Review Article

Punica granatum and its therapeutic implications on breast carcinogenesis: A review

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Abstract

Punica granatum has a recorded history of pharmacological properties which can be attributed to its rich reservoir of phytochemicals. Investigations in recent years have established its tremendous potential as an antitumorogenic agent against various cancers including breast cancer, which is the second leading cause of cancer-related deaths in women. The plausible role of *Punica* as a therapeutic agent, as an adjuvant in chemotherapy, and its dietary implications as chemopreventive agent in breast cancer have been explored. Mechanistic studies have revealed that *Punica* extracts and its components, individually or in combination, can modulate and target key proteins and genes involved in breast cancer. Our earlier finding also demonstrated the role of methanolic extract of pomegranate pericarp in reducing proliferation in breast cancer by binding to estrogen receptor at the same time not affecting uterine weight unlike estradiol or tamoxifen. This review analyses other plausible mechanisms of *Punica* in preventing the progression of breast cancer and how it can possibly be a therapeutic agent by acting at various steps of carcinogenesis including proliferation, invasion, migration, metastasis, angiogenesis, and inflammation via various molecular mechanisms. © 2015 BioFactors, 41(2):78–89, 2015

Keywords: *phytochemicals;* Punica granatum*; breast cancer; phytoestrogens; adjuvant; proliferation; invasion; angiogenesis*

1. Introduction

Punica granatum has a vast ethnomedical history and represents a reservoir of phytochemicals with high medicinal values. The different anatomical compartments of the tree, that is, fruit, seed, juice, peel, leaf, flower, bark, and roots, are known to have varied pharmacological activities and extracts of these parts are known to target a wide range of diseases including cardiovascular disorders, diabetes, male infertility, Alzheimer's disease, and AIDS [1]. Studies have shown that the extracts of the parts of the fruit also possess anticancer activity. The mechanisms of action are diverse and include interference with tumor cell proliferation, cell cycle, invasion, and inhibition of angiogenesis. Pomegranate is a fruit abundant in polyphenols, hydrolysable tannins punicalagin, pedinculgin, punicallin gallic and ellagic acids, esters of glucose anthocyanins and other polyphenols, and anthocynidins such as delphinidin, cyanidin, and pelargonidin. The concentration of polyphenols in pomegranate (3.8 mg/mL gallic acid equivalents) is observed to be higher than in any other fruit juice, for example, grape, blueberry, black cherry, apple, cranberry, or orange (0.46–2.6 mg/mL of gallic acid equivalents) [2]. Therapeutically significant constituents of pomegranate include ellagic acid, ellagitannins (including punicalagins), punicic acid, flavanoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones.

Evidences suggest that pomegranate extracts act against multiple human carcinoma, one of which is breast cancer, a highly heterogeneous disease and the fifth cause of death from cancer worldwide (globocan). Breast cancer is classified into different molecular subtypes. Studies have clearly shown that these cancer cells respond differently to the treatments depending on the molecular subtypes, pathways, and genomic aberrations when subjected to pathway-targeted therapy [3]. Current targets for breast cancer depend on its subtypes and the molecular targets of targeted therapy include tyrosine

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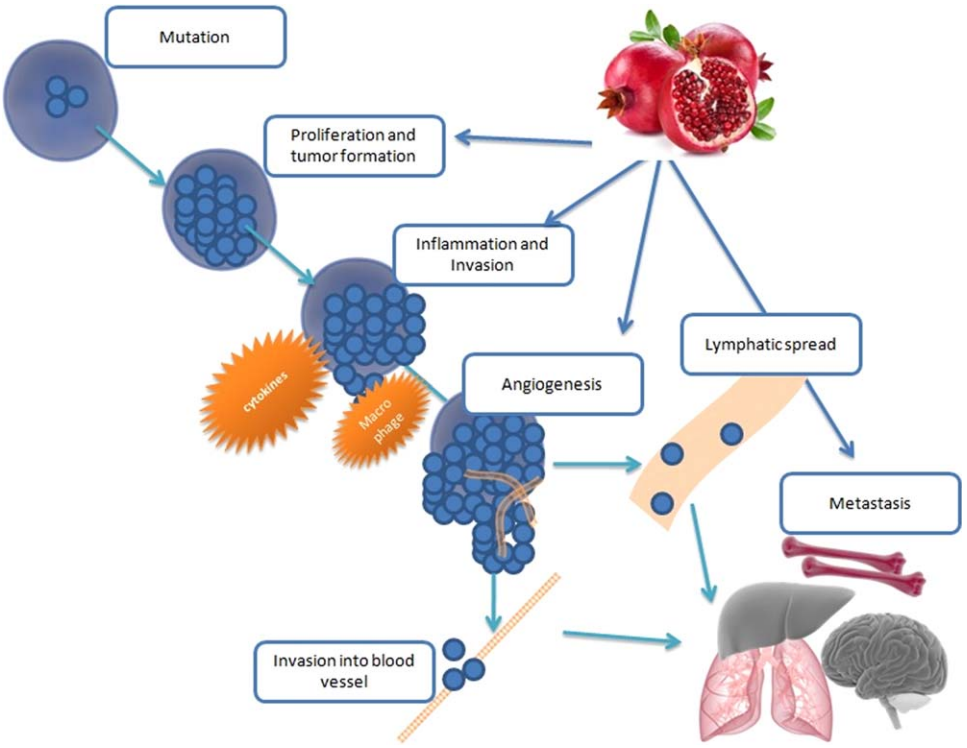

FIG 1 *Implication of* Punica *extract in breast cancer:* Punica *extract or its components individually or in combination is found to reduce proliferation, invasion, migration, angiogenesis, and its metastasis to various tissues.*

kinases, which encompass receptors like HER1, HER2, HER3, IGF receptor (IGFR), C-MET, FGF receptor (FGFR); molecules relevant to intracellular signaling pathways, such as PI3K, AKT, mammalian target of rapamycin (mTOR), and ERK; those that are involved in angiogenesis molecules associated with DNA repair and evading death by chemotherapeutic drugs [4]; and enzymes such as aromatase [5]. The chemotherapeutic drugs currently in use have several side effects. Volumes of research have demonstrated that phytochemicals present in fruit and vegetables can also act as potent chemopreventive agent. Hence, dietary modification could potentially be one of the ways to combat the disease. Also, many plant parts, which are not otherwise consumed, for example, the peel or the pericarp of certain fruits, have similar potent phytochemicals which can possibly be isolated and translated into marketable drugs. A few of the studies have pointed to relevance of these phytochemicals in overcoming multidrug resistance, which is a limiting factor in drug-based cancer treatments. A great deal of research, therefore, is currently focused on finding alternatives that have minimal side effects but lend themselves for use in the much-effective targeted or combinatorial therapies. *Punica granatum* or pomegranate is one such potential candidate. The plant has been shown, in several studies, to contain phytochemicals that target multiple molecules involved in various steps of carcinogenesis, making them potential candidates for use in targeted breast cancer therapies. The potent anticancer phytochemicals include tannins such as ellagic acid, gallic acid, punicalagin; flavanoids including flavones, flavanol, anthocynanidins, flavan-3-ols; alkaloids such as piperidines and pyrrolidines and organic acids like punicic acid and linoleic acid [6]. Many of these components, individually, or in combination as well as in the form of extract have been demonstrated to be effective against breast cancer. The major component of the juice and pericarp of *Punica* are polyphenols like anthocyanins such as delphinidin, cyanidin, and pelargonidin, which give the juice its red color [7], besides hydrolyzable tannins, such as punicalagin and gallagic acid [8,9]. Other polyphenolic components of potential interest include kaempferol, quercetin, and luteolin [10,11]. The seed oil, which comprises 65–80% conjugated fatty acids, also contains many compounds of interest with known anticancer activities. The predominant among these fatty acids is punicic acid [12,13], which has been proven to be effective against breast cancer.

This review focuses mainly on the potential of *Punica* extracts in fighting breast cancer, its ability to inhibit and interfere with steps that are of paramount importance to breast tumor progression from proliferation of the tumor to its migration, invasion, metastasis, angiogenesis, and inflammation as Fig. 1 indicates.

