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DNA Damage Response
and Breast Cancer: An Overview

Leila J. Green and Shiaw-Yih Lin
Department of Systems Biology,
The University of Texas M. D. Anderson Cancer Center, Houston, Texas,
USA

1. Introduction

The cells throughout the human body are constantly subjected to both internal forces and external insults that cause damage to their DNA. This damage to the DNA can be harmful to the overall integrity of the cell and the ability for replication. Accurate transmission of genetic information from one cell to its progeny is dependent upon mechanisms within the cell to monitor any defects within its genome and to repair these deficiencies so as not to pass them to subsequent generations. These mechanisms are mainly mediated through an array of DNA damage response proteins including DNA damage sensors, signal transducers and effectors. Sensors, such as ATM (ataxia telangiectasia mutated) and ATR (ATM-Rad3-related), have the ability to recognize areas of damage and activate signal transducers, which either activate or inactivate effectors. Effector proteins trigger cell cycle checkpoint and the cell may successfully repair the damage or proceed towards apoptosis if these damages are irreparable. These molecules are not only necessary for surveillance of occasional non-lethal DNA damage, but are also important for the survival of the cell and the organism. Moreover, mutations to these DNA damage response proteins may contribute to an unstable genome and the development of cancer.

In this chapter we will briefly review the cell cycle and relevant checkpoint proteins. We will also discuss in detail the DNA damage response signal transduction pathway and associated proteins: ATM, ATR, ChK1, Chk2, p53, BRCA-1, PARP-1, and BRIT-1,. Finally, we will discuss the future strategy in targeting the defects of these proteins in the treatment of breast cancer.

Key Words: DNA Damage Response, ATM, ATR, p53, BRCA-1, BRIT-1, PARP-1, PARP inhibitors, Triple Negative Breast Cancer

2. The cell cycle: A brief overview

The cell cycle, first described in 1979, has been accepted as the central dogma of cell replication and contains two main phases; Interphase and the Mitotic phase (Fig. 1.)

The G1 (Gap 1) phase of the cycle is the period in which the cell may grow and function normally. New proteins are synthesized and organelles that the daughter cells will need are created. The synthesis or S phase of the cell cycle follows the G1 phase and is the period of the cell cycle in which the genetic material of the cell is replicated. As stated before, accurate

DNA replication is needed to prevent genetic aberrations that may lead to cell death. The regulatory pathways and proteins that govern this event are highly conserved in eukaryotic cells.
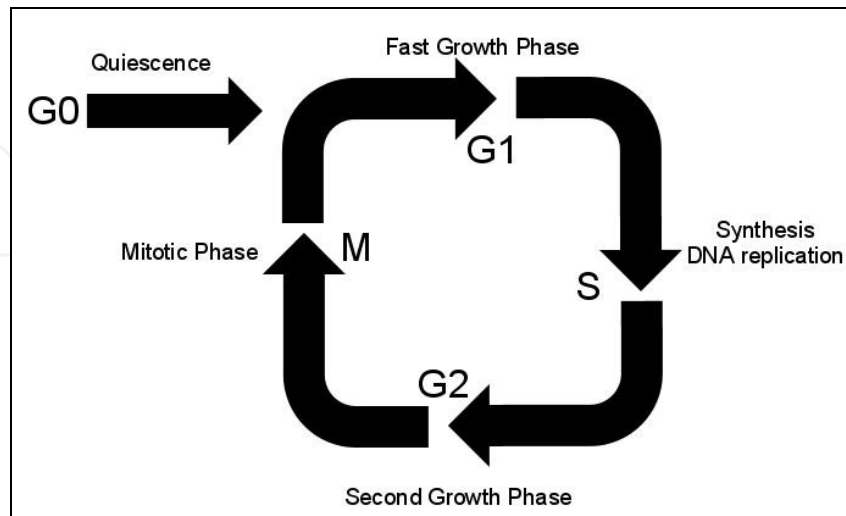

Fig. 1. The Cell Cycle

The G1/S phase transition is a major checkpoint in the cell cycle. The checkpoint response to DNA damage at the G1 phase is mediated by the ATM(ATR)/Chk2(Chk1)−p53/MDM1-p21 pathway, which will be discussed later in this chapter. Expression of ATM and Chk2 are relatively constant during the cell cycle while the concentrations of ATR and Chk1 increase closer to the G1/S transition (Kastan & Bartek, 2004). The end of the G1 Phase consists of the induction of Cyclin E and A, and CDC25A phosphatase, the activator of Cyclin E (A)/CDK2 kinase. In the event of DNA damage, Chk1 down-regulates CDC25A and in effect inhibits Cyclin E (A)/CDK2 kinase which stalls the transition from G1 to S. During the S phase of the cell cycle as the genome is replicated, intra S phase checkpoint networks can also be activated as a result of genotoxic insult. The two parallel branches of this checkpoint, controlled by the ATM/ATR signaling mechanism, will also be further discussed in the chapter.

