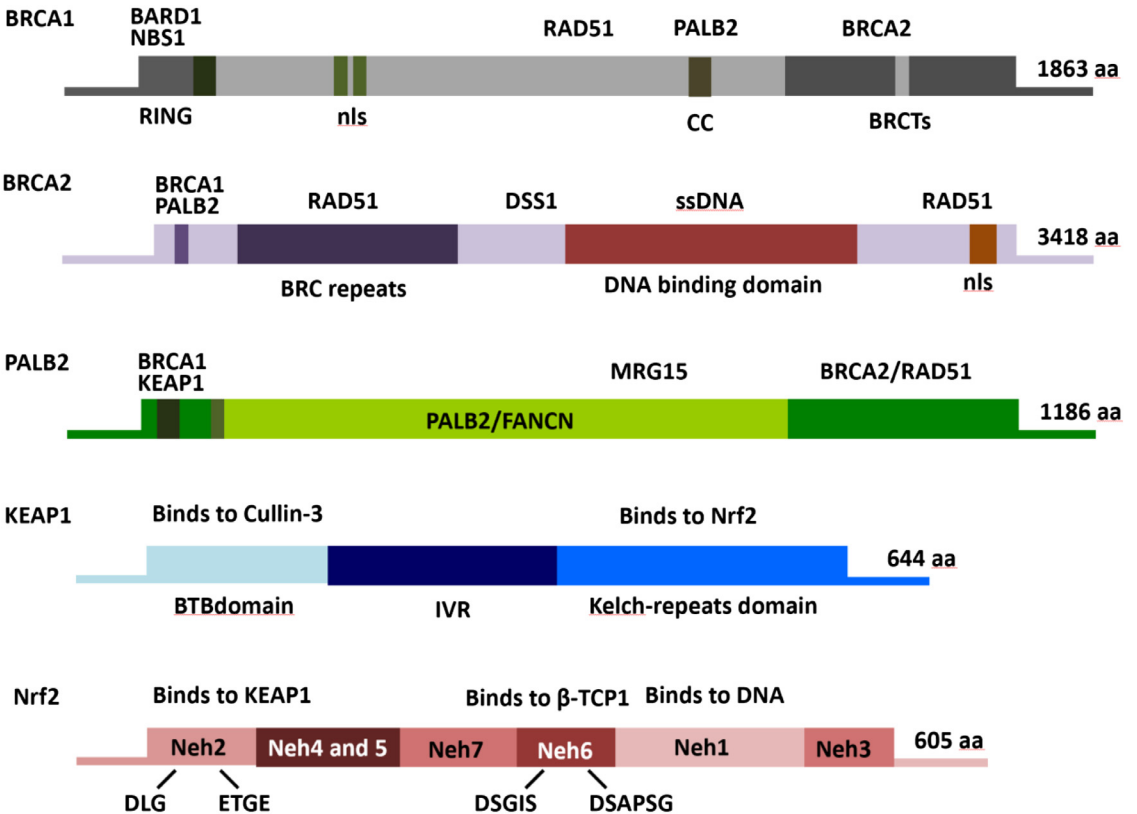

FIG 1. Identification of BRCA1, BRCA2, PALB2, KEAP1, and Nrf2.

