Am J Cancer Res 2020;10(5):1568-1591
www.ajcr.us /ISSN:2156-6976/ajcr0113159

Review Article

Breast cancer in low-middle income countries: abnormality in splicing and lack of targeted treatment options

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Received April 23, 2020; Accepted April 30, 2020; Epub May 1, 2020; Published May 15, 2020

Abstract: Breast cancer is a common malignancy among women worldwide. Regardless of the economic status of a country, breast cancer poses a burden in prevention, diagnosis and treatment. Developed countries such as the U.S. have high incidence and mortality rates of breast cancer. Although low incidence rates are observed in developing countries, the mortality rate is on the rise implying that low- to middle-income countries lack the resources for preventative screening for early detection and adequate treatment resources. The differences in incidence between countries can be attributed to changes in exposure to environmental risk factors, behaviour and lifestyle factors of the different population groups. Genomic modifications are an important factor that significantly alters the risk profile of breast tumourigenesis. The incidence of early-onset breast cancer is increasing and evidence shows that early onset of breast cancer is far more aggressive than late onset of the disease; possibly due to the difference in genetic alterations or tumour biology. Alternative splicing is a pivotal factor in the progressions of breast cancer. It plays a significant role in tumour prognosis, survival and drug resistance; hence, it offers a valuable option as a therapeutic target. In this review, the differences in breast cancer incidence and mortality rates in developed countries will be compared to low- to middle-income countries. The review will also discuss environmental and lifestyle risk factors, and the underlying molecular mechanisms, genetic variations or mutations and alternative splicing that may contribute to the development and novel drug targets for breast cancer.

Keywords: Breast cancer, *BRCA*, family history, HER2, ER, aberrant splicing

Introduction

Breast cancer is a significant issue globally. It is the most commonly diagnosed cancer in women with an estimated 2.1 million new cases being diagnosed each year and representing 24.2% of all cancer diagnoses among women. Breast cancer accounts for 1 in 4 cases in women globally and contributes to 15% of mortality [1]. Early-onset breast cancer is when women under the age of 50 years are diagnosed with the disease. Early onset breast cancer is typically aggressive and has a poor prognosis [2]. In comparison, late-onset breast cancer is less aggressive and is diagnosed in women aged 70 years or older [3-5]. The lifetime risk of developing breast cancer differs by country and ethnicity due to the exposure to risk factors. **Table 1** presents lifetime risk of breast cancer for women in the United States (U.S.) in different age groups; the lifetime risk varies compared to women in developing countries. For instance, the National Cancer Registry estimates the lifetime risk for women in South Africa is 1 in 27 [6].

Breast cancer is a complex and multifactorial disease that is attributed to both sporadic and familial factors. Risk factors that increase the probability of developing breast cancer include environment and lifestyle changes. Other contributing risk factors include reproductive and hormonal factors [7-9]. In familial or hereditary cases, genetic alterations increase the risk and play a vital role in the development of the disease. Genetic mutations that may initially arise

Table 1. Probability of developing breast cancer

Age	Probability of breast cancer Development in %*
Birth to 49	1.9 (1 in 52)
50 to 59	2.3 (1 in 43)
60 to 69	3.4 (1 in 29)
≥70	6.8 (1 in 16)
Birth to death	12.4 (1 in 8)

*Data from the United States between 2012 and 2014. Adapted from (193).

due to sporadic mutations can be transferred from generation to generation [10]. The incidence and mortality rates of breast cancer in different countries are determined by the economic development of the country, environmental factors and the ethnicity of the populace. The differences between incidence and mortality rates for breast cancer is observed when comparing data from developed countries to developing countries. For instance, the developed countries display high incidence and low mortality of breast cancer and comparatively, developing countries display low incidence and high mortality rates [11-13]. The lack of awareness and screening protocols, limited or no access to diagnostic centres in rural areas for early detection, and lower standards of healthcare facilities all contribute to higher mortality rates in developing countries [11, 14]. The incidence rates of breast cancer can differ by more than 10-fold among selected registries. The highest rates occur in Western Europe and the U.S. and the lowest rates are seen in Africa and Asia, which could be attributed to vast under reporting of the disease reflecting a false prevalence [15]; Israel is the exception having one of the highest incidence rates in the world [10].

This multinational review compares breast cancer in developed countries such as the U.S. and U.K with developing nations such as South Africa, Brazil, China and India. The review will focus on 1) differences in incidence and mortality rates in these countries, 2) the risk factors that contribute to the development of breast cancer 3) the molecular mechanisms and genetic factors underlying the disease 4) aberrant alternative splicing in breast cancer and 5) challenges with diagnosis and treatment.

Incidence and mortality

In over 100 countries, breast cancer is the most frequently diagnosed cancer and is a leading cause of cancer-related deaths in women [1]. The incidence rate of breast cancer is very high in Australia/New Zealand, Northern Europe (especially the U.K, Sweden, Finland and Denmark), Western Europe and North America [1]. The incidence of breast cancer remains high in developed nations compared to developing nations (**Figure 1**). Although, the incidence in underdeveloped regions is increasing and has a mortality rate that is marginally higher than developed regions [1, 13]. South Africa records the highest incidence rate for breast cancer on the African continent. Due to urbanisation and changes in lifestyle, the incidence rate is escalating [16].

In the United States of America, breast cancer is the second leading cause of death in women [17]. Breast cancer mortality rates are high in resource limited countries (**Figure 2**). It is the second leading cause of cancer-related death among women in Africa [18]. This is most likely due to the lack of awareness and limited access to healthcare facilities in rural regions. Reproductive risk factors linked to early menarche and late child-bearing also contribute to the rise in incidence in South Africa, however, this is observed predominantly among the white population [19, 20]. A similar trend of higher incidence in white women in Zimbabwe was reported by Vorobiof and group (2001) who examined breast cancer cases amongst women in Zimbabwe between 1990 to 1992 (19). The incidence of breast cancer among the studied population was found to be six times higher in white women as compared to black women [19, 20]. The prevalence of breast cancer is lower in black women compared to white women, however, black women generally develop an aggressive form of the disease at a much younger age [21]. Recent studies show that black women have a higher risk of developing late stage breast cancer and increased mortality compared to white women [22]. Other risk factors that may contribute to the prevalence of breast cancer in the Sub-Saharan region include having fewer childbirths, obesity, and socio-economic status [15, 23]. In Uganda and Algeria, the incidence rate for breast cancer has doubled in recent years. However, the

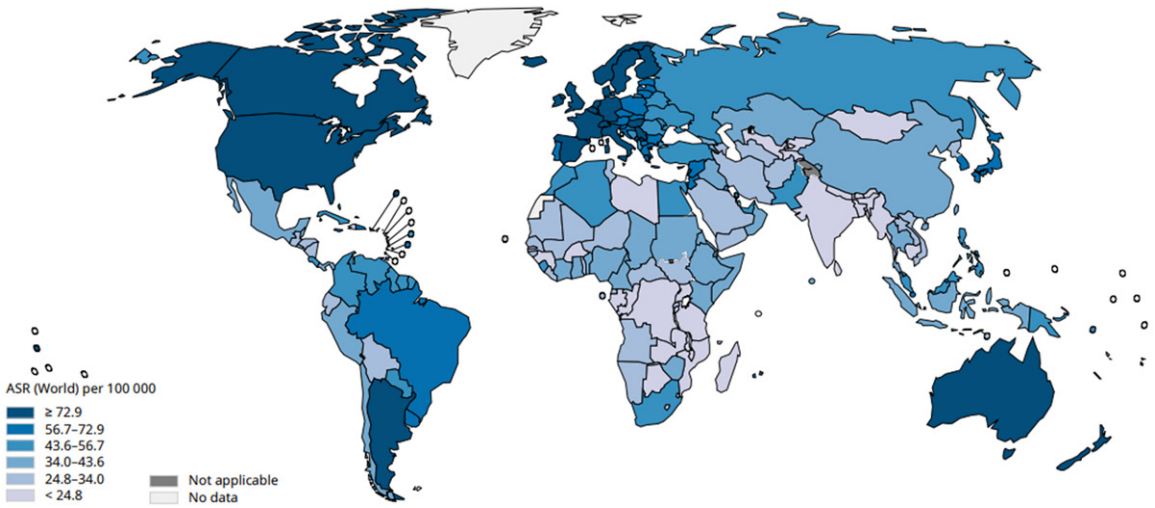


Figure 1. Global breast cancer incidence. Age-standardized rate (ASR) for breast cancer incidence including all ages [194].