Arguably, the most important phase of the cell cycle is the Synthesis and G2 phases. The G2 phase of interphase is the second growth period as the cell prepares for mitosis. Using a large number of highly conserved proteins, the G2/M checkpoint prevents cells from entering the mitosis should they experience DNA damage after the S phase, or if they should progress through G1-S-G2 with damage having occurred in previous phases that had been heretofore unrepaired (Kuntz & O'Connell, 2009). All forms of DNA damage, as well as incomplete replication, activate the checkpoint. The mitosis promoting Cyclin B/CDK1 kinase activity is a critical target of the G2 checkpoint. Cyclin B/CDK1 kinase is inhibited by the actions of ATM(ATR)/Chk2(Chk1) and/or p38 kinase mediated subcellular sequestration, degradation and inhibition of CDC25 family of phosphatases that normally activate CDK1 (Kastan & Bartek, 2004). The G2 checkpoint also relies on checkpoint mediators BRCA-1 and p53 which lead to the upregulation of cell cycle inhibitors p21 and GADD45a.

The G0 (Gap zero) phase of the cell cycle in which the cells enter a quiescent state. It can be viewed as an extended G1 phase or as a separate phase outside of the cell cycle. It is separate from apoptosis or senescence in that the cell is metabolically active may enter the G1 phase

and carry out the rest of the replicative cell cycle if needed. Should the cell complete interphase and not be ushered towards G0 or apoptosis the cell is allowed to enter the much shorter Mitotic phase consisting of Prophase, Metaphase, Anaphase and Telophase, the details of which will not be discussed in this chapter.

3. DNA damage

Sources of DNA damage may be endogenous or environmental. Reactive oxygen and nitrogen compounds are produced by macrophages and neutrophils at sites of inflammation and infection. These reactive species can attack DNA which leads to adducts that impair baseparing and/or block DNA replication and transcription, base loss, or cause DNA single strand breaks. Spontaneous alterations in DNA base chemistry and errors in the replication of DNA in the S phase may also contribute to endogenous DNA damage. The most pervasive environmental DNA-damaging agent is ultraviolet radiation. Ultraviolet A and B in strong sunlight can induce ~100,000 lesions per exposed cell per hour (Jackson & Bartek, 2009). Ionizing radiation can also generate various forms of DNA damage, and double strand DNA breaks (DSBs). Most environmental carcinogens operate by generating DNA damage and causing mutation. DSBs can also form in a programmed manner during development including meiosis (germ cell generation) and immunoglobin rearrangement.
DNA strand breaks occur after topoisomerase activity is aborted. Topoisomerase I and II are enzymes that unwind and wind DNA to control the synthesis of proteins and facilitate replication and damage repair. This action is targeted by chemotherapies such as Etoposide, which works by forming a complex with the Topoisomerase II enzyme preventing the re-ligation of the DNA Strands causing errors in DNA synthesis and promotes cell apoptosis.
DNA Mismatches occur via physiological processes such as DNA replication and are strand specific. They are recognized by DNA Mismatch repair proteins MSH1 and MLH2 and when theses proteins are deficient or mutated this may lead to micro satellite instability as seen in hereditary nonpolyposis colorectal cancers and Muir-Torre Syndrome.

4. DNA damage repair

A complex network of proteins and enzymes are designated to detect, signal the presence of, and repair DNA damage. This highly conserved DNA Damage Repair (DDR) signal transduction pathway (figure 2.) allows the cell to survive and maintain the integrity of the genome prior to replication or directs a cell with an overwhelming number of genomic defects towards apoptosis.

4.1 Non Homologous End Joining and Homologous Recombination

Non Homologous End Joining (NHEJ) seen in the repair of DSBs that are induced by radiation. Double strand breaks are recognized by the Ku protein, which then binds to and activates the protein kinase DNA pKcs. This leads to the recruitment and activation of end processing enzymes, polymerases, and DNA Ligase IV. While this mechanism is error prone, it has the advantage of the ability to occur in any phase of the cell cycle. Homologous recombination (HR) results in fewer errors as it uses sister chromatid sequences as the template to mediate faithful repair. HR is initiated by DSBs, stalled replication forks and single strand DNA (ssDNA), and is restricted to the S and G2 phases of the cell cycle. Single strand DNA recruited by RAD51, BRCA-1 and BRCA-2 invades the damaged DNA