2. Breast Cancer

Phytochemicals are one of the most important class of compounds in the human diet which have gained traction in the

therapeutic field because of their pivotal role in human health, their abundance, and lack of toxicity. Phytochemicals encompass tannins, flavanoids, organic acids, and alkaloids. Ample epidemiological data show that phytochemicals confer protection against different cancers including breast cancer [14–16]. Also few of these, such as soy phytochemicals, are understood since long to have preventive action in breast cancer [17]. Many phytochemicals like genistein, curcumin, resveratol, and epigallocatechin gallate are reported to act against breast cancer by interrupting or targeting intracellular signaling pathways [18]. Some of them are known to act as inhibitors of enzymes involved in estrogen biosynthesis, while others act as inhibitors of proliferation, migration, invasion, or angiogenesis. Few phytochemicals are reported to act as phytoestrogens and are known to modulate signal transduction via estrogen receptors (alpha and beta), aryl hydrocarbon receptor, the pregnane X-receptor, the constitutive androstane receptor, and the peroxisome proliferator-activated receptors (alpha and gamma) and also are reported to influence transport proteins, kinases, and enzymes like 11 beta-hydroxysteroid dehydrogenase, 1,17 beta-hydroxysteroid dehydrogenases, sulfatase, and sulfotransferase [19] They are also known to inhibit aromatases [20] and estrone sulfatase [21]. Reports also suggest that phytochemicals can inhibit CYP1B1 [22], an enzyme responsible for metabolizing E2 to toxic substances like 4-OHE [23]. It has been demonstrated that few flavanoids, a class of phytochemicals, can also help in overcoming one of the primary obstacles that limit the therapeutic efficacy of anticancer agents that is the outcome of producing apoptotic defects in cancer cells by targeting novel noncanonical cell death pathways [24,25]. A few of this category of phytochemicals are also found to affect epigenetic factors like HDAC [26] and thus disrupt chromatin remodeling in breast tumor cells [27]. They are understood to suppress triple negative breast cancer cells, a class of cell line which has emerged as a challenge because of its poor prognosis [28–30]. Among the phytochemicals, studies have identified some which can enhance the efficacy of chemotherapeutic drugs by inhibiting ATP-binding cassette (ABC) transporters like P-gp [31,32], ABCG2 [33–35], and MRP-1 [32], which are relevant to multidrug resistance. Another interesting study showed that flavanoids can suppress breast cancer stem cells which are highly tumorigenic and possess the capacity to self-renew by inhibiting NANOG, a gene associated with regulating cancer stem cells and enhanced tumorigenicity [35]. Similarly, studies pointing to the inhibition of ABCG2, which are also proposed markers of stemness, is an indicator that phytochemicals may effectively reduce stem cell population in breast cancer. Thus, these different classes of phytochemicals, by different mechanisms, are known to combat breast cancer. But several phytochemicals have been shown to have biphasic effect on cell survival, that is, they can aid inhibition as well as proliferation of breast cancer cells, depending on the concentration [36]. A major caveat for the effective action of phytochemicals is their bioavailability and distribution to tissues and organs which varies according to their category and might depend on molecular size, polarity, and solubility [37]. For these reasons, the effect of the treatment with flavanoids in cultured cells might vary greatly when extended to *in vivo* level. Hence, at the clinical level, further detailed studies on the bioavailability and metabolism of flavanoids might be required.

3. *Punica granatum,* a Plant with Potential for Breast Cancer Prevention and Treatment

Pomegranate extracts and their constituents are known to exert their activity by diverse mechanisms and are known to inhibit angiogenesis, proliferation, invasiveness, growth, and also to induce apoptosis [1]. These extracts (juice, seed oil, peel) individually and upon combinatorial treatment are reported to inhibit tumor growth. Interestingly, the combinatorial treatment was found to be more effective than treatment with single extract [38]. Several *in vivo* studies have also elucidated the potential anticancer mechanism of the extracts [8,39]. This review mainly emphasizes on the action of pomegranate extracts against breast tumor and its progression.

3.1. Pomegranate, as a Source of Phytoestrogens

Estrogen exposure over a long term is associated with increased risk of breast cancer. Although the precise mechanisms are not understood, the theories hold that it act in estrogen receptor (ER)-dependent and ER-independent manner. The postulates are estradiol (E2), acts via estrogen receptor alpha (ERα), and further stimulates cell proliferation; it might cause direct genotoxic effects by increasing mutation rates through a cytochrome P450-mediated metabolic activation [40], and also that it can cause induction of aneuploidy [41]. These cells proliferate further and over a period of time the mutations accumulate to induce neoplastic transformation. Laboratory and epidemiological data have demonstrated the role of estrogen in breast cancer development [40]. So limiting the exposure to estrogen by targeting ER, or by limiting the synthesis of estrogen, may help in preventing estrogen carcinogenesis in breast cancer. Kim et al. [42] carried out an *in vitro* study for assessment of the possible chemopreventive or adjuvant therapeutic potential of different extracts of pomegranate, which included fermented juice, aqueous pericarp extract, and cold-pressed or supercritical CO_2-extracted seed oil, on human breast cancer cell line. The focus of the investigation was the ability of these extracts to block endogenous active estrogen biosynthesis. The fermented juice and pericarp polyphenols, and whole seed oil were found to inhibit 17-β-hydroxysteroid dehydrogenase type 1 (17 β HSD). This enzyme is involved in the conversion of estrone (E1) to estradiol (E2) and is expressed in many steroidogenic tissues and breast cancer cell lines [43]. Thus, inhibition of this enzyme is likely to reduce estrogen carcinogenesis. The order of inhibitory potential of the extract was found to be as follows: seed

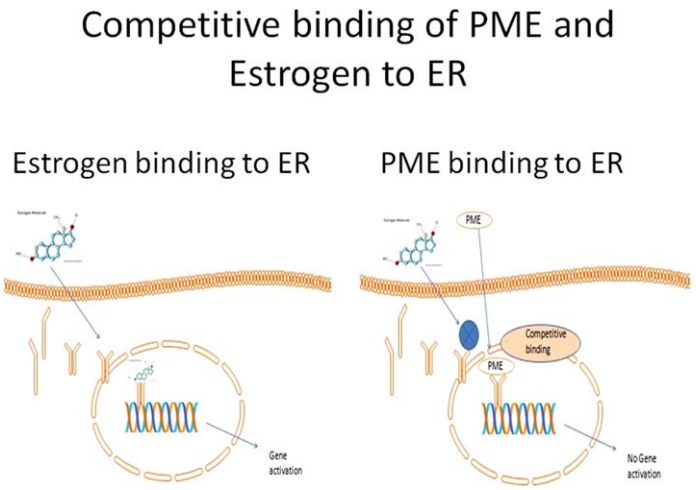

FIG 2 *Competetive binding of methanolic extract of pomegranate (PME) and estrogen to ER.* Punica *extracts can compete with estradiol (E2) to bind to estrogen receptor (ER) and thus have a plausible role in reducing estrogen carcinogenesis.*

oil > fermented juice polyphenols > pericarp polyphenols. The inhibition of cell growth was found to vary with cell lines using fermented juice and pericarp polyphenols, and the order was found to be as follows: estrogen-dependent (MCF-7) >> estrogen independent (MB-MDA-231) > normal human breast epithelial cells (MCF-10A). They also observed that polyphenols from fermented juice, pericarp, and oil inhibited aromatase activity by 60–80%. The research group further extended the study to understand the potential chemopreventive efficacy of purified fermented juice and also of whole pomegranate seed oil. They observed that these purified extracts showed higher reduction in tumor lesion than the crude extract of fermented juice polyphenols [39]. In agreement with this study, another investigation confirmed that ellagic acid derivatives isolated from pomegranate were potent inhibitors of aromatase [44]. Studies have demonstrated that ellagic acid, a major component in *Punica*, inhibits the cell proliferation induced by estradiol, and also that it increases the expression of c-fos and pS2. The expression was higher in comparison with estradiol treatment and was therefore an indicator of ER-mediated mechanism in MCF-7 [45]. Our investigation [46] on the tissue-specific estrogenic/antiestrogenic activity of methanol extract of pomegranate pericarp on different cell lines, including human breast (MCF-7, MDA MB-231) and normal breast fibroblast (MCF-10A) cells, suggested that the extract had ER-dependent activity. Competitive radioactive binding studies carried out confirmed that the methanolic extract of pomegranate pericarp interacts with ER and competes with estradiol to bind to ER and also that it suppresses the growth and proliferation of ER-positive breast cancer cells (as illustrated in Fig. 2) and also it did not increase uterine weight unlike estradiol or tamoxifen. The major conclusion of the study was that the extract displays a SERM profile. Most of the findings therefore indicate that pomegranate extracts can act as potent phytoestrogens and act via various means to reduce estrogen carcinogenesis, while few other studies demonstrated their proliferative activity leading to conclusion that the extracts have a concentration-dependent action on breast cancer. Further studies based on the same might be required to establish the range of concentration required for its antitumor activity against breast cancer.