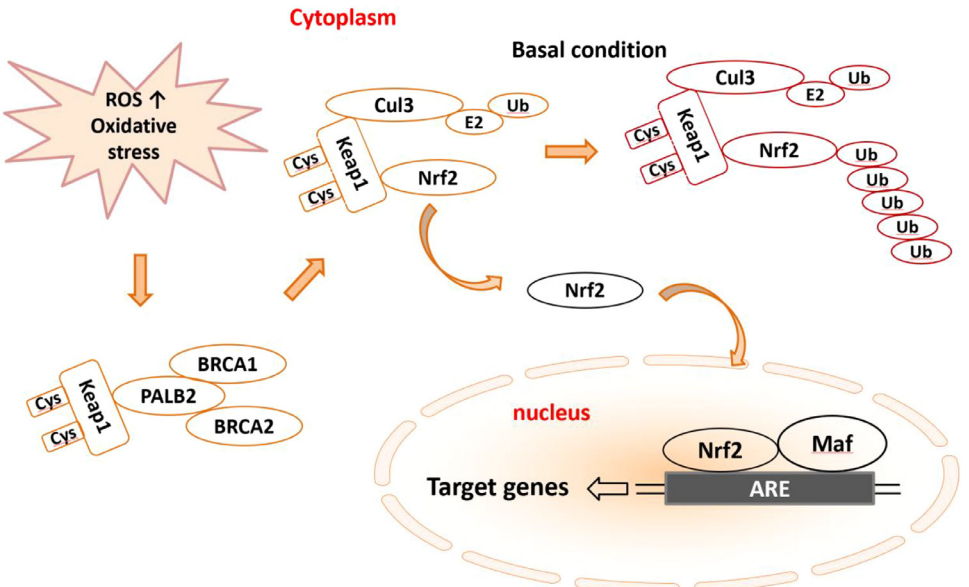

FIG 2. BRCA and Nrf2 involved in cardiac remodeling.

Given the importance of BRCA1 and BRCA2 in human diseases, how the expression of these 2 is regulated at baseline and/or in a diseased setting is also an interesting area of research. Early studies suggested that the expression of BRCA1 and BRCA2 was associated with the cell cycle progression.[95] However, some chemicals, such as DOX and camptothecin, as well as ultraviolet radiation substantially diminished the expression of these 2, which are not cell cycle-dependent.[96] At the transcriptional level, BRCA1 expression is governed by chromatin remodeling factors. For instance, CtBP and HDAC1 suppressed the expression of BRCA1 through inhibiting the histone acetylation of the BRCA1 promoter.[97] Interestingly, BRCA1 is also a transcriptional target of Nrf2. Nrf2, together with CBP and P300, were found to directly bind to an ARE located on the cis-regulator region of BRCA1 gene and activate its basal transcription.[98] However, whether Nrf2 can mediate the BRCA2 expression is not clear, and whether the regulation of BRCA1 expression and activity by Nrf2 is involved in the cardiac homeostasis and cardiovascular disorders merits further investigation.

Clinical Testing of BRCA1/2

Based on the above discussion, it appears very important to identify the population who has BRCA1/2 deficient (mutation) since these peoples are not only high risk for the development of cancers, and now may also CVD, Diabetes, but these people would more sensitive to the anticancer drugs that induces DNA damage. For instance, BRCA1/2 mutation carriers exhibit doubled risk of developing diabetes within the 15-year from the breast cancer diagnosis compared to the healthy carriers.[59]

If possible, all female cancer patients should be examined whether their BRCA1/2 gene is mutated. If so, these patients may not be given with anticancer drugs that provide cancer therapy via generating DNA damage and inhibiting DNA repair enzymes, such as DOX since these patients are highly susceptible to DOX-induced cardiac side-effect. For instance, one study with 401 female with breast cancer (232 BRCA1 and 159 BRCA2 patients; 10 with both mutations) showed the significant increase in the risk of cardiotoxicity in BRCA mutation carriers compared to general population and an overall increased risk of cardiotoxicity from anthracycline-based therapy.[38] However, there was also report that shows no increased risk of CVDs in breast cancer patients with BRCA1/2 mutation (39 cases) compared to those without BRCA1/2 mutation (42 cases) when both were treated with anthracycline-based therapy.[39] The contradictory outcome is mainly due to the lack of a systemic and comprehensive study with a large number of such patients since BRCA1/2 gene mutation examination has not been extensively applied into clinics before.

Although clinical sequencing to reveal variants of BRCA1 and 2 in patients with breast/ovarian cancers began many years ago, and the relevant database has been published online,[99] due to sequencing technical issues and high cost, the clinical testing of BRCA1 and 2 was not widely performed for a while. Recent advances in high-throughput sequencing technologies have promoted widespread application of sequencing of BRCA1 and 2, although major differences in the techniques used in many laboratories vary.[100] However, whether and how this clinical testing of BRCA1 and 2 can be used to assist in the diagnosis of cardiovascular diseases remains to be addressed.