template. DNA ligation occurs with nucleases, polymerases, and helicases. HR restart stalled replication forks and requires Fanconi Anemia Protein complex (FANC) to repair interstrand DNA crosslinks.
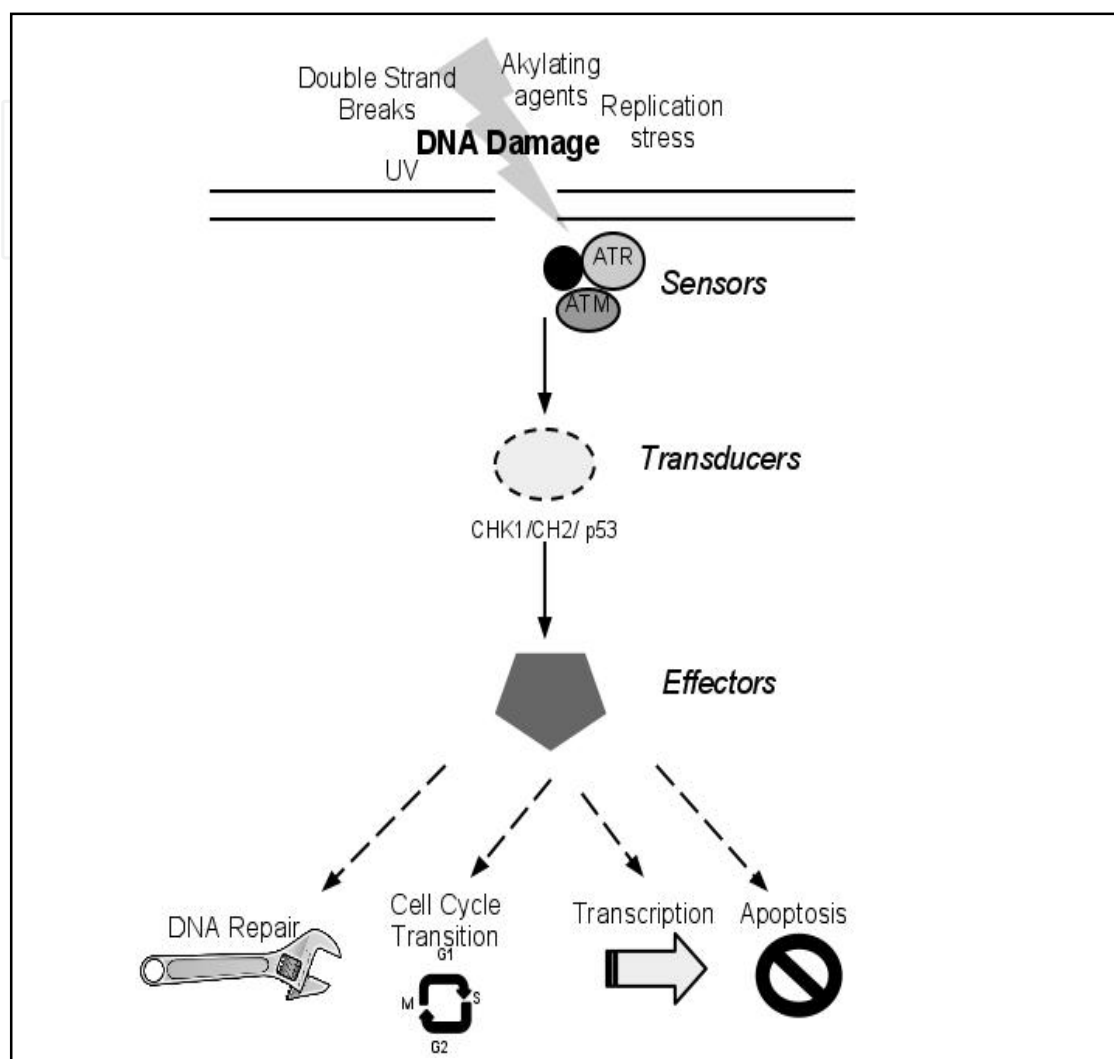

Fig. 2. The DNA Damage Response

4.2 The role of ATM and ATR

Two phosphatidylinositide-3-related kinases, Ataxia telangiectasia mutated (ATM) and ATM-rad3 related (ATR), are sensors integral to the DNA damage response. Their recruitment to DNA lesions is highly conserved and is required for PIKK-dependent DNA damage signalling (Falck et al., 2005). ATM responds to double strand breaks created by ionizing radiation, controlling the initial phosphorylation of several proteins including p53, Mdm2, BRCA-1, Chk2 and Nbs1. While ATR responds to stalled replication forks, ssDNA and DNA damage induced by UV damage. ATR also diminishes the G2/M checkpoint response induced by γ-radiation (Zhou & Elledge, 2000).

ATM and ATR are recruited to and activated by DSBs and replication protein A (RPA) coated ssDNA. ATM-ATR complexes with DNA dependent protein kinases, which phosphorylate Serine 139 on H2AX, a histone variant. Phosphorylated H2AX is referred to

as γH2Ax and recruits DDR factors and relaxes the chromatin actively marking the sites of DNA damage. The resulting immunostainable nuclear foci dubbed IRIFs (irradiation-induced foci) serve as a platform where checkpoint and DNA repair proteins accumulate to promote propagation of the damage signals and repair (Peng & Lin, 2008).

Effector protein kinases, Chk1 and Chk2, are the most understood targets of ATM and ATR respectively. They are serine/threonine kinases that are structurally unrelated but share overlapping substrate specificity (Zhou & Elledge, 2000). Chk2 is the homologue of yeast Rad53 and Cds1, and is phosphorylated in response to ionizing radiation in an ATR dependent fashion. Chk1 is phosphorylated on Ser 345 in response to hydroxyurea and ultraviolet light. The complex of ATM/ATR and Chk1/Chk2 leads to the reduction of Cyclin Dependent Kinases which, as previously described, slows/arrests the cell cycle at G1-S, intra S and G2-M check points, allowing for time to repair the damaged DNA prior to replication (Jackson & Bartek, 2009; Falck et al. 2001; Zhou & Elledge, 2000). Mutations of Chk2 alleles in irradiated cells are unable to undergo phosphorylation and fail to inhibit DNA synthesis after damage, making Chk2 a candidate tumor suppressor (Falck et al., 2001).

ATM and ATR also enhance DDR repair by inducing the DNA-repair proteins by modulating their phosphorylation, acetylation, ubiqutylation or SUMOylation (Jackson & Bartek, 2009). After cell cycle arrest, if the DSB gets repaired via NHEJ or HR, the DDR is inactivated and the cell is able to complete mitosis. If the DNA is unable to be repaired, chronic DDR signaling triggers cell death by apoptosis or cellular senescence.