3.2. Cyclooxygenase Inhibition

Early investigations into the antioxidant property of pomegranate have proven that fermented pomegranate juice and cold-pressed seed oil to are powerful antioxidants [47]. This free radical scavenging property might play a plausible inhibitory role in the initial stages of carcinogenesis. The same study demonstrated that cold-pressed pomegranate seed oil could also inhibit sheep cyclooxygenases, by around 31–41%. Out of the two isoforms of cyclooxygenases, COX1 is expressed in all human cells while COX2 is not expressed under normal conditions. Importantly, COX2 is found to be overexpressed in breast cancer [48] and its enforced overexpression in mammary gland epithelia of transgenic mice is found to result in breast tumor development [49]. It is also involved in breast cancer metastasis to bone [50] and reported to reduce Ki-67-positive cells, an indicator of proliferation [51]. One of the recent researches elucidates relevance of COX2 in EMT transition and invasiveness [52]. COX2 downregulation can also cause downregulation of aromatase because it catalyses the rate-limiting step in the synthesis of prostaglandin, including prostaglandin E2 (PGE2), which is found to downregulate aromatase [7]. Hence, COX2 can be considered to be a relevant candidate for targeted therapy. Stronger evidences were obtained with the finding that plasma isolated after oral ingestion of pomegranate fruit extract could inhibit both the COX enzymes *ex vivo*, the effect being more pronounced in the case of COX2 [53]. This further proved the potential of the metabolites present in plasma. More evidences are required to prove whether COX2 inhibition by pomegranate-derived components can cause a reduction in the proliferation of human breast cancer cells. Considering the relevance of COX2 in breast cancer, the COX2 inhibitory activity of pomegranate extract warrants more attention.

3.3. Migration, Invasion, and Metastasis

Of all the processes involved in carcinogenesis, local invasion and metastasis are clinically the most relevant and difficult to target. Excitingly, pomegranate appears to contain components capable of suppressing tumor cell invasion. Cold-pressed pomegranate seed oil was found to inhibit the invasion of MCF-7 human breast cancer cells *in vitro* across an artificial Matrigel™ membrane at doses less than 10 μg/mL [42]. Further investigations by Khan et al. [54] in breast cancer aggressive cell lines MDA-MB231 and SUM149, both of which are ER- and progesterone receptor (PR)-negative cell lines, illustrated that pomegranate fruit extract consisting of fermented juice and seed of pomegranate modulates NFκ-B. In

agreement with this observation, another investigation also demonstrated that pomegranate extract can downregulate SP1 which regulates NF kb through GC-rich binding site in NF-κB p65 subunit [55]. This transcription factor is involved in the regulation of cell survival, proliferation, tumorigenesis, and inflammation; hence, its regulation is vital. The extract also decreased the expression of RhoC and RhoA, proteins that are involved in cell motility. There are also evolving data on the crosstalk between NFκb and RhoC interaction [54]. A possible role of pomegranate extract in this interactive pathway is hypothesized by this research group. The bioactive constituents were mainly found to be ellagitannins and phenolic acids in the aqueous extract and conjugated octadecatrienoic acids in the lipid phase of pomegranate fruit extract derived from seeds. This study also helps to conclude that antimetastatic potential is not ER or PR dependent. Another research [56] in concurrence with this observation found a reduction in HMMR, a molecule that functions as a hyaluronan (HA) receptor which upon binding to HA causes stimulation of RhoA-activated protein kinases (ROCK) signal transduction pathway, leading to tumor. These results clearly suggest a role of these extracts in lowering the metastatic potential of aggressive breast cancer species. Interestingly, it was also found that pomegranate juice, or a combination of its components luteolin and ellagic acid, along with punicic acid (L + E + P) increase cancer cell adhesion by stimulating intercellular adhesion molecule-1 (ICAM-1), and myristoylated alanine-rich protein kinase C substrate (MARCKS) and upregulating E-cadherin. Both of these could also downregulate N-chimearin, which results in the loss of filopodia and reduced migration, as well as anillin and nexillin, actin-binding proteins that are known to be involved in the regulation of the structure of the cytoskeleton [56]. This combination of L + E + P was found to decrease the expression of TWIST, another gene involved in three epithelial-to-mesenchymal transition. These components also inhibited chemotaxis of the breast cancer cells to SDF1α, a chemokine that attracts breast cancer cells to the bone. All of the investigations connote the relevance of pomegranate extracts in promoting migration, invasion, and metastasis of breast cancer by different mechanisms.

3.4. Inhibition of Angiogenesis

The initiation and the development of new blood vessels (angiogenesis) are essential for supplying oxygen and nutrients for tumor growth and metastasis. The number of these blood vessels formed can be one of the deciding factors for a patient's prognosis [57]. For this reason, it is one of the main pharmacological targets both for the treatment of cancer. Interestingly, studies indicate that pomegranate extract possesses the ability to inhibit angiogenesis. Proangiogenic vascular endothelial growth factor (VEGF) was found to be potently downregulated in MCF-7, less so in MDA-MB-231 breast cancer cells [58] and most strongly in MCF-10A immortalized normal breast epithelial cells upon treatment with fermented pomegranate juice and supercritical CO_2-extracted pomegranate seed oil, and only mildly so by pomegranate peel extract and pressed pomegranate seed oil. The study also observed that the antiangiogenic migration inhibitory factor (MIF) was potently upregulated in MDA-MB-231 cells by fermented pomegranate juice and supercritical CO2-extracted pomegranate seed oil. In concurrence with the study, a recent investigation observed a downregulation of SP transcription factors (SP1, SP3, SP4) and the genes regulated by these factors, VEGF, VEGFR, and survivin [55]. Survivin is a multifunctional protein that controls cell proliferation, apoptosis, and the promotion of angiogenesis [59]. Survivin is found to be overexpressed in breast cancer and is associated with poor survival in breast cancer patients [60]. Hence, it has apparently surfaced as a hot target for therapy. *Punica* can therefore inhibit angiogenesis via survivin. Another interesting research observes that *Punica* could downregulate IL-8, one of the chemokines/proteins [56] released by tumor cells to promote angiogenesis [61]. This implies that *Punica* extracts can possibly prevent angiogenesis from its root. Though not many studies have been carried out in this area, this study unravels the potential of *Punica* extracts in preventing angiogenesis, a critical step in tumor progression.

3.5. Cell Survival and Proliferation

The ability to selectively inhibit proliferation of malignant, but not normal cells, is the hallmark of a promising anticancer therapeutic agent. Strikingly, pomegranate peel extracts have been reported to retard proliferation of cells in several different human cancer cell lines, including breast cancer cell lines [8]. Investigations have revealed that fermented pomegranate juice and pomegranate peel extract had most pronounced effect against estrogen-responsive MCF-7 cells, less so in estrogen-negative MDA-MB-231 cells, and was least effective against immortalized normal breast epithelial cells MCF-10A [42,58]. The study reported that the antiproliferative activity of fermented polyphenol of pomegranate was twice that of fresh pomegranate juice in both of these cell lines [42]. This pins down the conclusion that the inhibition of proliferation can be both by ER-dependent and ER-independent mechanisms. The study illustrated that pomegranate fermented juice could inhibit cancerous lesion induced by the carcinogen 7,12-dimethylbenz[a] anthracene (DMBA) in murine mammary gland organ culture. These studies suggest that whole, complex pomegranate products possess potential antiproliferative activity against breast cancer cells. The outcome of the investigation by Adams et al. [44] on the effect of ellagic acid derivatives isolated from pomegranate on breast cancer cells was in concurrence with the earlier studies, thus giving a stronger evidence on antiproliferative activity of pomegranate extracts. Furthermore, research has also revealed that pomegranate extract can also act via downregulating microRNAs155 and 27a and upregulating ZBTB10, which is sp-repressor [55]. The same study showed that pomegranate extract downregulated NF-kB in agreement with earlier investigation by Khan et al. [54]. In addition, the authors showed that pomegranate extract