Mechanistically the increasing evidence suggests the possibility for us to upregulate Nrf2 expression and activation of Nrf2 as one key pathway that plays critical role in protecting the patients against subsequent suffering from diabetes and anticancer drugs-induced systemic side effects since Nrf2 can be efficiently induced by sulforaphane, a derivative of cruciferous vegetables, which is a potent stimulator of Nrf2 activity or directly by broccoli sprout extracts, both which have been its clinical trials or completed for its safety and efficiency for various conditions (https://clinicaltrials.gov/).

Conclusions

Female carriers of germline mutations in either of the 2 BRCA genes are predisposed to breast and ovarian cancers. The currently available evidence shows that BRCA1/2 deficiency is implicated in the initiation and progression of various CVD, including ischemic heart disease, atherosclerosis, and chemotherapy-related cardiac muscle disorders (as shown in Fig 3, Tables 1 and 2). The involvement of BRCA1/2 in various

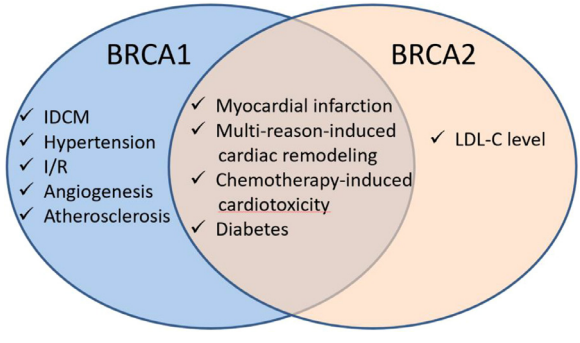

FIG 3. BRCA involved in different cardiac diseases.

TABLE 1. BRCA in animal and cell studies

BRCA gene	Change in expression	Target disease model (cell)	Pathology or function	Reference
BRCA1	↑	MI	DNA damage	15
		Spontaneously hypertensive rats	Oxidative stress	19
BRCA1	↓	H_2O_2 (HASMCs)	Oxidative stress	19
BRCA1	knockout	CM BRCA1$^{-/-}$ mice	Reduce cardiac fatty acid and glucose metabolism	25
BRCA1	Knockout	DOX-induced cardiac damage (CM BRCA1$^{-/-}$ mice)	Apoptosis	16
BRCA2	Knockout	DOX-induced cardiac damage (CM BRCA2$^{-/-}$ mice)	Apoptosis	17
BRCA1	Loss and gain	Angiogenesis and atherosclerosis	Oxidative stress and inflammation	52
BRCA1	knockdown	MCF7 human breast cancer cells	Increase in the fatty acid synthesis	60

TABLE 2. BRCA in human studies

BRCA gene	Change in expression	Target disease model (number of subjects)	Tissue	Reference
BRCA1	↑	Ischaemia and reperfusion (4-5)	Atrial biopsies	16
BRCA2	rs11571836; rs1799943	CVD (985)	Blood	21
BRCA1	↑	Idiopathic dilated cardiomyopathy (10)	Myocardial samples	24
BRCA1/2	mutation	Anthracycline treatment (81)	Heart function by echo	36
BRCA1	↓	Atherosclerosis (3-4)	Carotid artery samples	52
BRCA1/2	mutation	Diabetes (6052 women)	Blood	57
BRCA2	SNP	LDL-C GWAS meta-analysis (66,240)	Blood	58
BRCA1	↑	Type 2 diabetes	Adipose tissue samples	59

CVD is at least in part through mediating the activity of Nrf2 and its downstream targets that govern antioxidant signaling. The fact that BRCA1/2 mediates Nrf2 activity indicates important implications in the etiology and therapeutic intervention of BRCA-related cardiac dysfunction. Although further systemic investigation for these links, the potential application of the novel strategy to upregulate BRCA1/2 downstream

Nrf2 to prevent subsequently developing other noncancer diseases and protect from anticancer drugs-induced side effects systemically for these cancer patients with BRCA1/2 gene mutations.

Contributions of the authors

SZ, YZ, and LC conceived the idea of the review article. SZ and LC designed the layout of the review article. SZ, JJ, JW, SH, ZZ, YZ, and LC searched the literature search. Figures and Tables were created by SZ and JJ. The manuscript was written by SZ, JJ, JW, and ZZ. YZ and LC reviewed and edited the manuscript. All authors approved the final version.

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