Review

Resveratrol as MDR reversion molecule in breast cancer: An overview

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A R T I C L E   I N F O

Article history:
Received 26 January 2017
Received in revised form
5 March 2017
Accepted 13 March 2017
Available online 15 March 2017

Chemical compounds reported in this article:
Tamoxifen (PubChem CID: 2733526)
Daunorubicin (PubChem CID: 30323)
Doxorubicin (PubChem CID: 31703)
cis-resveratrol (PubChem CID: 1548910)
trans-resveratrol (PubChem CID: 445154)
Paclitaxel (PubChem CID: 36314)
Daidzein (PubChem CID: 5281708)

Keywords:
Phytoestrogens
Resveratrol
Breast cancer
Multidrug resistance

A B S T R A C T

Breast cancer is the most common cause of cancer mortality among women worldwide; therefore, a strategy to defeat breast cancer is an extremely important medical issue. One of the major challenges in this regard is multidrug resistance (MDR). Resveratrol, a well-known phytoestrogen, may be helpful as part of an overall strategy to defeat breast cancer. The mixed agonist and antagonist role of resveratrol for the estrogen receptor makes it a controversial but interesting molecule in cancer therapy, especially in hormone dependent cancers. Several in vitro investigations have suggested that resveratrol can reverse multidrug resistance. However, poor bioavailability of resveratrol is a potential limitation for resveratrol treatment and cancer outcome in vivo. Fortunately, combination therapy with other selected compounds improves resveratrol’s bioavailability and/or a delay in its metabolism. This review summaries the available published literature dealing with the effects of resveratrol on multidrug resistance in cancer and more specifically, breast cancer.

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Abbreviations: AC, Adenylyl Cyclase; BCRP, Breast Cancer Resistance Protein; cGMP, Cyclic GMP; cAMP, Cyclic AMP; cyt.c, Cytochrome c; DOX, Doxorubicin; ER+, Estrogen receptor positive; E2, 17β-estradiol; MAPKs, Mitogen Activated Protein Kinases; MMR, Mismatch Repair; MDR, Multidrug Resistance; MRP, Multidrug Resistance Associated Protein; Pgp, P-glycoprotein; PKA, Protein Kinase A; PKB, Protein Kinase B; PDE, Phosphodiesterase; RES, Resveratrol; SMnase, Sphingomyelinase; SM synthase, Sphingomyelin synthase; UV, Ultraviolet Irradiation; b.wt, body weight.

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1. Breast cancer: general aspects

Despite years of intensive research, breast cancer remains the most common cause of cancer mortality among women especially younger women (Ferlay et al., 2015). Major challenges in the treatment of breast cancer include, among other things, high heterogeneity from patient to patient, anti-cancer drug combinations leading to tremendous adverse effects, and long-time anti-cancer drugs administration along with drug resistance and recurrence of the cancer following treatment. In this regard, the use of natural compounds (Karimi et al., 2011; Razavi and Karimi, 2016) like phytoestrogens as pleiotropic molecules with fewer side effects and as potential drug resistance inhibitors may be an alternative to conventional therapy (Staaf and Ringner, 2015).

2. Phytoestrogens: promising source of phytoneutrity

Phytoestrogens have been a topic of research since the 1980s (Dixon, 2004). Among the many phytochemicals, phytoestrogens have been reported to contain several potential anticancer molecules and are mostly found in soy, vegetables, and fruits. These compounds can be classified into four main groups including isoflavonoids (genistein, daidztein, glycinein), flavonoids (luteolin, apigenin, kaempferol, quercetin), stilbenes (resveratrol), and lignans (matairesinol, pinoresinol) (Pilsakova et al., 2010).

Estrogen and its receptor play pivotal roles in the proliferation and malignancy of breast cancer cells (Azizi et al., 2010). Mechanistically, phytoestrogens have a strong affinity to bind to estrogen receptors (ERα and ERβ). Therefore, they can act as estrogen antagonists competing with estradiol at the receptor complex by modifying the ER conformation (Kuiper et al., 1998).

Epidemiological studies have suggested that breast cancer incidence and mortality rate among women in Asian countries is six folds lower than that among women in the West. It has been speculated that this difference may be because in Asian countries there is a higher intake of soy and soy products (approximately 50 g/day), major source of dietary phytoestrogens, than in Western women (Ganry, 2002; Zuljevic and O’Brien, 2016).