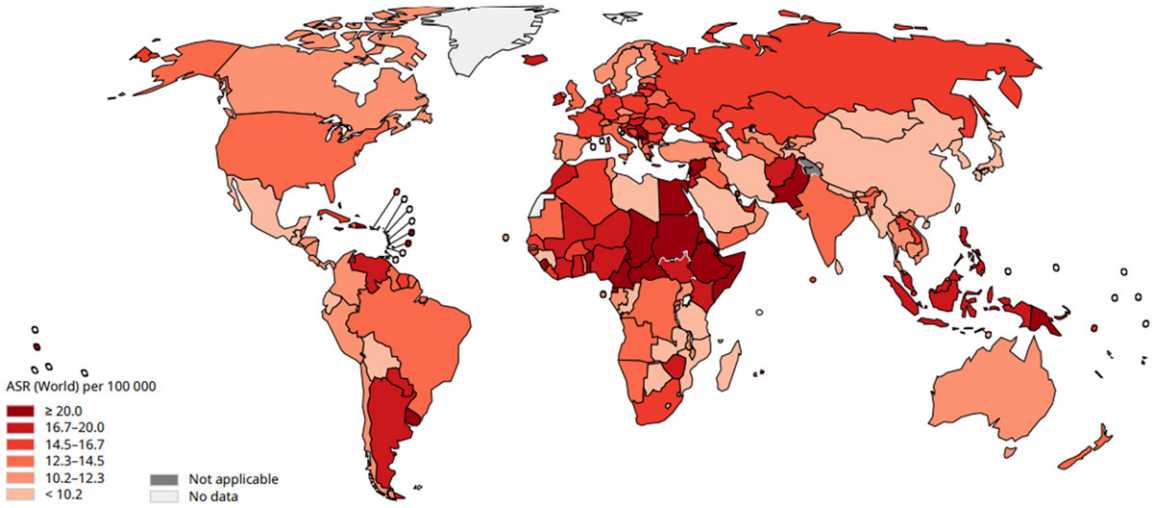


Figure 2. Global breast cancer mortality. Age-standardized rate (ASR) for breast cancer mortality including all ages [194].

incidence rate in these two countries is still much lower than in black women in the U.S. and other Western countries. Early-onset breast cancer is more common among black women in America [20, 24] and in the UK [20, 25]. Early-onset breast cancer has also been observed to be a common trend among black South African women compared to the other low- and middle-income countries [26].

Economically developing countries such as African, Asian and Central American countries have low incidence rates with high mortality rates as compared to developed countries such as Western Europe and North America, where high incidence and low mortality rates are seen [1, 10, 12]. Developing countries lack resources, have poor access to cancer screening and prevention programs; these countries are also lagging in the control of environmental factors contributing to the development of breast cancer. Access to early diagnosis and early treatment is vital to repressing mortality rates. **Table 2** shows the trends observed in breast cancer incidence and mortality rates worldwide. An estimated 60% of deaths worldwide attributed to breast cancer occur in economically developing countries such as Brazil

Table 2. Incidence and mortality rate of breast cancer in different developing countries

COUNTRY	INCIDENCE*	MORTALITY*
World	43.1	12.9
Europe		
Northern Europe	90.1	14.1
Southern Europe	80.3	13.3
Eastern Europe	54.5	15.5
Western Europe	92.6	15.5
America		
Northern America	84.8	12.6
South America	56.8	13.4
Central America	38.3	10.1
Caribbean	50.2	18.1
Africa		
Northern Africa	48.9	18.4
Southern Africa	46.2	15.6
Eastern Africa	39.2	8.6
Western Africa	37.3	17.8
Middle Africa	27.9	15.8
Asia		
South-Eastern Asia	38.1	14.1
South-Central Asia	25.9	13.6
Eastern Asia	39.2	8.6
Western Asia	45.3	13.6
Oceania		
Micronesia/Polynesia	58.2	19.1
Melanesia	49.7	25.5
Australia/New Zealand	94.2	12.6

*ASR: Age Standardised Rate (per 100,000 women); Data adapted from (1).

despite low incidence rates [12, 27-29]. Breast cancer is the predominant cancer in women and the leading cause of cancer-related deaths in Brazil [29, 30]. Recent data from Brazil shows that the breast cancer incidence in Southern and South-Eastern regions of Brazil are similar to that of developed countries [31]. Albeit, regional differences are largely observed in mortality rates [32].

Similar to the developing countries in South America and Africa, breast cancer ranks as the leading cancer in women and the second cause of cancer-related death in Asia. It has been estimated that 39% of all breast cancers diagnosed globally are in Asia; one fourth (25%) of these deaths are observed in China. Breast cancer incidence and mortality rates differ due to the large variations in socioeconomic disparities in countries and regions in Asia [33]. In a recent population study performed by Mubarik et al. [34], the highest breast cancer incidence was observed in Pakistan; China had the second highest breast cancer incidence in Asia, closely followed by India. Similar to other studies, Mubarik et al. reported prominent variations in the breast cancer incidence in Asia; the incidence rates may vary up to 10-fold across Asia [12, 34]. The age-standarised rate (ASR) of breast cancer in China varies from 7.9 per 100 000 to 46.6 per 100 000 based on how economically developed or urbanised the region may be. Comparatively, the average incidence in Europe is 80.3 per 100 000, in the US it is 92.9 per 100 000 and the world average ASR is 43.1 per 100 000 [33, 35]. The low incidence rates noted in Asia could possibly be due to poor reporting or under diagnosis attributed to the lack of advanced primary healthcare. Additionally, the variation in mortality rates amongst these countries are also prominent. Results from a population study, predictably, showed the incidence rate increased with age in China and India for women aged between 20-49 years. Albeit, the incidence risk decreased in women aged between 60-79 years [34]. To understand the mechanism underlying the difference in the risk of breast cancer in pre- and post-menopausal women, factors that cause molecular alterations leading to breast cancer need to be elucidated. Managing risk associated with breast cancer is crucial for the prevention of the disease.

Risk factors associated with breast cancer

Obesity and physical inactivity

Obesity is the most important modifiable cause of cancer after smoking. Being overweight and obese are associated with high risk of colorectal, postmenopausal breast and cancers of the endometrium, gall bladder, pancreas and liver [36]. The increase in obesity prevalence is thought to contribute to the incidence rate of breast cancer. Adipose tissue influences the tumour development. Adipocytes and inflammatory cells secrete adipokines and cytokines which in turn promotes tumour development [36]. Lipids from adipocytes in the tumour microenvironment supports tumour progression and uncontrolled growth. This implies that understanding the mechanisms of metabolic symbiosis between cancer cells and adipocytes may reveal new therapeutic possibilities. High-fat diets and physical inactivity are associated

with high risk of several cancers [37]. Reports indicate that the prevalence of obesity and physical inactivity are increasing in several African countries especially in urban areas. This results from an increase in consumption of calorie-dense food and reduced energy usage in daily life activities which is closely associated with breast cancer [38-41]. In Africa, obesity exists concomitantly with serious food shortages and hunger issues. The World Health Organisation (WHO) formed a global strategy to improve dietary patterns and physical activity through the development of national, regional, and/or community level policies and programs that are comprehensive and sustainable [20]. This could result in decreasing breast cancer risk by preventing obesity and breast cancer patients should be encouraged to perform physical activities.