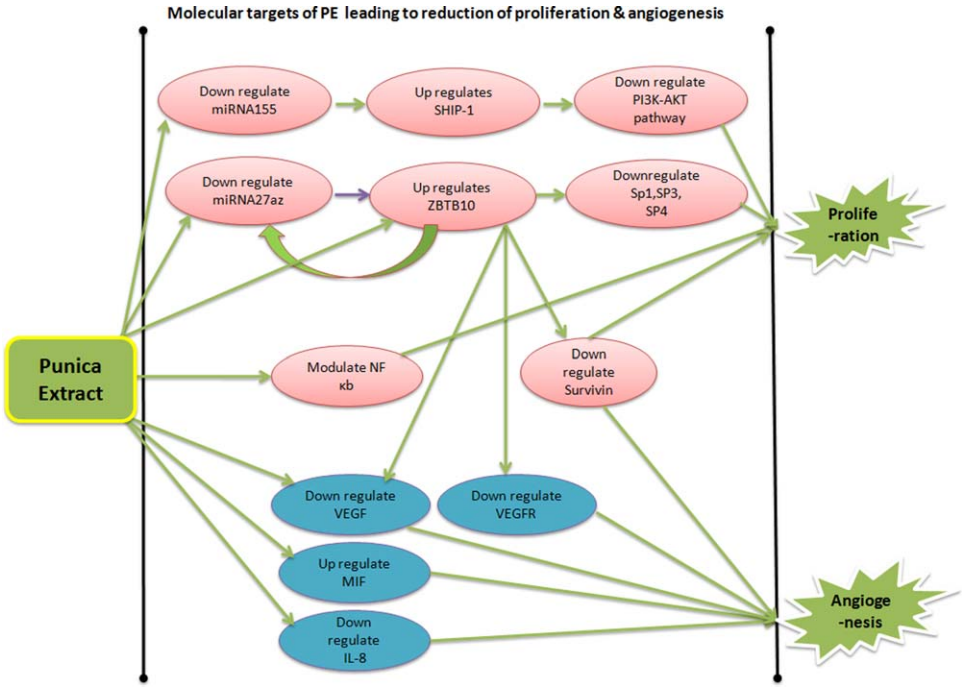

FIG 3 *Molecular targets of PE leading to reduction of proliferation and angiogenesis.* Punica *extract (PE) can modulate proteins and genes to reduce proliferation and angiogenesis.*

significantly decreased specificity protein (Sp) transcription factors (Sp1, Sp3, Sp4), which, as mentioned earlier, are involved in regulating genes involved in proliferation, survival, and angiogenesis in cancer cells. The same research group had earlier shown that these transcription factors are targets of mi RNA 27a for which ZBTB10 acts as repressor [62]. So the results obtained upon treatment with the extract are in accordance with this finding. Also it has been reported that decreased expression of sp proteins specifically SP1 is associated with reduced expression of ER alpha in ER-alpha-positive cell line MCF-7 [63]. This reduced availability of ER-alpha receptors can probably reduce carcinogenicity arising out of gene activation associated with binding of estrogen with ER-alpha receptor.

In addition, pomegranate extracts increased the expression of miR-155 target gene, inositol 5′-phosphatase SHIP-1, a critical negative regulator of phosphatidylinositol-3,4,5-trisphosphate (PI3K)/AKT signaling pathway, which promotes cell proliferation and survival and is commonly activated in various tumors [55]. The investigators extended their study to understand the clinical relevance of the cytotoxic activities of pomegranate extract by constructing an orthotopic model of breast cancer by using BT474 cells as xenografts in an athymic female nude mice. The results were very much in agreement with that obtained with *in vitro* treatments. A very recent research also illustrated the antiproliferative potential of pomegranate on breast cancer cells by performing cell viability assays [64].

Another finding of this study was that the extract could delay cell growth. Thus, studies on cell viability and proliferation emphasize the capability of pomegranate extract in suppressing these activities in breast cancer. *Punica* extracts can modulate varied proteins and genes resulting in inhibition of multiple steps in carcinogenesis. For instance, downregulation of survivin via miRNA27a can reduce angiogenesis as well as proliferation. This network is illustrated in Fig. 3.

3.6. Inflammation

Chronic inflammation is known to cause cancer. Inflammation is understood to play a prominent role in cancer progression also. It helps tumor cells to circumvent the host immune response as well as to facilitate its growth and also aids in the process of angiogenesis, metastasis, invasion, and o forth by inducing effector molecules like MMPs [65]. Suppressing the cytokines and chemokines might therefore reduce the chances of survival of tumor cells. Pomegranate extract was demonstrated to have this action. A few of the proinflammatory cytokines/chemokines associated with cancer progression and metastasis were found to be downregulated upon exposure to pomegranate extract or the combination of punicic acid, ellagitannins, and luteolin. The levels of IL-8, a leukocyte chemoattractant RANTES, a chemotactic factor for T cells, monocytes, and dendritic cells, and PDGFB, mitogenic and proangiogenic factor were observed to be significantly reduced in both ER-positive and ER-negative cells. These point out to the possibility of *Punica*'s potential to decrease inflammation and its impact on cancer progression [56].

3.7. Cell Cycle Regulation and Apoptosis

Apoptosis aids mature cells to establish a balance between cell death and renewal and helps to remove damaged or excess

cells. Cancer cells often tend to have reduced apoptosis. Hence, the induction of apoptosis is one of the ways cancer therapies work. Pomegranate extracts have been reported to induce apoptosis. A major component of *Punica* extract, ellagic acid, has been found to induce apoptosis in MDA MB 231 cell lines via intrinsic pathway and decrease the expression of bcl-xL. But this was not the case observed with ellagic acid on MCF-7 [45]. Another component, ursolic acid, in the seed of pomegranate was also found to cause apoptosis in MCF-7 cells via P53 [66]. This protein or tumor repressor can activate the transcription of the genes like p21WAF1 and Bax involved in the apoptotic process. It follows that ellagic acid and ursolic acid might be two components responsible for inducing apoptosis. Studies with the extract as a whole also have produced positive results with regard to apoptosis. Around 50 mg/mL pomegranate seed oil led to 54% greater extent of apoptosis in MDA-MB-435 estrogen receptor negative, metastatic human breast cancer cells [42]. Adding evidence to the apoptotic potential was an investigation that demonstrated that pomegranate fruit extract could induce twofold to threefold apoptosis in aggressive cell lines like MDA MB 231 and SUM14 in comparison with MCF-10A [54]. Supporting this finding is an investigation showing reduced cell viability in a concentration-dependent manner and accompanied by caspase-3 activation and cleavage of poly(ADP-ribose)-polymerase-1 (PARP-1) in breast cancer cells when compared with normal breast cell line [55]. Supplementing this was another research demonstrating that pomegranate extract and punic acid derived from pomegranate induced apoptosis in both breast cancer cell line (MDA-MB-231) and an estrogen-sensitive cell line developed from MDA-MB-231 cells (MDA-ERalpha7) through lipid peroxidation and the protein kinase C (PKC) signaling pathway [67]. They were also found to cause disruption of cellular mitochondrial membrane [67]. As mentioned earlier in this review, pomegranate extracts have been shown to downregulate NF-κb, which is likely to sensitize cells to apoptosis. Several genes known to function via blocking apoptosis pathways like Bcl-2, Bcl-XL, CIAP, survivin, TRAF1, and TRAF2 have understood to be regulated by NF-kB [61]. This marks another molecular mechanism responsible for apoptosis. Recent investigations into the effect of pomegranate extract on breast cancer line MCF-7 also proved that pomegranate extract treatment could lead to apoptosis [64]. The loss of cell cycle regulation is the hallmark of cancer. Dysregulation of the cell cycle checkpoint leads to overexpression of growth-promoting proteins eventuating in tumorigenesis. Few researches have shown that pomegranate extract is associated with blocking this deregulation. A study demonstrated that the extract could cause G0/G1 arrest in mouse mammary cell line [68]. Another investigation illustrated that pomegranate extract could induce G2/M arrest in MCF-7 cell line [64]. Earlier studies have shown that miRNA27a reduction with antisense miRNA27a and ZBTB10 overexpression both cause a GO/G1 to S phase arrest in MCF-7. The case was different with MDA-MB 231, miRNA27a reduction caused a G2-M arrest and ZBTB10 overexpression caused G0/G1 to S phase arrest. G2-M arrest upon reduction in miRNA27a was attributed to the increased expression of Myt-1, a target of miRNA27a, and a G2-M regulation [7]. However, a G0/G1 to S phase arrest in both the treatments, that is, transfection with antisense miRNA27a and ZBTB10 overexpression, attributed to insufficient induction of the gene and dominance to ZBTB10/Sp1-regulated response. A possible explanation for the G2-M arrest in MCF-7 may be dominance activation of Myt-1 upon treatment with pomegranate extract. But this requires further studies. Researchers have thus proven the ability of pomegranate extract in inducing apoptosis, which is otherwise reduced in cancer cells.

3.8. DNA Damage and Repair Machinery

DNA repair pathways can reduce the efficacy of chemotherapeutic drugs by aiding in the survival of tumor. Thus, combination therapy, targeting specific DNA repair pathway, along with chemotherapeutic drugs, might be beneficial. Microarray studies on genes upon treatment with pomegranate extract showed a downregulation of genes involved in DNA damage response and repair, including MRE11, RAD50, NBS1, RAD51, BRCA1, BRCA2, RCC3, and MSH6 [64]. An interesting observation was that most of the downregulated DNA repair genes specifically targeted homologous recombination genes and thereby sensitized cells to DSBs, cell cycle arrest, and apoptosis. This investigation is the first of its kind and throws light on the effect of pomegranate extract on DNA repair mechanism in breast cancer cell lines, proving that it can potentially be effective in combinatorial therapies.