Phytoestrogens appear to have steroidal potency not only by acting on steroid receptors but also by modulating steroidogenesis enzymes. These compounds have appeared to inhibit some of the important enzymes (17β- and 3β-hydroxysteroid dehydrogenase (HSD)) involved in estrogen biosynthesis and metabolism. The concentration of phytoestrogens required for inhibition of these enzymes may be achieved physiologically from a diet rich in phytoestrogens (Costa et al., 2016) and thus these compounds may play a chemo-preventive role in hormone related cancers, especially breast cancer (Zhao and Mu, 2011).

3. Resveratrol: well-known monomeric stilbene

Stilbenes, in particular trans-resveratrol and its glucoside, are widely reported to be beneficial to human health, having been shown to possess antioxidative, anticarcinogenic, antitumor properties and estrogenic/anti-estrogenic activity (Stagos et al., 2012; Apostolou et al., 2013; Carter et al., 2014). In plants, this compound acts as a phytoalexin, a class of defense molecules that protects against infection (Botrytis cinerea) and damage from exposure to ultraviolet irradiation (UV) (Juan et al., 2012; Bartolacci et al., 2017). Resveratrol was first found in the roots of the medicinal plant Polygonum cuspidatum, traditionally used in Chinese and Japanese medicines as an anti-inflammatory and anti-platelet agent (Rieder et al., 2012). Major dietary sources include grapes, wine, peanuts, and soy. It can also be introduced into the diet through Itadori tea, which has long been used in Japan and China as a traditional herbal remedy for heart disease and strokes. The chemical structure of resveratrol (trans- 3, 5, 4’-trihydroxystilbene) is depicted in Fig. 1.

It is present in dietary products as both isofoms of cis and trans but mostly in the glycosylated form, piceids (3-O-h-d-glucosides). Although resveratrol and other polyphenols have very low bioavailability which may be a major limitation with the in vivo use of resveratrol, glycosylation prevents enzymatic oxidation of this molecule and therefore, its biological activity and bioavailability is improved. Additionally, combination therapy with other natural compounds or combined as part of a nanoparticle-mediated delivery system or by using resveratrol analogues, or by the use of conjugated metabolites of resveratrol may improve its bioavailability or possibly delay metabolism of resveratrol in in vitro and in vivo models (Regev-Shoshani et al., 2003; Androutsopoulos et al., 2011; Du et al., 2016).

In addition to its antimicrobial effects (Stagos et al., 2012b), resveratrol has been reported to have psychological activity including cognitive capability associated with dementia, and antioxidant activity as well as cardiovascular health (Goutzoulas et al., 2015; Fenga et al., 2016; Hashemzaei et al., 2016a; Sinha et al., 2016; Razavi-Azarkhiavi et al., 2016).

Resveratrol is particularly interesting as an antitumor agent in breast cancer (Fenga et al., 2016) because it affects several intracellular mediators involved in the initiation, promotion and progression of cancer, apoptosis and cell-cycle arrest (Stagos et al., 2012; Huang et al., 2016; Chimento et al., 2016).
dealing with various aspects of resveratrol and its potential use as an anti-cancer drug.

### 3.1. Estrogen receptor modulatory effects of resveratrol

Resveratrol acts as a mixed agonist/antagonist for estrogen receptors in a dose dependent way. Levenson et al. (2003) suggested that resveratrol may act as a super-agonist at moderate concentrations (10–25 µM) by activating hormone receptor mediated gene transcription because the structure of diethylstilbestrol, a synthetic estrogen, is similar to E2.

The effects of resveratrol alone, and in combination with 17-β-estradiol, were evaluated in MCF-7, T47D, LY2 and S30 cell lines. In MCF-7 cells, resveratrol displayed mixed estrogen agonist/antagonist activities in the absence of 17-β-estradiol, but acted as an anti-estrogen in the presence of 17-β-estradiol (1 nM). In addition, similar mixed effects (estrogenic/anti-estrogenic) were reported in S30 cells, whereas resveratrol acted as a pure estrogen antagonist in T47D and LY2 cells. Of note, anti-estrogen activity of resveratrol has been observed at low (0.1–1 µM) concentrations by triggering parallel pathways that inhibit cellular estrogen dependent effects, such as tumoral proliferation and transformation (Bhat et al., 2001). It is important to note that the estrogen activity of resveratrol is only for ERα and not for ERβ subtype (Park et al., 2011). In another study, Nakagawa et al. (2001) reported that low concentrations of resveratrol caused cell proliferation in ER positive human breast cancer cell lines (KPL-1, <22 µM; MCF-7, ≤4 µM), whereas it inhibited cell growth at high concentrations (>44 µM). Based on Basly et al. (2000) study, the anti-estrogenic effects of resveratrol are indicated in transfected estrogen response element (ERE)-luciferase reporter experiments, in MCF-7 (Michigan Cancer Foundation-7) cells.