Reproductive and hormonal factors

Reproductive risk factors are associated with breast cancer and they are possibly mediated by alterations in sex hormones [42]. Elevated circulating concentration of sex hormone are associated with breast cancer risk [43, 44] and high levels are observed in women with a higher body mass index (BMI). Estrogen circulating levels are reported to be higher in women with higher BMI when compared to women with lower BMI. [43]. Long term exposure to estrogen has been shown to elevate risk; however, increased exposure to estrogen during pregnancy has a protective function against breast cancer [45, 46].

Women are exposed to ovarian hormones throughout life caused by the onset on menarche until the cessation of the menstrual cycle which occurs at menopause [42]. The exposure to these hormones confers certain risk profiles to breast cancer. There are two possible mechanisms that provides support for a dose-dependent relationship between ovarian steroid hormones and breast cancer risk. The levels of natural estrogen are the first mechanism. Russo and Russo (2006) demonstrated that the carcinogenicity of estrogen is dependent on 3 mechanisms: i) the activity of the hormone is mediated by receptors, ii) metabolic activation that is mediated by the P450 cytochrome and iii) aneuploidy induction. These mechanisms stimulate direct genotoxic effects and thereby increases mutation rates [47]. Estrogen is reportedly mutagenic through a genotoxic mechanism-formation of depurating estrogen-DNA adducts. This is performed by the reaction of catechol estrogen quinones with DNA [48, 49]. The second mechanism may involve the stimulatory effect of estrogen and progesterone on cell proliferation in the breast, potentially via breast tumour stem cells [50, 51]. Estrogen plays a key role in facilitating the development and progression of normal breast tissue, although, this function is not confined to healthy breast tissue alone; estrogen increases the proliferation of malignant breast cells as well [47]. Furthermore, progesterone induces cell proliferation by activation of the progesterone receptor (PR). Excess PR contributes to breast malignancy and promotes development of invasive tumours [52, 53]. In both mechanisms, it is important to scrutinize and alter potential lifestyle factor that increases hormonal exposure.

Menopausal hormone therapy (MHT) is associated with an increase in breast cancer risk and it is correlated with the length of therapy. Use of estrogen plus progestin therapy for less than three years does not increase breast cancer risk significantly, however, it can impede the identification of early stages of breast cancer [7]. The combination of estrogen plus progestin MHT has an elevated risk of breast cancer when compared to only estrogen MHT [54]. Comparatively, breast cancer risk is reduced in women using estrogen MHT post hysterectomy [55, 56]. Reduction in MHT usage was linked to declining incidence rates in the early 2000s in the U.S., France, Australia and U.K [57-62].

Alcohol consumption and smoking

Alcohol is known as the main environmental risk factor in all cancers. According to reports, higher alcohol intake may increase the risk of breast cancer among women with both family history of the disease and folate intake. Based on existing literature, the association of folate intake, dietary or supplemental, with breast cancer risk has remained controversial; as some studies show it reduces breast cancer risk while others prove the opposite to be true [5, 63, 64]. Heavy alcohol intake disrupts the adsorption and metabolism of folate and may also increase the folate intake requirements [64-66]. As a result, DNA hypomethylation and disruption of DNA synthesis can increase the

risk of cancer due to low folate intake [67-69]. A study by Islam et al. (2013) showed a directly proportional relation between alcohol consumption and breast cancer risk; an inversely proportional relation of folate intake and breast cancer risk was also observed. They showed that breast cancer risk increases with alcohol consumption in Japanese women but the risk reduces with higher intake of folate [70]. There is a higher prevalence of alcohol consumption in women in America and Europe compared to women in Africa and Asia [71, 72]. Studies arising from Africa show a positive association with alcohol consumption and elevated risk of breast cancer. Altering the amount and frequency of alcohol consumption may modify associated breast cancer risk [73].

Smoking is a well-established risk factor of many cancers. Tobacco smoking creates free radicals that damages DNA by oxidative damage resulting in tumourigenesis. Various cohort studies have shown evidence of risk associated with smoking and developing breast cancer. Smoking has become very prevalent in developed countries. It is reported that 16% of all-American adults are smokers. Studies have investigated the direct link between smoking and increase in risk. Current smokers are shown to have an increased risk of 16%. An extensive smoking history increases an individual's risk by 37% [74]. Recent reports from the UK show increased risk in women with a family history of cancer and smoking [75]. In Africa, the prevalence of cigarette smoking is low; it is assumed that the prevalence may increase due to disposable income and adoption of a Western lifestyles driven by images such as films that portray smoking as a desirable activity [76, 77]. Evidence arising from India shows the mean age of diagnosis of passive smokers is 46 years of which 60.9% were premenopausal women and 39.1% were postmenopausal women [78]. In contrast, postmenopausal women in China have a greater risk of breast cancer with smoking [79]. Regardless of the economic status of the country, smoking is a global risk factor. Evading smoking practices can modify the risk of breast cancer.

Radiation exposure

The correlation of breast cancer risk and exposure to radiation is well-documented. Ionising radiation is medically utilised for diagnostics and treatment. There is a high prevalence of breast cancer in young women who have been diagnosed with Hodgkin's lymphoma. These young women often require radiotherapy to the chest area. The radiation exposure to the chest area increases the risk of breast cancer and its prevalence [80-83]. The dose of radiation used for diagnosis or therapy is directly proportional to the breast cancer risk. Age is a modifier with younger women primarily at higher risk of breast cancer when compared to older women. Despite gender, *BRCA1/2* mutation carriers are highly sensitive to radiation. Women with BRCA1/2 mutations undergoing mammography or other radiation related diagnosis or treatment should proceed with caution [84]. Consequently, radiation exposure is a factor that plays a role in elevating breast cancer risk.

Hereditary/familial breast cancer and breast cancer susceptibility genes

While breast cancer mutations can occur sporadically, there is a strong hereditary or familial component. Of all breast cancer cases, 15-25% have a family history of breast cancer and about 10% are hereditary with a considerably higher risk in *BRCA1/2* mutation carriers [85-88]. *BRCA1* and *BRCA2* are high penetrance genes with a lifetime risk of 40-70% [89, 90] and 20-57% [90, 91], respectively. Mutations in other DNA repair genes also confer risk that may be low, moderate or high [92-94]. Family history is a significant breast cancer risk factor. For women with family history of the disease, the risk is increased by 2-fold with a first or second degree relative that has breast cancer. The associated risk increases by 3.5-fold when two or more first degree relatives have breast cancer [95]. Obtaining a comprehensive family history is imperative in assessing risk of the individual.

Hereditary breast cancers generally have a low age at diagnosis [96, 97]. The genetic susceptibility accounts for about 12-25% of early-onset breast cancers [98, 99]. Genetic susceptibility is partly represented by family history. To fully understand the aetiology of the disease in young women, the exogenous exposures should be studied along with genetic factors [100]. According to Kim et al. (2017), amid women with family history of breast cancer, reduction of alcohol consumption and increase folate intake may reduce the risk of breast cancer; this

suggests that there is an association between environmental risk factors and genetic factors [100]. Some gene mutations are sporadic suggesting exposure to carcinogens which finally result in breast cancer. Since environmental factors can cause or modify gene alterations to increase cancer risk, certain environmental factors (alcohol consumption, smoking etc.,) known to cause breast cancer can be avoided to decrease the risk of disease.

