Chapter

Cell Cycle and Factors Involved in Inhibition or Progression of Breast Cancer

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Abstract

Cell cycle progression is driven by the sequential activation of a family of cyclin-dependent kinases (CDKs), which phosphorylate and activate proteins that execute events critical to cell cycle progression. Cell cycle checkpoints are scrutiny points that display the order, integrity, and fidelity of the major proceedings of the cell cycle. These comprise development to the correct cell size, the replication, integrity of the chromosomes, and their precise separation at mitosis. Many of these mechanisms are prehistoric in origin and highly preserved and hence have been deeply well versed by studies in model organisms such as the yeasts as well as in higher organisms. These molecular mechanisms switch alternative cell fates with substantial impact on tumor suppression. In the present study, we have explained different checkpoint pathways and the consequences of their dysfunction on cell fate in cancer.

Keywords: cell cycle, breast cancer, drug resistance, tumor suppressor genes, oncogenes

1. Introduction

Deregulation in cell cycle is a remarkable feature of tumor cells [1, 2]. Cell cycle helps to maintain homeostasis of normal cell growth and viability in a compact process. Cell cycle occurs in four phases as follows:

- preparation for cell division in G1 phase;
- process of DNA synthesis in S phase;
- G2 phase for cell growth and enzyme production; and
- mitosis: M phase, which is regulated by several controlled events, directs the replication of DNA and cell division [3].

The transitions between G1 to S and G2 to M phase are governed by changes in the kinase activity of CDKs [4]: Cdk1, Cdk2, Cdk4, Cdk6, and cyclins. Cyclins are the regulatory units, and CDKs contain the catalytic subunit of an activated heterodimer. The cyclin binds to CDKs and gets activated by forming CDK/cyclin complex by phosphorylation leading the dividing cell into next phase of cell cycle. During G1 phase, the predominant cyclin-CDK complexes are cyclin D-Cdk4, 6,

cyclin E-Cdk2, cyclin A-Cdk2 during S phase, cyclin A-Cdk1, and cyclin B-Cdk1 during G2/mitotic phases. The control of the G2/M transition is important in all cancers resulting in chromosomal aberrations, but the G1/S transition involves many of the important cell-cycle events that may be altered in breast cancer. G1/S transition involves functions of the oncogenes/tumor suppressors cyclin E, cyclin D1, and p27 [5]. The oncogenic processes do occur by targeting the regulators of G1 phase progression [6]. During the G phase, cells respond to extracellular signals by either going into next division or withdraw from the cycle and thus are arrested in a state (Go). G1 progression is dependent on stimulation by mitogens or growth factors and can be blocked by anti-proliferative cytokines, but cancer cells do not obey these controls. Cancer cells remain in a continuous cycle of cell division by halting maturation and terminal differentiation. Once the cells pass a restriction point late in G1, they do not respond to extracellular growth regulatory signals and commit themselves to the independent program that causes cell division. The cells, which pass through the restriction point G1 and enter into S phase, which is controlled by cyclin-dependent protein kinases (CDKs) that are regulated by cyclins D, E, and A, are committed to divide.

Cyclin D acts as growth factor regulator in response to extracellular signals in the cell cycle. The activity of Cyclin D depends on mitogenic stimulation upon binding with CDK4 and CDK6. Once bound, the catalytic activity of this complex is maximum in G1-S phase transition, but withdrawal of mitogen causes stoppage of cyclin D synthesis, and thus, the D cyclins holoenzyme activities decay rapidly, and the cells exit the cycle [7]. So, if cyclin DI-dependent kinase activity is lost before the restriction point, it prevents cells from entering S phase [8]. To pass through first check point – G1 phase that occurs in normal cell cycle, cyclin D-dependent kinases should phosphorylate protein retinoblastoma tumor suppressor protein (RB) [9]. As the hyper-phosphorylated RB gets dissociated from E2f, DP1, RB complex will result in the activation of E2F genes and leads to transcription of several genes such as cyclin E, cyclin A, and DNA polymerase. RB and other RB-like proteins (pI30, P107), which regulate gene expression which in turn are regulated by a family of heterodimeric transcriptional regulator E2Fs [10], which can transactivate genes and are required for S phase entry [11]. INK4 proteins inhibit (inhibitors of CDK4 and CDK6 INK4 proteins can directly block cyclin D-dependent kinase activity and cause G1 phase arrest) cyclin D-dependent kinases that phosphorylate RB. RB pathway does not functioning normal, which is feature of cancer cells [12]. The RB is inactivated by phosphorylation or by DNA damage; RB gene causes shrinking of G1 phase, and cell size is decreased. The mitogens and other signals required for cell are still present [13]. As the RB negative cells have few requirements for growth factors, these factors in addition to RB phosphorylation work for restriction point control [14]. As the cell cycle proceeds, the cyclin A- and cyclin B-dependent kinases keep RB in its hyperphosphorylated state. RB is not dephosphorylated until mitosis is completed and again renters the GI phase or Go. The activity of cyclin A synthesis occurs later in GI and is important for the G1-S transition, as blockage of cyclin A function in cells can also block S phase entry. For cell cycle progression, the inactivation of cyclin E and E2F is important when the cell enters S phase [15]. Cyclin B, B-cdc2 complex leads to stimulation of nuclear envelope and initiation of prophase, but its deactivation leads the cell to exit mitosis [16].

2. Drug resistance

Hindrance in treating cancer patient mainly occurs by drug resistance [17]. Targeted therapies on breast cancer depend on the type of receptor being

implicated by the expression of estrogen receptor (ER), progesterone receptor (PR), and overexpression of Her2/neu [17]. The therapeutic agents given in such cases, which eventually develop resistance as an acquired drug resistance, are clinically bigger challenge to treat. Thus, patient's resistant to chemotherapy has poor prognosis and overall poor survival [18].

2.1 Cell cycle regulation in tamoxifen-resistant breast cancers

The main drug given to patients expressing estrogen receptor is tamoxifen, which is the first therapeutic agent for the estrogen or progesterone receptor expressing breast cancers, mostly in premenopausal women with or without conventional chemotherapeutic agents [19]. The estrogen receptor (ER) induction occurs during G0/G1 phase in MCF-7 cells and breast cancer cells [19]. In the late S-phase, a rapid increase in ER has been reported [19]. A tamoxifen-resistant phenotype was developed by long-term exposure of MCF-7 xenografts to tamoxifen resulted in an altered expression of ER during the G0/G1 phase [19]. It has been shown that tamoxifen-resistant MCF-7 cells express higher levels of cell cycle regulators cyclin E1 and CDK2 than parental cells [20]. It has been shown that cyclins E1 and E2 were overexpressed in the tamoxifen-resistant cells when compared with parental MCF-7 cells [20]. TAMR cells may be dependent on cyclin E more than MCF-7C, which indicates that CDK2 is inhibited and is a potential therapeutic marker in endocrine-resistant breast cancer [20]. Cyclin E2 downregulation is required for anti-estrogen inhibition of cell proliferation; cyclin E2 overexpression is associated with endocrine resistance in breast cancer providing reason for deregulation of the cell cycle in endocrine resistance [21–24].