4.3 p53

TP53 is a tumor suppressor gene that is known as the custodian of the genome. Ironically, it is also the most frequently mutated gene in human cancer. The p53 gene encodes a nuclear phosphoprotein that normally activates G1 cell cycle arrest in response to DNA damage (Fan et al. 1995). This arrest extends the time available before S phase entry. Cells with mutant p53 fail to arrest in G1, but rather accumulate in the G2 phase. P53 can also activate an apoptotic response to DNA damage, especially in hematopoetic and lymphoid cells. In breast carcinomas, 15-40% of tumors present an altered TP53 gene and are associated with aggressive disease and poor overall survival. Although p53 mutations are less frequent in hormone expressing tumors, the prognostic value of TP53 function is relevant in determining the patient's response to chemotherapy.

Focal adhesion kinase, FAK/PTK, is a tyrosine kinase that is over expressed in a variety of human cancers. FAK is a regulator of adhesion and motility and its upregulation is associated with increased metastatic potential. FAK has been shown to contain p53 responsive elements that are down regulated by DNA damage in a p53 dependent manner. When p53 is defective in estrogen receptor positive breast cancer cell lines, loss of FAK down-regulation is associated with increased proliferation and invasion when these cells are exposed to estradiol (Anaganti, 2011). This suggests that the loss of p53 function not only promotes tumorigenesis but also contributes to the metastatic potential of estrogen-responsive tumors.

4.4 BRCA-1 and BRCA-2

The breast cancer genes, BRCA-1 and BRCA-2, encode a nuclear phosphoprotein that acts as a tumor suppressor and plays a role in maintaining genomic stability and are located on chromosome 17 and 13 respectively. The *BRCA-1* gene was identified in 1994 as a cause of

hereditary breast cancer and has since been shown to function in the complex HR pathway of DNA repair. The encoded protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA-1-associated genome surveillance complex (BASC). BRCA-1 promotes cell cycle arrest with p53 and associates with DSBs marked by RAD51 foci. ATM and BRCA-2 gather with BRCA-1 and RAD51 at the sites of double-strand DNA damage. BRCA-2 does not seem to play as important a role in the cell cycle checkpoint responses to DNA damage as BRCA-1, however it has been implicated in the mitotic spindle checkpoint, homologous recombination, and chromatin remodeling.

Mutations in breast cancer genes are responsible for approximately 40% of sporadic breast cancers and more than 80% of inherited breast and ovarian cancers. In the case of BRCA-1 and BRCA-2, heterozygous BRCA-1 mutation carriers have a high lifetime risk of breast and ovarian cancer, and patients with a BRCA-2 mutation will also have a high lifetime risk, though with later onset. The majority of pathogenic BRCA-1 and BRCA-2 mutations are small insertions, deletions or nonsense mutations that result in premature stop codons and a shortened, non-functional BRCA protein.

Women carrying a heterozygous deleterious mutation in the *BRCA-1* gene carry a 57% cumulative risk of developing breast cancer by the age of 70 years and a 40% risk of developing ovarian cancer (Annunziata & Bates, 2010). In cells lacking functional BRCA-1 or BRCA-2, HR is deficient and DDR may proceed through more error-prone NHEJ. This, when applied with a PARP inhibitor, may lead to further propagation of DNA damage and the death of the cell as will be detailed below. It is unclear why breast or ovarian epithelial cells are more susceptible to the oncogenic outcome of BRCA deficiency.

4.5 PARP-1 and PARP inhibitors

Poly (ADP-Ribose) polymerase is an abundant nuclear protein and is a key regulator of base excision repair process. PARP1 detects single strand breaks arising from reactive oxygen species and is also involved in the repair of DSBs. The poly ADP ribose activates histones, transcription factors, and signalers (NFkB, DNAPk, Laminin B) and has an N-terminal DNA binding domain that contains two zinc fingers, which bind to both SSBs and DSBs. PARP activates itself to recruit other component of SSB repair. When PARP-1 is inhibited, normal cells proceed towards homologous recombination repair of the DNA damage. As stated above, when BRCA-1 and BRCA-2 is mutated, cells are unable to proceed towards HR. When PARP-inhibitors are applied, the cell cannot repair the DNA damage and becomes apoptotic, leaving the normal cells unaffected (Liang et al. 2009) as seen in figure 3. This inhibition of PARP-1 also sensitizes tumor cells to chemotherapy and radiation in vitro. Currently, clinical trails are being conducted to assess the efficacy of PARP-1 inhibitors, such as Olaparib, in the treatment of patients with breast and ovarian cancer with BRCA-1/BRCA-2 mutations and triple negative receptor breast cancers (Annunziata & Bates, 2010).