Africa is an understudied population with unique genetic makeup. A limited number of studies in Africa, compared to the U.S. and U.K., have reported on recurring and novel mutations in breast cancer susceptibility genes. Previous South African studies reported germline mutations in *BRCA1* in about 20% of the familial breast cancers [101]. Based on patient selection criteria, these results differ vastly. By screening 52 South African families with history of hereditary breast cancer, Sluiter and van Rensburg (2010) identified large genomic arrangement in the *BRCA1* gene in 1 family [102]. By utilising a more sensitive Next generation sequencing method, Francies et al. (2015) identified 12.8% *BRCA1/2* mutations in a South African cohort of a triple negative or young breast cancer cohort [98]. In China, the prevalence of *BRCA1/2* mutations ranges between 8-13.5% in familial breast cancers. In early-onset breast cancer patients, the data is relatively similar to that of familial breast cancer patients ranging between 8.7-11.4%. The prevalence of familial and early-onset breast cancer was in the range of 2.9-28% and 2.8%, respectively in India. Compared to China, India has a higher prevalence of *BRCA1/2* mutations in familial breast cancers and a much lower prevalence in early-onset breast cancer [103].

In developed countries, genetic testing is offered subsequent to genetic counselling of high-risk individuals. In the US, the rate of *BRCA1/2* genetic testing has drastically increased. The UK has a prevalence of 20% positive *BRCA1/2* deleterious mutations. Europe is home to the majority of the Ashkenazi Jewish population where the *BRCA1/2* founder mutation effect is extensively described, this is evident in both low and high risk Ashkenazi Jewish patients [104, 105]. Additionally, a regional recurring effect is observed in Europe and could add to the increase in prevalence of mutations detected [105]. Assessing genetic risk prior to screening is essential to categorise relatively high and low risk patients such as family history, Ashkenazi ancestry and benign breast disease.

Molecular mechanisms of breast cancer

The breast cancer incidence and mortality rates are dictated by the biological profile and molecular subtype of the tumour. This can be determined through immunohistochemical and genetic analysis. Deciphering the molecular subtypes of the tumour are beneficial for evaluating treatment options and for prognosis. A number of breast cancers are associated with a heritable component. Genetic analysis is, therefore, recommended for patients with family history of the disease amongst other important criteria. Various DNA repair genes are associated with breast carcinogenesis. These mutated genes lead to protein modifications in breast tumours; proteins such as BRCA1/2, c-Met, STAT3 and p53. Genetic mutations may be diverse based on ethnicities as well as differences in exposure to breast cancer environmental risk factors in different countries. A brief summary of the role played by some important proteins in breast cancer is detailed below. These proteins may serve as targets for future drug development as well as diagnostic and prognostic markers.

BRCA1 and BRCA2

The tumour suppressor genes, *BRCA1* and *BRCA2*, are closely linked to breast cancer and play a vital role in maintaining genomic stability, DNA repair pathways and transcriptional regulation (**Figure 3**). The interaction of BRCA1 and BRCA2 proteins with recombination proteins in the Rad protein family is vital in the DNA damage response pathway [106]. Both BRCA1 and BRCA2 proteins form a complex with the RAD51 protein to facilitate the error-free homologous recombination (HR) repair of double-stranded breaks (DSBs). The regulation of DNA repair of single-strand breaks (SSBs) and DSBs, and DNA recombination is regulated by the pivotal interaction between BRCA2 and RAD51 interaction [107]. In response to DNA DSBs, BRCA1 and RAD51 are co-localised at the site of damage forming a nuclear focus. Defects in BRCA1/2 cause failure of foci formation of RAD51 protein. Defects in HR repair pathway and BRCA-mutated tumours is shown by the

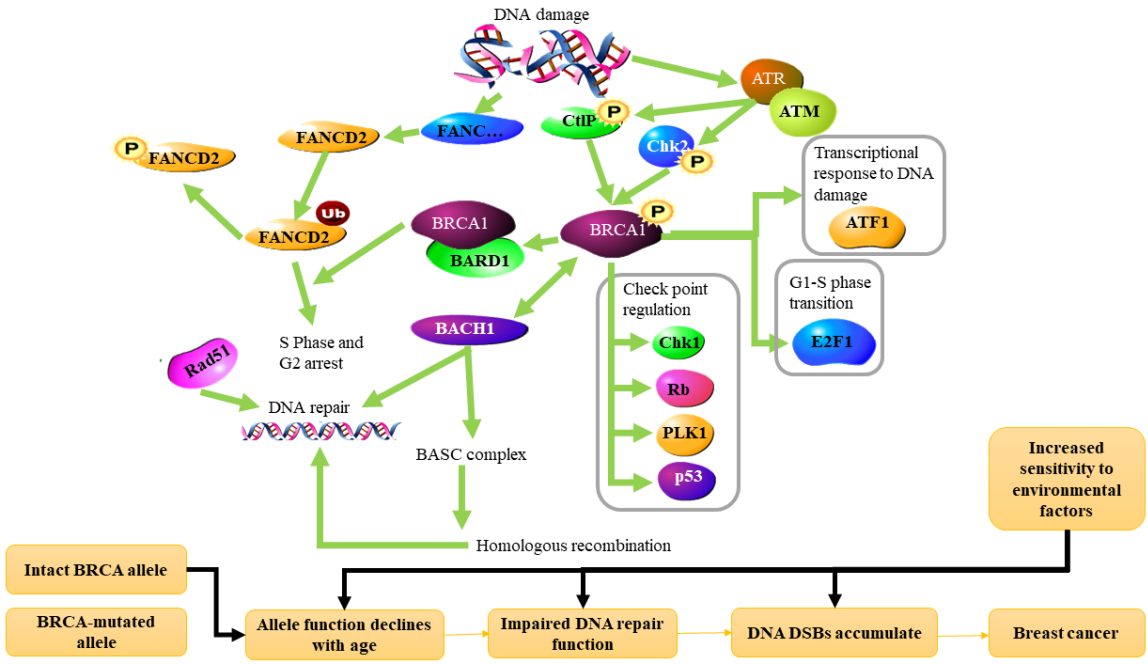


Figure 3. Schematic representation of the BRCA pathway. BRCA1/2 interacts with numerous proteins in response to DNA damage. A mutated *BRCA1/2* can lead to genomic instability resulting in cancer [115].

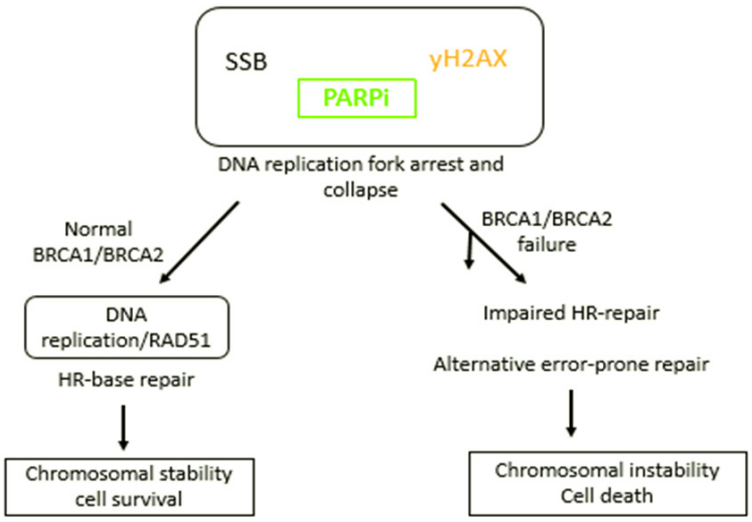


Figure 4. Mechanism of PARP inhibitors and tumour selective synthetic lethality [108].

absence of RAD51 foci in the nucleus which acts as a biomarker [108, 109]. Defects in *BRCA1/2* genes or reduced expression of its proteins by BRCA1 promoter methylation induces impaired DNA repair that contributes to genetic instability, cell cycle irregularities and enhanced probability of cancer [106].