3. Genes associated with breast cancer development and its progression

There are various genes associated with breast cancer, and how these respond to different treatments are mentioned as follows:

3.1 EGFR

Epidermal growth factor receptor EGFR (ERBB1 or HER1) is from ERBB family of cell-surface receptor tyrosine kinases including HER2, also known as NEU or ERBB2 [25, 26]. The epidermal growth factor receptor family consists of four cell surface receptors; EGF receptor also called as HER1, HER2 or neu, HER3, and HER4. Epithelial growth factor binds to the receptor and stimulates homo/hetero dimerization of receptor with other ERBB member like HER2, receptor phosphorylation, which makes binding sites available for cytosolic proteins containing *src* homology 2 (SH2) domains [27]. EGFR growth factors cause activation of downstream effectors such as RAS-RAF-MEK-ERK-MAPK and PI3K-AKT-mTOR pathway. PI3K-AKT-mTOR sources irreversible entry of the cell in S phase of cell cycle resulting in cell proliferation [28]. There are various EGFR ligands such as transforming growth factor-α (TGF-α), amphiregulin, epigen, betacellulin, heparin-binding EGF, and epiregulin [29]. EGFR has an important involvement in cellular differentiation, motility, survival, and tissue development [30]. There are many copies of the EGFR gene in some of the breast cancer cells termed as EGFR amplification, which effects on behavior and response of cancer cell. There are other EGFR-positive cancers like colon cancer, which respond to medicines that target EGFR-positive cancers [31]. In a clinical study, there was an increase in EGFR gene copy number in about 6% of breast cancers and protein overexpression in

7% of breast cancers [32]. The study also showed that increased EGFR gene copy number changes, and protein overexpression was seen mostly in ER negative, PR negative, and HER2 negative (triple negative) cases with three exceptions that are HER2-positive cases in total of 175 cases (reference). The study was similar to that of gene expression profiling studies, which have identified EGFR expression mainly in basal-like breast carcinomas (reference). There was another subtype of breast carcinoma that showed an increase in EGFR copy number, or EGFR protein expression is the heterogeneous category of metaplastic carcinoma. These tumors are subtypes called as basal-like breast carcinomas [33] and were seen in 47 metaplastic breast carcinomas in which EGFR protein overexpression was in 32 cases, but gene amplification (as >5 EGFR gene copy number) was seen only in 11 of these 32 (34%) cases [33]. There are other studies that have shown that EGFR gene amplification and EGFR protein overexpression in various organ systems have found similarity in approximately 50% cases [34]. EGFR protein expression is the result of multiple genomic processes of which EGFR gene amplification is only one of them [34]. EGFR protein expression is seen in breast carcinoma, which is mostly triple negative, and there exists a relation between EGFR gene copy number and protein expression, but relation is not as strong as seen with HER2 [34]. The therapeutic use of EGFR as a responsive marker in breast carcinoma is being studied and needs to identify breast cancer patients that will respond to EGFR-related therapies. A new mechanism-based inhibitors and combination therapies are being worked to overcome therapeutic resistance in tumors [34–36].

3.2 HER2

HER2 gene is amplified in breast cancers by about 20% categorized as HER2-positive breast cancers [37], the extra HER2 protein leads to increase in activation of signal pathway, which results in uncontrolled growth and occurrence of cancer. Breast tumors having HER2 overexpressed proteins are more aggressive than other breast tumors [37]. This results in poorer prognosis in patients and decreased survival rate compared with patients whose tumors do not overexpress HER2 [37]. The inhibition of HER2 signaling will provide a tool to reduce breast cancer. Monoclonal antibodies like lapatinib are used to inhibit the signaling [37]. Herceptin (trastuzumab) is also a monoclonal antibody binding to HER2 [37]. This stops receptor from activating the signaling pathways, which is responsible for proliferation and survival of breast cancer cells [37]. Herceptin also causes inhibition of cancer cell growth by activating an immune response, which will damage nearby cells [37]. It is used to treat breast cancer only in tumor overexpressing HER2 with at least one high risk like estrogen receptor or progesterone receptor negative, pathologic tumor size greater than 2 cm, Grades 2–3, age less than 35 years [37]. Herceptin is used in combination with paclitaxel for first-line treatment of HER2-overexpressing metastatic breast cancer [37].

And as a single agent for treatment of HER2-overexpressing breast cancer in patients who have received one or more chemotherapy regimens for metastatic disease [33, 37, 38].

3.3 HSP27

Heat shock protein 27 (HSP27) is the small molecular weight heat shock protein (HSP) family (12–43 kDa). HSP27 and different members of small HSP family possess a conserved c-terminal domain, the α-crystallin domain, which is similar to the vertebrate eye lens α-crystallin [39]. HSP27 was originally characterized in response to heat shock as a protein chaperone making proper refolding of damaged proteins [40].

It has been seen that HSP27 protein also responds to cellular stress such as oxidative stress and chemical stress. HSP27 is a protein, which functions as a protein chaperone, as an antioxidant helps in inhibition of apoptosis and actin cytoskeletal remodeling, regulation of cell development, cell differentiation, and signal transduction [41]. The oligomerization state of HSP27 is due to chaperone activity as the aggregates of large oligomers have high chaperone activity, whereas dimers have no chaperone activity. Large aggregates are formed under heat shock [40]. Hsp27 occurs in all cell types, mostly of muscle cells. It is located mainly not only in the cytosol but also in the perinuclear region, endoplasmic reticulum, and nucleus. It is overexpressed during cell differentiation and development. It has an essential role in the differentiation of tissues [41].