4.6 BRIT-1

The BRCT-Repeat Inhibitor or hTET expression/Microcephalin or BRIT-1 gene is located on chromosome 8 (8p23.1) and is a damage response protein that is physically recruited to sites of damaged DNA. The most distinct role of BRIT-1 is to stop the G2-M transition in the cell cycle of damaged cells. ATM and ATR act on BRIT, However, BRIT is NOT required to phosphorylate ATM but is needed to recruit pATM to the damaged DNA and also

positively regulates CHK1 and BRCA-1 expression. BRIT binds to chromatin and forms nuclear foci after exposure to ionizing radiation or UV rays as short as 2 minutes after the radiation occurs (Chaplet et al., 2006). This chromosome region is frequently deleted in breast, ovarian and prostate cancers. Microarray data from a public database show that BRIT1 mRNA levels are markedly decreased in 19 of 30 cases; 63% of ovarian cancer specimens relative benign ovarian tissue specimens and 72% or the 54 breast cancer cell lines tested also showed a decrease in the BRIT1 gene copy number (Lin et al. 2010).
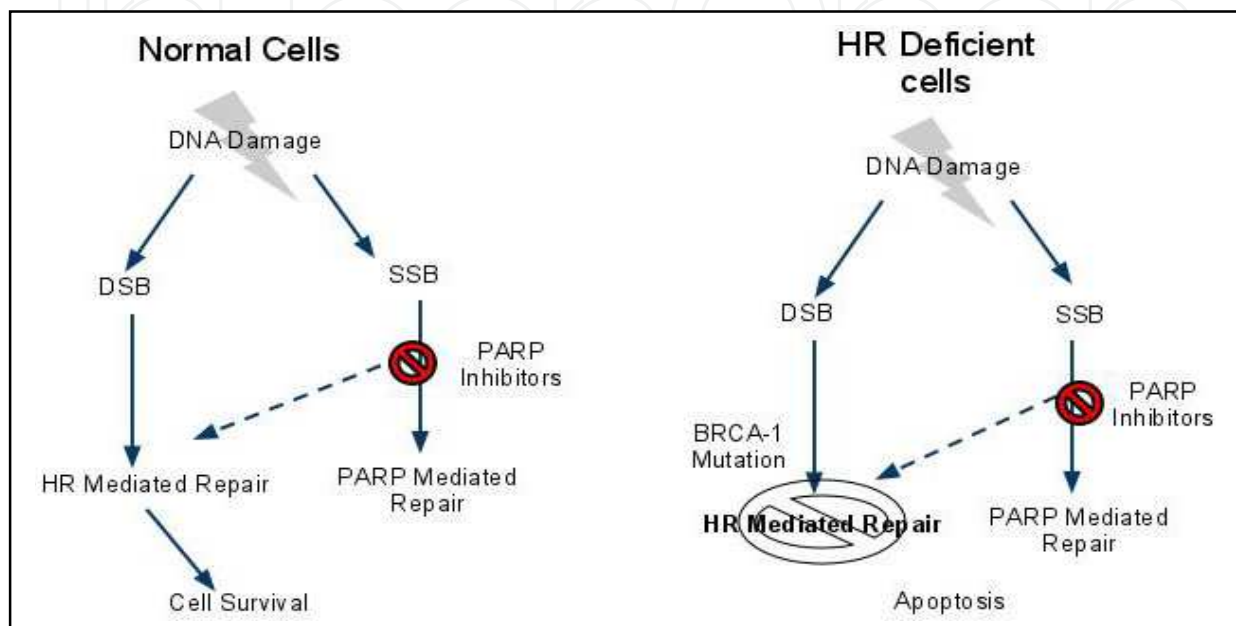

Fig. 3. Action of PARP–inhibitors in HR deficient cells.

When wild type and BRIT knock down cells are irradiated, BRIT deficient cells continue to enter mitosis at a greater rate than the wild types cells (Chaplet et al., 2006). This supposes that the DNA damage that was created during irradiation is not being sufficiently repaired in the BRIT1- group and thus genome mutations are propagated through subsequent generations. BRIT1 has also been clearly demonstrated to be crucial for maintaining genomic stability *in vivo*. Using gene targeting technology to create BRIT1 knock out mice, the BRIT$^{-/-}$ mice were able to survive to adulthood but they were growth retarded and hypersensitive to IR when compared to wild type and heterozygous mice (Liang et al., 2010). Our data suggest that breast tumors can be induced in the mammary glands of mice with conditional BRIT1 deficiency via irradiation, but not in the control littermates. BRIT knock down cells also show reduced expression of BRCA-1 and Chk1 (Lin et al. 2005). The next step will be to investigate if, and to what extent, BRIT1 deficiency may contribute to the initiation and progression of cancer with the existence of oncogenic or genotoxic stress.

5. DNA damage repair and triple negative breast cancer

5.1 Breast cancer epidemiology

Breast cancer is the most common female malignancy and second to lung cancer in terms of mortality. It is the leading cause of death among women aged 40 to 50 years. According to the Surveillance Epidemiology and End Results (SEER) Program of the National Cancer Institute, based on rates from 2005-2007, 12.15% of women born in the United States today

will be diagnosed with cancer of the breast at some time during their lifetime. The estimated new cases of breast cancer in 2010 were 207,090 (female); 1,970 (male) and deaths were 39,840 (female); 390 (male) (Altekruse et al., 2010). The SEER data also show that from 2003-2007, the median age at diagnosis for cancer of the breast was 61 years of age with 0.0% were diagnosed under age 20; 1.9% between 20 and 34; 10.5% between 35 and 44; 22.6% between 45 and 54; 24.1% between 55 and 64; 19.5% between 65 and 74; 15.8% between 75 and 84; and 5.6% 85+ years of age The age-adjusted incidence rate was 122.9 per 100,000 women per year. These rates are based on cases diagnosed in 2003-2007 from 17 SEER geographic areas. (Altekruse et al., 2010).