In addition to DSBs, accumulation of SSBs can be lethal. SSBs are primarily repaired by base-excision repair, a repair pathway that contains crucial enzymes called Poly (ADP-ribose) polymerases (PARPs). PARPs constitute a family of enzymes that are involved in base-excision repair. SSBs activate PARP1 and PARP2 to aid in repair. A defective PARP1 can sustain the accumulation of SSBs that are converted to DSBs during replication or repair. This induces the formation of the nuclear RAD51 foci owing to the increase in DNA DSBs to be repaired by the HR pathway (**Figure 4**). There is evidence to demonstrate that BRCA-deficient tumours are hypersensitive to PARP inhibitors. Inhibition of PARP in BRCA-deficient cells may cause accumulation of DNA lesions that would repair inadequately and lead to apoptosis [108]. Based on this theory of PARP inhibitors, it may likely be a candidate drug for clinical use to target breast cancers that have mutated *BRCA1/2* [109, 110].

The concomitant occurrence of *BRCA1/2* germline mutations and triple negative tumours is prominent and associated with bilateral breast

cancer. A significant number of triple negative patients are *BRCA1* mutation carriers and most likely to have a higher graded tumour [111, 112]. This trend is predominantly seen in early-onset breast cancer patients [113, 114]. It is also proposed that environmental factors play an important role in BRCA alteration in breast/ovarian cancer [115]. The loss of function in the *BRCA1* gene seems to occur much earlier when compared with the *BRCA*2 gene. This loss of function of *BRCA1* is correlated with the earlier breast cancer risk and the risk is more prominent in ovarian cancer [116]. Attesting to the sensitivity of *BRCA* mutations and cigarette smoking have a dose-dependent, addictive effect on earlier menopause [117].

c-Met

According to reports, c-Met protein was identified in 1991 as a receptor for hepatocyte growth factor (HGF), a protein previously known to promote hepatocyte growth in culture [118, 119]. The dysregulation of c-Met signalling has been found in various and pre-malignant lesions such as lung, breast, stomach, pharynx, colorectum and cervix [120-123]. In breast cancer, overexpressed c-met is associated with higher grade tumours, large tumour size and metastasis. The overexpression of c-met is closely related to triple negative tumours. This renders c-met as a potential target for novel breast cancer drugs, particularly the triple negative phenotype that lacks targeted therapy [124].

c-Met is a 170 kDa precursor that undergoes proteolytic cleavage leading to the generation of a 50 kDa α-subunit and a 145 kDa β-subunit [125]. The extracellular α-subunit adheres to the β-subunit by a disulphide bond (Trusolino et. al, 2010). The intracellular part has three domains, a juxta membrane region which has a downgraded kinase function following Ser 975 phosphorylation; a catalytic domain that contains the Y1234 and Y1235 residues and a multifunctional carboxyl-terminal docking site [126]. HGF is the known mammalian agonistic ligand for c-Met. The binding of HGF ligand to c-Met, results in the receptor undergoes autophosphorylation of the Y1234 and Y1235 residues in the kinase domain [125]. Also, tyrosine residues, Y1349 and Y1356, are phosphorylated allowing the binding of adaptor molecules including growth factor receptor-bound protein 2, growth factor receptor bound protein 2-associated binder 1 and Src-homology-2 domain-containing transforming protein (SHc) [125, 126]. This leads to the facilitation of the downstream signalling through several pathways and these pathways regulate cellular proliferation, motility, migration, invasion and tubulogenesis [127]. The mechanisms of c-Met signalling in breast cancer may be through gene mutation, gene amplification, autocrine signalling, paracrine signalling and phosphorylation (c-Met activity) [119]. Further investigation of c-met is highly warranted to identify the protein interactions that could lead to the cause of breast cancer. Furthermore, c-met inhibitors could reverse the function of overexpressed c-met.

STAT

The signal transducer and activator of transcription (STAT) is a family of transcription factors that integrate cytokine and growth factor signalling to transcriptionally regulate some cellular processes. STAT is implicated in tumour initiation and progression. One of the STAT family, STAT3 is aberrantly activated in 70% of breast cancers. However, STAT3 is associated with triple negative tumours which lack the expression of the estrogen (ER), progesterone (PR) and the human epidermal growth factor receptor 2 (HER2)/neu receptors [128-130]. STAT3 has been extensively investigated for its interaction with the hallmarks of cancer. According to Walker et al. (2014), evidence reveals a significant role of STAT3 in apoptosis, angiogenesis, cell proliferation, immune response and metastasis in breast cancer [129]. The activation of STAT3 is triggered by phosphorylation of its tyrosine and serine residues as a result of upstream signalling [131], which lead to the induction of dimerization of two STAT3 molecules [132, 133]. STAT3 has become especially important as a potential biomarker and target for triple negative tumours. Given the involvement of STAT3 in a range of cellular processes in breast and other cancers, it is a suitable target for cancer drug development [134].

p53

The p53 protein is highly conserved across animal species and is encoded by the *TP53* gene located in chromosome 17 [135]. The protein composition has an N-terminal region and a

region rich in proline. The activation of the protein could induce different effects. As a transcriptional factor, p53 is involved in the control of G_1/S and G_2/M phase transition, in DNA repair, induction of senescence, apoptosis, autophagy, mitotic catastrophe and angiogenesis [136]. p53 is an important tumour suppressor that regulates DNA damage and is mutated in greater than 50% of human cancers. Triple negative breast cancers have a high prevalence of p53 mutations [137]. Evidence indicates that the function of p53 is compromised in several cancers such as ovarian, colorectal, lung, brain, liver and cervical cancer [138, 139]. In response to a variety of cellular stress signals, p53 induces a complex network of hundreds of genes which in turn trigger context-dependent anti-proliferative cellular responses including cell cycle arrest, apoptosis, autophagy and senescence [139-141]. The p53 protein has a network which encompasses direct effectors of these processes; these include the i) cyclin-dependent kinase inhibitor 1A (p21), ii) p53 upregulated modulator of apoptosis (PUMA) and iii) Bcl2-associated X protein. p53 also participates in indirect effects and cross-talks with (proto-) oncogenes and other tumour suppressors [142]. As a result of this cross-talk, p53 mutations and loss of function has ramifications on cell migration, angiogenesis and cell metabolism [141]. Finally, p53 activities adapt cellular functioning in pro-oncogenic stress conditions and protect normal cells from turning malignant [141, 143-145].

Alternative splicing in breast cancer

A common feature observed in breast cancer and most other cancers is aberrant alternative splicing. Alternative splicing produces a range of protein isoforms of the same gene with diverse structure and functions achieved by splicing identical pre-mRNA. These multiple protein isoforms contribute to a range of diverse phenotypes that function in normal development and differentiation. Aberrant splicing, however, produces oncogenic phenotypes that promote tumour progression, malignant cell invasion, metastases, poor survival and, importantly, resistance to treatment. Breast cancer cells adapt their natural environment to aid in proliferation achieved through aberrant alternative splicing that plays a crucial role in cancer progression [146, 147].

A number of protein isoforms arising from aberrant alternative splicing have been implicated in breast cancer tumour progression such as BRCA1 and BRCA2, Cyclin D-binding myb-like transcription factor 1 (DMTF1), Ras-related C3 botulinum toxin substrate 1 (Rac1), Krüppel-like zinc finger factor 6 (KLF6), survivin, TP53, HER2 and, ERα and ERβ [147-149]. As such, these isoforms may also serve as potential targets for breast cancer therapy.