Taken together, these studies show that resveratrol possesses an ER-mediated proliferating effect on ER+ breast cancer cells at low to moderate concentrations and anti-estrogen activity at very low or high concentrations. Due to the dual role (estrogen and anti-estrogen activities) of this phytoestrogen, dose dependency is very important for its anti-tumor effect, especially in breast cancer.

### 3.2. Human studies: brief review

The overwhelming evidence from the peer reviewed literature acknowledges that the vast majority of publications regarding resveratrol have been conducted in laboratory models and not in humans. In laboratory animals and in vitro human cancer cells, resveratrol has shown positive effects, but there are still few reports in humans. Perhaps, one of the limitations is its poor bioavailability when taken orally (Smoliga et al., 2011). Despite the combination therapy with other compounds or resveratrol synthetic analogs with more desirable bioavailability, many factors such as tumor model, dose and route of administration should be considered for human cancer patients.

There is some evidence that resveratrol and its metabolites accumulate within human cells in a tissue-specific manner which is highly dependent on dosage. Following eight days of resveratrol administration (0.5 or 1 g/day) to twenty prostate cancer patients, only resveratrol metabolites aggregated in normal prostate tissues (Patel et al., 2010). With increasing knowledge about the estrogen modulatory preclinical effects of resveratrol, a case-control study was done between 1993 and 2003 in the Swiss Canton of Vaud on 369 cases (breast cancer cases with resveratrol intake) and 602 controls (breast cancer without resveratrol intake), and there is an inverse relationship between resveratrol and breast cancer (Levi et al., 2005). Furthermore, a pilot clinical study of resveratrol in postmenopausal women with high body mass.

Index (BMI ≥ 25 kg/m2) was published in 2014. This study showed that twelve weeks administration of resveratrol (1 g) in postmenopausal women with high adiposity, did not change the serum estrogen and testosterone concentrations, but significantly increased the SHBG concentrations, which has been inversely associated to breast cancer risk. However, further studies are needed to confirm the effect of resveratrol-induced hormonal alterations on breast cancer risk modulation (Chow et al., 2014).

In another study, Singh et al. (2015) reported the effects of resveratrol on methylation of certain breast cancer related proteins in women who were at increased risk for breast cancer. The effects of either 5 or 50 mg of trans-resveratrol twice per day (for twelve weeks) was studied on methylation of certain genes, as compared to placebo. Methylation reduction of RASSF-1α with increasing levels of trans-resveratrol and resveratrol-glucuronide in circulation, and decreasing prostaglandin E2 (PGE2) expression in the breast were demonstrated in this study. The daily intake of appropriate amounts of selective estrogen receptor modulators (SERMs) like resveratrol could be beneficial for hormone dependent tumors like breast and prostate cancers (Abdal Dayem et al., 2016).

Overall, resveratrol’s safety and benefit profile needs additional investigation in humans, particularly in combination with other substances.

### 3.3. MDR mechanisms

A major obstacle to successful chemotherapy, especially in breast cancer, is multidrug resistance (MDR) (Li et al., 2016a, b). This important issue has a variety of molecular mechanisms such as increased drug efflux, alteration in drug targets, the activation of detoxification systems, activation of DNA repair mechanisms, alterations in cell cycle regulation and evasion of apoptosis (Garofalo and Croce, 2013; Alamolhodaei et al., 2016). Many of these pathways remain poorly understood. However phytochemical compounds may be helpful as part of an overall strategy to defeat multidrug resistance (Mahdizadeh et al., 2016).

#### 3.3.1. Overactivity of drug detoxifying enzymes

Cytochrome P450 enzymes (CYPs) and epoxide hydrolases are the basic agents for phase I or oxidative metabolism of many drugs (Brown et al., 2008).

In vitro, resveratrol inhibits the enzymatic activity of various CYPs including CYP1A1 and CYP1B1, suggesting that resveratrol may reduce CYP over expression and thus, reduce the exposure of cells to carcinogens (Baur and Sinclair, 2006).

Casper et al. (1999) demonstrated that resveratrol is a competitive antagonist for the AhR and efficiently blocks CYP 1A1 induction ex vivo and in vivo in various organs. Furthermore, AhR down-regulation by resveratrol and its methoxy derivatives modulated the expression of estrogen metabolism enzymes in breast epithelial cells (Licznerska et al., 2017).