5.2 Breast cancer risk factors

The most important risk factor for the development of breast cancer is gender with the female to male ratio being 100:1. Further risk factors for breast cancer and relative risks as elegantly described by Singletary in 2003 are shown in table 1. A personal history of breast cancer is a significant risk factor for the development of contralateral breast cancer with an incidence of 0.5%-1.0% per year. As stated previously in the chapter, exposure to ionizing radiation, such as for the treatment of Hodgkin's Lymphoma, has been associated with an increased risk of breast cancer, especially if it occurs prior to age 30, however the risk is less in the first 15 years after treatment than after 15 years (Zager et al, 2006).

Risk Factors	Category at Risk	Relative Risk
Alcohol Intake	2 drinks per Day	1.2
Body Mass Index	80th percentile, age 55 or older	1.2
Hormone replacement therapy with estrogen AND progesterone	Current user for at least 5 Years	1.3
Ionizing radiation exposure	Treatment for Hodgkin's Disease	5.2
Age at first Childbirth	Nulliparous or 1st Child after 30	1.7-1.9
Current age	65 or older	5.8
Past History of Breast Cancer	Invasive Breast Cancer	6.8
Family History	First Degree relative with premenopausal breast cancer	3.3
Family History	First Degree relative with postmenopausal breast cancer	1.8
Germline Mutation	Heterozygous for BRCA1 age <40	200
Germline Mutation	Heterozygous for BRCA1 age 60-69	15

Table 1. Risk Factors for Breast cancer (Singletary, 2003).

Breast diseases such as adenosis, fibroadenomas, apocrine changes, duct ectasias and mild hyperplasias carry no increased risk for breast cancer as they are non-proliferative. However, proliferative diseases, such as atypical ductal or lobular hyperplasias do carry an increased risk. Hormone replacement therapy used for the treatment of postmenopausal symptoms have been shown to increase a woman's risk of developing breast cancer, yet patients may weigh this risk with the benefits of hormone therapy such as a decrease in postmenopausal symptoms and increased bone density.

The age of the patient is also a risk factor for breast cancer. For women who experience menopause after the age of 55 the risk of breast cancer is twice that of women who enter menopause before age 44. Women who experience regular ovulatory cycles prior to age 13 have a 4 fold increased risk of breast cancer than women who had up to a 5 year delay in the development of regular cycles. Women who menstruate for more than 30 years are also at a greater risk for developing breast cancer than women who menstruate for less than 30 years (Zager et al. 2006).

Family history of breast cancer is very important in determining the risk of breast and other cancers. Though the majority of breast cancers are of the sporadic type, the highest risk is seen in patients with a young (premenopausal) first-degree relative with bilateral breast cancer (Zager et al. 2006). Overall risk is determined by the number of relatives with cancer, their ages at diagnosis and laterality of their disease (unilateral or bilateral). The above risk factors have been used in the Gail breast cancer risk model, which will be discussed later in this chapter.

Genetic abnormalities have also been seen to predispose individuals to breast and ovarian cancers. Autosomal dominant mutations such as Li-fraumeni syndrome, Muir-torre Syndrome and, of course, BRCA-1 and BRCA-2 mutations which predispose patients to breast cancer have been well described and are diagrammed in table 2. As seen in the previous table, heterozygosity for BRAC-1 germline mutation carries a relative risk of 200 for the development of breast cancer.

Syndrome	Site of Mutation	Increased Malignancies of:
BRCA-1	Chromosome 17q	Breast, Ovarian, Prostate, Colon
BRCA-2	Chromosome 13q	Breast (including male), Ovaries, Prostate, larynx, pancreas
Li- Fraumeni	p53 gene on chromosome 17p	Breast, brain, adrenal glands, soft tissue sarcomas
Muir-Torre	DNA Mismatch repair genes hMLH1 and hMSH2 on chromosome 2p	Breast, gastrointestinal tract, genitourinary tract, sebaceous tumors, keratoacanthomas
Cowden Disease	PTEN gene on chromosome 10q	Breast, Colon, Uterus, thyroid, lung, bladder. Hamartomatous polyps in the GI tract.
Peutz-Jegers	STK11 gene on chromosome 19p	Breast, Pancreas, mucocutaneous melanin deposition Hamartomatous polyps in the GI tract.

Table 2. Autosomal dominant mutations and syndromes associated with increased risk for breast cancer (Zager et al, 2006).

5.3 Luminal and basal sub-typing

Breast cancers are often described in terms of luminal and basal subtypes corresponding to their likely origin based on hormone receptor status. The luminal subtypes A and B are characterized by estrogen receptor (ER+) expression and expression of genes associated with luminal epithelial cells. Luminal A tumors are often low grade while luminal B tumors are often high grade. Human epidermal growth factor Receptor 2 receptor positive tumors (also known as HER2/neu or ERBB2) encode a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. These tumors respond to trastuzumab, a monoclonal antibody that binds to the domain IV of the extracellular segment of the HER2/neu receptor causing arrest during the G1 phase of the cell cycle. While the HER2 phenotype is aggressive, this response to trastuzumab makes for a favorable prognosis.