The role of deleterious BRCA1/2 in the development of breast cancer are well-established. Alternative splicing is a common process in normal breast tissue, but aberrant alternative splicing signatures have identified frequent hotspots. Identified hotspots that are frequently associated with BRCA1 splice variants and breast cancer are exon 2, 3, 9, 10 and 11 [149]. Recent literature shows the role of BRCA1/2 pathogenic splice variants in tumourigenesis and drug resistance [147, 149, 150]. For instance, mutated BRCA1 has 3 different isoforms at the hotspot of exon 11 arising from alternative splicing. Splice variant 1 includes all coding regions of exon 11, variant 2 partially skips regions of exon 11 and variant 3 splices the entire coding region of exon 11 out of the coding mRNA (**Figure 5A**). Literature shows evidence of poor prognosis and survival in patients who harbour deleterious mutations in exon 11 compared to BRCA1 mutations in other regions. Additionally, mutations in this region in BRCA1, particularly the variant 2 isoform with partial exon 11 skipping, are associated with acquired drug resistance to PARP inhibitors and cisplatin [150].

Tumour suppressor genes are the guardians of the genome by maintaining the integrity of DNA. An important tumour suppressor gene is TP53 that has a range of functions including apoptotic induction. However, it is absent or the most commonly mutated gene in most cancer types. Breast cancer has differentially expressed isoforms of TP53; the DNA-binding domain or the regulatory domain may be truncated in the splice variants (**Figure 5B**). These isoforms have reduced tumour suppressor activities and have an impact on survival on patients harbouring mutations in TP53 [149]. Alternative spliced variants can be useful as a prognostic marker for breast cancer. A number of variants identified are closely associated with poor prognosis and survival of breast cancer pati-

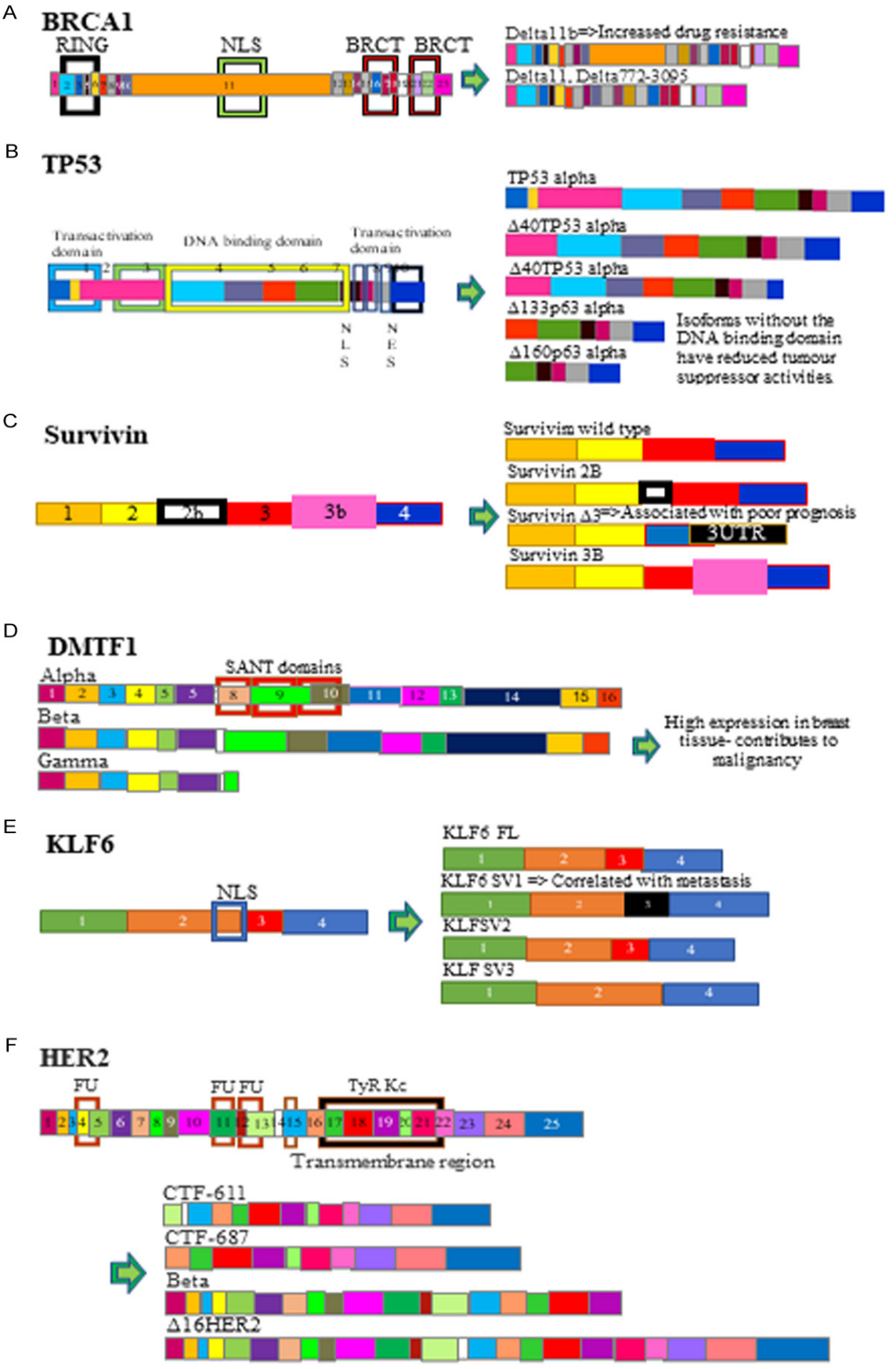

A
BRCA1
RING
NLS
BRCT BRCT
Delta11b=>Increased drug resistance
Delta11, Delta772-3095
B
TP53
Transactivation domain
DNA binding domain
Transactivation domain
NLS
NES
TP53 alpha
Δ40TP53 alpha
Δ40TP53 alpha
Δ133p63 alpha
Δ160p63 alpha
Isoforms without the DNA binding domain have reduced tumour suppressor activities
C
Survivin
1
2
2b
3
3b
4
Survivim wild type
Survivin 2B
Survivin Δ3=>Associated with poor prognosis
3UTR
Survivin 3B
D
DMTF1
Alpha
SANT domains
Beta
Gamma
High expression in breast tissue- contributes to malignancy
E
KLF6
NLS
KLF6 FL
KLF6 SV1 => Correlated with metastasis
KLFSV2
KLF SV3
F
HER2
FU
FU FU
TyR Kc
Transmembrane region
CTF-611
CTF-687
Beta
Δ16HER2

Figure 5. The alternative splicing of genes with isoforms that play a role in the development and progression of breast cancer. A. *BRCA1* is alternatively spliced resulting in truncation or omission of exon 11. B. *TP53* is alternatively spliced to give rise to a number of isoforms. Omission of exons coding for the DNA binding domain give rise to isoforms that function as negative regulators of TP53. C. Survivin is alternately spliced to give rise to an isoform that lacks exon 3. D. DMTF1 is alternatively spliced to give rise to an isoform lacking exon 7 which codes for one of the SANT domains. These domains allow interaction with histones to facilitate chromatin remodelling. E. KLF6 is alternately spliced to give rise to isoforms without exon 3 or with a truncated exon 2 which lacks the nuclear localisation signal. F. HER2 is alternatively spliced to give rise to isoforms lacking exon 20. This disrupts the Tyrosine kinase phosphotransferase domain.

ents. In contract to TP53, survivin negatively regulates apoptosis. Splice isoforms of survivin identified in breast cancer are each associated with grading and size of the tumour, lymph nodes, expression of ER and metastases [149]. Increased expression of survivin is correlated with drug resistance, radioresistance and poor prognosis [146, 151]. With the deletion of exon 3, this splice variant of survivin is associated with poor prognosis and adverse clinical outcomes [152] (**Figure 5C**). Targeting wild type survivin and its splice variants, therefore, has been proposed as a candidate biomarker for early diagnosis of breast cancer and survivin inhibitors as potential therapeutic targets [146, 149, 151].