HSP27 functions as an antioxidant during oxidative stress, which lowers the levels of reactive oxygen species (ROS) by increasing the levels of intracellular glutathione and lowering the levels of intracellular iron [40, 42]. During chemical stress, protein acts as an anti-apoptotic agent through mitochondrial-dependent and -independent pathways of apoptosis [43]. During Fas-FasL-mediated apoptosis, HSP27 binds DAXX and stops the binding of Ask1 by DAXX [43]. HSP27 also interplays with Bax and cytochrome c stopping mitochondrial-dependent apoptosis [43]. HSP27 is mostly useful in protection from programmed cell death by inhibition of caspase-dependent apoptosis [43]. The anti-apoptotic properties of HSP27, which occur due to chemical stress, have been useful in chemotherapies such as doxorubicin and gemcitabine [44, 45]. HSP27 regulates actin cytoskeletal dynamics during heat shock and stress conditions, promotes both actin polymerization, and concerts as an actin capping protein. The upregulation of HSP27 is a biomarker acting in some of the disease subsequently, a cell safeguards itself from death or reduces oxidative stress by the help of HSP27 [46].

In vitro studies have shown that HSP27 acts as an ATP-independent chaperone by inhibiting protein aggregation and stabilizing partially denatured proteins, which ensures refolding by the Hsp70-complex [46]. It also preserves the focal contacts fixed at the cell membrane [46]. The main function of Hsp27 is to provide thermo tolerance in vivo, cytoprotection, and support of cell survival under stress conditions [46]. Another function of Hsp27 is the activation of the proteasome. It speeds up the degradation of irreversibly denatured proteins and junk proteins by binding to ubiquitinated proteins and to the 26S proteasome [46]. Hsp27 enhances the activation of the NF-κB pathway that controls a lot of processes, such as cell growth and inflammatory and stress responses [47]. Various reports have confirmed that cytoprotective properties of Hsp27 have been attributed to its ability to modulate reactive oxygen species production and to raise glutathione levels [47]. Hsp27 is known to play a role in the process of cell differentiation [47].

Hsp27 expression is varied in several cells such as Ehrlich ascites cells, embryonic stem cells, normal B cells, B-lymphoma cells, osteoblasts, keratinocytes, and neurons [47]. The upregulation of Hsp27 relates with the rate of phosphorylation and with an increase in large oligomers [48]. It is possible that Hsp27 plays a crucial role in the termination of growth [40].

3.4 HSP27 in breast cancer

Tumor cells show an increase in transcription of heat shock proteins (HSPs) due to loss of p53 functions and increased expression of proto-oncogenes such as HER and c-Myc, which is important for tumorigenesis [49]. Cellular protection and protein folding being the entailed function of HSPs are operative during oncogenesis [49]. Since tumor cell growth and survival are enabled by the increased expression of HSPs. HSP27 is mostly the important heat shock protein involved in protection

from programmed cell death by inhibiting caspase-dependent apoptosis [45]. Since HSP27 has been associated with poor prognosis in many types of cancers such as gastric, liver, and prostate carcinoma, osteosarcoma, rectal, lung, and breast cancer [45]. HSP27 has been reported to play an important drug resistance in breast cancer, and some other cancers, HSPs, are involved in immune tolerance by cancer cells and are important targets for cancer therapy process [50]. A study done by Langer et al. in 2008 found that the protein expression profiles in patients with esophageal adenocarcinomas from two groups have shown as responsive and nonresponsive to neo-adjuvant platin and 5-fluorouracil based chemotherapy. The study showed that low HSP27 expression has a correlation with nonresponsiveness to the chemotherapy application [49]. It shows that low levels of HSP27 expression are associated with a negative outcome in cancer. HSP27 levels were studied in patients having colon or rectal cancer [49]. It showed that high HSP27 expression level results in incomplete resection margins in rectal cancer and poor survival.

HSP27 expression was not having any role in survival of colon cancer group but was related to poor survival in the rectal cancer group [49]. The metastatic breast cancer cell lines, which overexpress Her2 and are resistant to Herceptin (SK-BR3 HR), also overexpress HSP27 [49]. The downregulation of HSP27 protein levels was shown by transfecting with siRNA, where Herceptin resistance was also reduced in SK-BR3-HR cells [49]. HSP27 could form a complex with Her2, resulting in a potential mechanism by which the protein potentiates [51]. Her2 overexpressing breast cancer tumors have showed increased expression of phosphorylated HSP27, particularly at serine 78 [51, 52].

3.5 H2AX (H2A histone family, member X)

Histone H2A is programmed by a gene H2AFX (H2A histone family, member X), as H2A is one of the four core histones of DNA in humans and eukaryotes [53]. H2AX helps in nucleosome formation and in the structure of DNA. DNA known as the most stable material present, whose damage occurs due to ionizing radiation, hypoxia, reactive oxygen species, chemicals, and replication or transcriptional errors, which causes activation of DNA repair pathway [53]. Different materials can cause different types of damage in DNA-like double stranded breaks (DSBs), where both DNA strands have been cleaved [53]. If the damage occurred is not repaired, DSBs are lethal for the cell. DNA damage leads to an activation of the DNA damage repair pathway, phosphorylation of histone H2AX on serine 139. Activation of downstream pathway is done by the kinases of the PI3 family (ataxia telangiectasia mutated, ATR, and DNA-PKcs), which are responsible for this phosphorylation, especially ATM. γ-H2AX enrolls other factors, for example, 53BP1, BRCA1, MDC1, and the MRE11-RAD50-NBS1 (MRN) complex to sites of damage [53]. The DSBs, which are not repaired due to irradiation induced γ-H2AX foci, have been used in tumors as a biomarker for sensitivity to radiotherapy [54]. Endogenous γ-H2AX foci are present in normal primary human cells and tissues [54]. In tumor cells, phosphorylated H2AX exists in different levels in the absence of exogenously DSBs [54]. Instability of chromosomes occurs in cells having more endogenous foci [41]. The colocalization of these endogenous foci in association with other DNA repair factors, for example, 53BP1, MRN complex shows that DNA repair occurs at these sites [54]. The endogenous expression of γ-H2AX is present not only in tumor cell lines but also in cancer tissues and in their precursor lesions, which gives an insight into activated DNA damage repair in tumorigenesis [55]. The endogenous expression of DNA damage response factors is also due to damaged, shortened telomeres and hypoxia [56, 57].

Constitutive γ-H2AX expression is higher in triple negative and in BRCA1 and p53-mutated breast cancer cell lines, which makes a relation between endogenous

γ-H2AX expression and 53BP1 expression in breast cancer tissue [55]. Other studies have also supported that triple negative breast cancers are having more endogenous γ-H2AX expression and have higher chances of carrying errors in components of the DNA damage repair pathway [55, 57]. Higher occurrence of γ-H2AX positive is present in basal like and triple negative tumors that are BRCA1 mutation carriers. So, the breast cancer patients having high endogenous γ-H2AX or 53BP1 expression showed a subset of triple negative tumors with poor prognosis. The expression of endogenous γ-H2AX in cancers is due to telomeres (protective structures that form the chromosome ends in eukaryotes) [57].