The basal-like subtype is characterized by the expression of basal keratins 5 and 17, laminin and fatty acid binding protein 7. Large portions of basal subtype tumors are also lacking expression of ER, Progesterone Receptor (PR) and HER2. These tumors are termed triple negative breast cancer due to their lack of the three receptors (Jaspers et al. 2009).

5.4 Triple negative breast cancer

Triple negative breast cancers (TNBC) show the poorest survival of all US groups and are larger than and show a higher rate of node positivity at the time of diagnosis. Triple negative breast cancers have a high prevalence in young, obese women; and pre-menopausal African-American women, compared to post menopausal African-American and non-African-American women. They also present as interval cancer with a weak association between tumor size and lymph node involvement. TNBCs have a high risk of early recurrence with a peak recurrence rate seen between the first and third years after diagnosis with metastasis rarely preceded by local recurrence and most mortalities occurring in within the first 5 years, further highlighting the need for close clinical follow-up (Chacón & Constanzo, 2010).

Like basal subtype tumors, TNBC patients have significantly higher rates of pathological complete response following neoadjuvant chemotherapy, with increased frequency of distant metastasis formation but not local relapse (Jaspers et al. 2009). The majority of BRCA-1-related breast tumors share phenotypic features with TNBCs and basal- like tumors as they mostly lack expression of ER and HER2, and have epidermal growth factor over-expression and p53 mutations. These similarities suggest that basal-like breast cancers arise through common genetic pathways as BRCA-1 mutated cancers. Recently, an unselected cohort of patients with TNBC was found to have a 19.5% incidence of BRCA mutations with 12.6% in BRCA-1 and 3.9% in BRCA-2 (Gonzalez-Angulo et al., 2011). This suggests that TNBC and basal- like breast cancers may be similar to BRCA-1 mutated tumors in their pathogenesis and that they may be susceptible to PARP-1 inhibitors and other chemotoxins that are designed for BRCA-1 mutated tumors. These findings also suggest that genetic counseling may be indicated in patients presenting with triple receptor negative breast cancer.

6. Targeting DNA damage repair defects in the treatment of breast cancer

6.1 Development of new treatment modalities

For patients with breast cancer, treatment consists of surgery, radiation, hormonal and chemotherapy. Commonly, clinicians employ a combination of all of these modalities to prevent loco-regional recurrence or to treat distant metastasis. Radiation and some

chemotherapeutics rely on the inherent DDR impairment of tumor cells to induce apoptosis. Cancer cells, which proliferate more rapidly than most normal cells, are thought to be especially vulnerable DNA damage exposure, which occurs during S phase of the cell cycle. Chemotherapeutics that induce DDR inhibition might enhance the effectiveness of radiotherapies and DNA damaging chemotherapies.

The status of DNA damage repair proteins may also allow for the prediction of breast cancer sensitivity to neoadjuvant chemotherapy. In a recently published study (Asakawa et al. 2010) sixty patients with primary breast invasive ductal carcinoma consecutively underwent neoadjuvant chemotherapy with Epirubicin and Cyclophosphamide (EC), two chemotherapies that induce DSBs, followed by treatment with docetaxel. The investigators were able to correlate focus formation of BRCA-1, γH2AX, and RAD51 prior to treatment and RAD51 focus formation after treatment with mean tumor volume reduction and tumor response rate. When cells showed a high percentage of DDR nuclear foci staining shortly after the first EC treatment, they showed poor tumor response when evaluated for mean tumor volume reduction, which, in breast cancer, is a major goal of neoadjuvant chemotherapy. These findings may lead to the possibility of using DDR status clinically to determine which patients will respond to chemotherapy.

Targeting specific DDR proteins to inhibit is another way to develop tailored treatment modalities. As stated prior in the case of PARP-1 inhibitors, breast and ovarian cancer patients with BRCA-1 and BRCA-2 mutations may be more likely to respond favorably than those patients with functioning BRCA-1. This could be a great breakthrough for these patients as they generally have more aggressive disease with high rates of loco-regional recurrence, elevated risk of contralateral breast cancer formation and are notoriously resistant to the chemotherapeutics available today. We can further these efforts to investigate patients with deficiencies in other DDR associated proteins.

DDR targeted cancer therapies currently in clinical trails include those targeting the Chk1 and Chk2 proteins. The inherent resistance to radiation of cancer stem cells is another challenge to overcome and inhibiting the Chk1 auto phosphorylation may increase the toxicity of DNA damaging agents that are currently in use and may improve radiosensitvity. In the case of p53, which is functionally reduced in 50% of cancers, efforts have been made to restore the wild type p53 activity via recombinant adenovirus encoding p53 or by using small compounds and short peptides to restore the function. (Bolderson et al. 2009)

6.2 Development of new cancer screening modalities and risk assessments

As stated previously, women who have BRCA-1 germline mutations have a higher risk of developing breast cancer than the general population, and the cancer that they develop is exceedingly aggressive with a high rate of recurrence, development of contralateral disease and resistance to current chemotherapies. Genetic testing is now being used to identify patients with the genetic predispositions to develop tumors. This testing allows clinicians to better define a patient's effective risk and to allow for preventive treatment strategies such as prophylactic surgery or hormonal therapies. In the case of multiple family members presenting with breast cancer, genetic counseling should be offered to the patient affected with the disease first to determine what type of mutation is being expressed before testing any unaffected relatives for the disease. In the case of BRCA-1 and BRCA-2 mutation screening, commonly used methods their advantages and disadvantages were described in

the 2006 paper by Palma and is reproduced here in table 3. These methods include: Direct Sequencing (DS); Single Strand Conformation Polymorphism Analysis (SSCA); Heteroduplex Analysis (HAD); Denaturing Gradient Gel Electrophoresis (DGGE); Chemical Cleavage Mismatch (CCM); Protein Truncation Test (PTT); and Denaturing High Performance Liquid Chromatography (DHPLC).