In contrast, Ciolino and Yeh (1999) showed inhibition by resveratrol of aryl hydrocarbon induced cytochrome CYP 1A1 enzyme activity and CYP1A1 expression. This study failed to establish a link with AhR competitive binding. Moreover, the family of CYP1 enzymes increased the antiproliferative activity of dietary flavonoids in breast cancer cells such as MDA-MB-468 (Androutsopoulos et al., 2005). Based on the above, it is not possible to conclude whether resveratrol efficiently blocks CYP 1A1 induction or not.

In estrogen dependent diseases such as breast and ovarian cancers, CYP1A1 and CYP1B1 mRNA expression increased compared with healthy subjects (Piccinato et al., 2016); therefore, such therapeutic agents often are metabolized in the target tissue
and consequently chemo-resistance may occur.

Phase II enzymes such as glutathione s-transferase (GST) and glucuronosyltransferase (UGT) transform reactive species into hydrophilic, often nontoxic metabolite conjugates which then are effluxed by transporters such as the ABC transporter family (Rauf et al., 2016).

The transcription of these enzymes is induced through the ARE/EphRE (antioxidant/electrophile responsive element). Resveratrol inhibits XRE (xenobiotic responsive element) mediated transactivation but is inactive on the ARE-based mechanism. Therefore, the production of detoxifying enzymes is not affected by resveratrol treatment (Casper et al., 1999). In contrast, in another study, resveratrol was shown to enhance expression of phase II enzymes in vitro and increased resistance to oxidative and electrophilic cell injury (Cao and Li, 2004).

3.3.2. Activation of DNA repair mechanisms

If DNA damage is too severe, without repair, the cell will enter one of the following states: senescence, apoptosis or necrosis. There are several important repair pathways for DNA including mismatch repair (MMR), nucleotide excision repair (NER), and base excision repair (BER), and homologous recombination (HR) pathways (Liu et al., 2016). A number of anticancer drugs induce extensive DNA damage that can lead to cell cycle arrest and eventually cell death. Thus, the efficacy of such therapeutic agents can be significantly reduced by the cells ability to repair DNA damage (Sarkaria et al., 2008).

The mismatch repair pathway appears to be the most important mechanism of chemo-resistance by involving the DNA repair enzyme O6-methylguanine methyltransferase. Tumor cell lines deficient in mismatch repair are resistant to alkylating agents. The MMR pathway is critical for mediating the cytotoxic effect of O6-methylguanine. Among the 60 cell lines in the National Cancer Institute tumor panel, five are deficient in hMLH1 activity, and all are resistant to temozolomide (Peña-Díaz and Rasmussen, 2016).

Notably, the DNA repair enzyme O6-methylguanine methyltransferase decreases its cytotoxic substrate, O6-methylguanine adducts, which can break double strand DNA and thus, allow for tumor cell survival.

Gatz and Wiesmüller (2008) have reported that resveratrolin concentrations of 50–200 μM together with Cu (II) leads to DNA breakage by oxidative damage through endogenous copper mobilization. Hence, endogenous copper mobilization could be involved in DNA damage induction by resveratrol.

Overall, resveratrol induced DNA breakage can reverse mismatch repair deficiency in certain resistant cell lines.

3.3.3. Alterations in cell cycle regulation

In the cell cycle, checkpoints (Chk), cyclins and cyclin dependent kinases (CDKs), are a network of regulatory proteins that monitor and determine the progression of the cell. Deregulation of one of these checkpoints correlated with poor outcome in breast cancer treatment (Li et al., 2016a, b). Resveratrol has been shown to enhance the chemo-sensitivity of tumor cells by arresting cells at different stages of the cell cycle, mostly in an irreversible way by down-regulating the genes involved in cell proliferation (Apostolou et al., 2013; Androustopoulos and Tsatsakis, 2014; Thomas et al., 2016). For example, combination therapy with resveratrol in chemo-resistant tumor cells enhanced doxorubicin and paclitaxel anti-proliferative potential by the down-regulation of cyclin D1 and the over expression of p21/waf1 leading to decreased chemo-resistance (Gatouillat et al., 2010).