3.9. Inhibition of Proliferation of Mammary Cancer Stem Cells

An investigation [68] related to the study of the effects of a standardized extract of pomegranate on a mouse mammary cancer cell line WA4 derived from mouse MMTV-Wnt-1 mammary tumors characterized to contain a major proportion of cells possessing stem cell characteristics reported that the extract inhibited the proliferation of WA4 cells in a time- and concentration-dependent manner. This was found to be due to an arrest of cell cycle progression in the G0/G1 phase. Several individual phytochemicals derived from the extract of pomegranate, like caffeic acid, ellagic acid, ursolic acid, and luteolin, were tested on WA4 cells of which all the components except caffeic acid were found to reduce proliferation and viability in a time- and concentration-dependent manner, suggesting that they contribute to the inhibitory effect of pomegranate extract. Cancer stem cells, which are highly resistant to conventional chemotherapeutic agents, are thought to be the origin of both primary and secondary breast tumors, and thus are a critical target in both breast cancer therapy and prevention. These data imply that pomegranate extract, a proven and safe dietary supplement, can prevent the proliferation of cancer stem cells and thus further aid in the treatment of breast cancer as well as possibly reduce the relapse of the disease [68].

3.10. *Punica* as an Adjuvant in Chemotherapy

Multidrug resistance is major hindrance to chemotherapy in all cancers and is relevant to breast cancer as well. Overcoming this problem will make chemotherapy more effective. ATP-binding cassette transporters (ABC) like P-gp, ABCG2, and MRP1 are significant with respect to breast cancer [69]. A recent report demonstrates that pomegranate could downregulate survivin mRNA as well as its protein, as mentioned earlier in this review [55]. Survivin is associated with chemotherapy resistance and specifically with MRP1/p-gp overexpression [59]. Thus, this might be an indicatory result to highlight the importance of pomegranate extracts pertaining to chemotherapy resistance. Quericitin, a component in the juice and pericarp of pomegranate, has long ago been reported to reverse adriamycin (ADR) resistance on MCF-7 ADR-resistant human breast cancer cells in a dose-dependent manner, and this study also pointed out a possible mechanism of inhibition of p-gp by cytofluorographic efflux experiments with the fluorescent dye rhodamine 123 (Rh 123) [70]. Similarly, kaempferol present in the pericarp of pomegranate is reported to be a P-glycoprotein inhibitor. Kitagawa et al. [10] showed that kaempferol (at 100 μM) increased rhodamine 123 accumulation in P-gp-overexpressing KB-C2 cells more than twofold. The kaempferol or the extract might aid in chemotherapy by inhibiting the pump responsible for drug efflux and increase localization of drug inside the cell. *Punica* therefore has potential as an adjuvant in chemotherapy and in increasing drug efficacy. Nonetheless, more research need to be done to establish its exact role in reverse multidrug resistance.

4. Other Specific Constituents of Pomegranate with Known Anti-Breast Cancer Activity

Many of the components in pomegranate fruit extract obtained from other plants have already been demonstrated to have anticancer activity, and in few cases the mechanisms of action have also been elucidated. For instance, luteolin, a constituent in the pericarp of pomegranate, is reported to inhibit tumor formation *in vivo* through its anti-oxidant and anti-inflammatory activity. Luteolin is also reported to reduce the proliferation of IGF-stimulated MCF cells in ER-alpha-dependent manner and affects the PI3K-AKT pathway downstream [71]. Luteolin is also known to act against MDA MB 231 breast cancer cell line in concentration [72]. Quericitin, a flavonoid present in the juice and pericarp of pomegranate, is known to be effective against breast cancer as a single compound and synergistically with other compounds [73,74]. The compound could also enhance the efficacy of chemotherapeutic agents and other flavanoids. For instance, several recent reports showed that quericitin in combination with doxorubicin, tamoxifen, resveratrol, and catechin, respectively, was more potent than either agents alone in suppressing breast cancer growth [74]. A study of the action of quericitin on the proliferation of an adriamycin-resistant estrogen-receptor-negative human breast cancer cell line (MCF-7 ADRr) showed a dose-dependent effect on cell proliferation. Punicic acid, an ω-5 long-chain polyunsaturated fatty acid found in pomegranate seed oil, was demonstrated to potentially inhibit proliferation as well to cause apoptosis of both MDA-MB-231 and an estrogen-sensitive cell line developed from the MDA-MB-231 cells (MDA-ERα7) [13]. The compound also disrupted cellular mitochondrial membrane potential. The results also suggested that punicic acid action against breast cancer cell lines is dependent on lipid peroxidation and the PKC pathway. Punicic acid has also been reported to inhibit prostaglandin production and is hypothesized to be involved in COX2 inhibition [75]. Γ-tocopherol, though not a major component, is present in pomegranate seed and studies on treatment with γ-tocopherol isolated from different plants have reported its ability to reduce cell proliferation and tumor burden. Studies have shown that γ-enriched mixed tocopherols inhibit the development of mammary hyperplasia and tumorigenesis in animal models [76]. It has been shown to inhibit ER-positive cell proliferation and work as an antagonist of estrogen signaling in MCF-7 and T47D breast cancer cells. It is also understood to cause cell cycle arrest and apoptosis in these cell lines [76].

Thus, *Punica* can act via diverse molecules at different steps of progression in breast cancer and the molecular targets at a glance is illustrated in Fig. 4.

5. Bioavailability

Bioavailability of a compound encompasses essential features like how fast the drug enters the systemic circulation (the rate of absorption) and how much of the nominal strength enters the body (the extent of absorption). This is a paramount factor in drug availability as it decides the physiological availability of a compound. Potential health benefits have prompted researches to analyze the bioavailability of pomegranate components. An *in vitro* study examining the fate of pomegranate juice showed that pomegranate phenolic compounds are available in increasing amounts during digestion, whereas the anthocyanins are largely metabolized to some noncolored forms, oxidized, or degraded into other chemicals [77]. The first report on metabolism and bioavailability of components analyzed punicalagin, an ellagitannin pomegranate juice. In this study, a number of colonic microflora metabolites, ellagic acid derivatives, and ellagitannins were detected in rat urine, plasma, and feces [78]. This research group further studied the bioavailability and metabolism of these ellagitannins in humans and proved that the potential systemic biological effects could be attributed to the colonic microflora metabolites rather than to the polyphenols present in the juice [79]. Supplementing data for the investigation was another study that demonstrated that ellagitannins from pomegranate persist in plasma for around 48 h and that urolithins, formed by intestinal bacteria, may contribute to the biological effects of