Cell having DNA damaged after duplication of DNA results in shortened telomeres after every cell cycle, so in precancerous event, there occurs shortening of telomeres and activation of telomerase, an enzyme necessary for telomere lengthening [58]. Telomere shortening in normal process is an action for replication arrest and replicative senescence, but in the absence of a telomeric structure, chromosome ends are not stable and are likely either to undergo degradation, combining with other chromosomes resulting in genomic instability or having DNA double-stranded breaks [58]. A good number of endogenous γ-H2AX foci present, which do not have actual double-stranded breaks, are in fact uncapped telomeres, but the DNA damage and phosphorylation of H2AX at these sites occur due to nonfunctioning of telomere [58]. Telomere-associated chromosomal rearrangements may lead to a tumor phenotype with the associated immortality and replicative potential without any barrier [58, 59].

4. PARP poly ADP ribose polymerase

4.1 PARP in normal cells

PARP1 (protein) repairs single-strand breaks of DNA in a normal cell when it is damaged or mutated, and the cell survives when its DNA repaired, but sometimes when the DNA repair mechanism fails, the cell undergoes suicidal apoptotic process, subsequently that the damaged DNA is not passed to progeny cells [60]. When DNA is damaged or requires repair, one of the proteins, which is involved in repairing damaged DNA, is poly (ADP ribose) polymerase 1, or PARP1 moves at the site of damage, gets activated, and enables various DNA repair proteins to repair the broken strand of DNA, but if the breaks in DNA are not repaired until DNA replication occurs, then the replication itself causes double-strand breaks to form [60]. Inhibition of PARP1 is done by number of drugs, which causes double-strand breaks. Tumors having BRCA1, BRCA2, or PALB2 mutations where repair is not done, the double-strand breaks cannot be repaired causing death of cells [61]. In normal cells, DNA replication is not as frequent as in cancer cells, and they also do not have mutated BRCA1 or BRCA2 but have homologous repair mechanism, which makes them to survive the inhibition of PARP [61]. There are cancer cells that lack the tumor suppressor PTEN and maybe sensitive to PARP inhibitors because of downregulation of Rad51, a critical homologous recombination component [33]. A study has shown that PARP inhibitors may also be effective against PTEN-defective tumors like prostate cancers. Most of the tumors are sensitive to PARP inhibitors [61].

4.2 PARP in breast cancer cells

DNA damage in dividing cells or tumor cells is caused mainly by the chemotherapeutic drugs and radiation therapy, but if PARP repairs the damage caused by these agents, the tumor cells survive and grow.

4.3 Inhibiting PARP: mechanism of action

The preclinical studies have shown that standard therapies alone are not as effective as in combination with PARP inhibitors; they are used in cancer cells, which make protein unable to function during chemotherapy, which results in apoptosis of the cell where DNA is unrepaired [61]. In inherent DNA repair defects, such as breast tumors with mutations in the DNA repair proteins BRCA1 or BRCA2, PARP inhibitors are effective as single agents, and they undergo an arrest of the cell cycle and apoptosis on exposure to PARP inhibitors, whereas cells with normal BRCA proteins survive and continue to grow [61]. It has also been predicted that cancer cells with BRCA1 or BRCA mutations are more sensitive to PARP inhibitors to undergo growth arrest and apoptosis than cells with normal BRCA1 or BRCA2 [61]. This occurs due to combination of PARP and loss of BRCA1 or BRCA2 function causing inactivation of two major forms of DNA repair [61], and the damaged cells are not able to maintain the integrity of their genome and become more prone to apoptosis [37, 38, 62].

Function of PARP inhibitors is to block PARP enzyme activity, which stops the repair of DNA damage and causes the cell death [62]. In one of a clinical study that has shown the PARP inhibitors localize PARP proteins near the site of DNA damage, which suggests its role in antitumor activity [62]. The PARP inhibitors are able to trap PARP proteins on damaged DNA, and this function varies among inhibitors, for example, PARP family of proteins in humans includes PARP1 and PARP2, which are used in binding of DNA and protein repair action [63]. DNA damage causes activation of these proteins; they recruit other proteins that are actually involved in repairing DNA [63]. In normal condition, PARP1 and PARP2 are released from DNA when the repair mechanism is in process [64]. The study shows that when they are attached to PARP inhibitors, PARP1 and PARP2 become trapped on DNA [38]. The trapped PARP-DNA complexes are more harmful to cells than the single-strand DNA breaks, which are not repaired that accumulate in the absence of PARP activity resulting in harmful action of PARP inhibitors [38, 62]. There may be two classes of PARP inhibitors, catalytic inhibitors that block PARP enzyme activity and do not trap PARP proteins on DNA and dual inhibitors that both inhibit PARP enzyme activity and act as PARP poison [38, 63, 64]. Various PARP inhibitors are used in clinical trials as shown in **Table 1**.

4.4 PARP in combination with chemotherapy

A number of studies have been done to see the toxicity with PARP inhibitors as monotherapy in tumors with homologous recombination defects and have been compared with chemotherapy [68]. Moreover, in some studies, combination of

PARP inhibitors	Cancer types	References
BMN-673	BRCA mutated breast cancer	[62]
Olaparib	Breast, ovarian, and colorectal cancer	[15]
Veliparib	Melanoma and breast cancer	[65]
CEP 9722	Non-small-cell lung cancer	[66]
MK 4827	Inhibitor of PARP1 and PARP2	[67]
3-aminobenzamide	PARP inhibitor	[67]
Rucaparib	Metastatic breast and ovarian cancer	[15]

Table 1.
PARP inhibitors used in various types of cancers.

PARP inhibitors is used in combination with chemotherapy. PARP inhibitors as known can act as chemo sensitizing agents [68]. The resistance to chemotherapy drugs occurs when PARP repairs the DNA damage caused by these agents, for example, temozolomide is an alkylating agent, which causes DNA damage in which PARP inhibitors act as potential anticancer agents [68].

4.5 PARP in combination with radiotherapy

Radiotherapy causes DNA strand breaks leading to DNA damage and cell death; however, it kills all of the targeted cells, having side effects [68]. Combining radiation therapy with PARP inhibitors has been used to overcome the side effects as these inhibitors form double-strand breaks from the single-strand breaks generated by the radiotherapy in tumor tissue with BRCA1 or BRCA2 mutations. In such cases, the combinatorial therapy leads to better and efficient response with less dose of radiation [68].