Technique	Principle for Detection	Advantages/Disadvantages
DS	Direct sequencing of DNA fragments	Best sensitivity ~100%, Exact location and nature of deletion . Labor intensive and expensive
DHPLC	Detects heteroduplex through their chromographic elution profiles	High sensitivity ≤ 100%, rapid and precise Initial investment in equipment is high. However, low cost single analysis
PTT	Detects pre-terminal in vitro synthesized protein products	98% sensitive, gives approximate location of pathogenetic mutations in large fragments. Only detects sequence alterations responsible for truncated proteins. Cannot analyze small exons.
CCM	Detects heteroduplex through chemical cleavage at the site of DNA mismatch	Good sensitivity (>95%) Scans large fragments. Approximates location of damage. Time consuming and labor intensive.
DGGE	Altered Electrophilic mobility of heteroduplex based on their melting behavior (denaturing)	Rapid and easy after initial planning. Low detection rate. Difficult to set up technique.
HAD	Altered Electrophilic mobility of heteroduplex (non-denaturing)	Rapid and easy to carry out, Detects insertion/deletion mutations in large fragments. Low detection rate (80%) poor for point mutations
SSCA	Altered Electrophilic mobility of single stranded DNA	Rapid and easy to carry out Low detection rate (70-80%) scans short fragments.

Table 3. Commonly used methods for BRCA1 / 2 mutational screening in descending order of sensitivity. (Palma et al., 2006)

In the interpretation of these genetic tests, patients who test negative and have a known affected relative who is positive for a specific mutation are thought of as "real negatives" and have a risk of that of the general population. "Non informative" tests are from patients who test negative but who do not have a previously identified familial BRCA-1 or BRCA-2 alteration. There may be a mutation in another gene or a low penetrance gene. The subsequent treatment of family members who test positive for a BRCA-1 or BRCA-2 mutation depends on the age of the patient and the desire of child bearing. Prophylactic

bilateral mastectomies reduce the risk of breast cancer by up to 90%, and while the risk of ovarian cancer is lower than the risk for breast cancer in the context of BRCA mutation, the absence of early detection screening for ovarian cancer has increased the necessity for prophylactic oophorectomy (Palma et al., 2006).

DNA damage repair protein function may be another useful tool to incorporate into our risk stratification models. The Gail Breast Cancer Risk Assessment model has been in use since 1989 (Gail, et al. 1989). This model, adopted by the National Cancer Institute, uses individual risk factors of age; family breast cancer history (amongst first degree relatives); age of first menarche and first live birth; personal medical history including number of previous breast biopsies and confirmed atypical hyperplasia; and race. A 5-year risk estimate and lifetime risk are calculated via logistic regression of the risk factors and converted into absolute risk based on epidemiological data for breast cancer incidence and other risks. A Gail 5-year risk score greater than or equal to 1.66% has been seen to be an important tool to identify women who have an increased risk for developing breast cancer.

This method is not without shortcomings, including having a lower sensitivity for women of color, having been based on a cohort of Caucasian women, and has been challenged in the use of patients younger than age 40 (MacKarem et al., 2001). However when these clinical features of the Gail model are combined with genetic information they may increase the power of the test. Single-nucleotide polymorphisms (SNPs) are reproducibly associated with breast cancer risk in women of European and other racial or ethnic backgrounds. Investigators located seven SNPs associated with breast cancer risk from the literature and genotyped in white non-Hispanic women in a nested case- control cohort of 1664 case patients and 1636 control patients within the Women's Health Initiative Clinical Trail. SNP risk scores were calculated and combined with Gail risk estimates. These investigators found that the SNP risk score was nearly independent of Gail risk with good agreement between predicted and observed SNP relative risks. Combining validated genetic risks factors with clinical risk factors modestly improved classification of breast cancer risk in these women (Mealiffe et al., 2010). This may be able to be extrapolated to other genetic mutations further combining what we know to be absolute clinical risk factors with DNA damage repair gene germline mutations to create a more inclusive model for the prediction of breast cancer development.

7. Conclusion

The cells throughout the human body are constantly subjected to both internal forces and external insults that cause damage to their DNA. Understanding the DNA damage response pathway is key to the understanding breast cancer tumorigenesis and to the creation of superior chemotherapeutics.

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9. References

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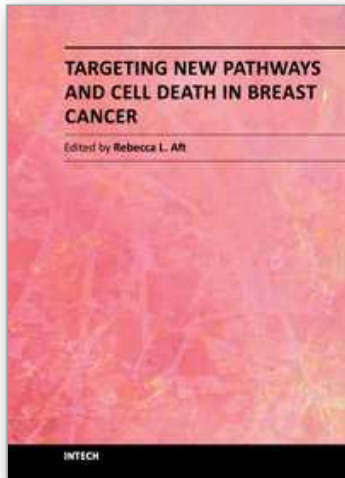
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