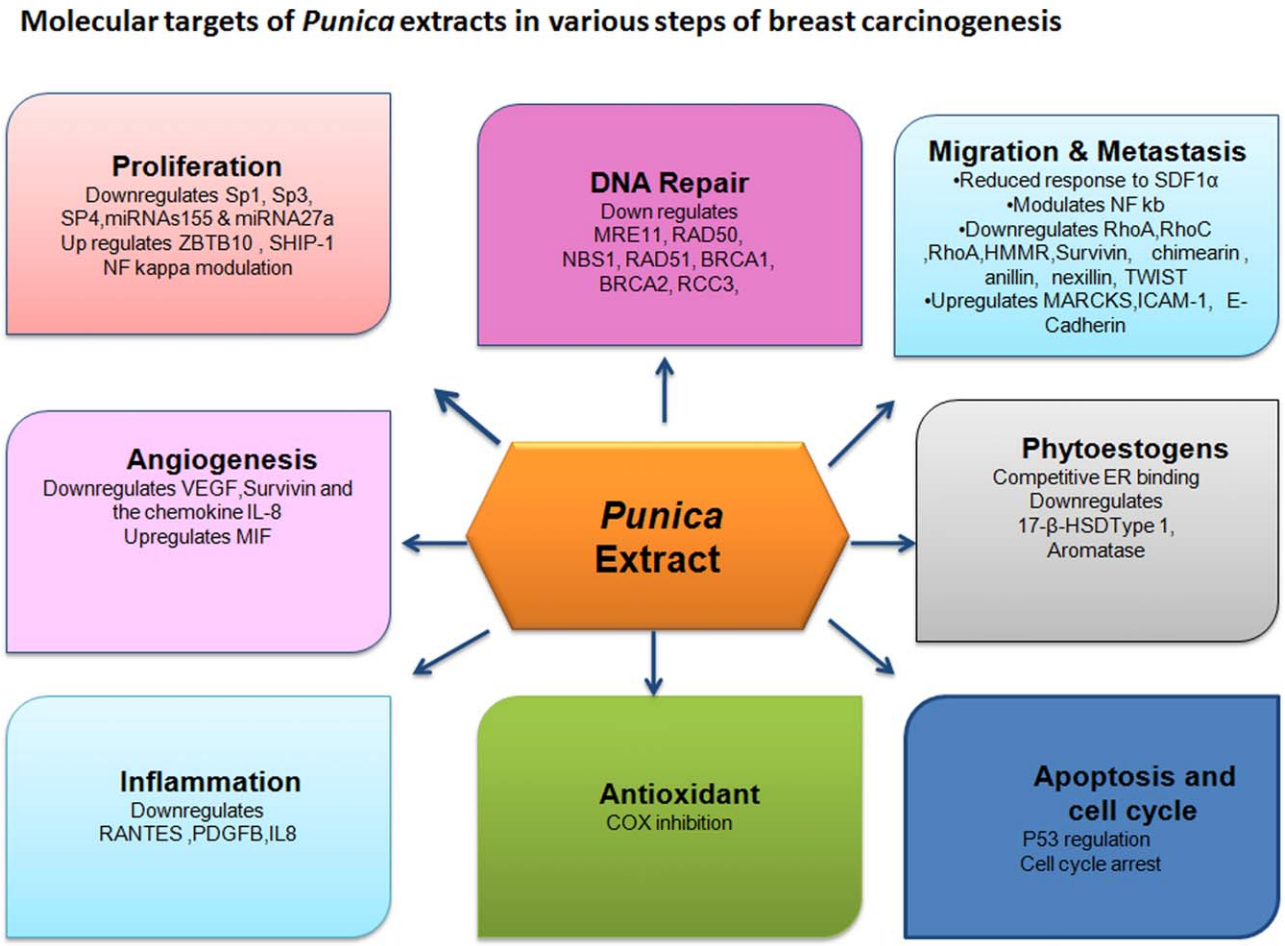

FIG 4

Molecular targets of Punica *extract in various process of breast carcinogenesis.*

pomegranate juice and might give a plausible explanation for some of the health benefits noted after extended consumption of pomegranate juice [80]. Seeram et al. [81] expanded the research to understand whether the bioavailability of the extract varied among different forms of the extract, namely pomegranate juice, pomegranate polyphenol liquid extract, and pomegranate polyphenol powder extract (1,000 mg). No difference in bioavailability was found among pomegranate juice, liquid, or powder extract forms of treatment with similar levels of total polyphenols standardized as gallic acid equivalents. Taken together, these studies indicate that pomegranate polyphenolic compounds can act in multiple ways and undergo different fates. A few of the compounds get absorbed and enter the bloodstream to act as antioxidants, and the remainder get digested by the colonic microflora to produce other biologically active substances [82]. Though these researches did not study the implication of the bioavailability of pomegranate juice constituents with respect to their anticancer activity, these might give a possible explanation related to the *in vivo* activity in relation to cancer.

6. Conclusion

Taken together, these studies connote the potential of pomegranate extract in fighting breast cancer and underpin the ability of the extract to act in various steps of breast cancer, from initial growth to proliferation, metastasis to angiogenesis and escape of immune surveillances, and its ability to target many levels of regulation of cell growth and apoptosis. Thus, it can possibly act as an adjuvant in chemotherapy. A plethora of action of *P. granatum* have been reported against breast cancer and many of these have targets coinciding with the current targeted therapies of breast cancer mentioned earlier in this review, pointing to its relevance in breast cancer treatment and prevention. Researchers have also attempted to understand the bioavailability and metabolism of different components in pomegranate juice. A good many researches have focused on the phytoestrogenic potential of pomegranate extract, which has been found to inhibit enzymes like 17-β-hydroxysteroid dehydrogenase type 1 and aromatase which are relevant to estrogen biosynthesis. It is also found to

compete with estrogen to bind to its receptor. The extracts have also been reported to downregulate estrogen-responsive genes at specific concentrations. The antioxidant potential of the fruit has been extensively studied. One of the implications of the studies with regard to breast cancer is its possible ability to inhibit the enzyme COX, a relevant enzyme in breast cancer growth and metastasis. *Punica* extracts have also been demonstrated to inhibit angiogenesis via VEGF and MIF and survivin. Extracts of the fruit have been found to reduce invasiveness, migration, and the metastatic potential of breast cancer by acting via different molecular mechanisms, including enhancing the expression of adhesion proteins, reducing migratory proteins, decreasing response to chemokines responsible for metastasis, and downregulating/upregulating transcription factors involved in migration and metastasis. Pomegranate extract can also downregulate molecules like NF κb, a transcription factor involved in tumor progression, survival, and angiogenesis. A handful of investigations have demonstrated the antiproliferative activity of the extract. Many investigations have also brought to light the role of these extracts in inducing apoptosis and arresting cell cycle. Very recent research brought to attention the mechanisms and molecular targets by which it targets DNA repair pathway. Researches also demonstrate that pomegranate extract can reduce inflammatory responses that help in tumor survival. The components responsible for these actions are mainly punicic acid, ellagitannins, and luteolin. It also has possible contributions with respect to overcoming MDR resistance and thus has a plausible role in aiding chemotherapy by a combinatorial treatment. Studies claim that *Punica* extracts can induce cytotoxicity and reduce proliferation in mammary cancer stem cells. Few of the active ingredients include ursolic acid, ellagic acid, luteolin, and punicic acid. Few others luteolin γ-tocopherol and quericitin are components present in *Punica* that are already reported to have anti-breast cancer activity when isolated from other plants. Bioavailability studies point out that the *in vivo* effect of pomegranate extracts is predominantly due to the presence of colon microflora that metabolize the components, especially compounds like ellagitannins and bring health benefits associated with the same. Understanding the bioavailability of the relevant components of the extract might further help in bypassing their limitations.

Collectively, the findings indicate that *Punica* can be considered a promising candidate for preventing and inhibiting the progress of breast cancer. However, investigations at a clinical level have not gained the required spotlight of research. More studies at clinical level are warranted to measure and evaluate the actual potential of these extracts and their specific components, both individually and in combination.

Chemotherapy has always been associated with number of side effects. Targeted therapy approach in treating different cancer is reducing the side effects associated with generic drugs. Suitable adjuvants might make chemotherapy more effective. *Punica granatum* might serve to be one such ideal adjuvant for breast cancer therapy.

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References

[1] Sreekumar, S., Sithul, H., Muraleedharan, P., Azeez, J. M., and Sreeharshan, S. (2014) Pomegranate fruit as a rich source of biologically active compounds. Biomed Res. Int. 2014, 686921.

[2] Seeram, N. P., Aviram, M., Zhang, Y., Henning, S. M., Dreher, M., et al. (2008) Comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States. J. Agric. Food Chem. 56, 1415–1422.

[3] Heiser, L. M., Sadanandama, A., Kuoa, W. L., Benzb, S. C., Goldsteinb, T. C., et al. (2012) Subtype and pathway specific responses to anticancer compounds in breast cancer. Proc. Natl. Acad. Sci. USA 109, 2724–2729.

[4] Higgins, M. J. and Baselga, J. (2011) Targeted therapies for breast cancer. J. Clin. Invest. 121, 3797–3803.

[5] Chumsri, S. and Brodie, A. (2012) Aromatase inhibitors and breast cancer. Horm. Mol. Biol. Clin. Invest. 2, 119–126.

[6] Wang, R., Ding, Y., Liu, R., Xiang, L., and Du, L. (2010) Pomegranate: constituents, bioactivities and pharmacokinetics. Fruit Veg. Cereal Sci. Biotechnol. 4, 77–82.

[7] Wang, L. and Martins-Green, M. A. (2013) The potential of pomegranate and its components for prevention and treatment of breast cancer. Agro Food Ind. Hi Tech 24, 58–61.

[8] Lansky, E. P. and Newman, R. A. (2007) *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J. Ethnopharmacol. 109, 177–206.

[9] Gomez-Caravaca, A. M., Verardo, V., Toselli, M., Segura-Carretero, A., Fernandez-Gutierrez, A., et al. (2013) Determination of the major phenolic compounds in pomegranate juices by HPLC–DAD–ESI-MS. J. Agric. Food Chem. 61, 5328–5337.

[10] Kitagawa, S., Nabekura, T., Takahashi, T., Nakamura, Y., Sakamoto, H., et al. (2005) Structure-activity relationships of the inhibitory effects of flavonoids on P-glycoprotein-mediated transport in KB-C2 cells. Biol. Pharm. Bull. 28, 2274–2278.