5. ROS

Reactive oxygen species (ROS) are reactive molecules containing oxygen [69]. These molecules are formed when a chemical reaction takes place, for example, between oxygen ions and peroxides [69]. ROS plays an important role in cell signaling and homeostasis, due to environmental stress like UV or heat exposure, and ROS levels are increased, which damage cell structure and its function [69].

5.1 ROS in cancer

ROS being secondary messengers in cell signaling are required for various biological processes in normal cells; any dysfunction in redox balance results in human cancers [27]. ROS are increased mostly in cancer cells when oncogenes are activated, and there is lack of blood supply, which initiates progression and metastasis of cancer [27]. ROS levels decide the difference between tumor and nontumor cells [27]. Generation and elimination of ROS at the same time in the system are the expenditure to operate regulatory pathways in a normal physiological condition of cell, and this process is balanced by scavenging system [27]. When oxidative stress occurs, ROS are generated more, which cause carboxylation of cellular proteins, peroxidation of lipids, and DNA damage leading to dysfunction of cell resulting in carcinogenesis, while in cancer cells, ROS stress causes increased metabolism and mitochondrial dysfunction [27]. Consequently, ROS have dual function, on one side, it helps in survival of cancer cell, as cell cycle progression, which is regulated by growth factors via receptor tyrosine kinase activation and chronic inflammation, is regulated by ROS [62]. On an altered side, an increase in ROS level suppresses tumor growth by activating cell cycle inhibitors, which induces cell death and senescence by damaging macromolecules [62]. This dual mechanism helps in chemotherapy and radiotherapy, where cancer cells are killed by ROS stress. The cancer cells are able to differentiate between ROS as survival or apoptotic signal because of the dosage, duration, type, and site of ROS production [66]. ROS is used for survival of cancer cells in moderate level and kills cancer cells in excessive level [66]. Effects of ROS are maintained by cell metabolism by producing antioxidant molecules such as reduced glutathione (GSH) and thioredoxin (TRX), which depend on the reducing power of NADPH to maintain their function (reference) [27]. Sometimes tumor cells overproduce ROS because the NADPH oxidase is regulated by the GTPase Rac1, which is a downstream of proto-oncogene Ras [27].

ROS when associated with cancer activate various transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells—NF-κB, activator protein-1—AP-1, hypoxia-inducible factor-1α, and signal transducer and activator of transcription 3—STAT3, which cause protein expression for inflammation, cell transformation, tumor cell survival and proliferation and invasion, angiogenesis, and metastasis (reference). ROS also control the expression of different tumor suppressor genes such as p53, retinoblastoma gene (Rb), and phosphatase and tensin homolog (PTEN) [70–73].

The several causes for oxidative stress in breast cancer cells are as follows:

- Thymidine phosphorylase induction in cancer cells is caused by oxidative stress, an enzyme that is overexpressed in breast cancer. Thymidine phosphorylase catabolizes thymidine to thymine and 2-deoxy-D-ribose-1-phosphate, which is a very powerful reducing sugar that rapidly glycates proteins, generating oxygen radicals within the cancer cell [74].

- Glucose deprivation and hypoxia is caused by continuously usage of blood supply which causes increased cellular oxidative stress in MCF-7 breast cancer cell line but does not increase in nontransformed cell line, reason being glucose deprivation depletes intracellular pyruvate in breast cancer cell, which prevents the decomposition of endogenous oxygen radicals [75]. Study done [76] supports that breast tumor increases its blood supply, leading to glucose deprivation and hypoxia thus causing glucose deprivation, which rapidly induces cellular oxidative stress within the MCF-7 breast carcinoma cell line, although it does not cause oxidative stress in nontransformed cell lines [75]. Cancer cells like breast cancer cells cause increase in blood vessel development (angiogenesis process), where blood flow causes hypoxia followed by reperfusion, which leads to myocardial infarction leading to generation of ROS which leads to oxidative stress in breast cancer [75].

- Breast tumors are accompanied by macrophage population [75]. Oxygen radicals are produced by macrophages; it causes oxidative stress in murine mammary tumor cells. Also, tumor necrosis factor is secreted by macrophages, which also cause cellular oxidative stress [67, 77].

5.2 Effects of ROS in breast cancer

Increase in mutation rate and tumor progression is caused mainly by ROS in which oxygen radicals cause DNA damage, which result in strand breaks, alterations in guanine and thymine bases, and sister chromatid exchanges [78]. ROS lead to inactivation of tumor suppressor genes in tumor cells and increase expression of proto-oncogenes; thus, genetic instability due to persistent oxidative stress in cancer cell will increase malignancy of the tumor [79]. In vitro ROS cause initiation of growth sensing signaling pathways due to cell proliferation in response to hydrogen peroxide because of activation of mitogen-activated protein kinases (MAPKs), like HeLa cells when treated with hydrogen peroxide lead to activation of all three MAPK pathways, extracellular signal-related protein kinase, c-Jun amino-terminal kinase, and stress-activated protein kinase and p38 [79]. Hyper-phosphorylation of c-Jun by oxidative stress activates activator protein-1 in MCF-7 breast cancer cells, which stimulates proliferation [80]. Multidrug-resistant human breast carcinoma cells lead to activation of extracellular signal-related protein kinase-2 when stressed by glucose deprivation [76]. ROS may also cause stimulation of mitosis by

MAPK-independent mechanisms. Oncogenic Ras causes ROS production by activating Rac1 and NADPH-oxidase. It has been also seen that in Ras-transformed human fibroblasts, ROS control cell cycle progression without the activation of MAPK pathways [81, 82].

5.3 Resistance to therapy

Apoptosis is caused by oxidative stress, which is induced depending on p53 in both mouse and human cells [83]. Resistance to apoptosis is caused by persistent oxidative stress [84], whereas the resistant to cytolysis by hydrogen peroxide may be explained by an upregulation of anti-ROS mechanism in cancer cells. Hydrogen peroxide activates anti-apoptotic Akt (protein kinase B) leading response to chronic oxidative stress that can be used for anticancer therapy though in radiotherapy, photodynamic therapy, and other chemotherapies generating oxygen radicals showing antitumor activity [85]. This is due to the induction of tumor cell apoptosis in response to oxidative stress and oxygen radical prompted DNA damage [85]. This results in persistent oxidative stress within carcinoma cells causes resistance to therapy that is further increased by oxygen radicals leading to an increasing carcinoma cell expression of P-glycoprotein, the multidrug-resistance efflux pump [86].