Aberrantly spliced variants that have carcinogenic properties can lead to neoplastic transformation which has been observed in mouse models. The deleterious variant of DMTF1 that contribute to malignancy is the DMTF1β with increased expression in approximately 60% of breast tissue compared to normal tissue [148] (**Figure 5D**). Similarly, when bound to GTP, the activated Rac1 which is part of the GTPase family, has the capability to promote the development of malignant tissue. There is evidence that shows increased upregulation of Rac1 in breast cancer cells [149]. Comparatively, the wild-type KLF6 protein acts as a tumour suppressor with decreased levels observed in breast cancer patients. Through alternative splicing, KLF6 produces a number of splice variants that promote tumourigenesis. Three important isoforms have been identified that are closely associated with breast cancer development and aids metastasis-KLF6-SV1, KLF6-SV2, and KLF6-SV3 [147] (**Figure 5E**). The splice variant KLF6-SV1 is especially correlated with metastasis. This is attributed to its association with the epithelial-mesenchymal transition in numerous breast cancer tumours [148]; targeting this slice variant in invasive cancers could have therapeutic benefits.

Alternatively spliced variants in cancer are often expressed at a significant level compared with the levels of accurately spliced variants. These variants are generally target candidates for cancer therapeutics. The most common genes that are frequently overexpressed in breast cancer is ER and HER2. Overexpression of these genes are frequently associated with aggressive tumour subtypes and metastasis [147, 148]. The overexpression of HER2 is present in 30% of breast cancers and is associated with aggressive tumours, metastasis and poor prognosis. HER2+ breast cancers have an overexpressed splice variant, Δ16HER2 [148] (**Figure 5F**). The co-existence of the wild type HER2 and the splice variant Δ16HER2, which lacks exon 20, are closely related to drug resistance, especially trastuzumab. Evidence suggest that the drug resistance in HER2 positive breast cancer can be attributed to the Δ16HER2 isoform [147].

Compared to the expression of HER2, ER mediates its biological effects through two receptors - ERα and ERβ. ERα is known to stimulate proliferation of the breast cancer cells while ERβ is a tumour suppressor and an antagonist of ERα; association of ERβ and oncogenic activity has been shown. It has also been implicated with favourable and poor prognosis [153]. ER-positive breast cancers expressing ERα account for about 70% of all breast cancers [154]. ERα and ERβ co-express in breast cancer cells, however, with varying ratios. The proportion of ERβ in normal mammary tissue is higher than ERα. This ratio reverses as tumours progress from pre-invasive to invasive. Despite the elevated amounts of ERα in malignant tissue, a certain proportion of both ERα and ERβ are present in some breast cancers [155, 156]. ERα expression levels in cancer tissues are associated with tumour diameter, TNM stage, while ERβ expression levels in cancer tissues are not correlated with clinicopathological factors of breast cancer [157]. Expression of the splice variants of ERα is dependent on the tis-

sue and disease type. The splice variant ERα46 has been documented to promote breast cancer and drug resistance. In contrast, the ERβ1 isoform has contradictory functions in breast cancer. Research shows that ERβ1 favours apoptosis in breast cancer cells [158] as well as decrease overall survival rates [159]. Exploring the role of ERα and ERβ could provide further therapeutic strategies for breast cancer management.

Modern medicine targets a number of genes and variants to treat breast cancer. These targets have various cellular functions such as regulating apoptosis, involved in DNA damage response and drug metabolism. Altering the expression levels of the targets, through alternative splicing, can determine the effectiveness and efficiency of drugs. Aberrantly spliced variants displaying pathogenic properties can be modified by introducing antisense oligonucleotides, typically 15-20 bases in length, to reverse the pathogenic activities and establish a non-pathogenic variant [148]. This approach can be implemented in previously identified pathogenic variants in breast cancer such as BRCA1-Δ11q variant resistant to PARP inhibitors and the trastuzumab resistance of Δ16HER2 variant [147, 148]. Emerging research shows that BRCA1 splice site has been the target using antisense oligonucleotides to reverse the function of BRCA1-Δ11q; the efficacy of PARP inhibitors are enhanced with antisense oligonuceloties as a combination treatment approach [160].

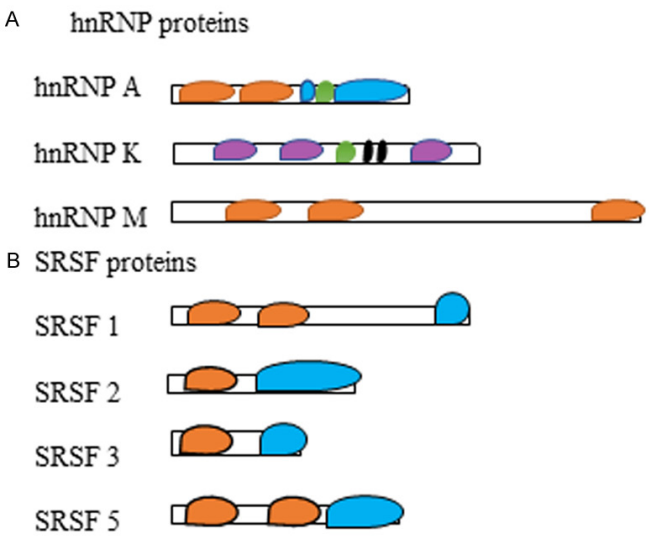
Spliceosomal proteins and breast cancer

Spliceosomal proteins have the capability to activate or block alternative splicing. Spliceosomal proteins bind to RNA sequences; in this way, the binding of spliceosomes to pre-mRNA is blocked. They are divided into two subtypes-the serine/arginine-rich proteins (SR) and heterogeneous nuclear ribonucleoproteins (hnRNP). By binding to the splicing regulatory elements, SR primarily functions to promote alternative splicing. While in comparison, the hnRNP utilises the exonic or intronic splicing silencers to inhibit alternative splicing. Pathogenic splice variant expression is induced when expression of SR or hnRNP is altered leading to cancer progression [149]. Splice variants that gain pathogenic properties are tissue-specific. For instance, the SR splice factor 3 is attributed to the development of breast cancer and acts as a suppressor for hepatic cancer. An estimated 52% of breast cancers report aberrations in SR proteins [161]. Breast cancer metastasis is enhanced by hnRNP. One way this is achieved is by upregulating CD44 alternative splicing. CD44 functions in the epithelial to mesenchymal transition process that is a hallmark of cancer metastasis. This is relevant in triple negative breast cancers where the CD44 alternative splicing is switched by both SR and hnRNP. This is often observed in aggressive breast cancer subtypes [156, 162]. It is evident that splice variants of SR and hnRNP regulate breast cancer biology (**Figure 6**) and progression. Exploring splice variants related to breast cancer may lead to the advancement of novel therapies.

Challenges with diagnosis and treatment of breast cancer

Early diagnosis of breast cancer is crucial in the management of the disease for improved prognosis. Early detection can be achieved by regular screening processes that include self- and clinical examinations, mammography and ultrasound. Mammography is the mainstream method for breast cancer detection [163, 164]. Malignant tissue can be missed when utilising mammography to screen women with dense breasts which is mostly observed in young women and therefore, an ultrasound is recommended [165, 166]. Breast cancer mortality rates can be reduced by implementing national mammography screening to detect malignancies earlier. Although, high-end mammography equipment with well-trained personnel and effective healthcare infrastructure are required to implement successful screening and patient follow-up. Low- and middle-income countries lack the necessary resources and an efficient healthcare infrastructure to conduct population-based screening for cancer control; therefore, women are diagnosed at a later stage and have poor survival outcomes. Although, clinical and self-breast examinations are not an effective strategy for diagnosis, it is still recommended in resource limited countries as part of routine examinations in the absence of other screening methods available [27].