[11] Ackland, M. L., Waarsenburg, S. V. D., and Jones, R. (2005) Synergistic antiproliferative action of the flavonols quercetinand kaempferol in cultured human cancer cell lines. In Vivo 19, 69–76.

[12] Gasmi, J. and Sanderson, J. T. (2010) Growth inhibitory, antiandrogenic, and pro-apoptotic effects of punicic acid in LNCaP human prostate cancer cells. J. Agric. Food Chem. 58, 12149–12156.

[13] Grossmann, M. E., Mizuno, N. K., Schuster, T., and Cleary, M. P. (2010) Punicic acid is an ω-5 fatty acid capable of inhibiting breast cancer proliferation. Int. J. Oncol. 36, 421–426.

[14] Johnson, I. T. (2007) Phytochemicals and cancer, Proc. Nutr. Soc. 66, 207–215.

[15] Romagnolo, D. F. and Selmin, O. I. (2007) Flavonoids and cancer prevention: a review of the evidence. J. Nutr. Gerontol. Geriatr. 31, 206–238.

[16] Takemura, H., Sakakibara, H., Yamazaki, S., and Shimoni, K. (2013) Breast cancer and flavanoids—a role in prevention. Curr. Pharm. Des. 19, 6125–6132.

[17] Mourouti, N. and Panagiotakos, D. B. (2013) Soy food consumption and breast cancer. Maturitas 76, 118–122.

[18] Surh, Y. J. (2003) Cancer chemoprevention with dietary phytochemicals. Nat. Rev. 3, 768–780.

[19] Deluca, D., Krazeisen, A., Breitling, R., Prehn, C., Moller, G., et al. (2005) Inhibition of 17beta-hydroxysteroid dehydrogenases by phytoestrogens: comparison with other steroid metabolizing enzymes. J. Steroid Biochem. Mol. Biol. 93, 285–292.

[20] Adams, L. S. and Chen, S. (2009) Phytochemicals for breast cancer prevention by targeting aromatase. Front. Biosci. 14, 3846–3863.

[21] Huang, Z., Fasco, M. J., and Kaminsky, L. S. (1997) Inhibition of estrone sulfatase in human liver microsomes by quercetin and other flavonoids. J. Steroid Biochem. Mol. Biol. 63, 9–15.

[22] Androutsopoulosa, V. P., Ruparelia, K., Arroo, R. R. J., Tsatsakisa, A. M., and Spandidos, A. M. (2009) CYP1-mediated antiproliferative activity of dietary flavonoids in MDA-MB-468 breast cancer cells. Toxicology 264, 162–170.

[23] Fernandez, S. V., Russo, I. H., and Russo, J. (2006) Estradiol and its metabolites 4-hydroxyestradiol and 2-hydroxyestradiol induce mutations in human breast epithelial cells. Int. J. Cancer 118, 1862–1868.

[24] Yang, P. M., Tseng, H. H., Peng, C. W., Chen, W. S., and Chiu, S. J. (2012) Dietary flavonoid fisetin targets caspase-3-deficient human breast cancer MCF-7 cells by induction of caspase-7-associated apoptosis and inhibition of autophagy. Int. J. Oncol. 40, 469–478.

[25] Zhang, X., Wei, H., Liu, Z., Yuan, Q., Wei, A., et al. (2013) A novel protoapigenone analog RY10-4 induces breast cancer MCF-7 cell death through autophagy via the Akt/mTOR pathway. Toxicol. Appl. Pharmacol. 270, 122–128.

[26] Rajendran, P., Ho, E., Williams, D. E., and Dashwood, R. H. (2011) Dietary phytochemicals, HDAC inhibition, and DNA damage/repair defects in cancer cells. Clin. Epigenet. 3, 4.

[27] Sun, X., Huo, X., Luo, T., Li, M., Yin, Y., et al. (2011) The anticancer flavonoid chrysin induces the unfolded protein response in hepatoma cells. J. Cell. Mol. Med. 15, 2389–2398.

[28] Rowe, D. L., Ozbay, T., O'Regan, R. M., and Nahta, R. (2009) Modulation of the BRCA1 protein and induction of apoptosis in triple negative breast cancer cell lines by the polyphenolic compound curcumin. Breast Cancer 3, 61–75.

[29] Seoa, H. S., Jua, J. H., Janga, K., and Shin, I. (2011) Induction of apoptotic cell death by phytoestrogens by up-regulating the levels of phospho-p53 and p21 in normal and malignant estrogen receptor α–negative breast cells. Nutr. Res. 31, 139–146.

[30] Wang, S., Liu, Q., Zhang, Y., Liu, K., Yu, P., et al. (2009) Suppression of growth, migration and invasion of highly-metastatic human breast cancer cells by berbamine and its molecular, mechanisms of action. Mol. Cancer 8, 81.

[31] Zhang, S., Sagawa, K., Arnold, R. D., Tseng, E., Wang, X., et al. (2010), Interactions between the flavonoid biochanin A and P-glycoprotein substrates in rats: in vitro and in vivo. J. Pharm. Sci. 99, 430–441.

[32] Alvarez, A. I., Real, R., Pe'rez, M., Mendoza, G., Prieto, J. G., et al. (2010) Modulation of the activity of ABC transporters (p-glycoprotein, MRP2, BCRP) by flavonoids and drug response. J. Pharm. Sci. 99, 598–617.

[33] Zhang, S., Yang, X., and Morris, M. E. (2004) Flavonoids are inhibitors of breast cancer resistance protein (ABCG2)-mediated transport. Mol. Pharmacol. 65, 1208–1216.

[34] Tan, K. W., Li, Y., Paxton, J. W., and Birch, N. P. (2013) Scheepens A: identification of novel dietary phytochemicals inhibiting the efflux transporter breast cancer resistance protein (BCRP/ABCG2). Food Chem. 138, 2267–2274.

[35] Liao, W. Y., Liaw, C. C., Huang, Y. C., Han, H. Y., Hsu, H. W., et al. (2013) Cyclohexylmethyl flavonoids suppress propagation of breast cancer stem cells via downregulation of NANOG. Evid.-Based Complem. Altern. Med. 2013, 170261.

[36] Sina, Y., Pham, A., Ng, A., Nguyen, D., Lialiutska, T., et al. (2013) Profiling flavonoid cytotoxicity in human breast cancer cell lines: determination of structure-function relationships. Nat Prod Commun 9, 597–606.

[37] Manach, C., Williamson, G., Morand, C., Scalbert, A., and Remesy, C. (2005) Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am. J. Clin. Nutr. 81, 230S–242S.

[38] Jurenka, J. (2008) Therapeutic applications of pomegranate (*Punica granatum* L.): a review. Altern. Med. Rev. 13, 128–144.

[39] Mehta, R. and Lansky, E. P. (2004) Breast cancer chemopreventive properties of pomegranate (*Punica granatum*) fruit extracts in a mouse mammary organ culture. Eur. J. Cancer Prev. 13, 345–348.

[40] Yue, W., Yager, J. D., Wang, J.-P., Jupe, E. R., and Santen, R. J. (2013) Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. Steroids 78, 161–170.

[41] Russo J. and Russo, I. H. (2006) The role of estrogen in the initiation of breast cancer. J. Steroid Biochem. Mol. Biol. 102, 89–96.

[42] Kim, N. D., Mehta, R., Yu, W., Neeman, I., Livney, T., et al. (2002) A chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. Breast Cancer Res. Treat. 71, 203–217.

[43] Ayan, I., Maltais, R., Roy, J., and Poirier, D. (2012) A New nonestrogenic steroidal inhibitor of 17β-hydroxysteroid dehydrogenase type I blocks the estrogen-dependent breast cancer tumor growth induced by estrone. Mol. Cancer Ther. 11, 2096–2104.

[44] Adams, L. S., Zhang, Y., Seeram, N. P., Heber, D., and Chen, S. (2010) Pomegranate ellagitannin–derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells in vitro. Cancer Prev. Res. 3, 108–113.

[45] Kim, H. A., Lee, R. A., Moon, B. I., and Choe, K. J. (2009) Ellagic acid shows different anti-proliferative effects between the MDA-MB-231 and MCF-7 human breast cancer cell lines. J. Breast Cancer 12, 85–91.

[46] Sreekumar, S., Thankayyan, R., Baddireddi, S. L., and Sreeharshan, S. (2012) Pomegranate extract demonstrate a selective estrogen receptor modulator profile inhuman tumor cell lines and in vivo models of estrogen deprivation. J. Nutr. Biochem. 23, 725–732.