Angiogenesis, which may be one of the reasons for oxidative stress, leads to tumor growth in blood borne metastasis of breast tumor, where oxygen radicals cause tumor migration causing increased risk of invasion and metastasis by activation of p38 MAPK and subsequent phosphorylation of heat shock protein-27 by p38 MAPK causing changes in actin dynamics [46, 79]. Studies have shown that phosphorylated heat shock protein-27 promotes the migration of MDA-MB-231 breast cancer cells on laminin-5 in vitro [41]. Oxidative stress in breast tumors causes invasion and metastasis by activating MMPs as well as by inhibiting antiproteases. MMP-2 as gelatinase has a major role in breast cancer invasion and metastasis; once its levels are high, there is a poor prognosis in breast cancer patients [87]. Subsequently, MMP-2 is seen more in malignant than in benign breast tumors; therefore, it is ROS, which also activate MMP-2 due to reaction of oxygen radicals with thiol groups within MMP-2 [88]. Oxygen radicals inactivate protease inhibitors, such as α1-proteinase inhibitor and plasminogen activator by oxidation of methionine residues at their active sites [65], leading to protease activation, which increase invasion and metastasis processes, for example, plasminogen activator causes metastasis [65].

Cancer cells synthesizing ROS at a higher level in vitro and tumors in vivo are under persistent oxidative stress, as oxygen radicals lead to a poorer prognosis, antioxidants can be used for therapeutic role in breast cancer [89]. Various research studies have shown that human melanoma cells were transfected with cDNA encoding the antioxidant enzyme manganese superoxide dismutase leading to suppression of malignancy; cells not only lost their ability to form colonies on soft agar but also no longer formed tumors in nude mice [89]. Various anticancer therapies are there, which add to the oxidative stress within breast cancer such as chemotherapeutic agent's doxorubicin, mitomycin C, etoposide, and cisplatin, which are superoxide generating agents [85], radiotherapy, and photodynamic therapy, which generate oxygen radicals within the carcinoma cell, and anti-estrogen tamoxifen used in breast cancer therapy also induces oxidative stress within cancer cells in vitro [90]. Conversion of breast tumors to a tamoxifen-resistant phenotype that has been seen is associated with a progressive shift toward a pro-oxidant environment of cells as a result of oxidative stress [90].

6. P53

P53 protein, a tumor protein present in humans, which is encoded by the TP53 gene (tumor suppressor gene), functions to inhibit proliferation of cells and regulates cell cycle, thereby preventing cancer, also called as the guardian of the genome as it maintains the stability in a cellular process preventing genetic mutation, under normal cellular phenomenon the p53 signaling pathway is in static mode, whereas its activation occurs when there is a cellular stresses like DNA damage or oncogene activation [91, 92]. Post-translational modifications activate P53 protein for DNA binding, transactivating downstream effector genes whose activation depends on the nature of stress and its extent. After oxidative stress transcriptional coactivators, for example, apoptosis stimulating protein of p53 and BRCA1 promotes various cellular processes like apoptosis, other components of signaling pathway which are targeted for genetic and epigenetic changes in breast cancer, for example, activation of MDM2 which acts as a negative feedback regulator of the pathway by promoting the degradation of p53 [93].

6.1 P53 mutations in breast cancer

p53 being activator of apoptosis or cell cycle arrest is generated upon DNA damage, or cellular stress has a major role in cancer as it stimulates genomic stability and anti-angiogenic effects, manages tumor inflammation and immune response, and represses metastases [94]. TP53 is mutated mostly in 50% of all human cancers and in 20–30% of breast cancers with more than 15,000 different mutations, which makes P53 as a potential biomarker for breast cancer [94].

In one of a clinical study, it was studied that in premenopausal women, p53 mutation is associated with ER and PR tumors, but in postmenopausal women having breast cancer, the presence of a p53 mutation is associated with higher body mass index (BMI), higher-grade, and poorly differentiated tumors, so women having tumors as well as p53 mutations had a 2.4-fold increased risk of dying from their disease [53]. In an additional clinical study, it has been shown that TP53 mutated noninflammatory locally advanced breast carcinomas respond to doxorubicin-cyclophosphamide chemotherapy unlike TP53 wild-type tumors, due to senescence in TP53 wild-type tumor cell and in MMTV-Wnt1 mammary tumors, growth arrest and senescent phenotype were stimulated in TP53 WT tumors following doxorubicin treatment, and there was no apoptosis while the absence of arrest in mutant tumors caused aberrant mitosis, cell death, and a better clinical response [53, 95]. In ER-positive breast tumors, ER represses the p53-mediated apoptotic response induced by DNA damage, but in ER-negative TP53 mutated breast cancers, accumulation of genetic abnormalities may lead to mitotic catastrophe and better response [95].

6.2 P53 and chemotherapy

Previous clinical studies done on breast cancer patients [96] have seen that ER (+) tumors (mostly TP53 wild type) are mostly resistant to chemotherapy, while ER (−) tumors (mostly a TP53 mutated) are more chemo sensitive, but in another study, it was found that there was no association shown in sensitivity to classical doses of taxane-based therapy and mutated TP53 in breast tumor [97]. TP53 wild-type tumor cells in human breast xenograft models have the presence of senescence in breast cancers in response to the treatment [95] senescence induction and cell cycle arrest in TP53 wild type tumors showed tumor proliferation after the end of treatment while

genetic abnormalities and mitotic catastrophe would occur with further response to treatment in TP53 mutated tumors, which was also seen in MMTV-Wnt1 mammary tumors [95]. It has also been seen that growth arrest and senescent phenotype and no apoptosis were induced in TP53 wild-type tumors following doxorubicin treatment, while lack of arrest in R-172-H mutant tumors resulted in aberrant mitoses, cell death, and a better clinical response; wild-type tumors or mutant tumors, which were having a wild-type TP53 allele, did not show apoptosis and did not lose any volume as did TP53 mutant tumors [98]. In ER (+) breast cancers, it was shown that there is a functional interplay between p53 and ER on a genome wide scale and that ER represses the p53-mediated apoptotic response induced by DNA damage, and distinct TP53 gene signatures are also needed to evaluate prognosis and response to chemotherapy in ER-positive and ER-negative breast cancers [74].

Thus, these clinical findings provide a way how to study p53-mediated response to dose-dense doxorubicin-cyclophosphamide chemotherapy in breast carcinomas in ER (+) TP53 wild-type breast tumors and that ER-induced inhibition of p53 apoptotic response would result in tumor cell senescence and resistance to treatment. However, in ER (−) TP53 mutated breast carcinomas, mostly in those having lost both TP53 alleles, there is an increase in genetic abnormalities that lead to mitotic catastrophe and better response [74].