Compared to developing countries, breast cancers in high-income countries are typically diagnosed at early stages and have better progno-



	Domain		Function
	RRM	RNA Recognition motif	Binds single stranded RNA
	Gly	Glycine rich domain	Nuclear localization and protein binding
	KH	K Homology domain	Nucleic acid binding
	RGG box	Arginine glycine glycine box	RNA binding
	Pro	Proline rich domain	Protein interactions
	-RS	Arginine serine rich domain	Splicing activation

Figure 6. Spliceosomal proteins whose expression increases the risk of developing breast cancer. A. The hnRNPs that are oncogenic in terms of breast cancer development include hnRNPA, hnRNPK and hnRNPM. B. The SRSF proteins that are oncogenic in terms of breast cancer are SRSF1, SRSF2, SRSF3 and SRSF5. C. The structure of hnRNPK differs due to the presence of the KH domains rather than RRM domains. None of these pro-oncogenic hnRNPs contain zinc finger domains or Aspartame glutamine acidic domains. The oncogenic SRSF proteins contain only RRM domains and relatively short RS domains.

sis. Western countries have a declining or stable incidence rate and decreasing mortality rate that may be attributed to mammographic screening [167-169]. The overall 5-year survival rates for high-income countries such as the U.K, Canada, Australia, Northern Europe and Western Europe is estimated to be higher than 85%. The U.S. has a 5-year survival rate of 83.9% compared to low- and middle-income countries like South Africa (53%), Algeria (38.8%), India (60%), Brazil (58.4%) [27, 170]. Due to the rising incidence of breast cancer in young African women, mammography screening in SA is routinely recommended for women 40 years and above [171]. In China, Brazil and India, there is a poor quality of cancer care and limited medical treatment available for patients living in rural areas and this is evident in the increasing incidence rates. In most developing countries such as Brazil, mammography screening guidelines are absent [172]. By balancing the available medical resources, introducing efficient screening guidelines and effective measures by the government to address the need of breast cancer diagnosis may assist to bridge the gap [173, 174]. The incidence of breast cancer may increase due to lack of awareness among policy makers, the private or public health agencies in the countries concerning the magnitude of the current and future burden of breast cancer and its economic impact [20].

There are various modalities available to treat breast cancer. Treatment option are based on the biology of the disease. Local treatments directly affect the tumour such as surgically removing the tumours or radiation therapy. Systemic treatments are generally used to treat the spread of cancer throughout the body such as chemotherapy, hormonal and targeted therapy [175]. Management of the disease differs vastly in developed countries compared to developing countries where the poor healthcare systems are enormous. The tumour biology of these populations also differs. Therefore, treatment options from the affluent West cannot be extrapolated to Africa, Asia and other developing regions [176]. The Breast Health Global Initiative has recommended guidelines for treating the disease based on available resources in low- middle-income countries [177]. First-line treatment options to treat tumours are radiation and systemic therapy. This is not a feasible option in Africa. Apart

from South Africa, most African countries have poor or lack radiation facilities and drastic measures, such as mastectomy, are opted for. Therefore, despite the effective use of radiation in the management of breast cancer being well established, radiation therapy is not viable in the majority of Africa and it remains a massive challenge in Africa [178]. Depending on the biology of the tumour, patients who present with late stage disease may also benefit from systemic chemotherapy, endocrine and targeted therapy. These are particularly important in low- and middle-income countries where late stage disease presentation is common [179]. These factors contribute to the increased mortality rates in developing countries, such as South Africa where precision medicine is still in its infancy. Precision medicine is evidence-based medicine with focus on genetics of specific populations. It can provide important information about the studied population which can be utilised to enhance treatment options at an optimal dosage. However, large volumes of experimental data are required from a population and modern technology. The developed countries have large population-based datasets and healthcare infrastructure to practice precision medicine. The same is not true for low- and middle-income countries. Countries like Africa typically have understudied populations with a unique genetic-make-up. With a dearth of genetic and population-based information and efficient expertise available in Africa, precision medicine may be limited [180].

In addition to chemotherapy and surgery, radiation serves as an important treatment modality in the management of breast cancer. In low- and middle-income countries with vast numbers of patients presenting with late stages of the disease, radiotherapy is crucial for improved survival. The reality in these countries are, however, staggering. The distribution of radiotherapy facilities in Africa and Latin America are poor [181]. Excluding South Africa, one unit treats approximately 5 million cancer patients in most African and Asian countries, and about 30 countries in these regions lack radiotherapy units altogether as reported by the International Atomic Energy Agency (IAEA) [182]. An estimated 83% of women in high-income countries who are diagnosed with breast cancer could have access to radiotherapy [182]. With access to radiotherapy, women with early stage disease are candidates for breast conserving surgery. In low- and middle-income countries with lack of access to radiotherapy facilities, breast conserving surgeries are not feasible [183].

Breast conserving surgery serves as an alternative to mastectomy to preserve the breast tissue in women. Any residual tumour tissue will be eradicated by radiation therapy. In China, only 15-30% of hospitals perform breast conservation surgery and the rates still remain very low. Only a few selected hospitals offer this option to patients in India; however, some institutions offer breast conserving surgery to all or most early breast cancer patients [184-186]. This is due to late presentation of tumours as well as the economic restraints and high costs of surgery. Research data from South Africa report that only 20% of breast cancer patients undergo breast conserving surgery which is attributed to late stage disease presentation, patients not being suitable candidates for surgery due to older age and HIV comorbidity [187]. A number of patients can afford the available multi-modality treatments, a larger number do not have access to efficient and cost-effective health system. In Western or high-come countries such as the U.S. and Canada, all forms of modalities are available to patients. In Canada, mastectomy rates have decreased due to the early detection that renders patients eligible for breast conserving surgery. Systemic treatment will be combined with surgery [185]. These factors add to the steady decline in mortality rates in high-income countries like the US.

A global phenomenon of breast cancer is the delay from diagnosis to treatment. Due to the paucity of efficient diagnostic programs, women in developing countries, such as in Africa, present with late stage disease compared to developed countries. The late diagnosis further delays treatment for women in these countries that already lack feasible, cost-effective treatment modalities. Delayed treatment accounts for poor survival rates in women compared to those who begin treatment soon after diagnosis [188-191]. This trend is specially observed in developing countries in women with low socio-economic background [192].

Conclusion

Breast cancer constitutes an alarming burden worldwide that may rise due to an increase in growth and aging of population. In economically less developed countries, an increase in frequency of cancers such as breast cancer has been identified and the incidence in young women is rising. This burden is also influenced by a change of behaviour and lifestyle, factors that are known to play a vital role in breast cancer development. These behavioural and lifestyle factors can influence protein/genetic alteration to cause loss of normal function in certain pivotal proteins/genes. Early detection and promotion of physical activities and healthy dietary patterns are required. Extensive research to understand the mechanisms underlying the biological effects is warranted; alternative splicing offers a new avenue of research for drug discovery for breast cancer. By identifying splice variants that are highly expressed and/or suppressed in breast cancer tumours, may shed some light on candidate genes and proteins as biomarkers or therapeutic options that can be exploited for novel drug development for breast cancer. Breast cancer burden can be managed, in low-, middle- and high-income countries, by enhancing healthcare systems, government collaboration to change public health policies and provide adequate diagnostic systems, creating awareness of the disease and implementing effective guidelines for diagnosis and treatment.

Acknowledgements

We would like to thank the South African Medical Research Council (SA-MRC) for funding this research.

Disclosure of conflict of interest

None.

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