[47] Schubert, S. Y., Lansky, E. P., and Neeman, I. (1999) Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. J. Ethnopharmacol. 66, 11–17.

[48] Soslow, R. A., Dannenberg, A. J., Rush, D., Woerner, B. M., Khan, K. N., et al. (2000) COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 89, 2637–2645.

[49] Liu, C. H., Chang, S. H., Narko, K, Trifan, O. C., Wu, M. T., et al. (2001) Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J. Biol. Chem. 276, 18563–18569.

[50] Singh, B., Berry, J. A., Shoher, A., Ayers, G. D., Wei, C., et al. (2007) COX-2 involvement in breast cancer metastasis to bone. Oncogene 26, 3789–3796.

[51] Brandao, R., Veeck, J., Koen, K., Vijver, V., Lindsey, P., et al. (2013) A randomised controlled phase II trial of pre-operative celecoxib treatment reveals anti-tumour transcriptional response in primary breast cancer. Breast Cancer Res. 15, R29.

[52] Bocca, C., Ievolella, M., Autelli, R., Motta, M., Mosso, L., et al. (2014) Expression of Cox-2 in human breast cancer cells as a critical determinant of epithelial-to-mesenchymal transition and invasiveness. Exp. Opin. Ther. Targets 18, 121–135.

[53] Shukla, M., Gupta, K., Rasheed, Z., Khan, K. A., and Haqqi, T. M. (2014) Bioavailable constituents/metabolites of pomegranate (*Punica granatum* L) preferentially inhibit COX2 activity ex vivo and IL-1beta-induced PGE2 production in human chondrocytes in vitro. J. Inflamm. 5, 9.

[54] Khan, G. N., Gorin, M. A., Rosenthal, D., Pan, Q., Bao, L. W., et al. (2009) Pomegranate fruit extract impairs invasion and motility in human breast cancer. Integr. Cancer Ther. 8, 242–253.

[55] Banerjee, N., Talcott, S., Safe, S., and Mertens-Talcott, S. U. (2012) Cytotoxicity of pomegranate polyphenolics in breast cancer cells in vitro and vivo—potential role of miRNA-27a and miRNA-155 in cell survival and inflammation. Breast Cancer Res. Treat. 136, 21–34.

[56] Rocha, A., Wang, L., Penichet, M., and Martins-Green, M. (2012) Pomegranate juice and specific components inhibit cell and molecular processes critical for metastasis of breast cancer. Breast Cancer Res. Treat. 136, 647–658.

[57] Weidner, N., Folkman, J., Pozza, F., Bevilacqua, P., Allred, E. N., et al. (1992) Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J. Natl. Cancer Inst. 84, 1875–1887.

[58] Toi, M., Bando, H., Ramachandran, C., Melnick, S. J., Imai, A., et al. (2003) Preliminary studies on the anti-angiogenic potential of pomegranate fractions in vitro and in vivo. Angiogenesis 6, 121–128.
[59] Jha, K., Shukla, M., and Pandey, M. (2012) Survivin expression and targeting in breast cancer. Surg. Oncol. 21, 125–131.
[60] Zhang, S. Q., Qiang, S. Y., Yang, W. B., Jiang, J. T., and Ji, Z. Z. (2004) Expression of survivin in different stages of carcinogenesis and progression of breast cancer. Ai Zheng 23, 697–700.
[61] Aggarwal, B. B. and Shishodia, S. (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem. Pharmacol. 71, 1397–1421.
[62] Mertens-Talcott, S. U., Chintharlapalli, S., Li, X., and Safe, S. (2007) The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. Cancer Res. 67, 11001–11011.
[63] Li, X., Mertens-Talcott, S. U., Zhang, S., Kyoung, K., Ball, J., et al. (2010) MicroRNA-27a, indirectly regulates estrogen receptor expression and hormone responsiveness in MCF-7 breast cancer cells. Endocrinology 151, 2462–2473.
[64] Shirode, A. B., Kovvuru, P., Chittur, S. V., Henning, S. M., Heber, D. et al. (2014) Antiproliferative effects of pomegranate extraction MCF-7 breast cancer cells are associated with reduced DNA repair gene expression and induction of double strand breaks. Mol. Carcinog. 53, 458–470.
[65] Lu, H., Ouyang, W., and Huang, C. (2006) Inflammation: a key event in cancer development. Mol. Cancer Res. 4, 221–233.
[66] Zhang, W., Li, Y., Zhang, G., Lü, J., and Ou, H. (2005) Experimental study on MCF-7 cell apoptosis induced by ursolic acid. Zhong Yao Cai 28, 297–301.
[67] Rahimi, H. R., Arastoo, M., and Ostad, S. N. (2012) A comprehensive review of *Punica granatum* (Pomegranate) properties in toxicological, pharmacological, cellular and molecular biology researches. Iran. J. Pharmacol. Res. 11, 385–400.
[68] Dai, Z., Nair, V.; Khan, M., and Ciolino, H. P. (2010) Pomegranate extract inhibits the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells in vitro. Oncol. Rep. 24, 1087–1091.
[69] Kordestani, L. M., Bassevillea, A., Kurdziel, K., Fojoa, A. T., and Bates, S. E. (2012) Targeting MDR in breast and lung cancer: Discriminating its potential importance from the failure of drug resistance reversal studies. Drug Resist. Updat. 15, 50–61.
[70] Scambia, G., Ranelletti, F. O., Panici, P. B., Vincenzo, R. D., Bonanno, G., et al. (1994) Quercetin potentiates the effect of adriamycinin a multidrug-resistant MCF-7 human breast-cancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol. 34, 459–464.
[71] Wang, L. M., Xie, K. P., Huo, H. N., Shang, F., Zou, W., et al. (2012) Luteolin inhibits proliferation induced by IGF-1 pathway dependent ERα in human breast cancer MCF-7 cells. Asian Pac. J. Cancer Prev. 13, 1431–1437.
[72] Lee, E. J., Oh, S. Y., and Sung, M. K. (2012) Luteolin exerts anti-tumor activity through the suppression of epidermal growth factor receptor-mediated pathway in MDA-MB-231 ER-negative breast cancer cells. Food Chem. Toxicol. 50, 4136–4143.
[73] Schlachterman, A., Valle, F., Wall, K. M., Azios, N. G., Castillo, L., et al. (2008), Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model. Transl. Oncol. 1, 19–27.
[74] Huang, C., Lee, S. Y., Lin, C. L., Tu, T. H., Chen, L. H., et al. (2013) Co-treatment with quercetin and 1,2,3,4,6-penta-O-galloyl-βD glucose causes cells cycle arrest and apoptosis in human breast cancer MDA-MB-231 and AU565 cells. J. Agric. Food Chem. 61, 6430–6445.
[75] Sturgeon, S. R. and Ronnenberg, A. G. (2009) Pomegranate and breast cancer: possible mechanisms of prevention. Nutr. Rev. 68, 122–128.
[76] Smolarek, A. K. and Suh, N. (2011) Chemopreventive activity of vitamin E in breast cancer: a focus on γ- and δ-tocopherol. Nutrients 3, 962–986.
[77] Perez-Vicente, A., Gil-lzquierdo, A., and Garcia-Viguera, C. (2002) In vitro gastrointestinal digestion study of pomegranate juice phenolic compounds, anthocyanins, and vitamin C. J. Agric. Food Chem. 50, 2308–2301.
[78] Cerdá, B., Llorach, R., Cerón, J. J., Espín, J. C., Tomás-Barberán, F. A. (2003) Evaluation of the bioavailability and metabolism in the rat of punicalagin: an antioxidant polyphenol from pomegranate juice. Eur. J. Nutr. 42, 18–28.
[79] Cerda, B., Espin, J. C., Parra, S., Martinez, P., and Tomas-Barberan, F. A. (2004) The potent in vitro antioxidant ellagitannins from pomegranate juice are metabolized into bioavailable but poor antioxidant hydroxy-6H-dibenzopyran-6-one derivatives by the colonic microflora of healthy humans. Eur. J. Nutr. 43, 205–220.
[80] Seeram, N. P., Henning, S. M., Zhang, Y., Suchard, M., Li, Z., et al. (2006) Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 hours. J. Nutr. 136, 2481–2485.
[81] Seeram, N. P., Zhang, Y., McKeever, R., Henning, S. M., Lee, R. P., et al. (2008) Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects. J. Med. Food 11, 390–394.
[82] Syed, D. N., Chamcheu, J. C., Adhami, V. M., and Hasan, M. (2013) Pomegranate extracts and cancer prevention: molecular and cellular activities. Anticancer Agents Med. Chem. 13, 1149–1161.