6.3 P53 and ROS signaling

p53, a tumor suppressor protein being redox active transcription factor, organizes and directs cell function during various stresses that lead to genomic instability, on the other hand, reactive oxygen species (ROS) are products or byproducts generated by cells, which function either as signaling molecules or as cell toxicants (reference). Cellular concentration and distribution of p53 have a different cellular function as ROS act as both up-stream signal that causes p53 activation and downstream factor that results in apoptosis [99], subsequently if ROS level is increased due to oxidative stress in cancer cell, then p53 level may be increased to maintain its stability in the environment [99]. A balance is maintained in cellular concentration between oxidant and antioxidant molecules in normal cells, but when the oxidant part increases or when a disruption of redox signaling occurs, oxidative stress is caused in a redox reaction in the system, which results in damage to DNA, proteins, and lipids through oxidative modification resulting in number of diseases and chemotherapeutic cytotoxicity [15].

The genomic stability is maintained by tumor suppressor protein p53, but when there is a cellular stress like disruption of redox signals, which leads to damage DNA, proteins, and lipids. This tumor suppressor gene maintains transcription of various genes and directs cell for cell cycle arrest, senescence or apoptosis through various activation of target genes, many effector molecules like proteins, noncoding RNAs, for example, myc, Hcas or CSE1L, Hzf and miR-34 which help in selecting transactivation of p53 target genes leading to various cellular responses comes into play [85]. Thus, this oxidative stress is associated with p53-dependent cell cycle arrest, DNA repair, and apoptosis, but a clear understanding of the mechanisms of the interactions between ROS and p53 is still elusive [100].

In unstressed cell or normal cell, P53 has a small half-life and is present in low levels by continuous ubiquitination by Mdm2 COP1 (constitutively photomorphogenic 1) and Pirh2 (p53-induced protein with a RING-H2 domain) and derogation by 26S proteasome, where the physiological levels of p53 have different effects on cellular redox potential either it regulates the pro-oxidant and antioxidant genes or it modulates the cellular metabolism [86, 101].

6.4 Levels of p53 and ROS

Overexpression of p53 transactivates a series of p53-induced genes (PIGs) in which many of these PIGs encode redox active proteins including two ROS-generating enzymes, NQO1-quinone oxido-reductase, PIG3, and proline oxidase (POX, PIG6). The upregulation of these pro-oxidant enzymes will lead to oxidative stress and apoptosis [102, 103]. The pro-oxidant genes, which are upregulated, are BAX, PUMA, and $p66^{shc}$ in which BAX and PUMA stimulate uncoupling of mitochondria, which result in ROS generation from a less efficient electron transport chain, and $P66^{shc}$ is a downstream target of p53, which is present in cytoplasm and is translocated into mitochondria by prolyl isomerase 1 (Pin1) and mitochondrial heat shock protein (mtHsp 70), and pro-apoptotic stimulation of $p66^{shc}$ oxidizes cytochrome c, which produces H_2O_2 and opens mitochondrial permeability transition pore initiating apoptosis, and upregulation of these pro-oxidant enzymes leads to oxidative stress and consequently to apoptosis [102, 103]. More genes have been added to the list of p53-induced pro-oxidant genes, which include BAX, PUMA, and $p66^{Shc}$ of which BAX and PUMA can induce uncoupling of mitochondria, resulting in ROS being generated from a less efficient electron transport chain (ETC) [74, 104]. P53 has a downstream target known as $p66^{Shc}$, which predominantly exists in cytoplasm and is translocated into mitochondria with the help of prolyl isomerase 1 (Pin1) and mitochondrial heat shock protein 70 (mtHsp 70) [105, 106].

Oxidative stress is caused by suppression of antioxidant genes by p53, which increases cellular ROS, for example, MnSOD (manganese superoxide dismutase) is suppressed at the promoter level by p53 activation or overexpression [107].

6.5 Redox regulation of p53

The oxidative stress caused by ROS is related to various p53-mediated cell processes like cell cycle arrest, DNA repair, and apoptosis like increase in generation of ROS in mitochondria when treated with chemotherapeutic agent's results in apoptosis, while oxidative stress in the nucleus causes cells to p53-dependent DNA repair [86, 92]. A number of pathways operating to induce redox and p53 signaling select various p53 target genes that decide the final fate of the cell have been found significant in studies going on cisplatin and ginkgo bilobalide resulted in chemotherapeutics-induced ROS increase C-myc [108]. As soon as C-myc levels increase, it causes suppression of p53 transactivation of $p21^{Cip1}$ blocking cell cycle arrest but does not affect p53-transactivation of the pro-apoptosis gene PUMA leading to apoptosis [108]. The mechanism acts in a same manner as in pathogenic bacterium *Pseudomonas aeruginosa* induced cell death having azurin, a copper-containing redox protein excreted by *Pseudomonas aeruginosa* that binds to p53 and transactivates pro-apoptosis protein Bax resulting in apoptosis [108].

6.6 Redox modification

p53-mediated ROS generation is the most important cellular concentration and subcellular localization function as P53 is a redox sensitive protein, which undergoes redox modification and decides the cell fate on the basis of p53 target genes, and the other factors such as cell type, stress, and intensity of stimuli give an insight into the interaction between ROS and p53 [92, 95].

7. P38-MAP kinase

Response to extracellular stimuli is managed and regulated by intracellular signaling pathways by mitogen-activated protein (MAP) kinase pathways whose members are responsible for signaling cascades, mammalian p38s responses, inflammatory cytokines (TNF-& IL-1), growth factors (CSF-1), ultraviolet irradiation, heat shock, osmotic shock, function in cell differentiation, apoptosis, and autophagy [109]. In general, there are four MAP kinase family subgroups, namely extracellular signal-regulated kinases (ERKs), c-jun N-terminal or stress-activated protein kinases (JNK/SAPK), ERK/big MAP kinase 1 (BMK1), and the p38 group of protein kinases [110]. p38 (p38), a 38-kDa protein when phosphorylated by tyrosine as a responsive protein to LPS stimulation (Han et al. 1993) and its kinases are divided by Thr-Gly-Tyr (TGY) dual phosphorylation motif residues in a TXY (where X is Glu, Pro and Gly in ERKs, JNKs and p38 MAPKs) activation motif by a dual specificity, activation of p38 is not only due to responsive nature on stimulus, but on cell type as well., Insulin signaling is reported to activate p38 in 3T3-L1 adipocytes but downregulates p38 in chick forebrain neuron cells [110].

7.1 p38 in the cell cycle

p38 has been studied in G1, G2, and M phases of the cell cycle [30]. p38 MAPK controls both the G2/M and G1/S cell cycle checkpoint in retort to cellular stress corresponding to DNA damage [30]. It facilitates the cell survival processes and initiation/maintenance of cell cycle checkpoints in retort to particular stimuli [30].

7.2 p38 in senescence and tumor suppression

A number of studies have provided evidence of p38 role in tumorigenesis and senescence [28], when there is a loss of senescence in tumor cells, it has been found that its activation may be decreased in tumors and that its pathway units such as MKK3 and MKK6 are lost resulting in increased proliferation [111].

7.3 p38 role in breast cancer

p38 MAPK in survival of tumor cells functions independently of DNA damage and supplements to metastasis, but this effect is indirectly regulated by p38 MAPK through the mediation of factors responsible for survival or migration of cells, for example, basal stimulation of p38 MAPK in B-cell chronic lymphocytic leukemia (B-CLL) is required for the MMP-9 metalloprotease for survival of these cells grown in the presence of stroma cells [112]. However, *in vivo* studies found that the decreased basal as well as TGFβ1-induced MMP-9 activity in breast cancer cells cause inhibition of p38 MAPK pathway by genetic regulators and pharmacological compounds causing decreased bone metastases [113].

In cell division and cell survival, p38 is studied at checkpoint control [113]. Role of p38 in invasiveness in cultured cell has been seen, which shows that phospho-p38 level is increased in cultured invasive breast cancer cells [95, 114]; increased expression of P38 MAPK in breast cancer has been found in relation to poor prognosis and in invasiveness and metastasis [115]. Overexpression of phosphorylated-P38 MAPK has been seen in ~20% of primary breast carcinomas or in relation to HER2 amplification and tamoxifen resistance and is a potential prognostic marker in breast cancer [116]. Therefore, the role of P38 MAPK in breast cancer cell proliferation remains a subject

of study as it has dual function in survival and proliferation depending on the expression of mutant TP53 being present in most ER-breast tumors to develop P38 MAPK inhibitors for the treatment of TP53-mutated, ER-breast cancers, it is expressed at a higher level in ER+ in comparison with ER tumors without post-transductional activation as there was no change in the phosphorylation rate of P38 MAPK [116].

8. Role of tamoxifen resistance in breast cancer

A number of studies have reported the role of P38 MAPK in the resistance of ER+ breast tumors to endocrine therapy [117] and its relation between activated P38 MAPK levels and tamoxifen resistance [114, 118]. It has been reported that P38 MAPK leads to increased ER agonist activity through increased phosphorylation of ER and increased ER signaling through coactivator regulation [76]. There is switch in estrogen receptor signaling from its classical pathway to the AP1-dependent nonclassical pathway upon activation of MAPK by anti-estrogens apart from ER being their main target; thus, the activation of P38 MAPK can reduce the cellular response to endocrine therapy, which has been reported as a biomarker for resistance to endocrine therapy, and its detailed study of expression and its activation in breast tumors may provide a new approach to the resistance of breast cancer to endocrine therapy; also it has been reported that increased phospho-p38 levels have been associated with high expression of EGFR and ErbB2 in tamoxifen-resistant xenografts, where it acts to support nuclear functions of ER [76]. In matched primary and recurrent tamoxifen-resistant tumors (and a parallel study of a mouse xenograft in tamoxifen resistance), a link between phospho-p38 and increased ErbB2 with tamoxifen resistance was found [64, 69, 119, 120]. The graphic illustration of the pathway is displayed in **Figure 1**.

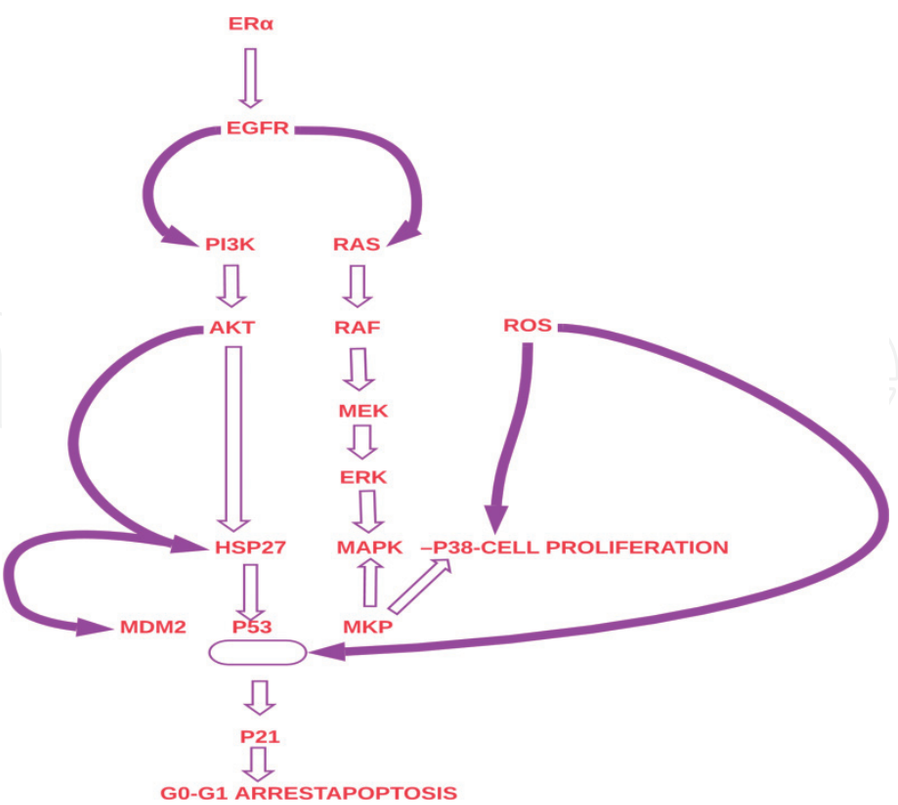

Figure 1.
Signalling pathway affecting estrogen receptor (ER) causing increase in EGFR level leading to cell proliferation during tamoxifen resistivity. P53 is increased during resistance and controls p21 function as well. ROS level is increased causing cell proliferation which in turn decreases Hsp27 and MAPK-P38 activity during stress lead by tamoxifen resistance.

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Conflict of interest

The authors declare no conflict of interest.

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