Resveratrol arrested the cell cycle in the G1 phase, through cyclin E/cdk2 complex inhibition and phosphorylation of pRb, a key step in this transition (Ahmad et al., 2001). Resveratrol has also been reported to stimulated cdk inhibitors such as p53-inducible p21cip1/Waf1and p27kip1 involving G1 arrest by inhibiting the cyclin D1/D2-cdk4 and cyclin D1/D2-cdk6 complexes, thuslicting an artificial checkpoint at the transition G1/S transition of the cell cycle (Venkatadri et al., 2016) and less frequently the G2/M phase (Hashemzaei et al., 2016a, b; Androustopoulos et al., 2011).

Nakagawa et al. (2001) found that growth suppression was indicated by G1-phase arresting and apoptosis by up-regulation of Bak and Bak proteins, down-regulation of the Bcl-XL protein and activation of caspase-3 in MCF7cell line.

It is important to note that resveratrol produced a transient increase in expression and kinase activity of positive G1/S and G2/M regulators in the MCF-7 cell line. p21cip1/WAF1 expression was significantly induced in the presence of high levels of p27kip1 and p53. It is interesting to note that although resveratrol inhibited cell proliferation in both MDA-MB-231 and MCF-7 cell lines, apoptosis occurred in a concentration and cell specific manner. In this regard, Pozo-Guisado et al. (2002) indicated that in the MDA-MB-231 cell line, the kinase activities of positive G1/S and G2/M cell-cycle regulators decreased and ribonucleotide reductase activity was hindered by resveratrol, without a significant effect on expression of the tumor suppressors p21cip1/WAF1, p27kip1 and p53. In fact, a non-apoptotic process in the lack of a remarkable change in cell cycle distribution was the cause of cell death.

Furthermore, resveratrol increased the cytotoxic effects of Melphalan on MCF-7 and MDA-MB231 cells in vitro and this cytotoxicity was higher in MCF-7 cells than MDA-MB231 cells. It was associated with p53 level elevation, procaspsase 8 reduction and the activation of caspases 7/9. Arresting of the cell cycle in the S phase with a decreased expression of cyclin A also was observed (Casanova et al., 2012).

Resveratrol strongly reduced the susceptibility of MDA-MB-435s, MDA-MB-231 and SKBR-3 cells to paclitaxel-induced cell death in vitro. This effect was not observed in MCF-7 cells or in athymic nude mice. Mechanistically, the reduced susceptibility to paclitaxel by resveratrol was partially attributable to its inhibition of paclitaxel-induced G2/M cell cycle arrest together with cyclinB/CDK1 elevation, suppression of paclitaxel-induced reactive oxygen species (ROS) accumulation and the inactivation of the anti-apoptotic Bcl-2 family of proteins (Fukui et al., 2010).

In a more recent investigation, resveratrol suppressed 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD)-induced proliferative structures in mammary glands as well as methylation of BRCA1 by inhibiting DNMT1, cyclin D1 and upregulating the aryl-hydrocarbon receptor repressor (AhRR) in the off spring of Sprague Dawley rats (Singh et al., 2014).

The anti-proliferative activity of resveratrol’s ability to affect cell cycle regulation may be dependent both on the resveratrol concentration and the characteristics of the target cells (Borska et al., 2016).

Taken together, cell cycle regulation activity of resveratrol such as marked inhibition of cyclin D/cdk4,6 complex, induction of p53, cdk inhibitor p21cip1/WAF1and p27kip1, inhibition of cyclin E/cdk2 complex and phosphorylation of pRb indicates that this molecule appears to be able to reverse Multidrug Resistance. Some pharmacological effects on cell cycle regulation by resveratrol are illustrated in Table 1.

3.3.4. Evasion of apoptosis

Disruption of apoptotic pathways is often a major obstacle in the success of chemotherapy. Resveratrol may be beneficial in overcoming this disruption. This fascinating product has been shown to mediate apoptosis through a variety of different routes including the fas pathway, mitochondrial passage, the adenyl-cyclase pathway, and RB-E2F/DP and p53 activation pathways (Joe et al., 2016).
Most investigations have demonstrated that resveratrol sensitizes cancer cell lines (neuroblastoma, glioblastoma, breast carcinoma, prostate carcinoma, leukemia, and pancreatic carcinoma) to chemotherapeutic agents such as doxorubicin, cytarabine (AraC), actinomycin D, taxol, and methotrexate by down-regulating survivin expression while increasing apoptosis, thereby leading to chemo-resistance reduced in a number of in vitro and in vivo malignancy models (Fulda, and Debatin, 2004).

The potential mediatory effect of resveratrol on apoptosis induction pathways is illustrated in Fig. 2.

### Table 1
Pharmacological effects of resveratrol on cell cycle regulation alone and in combination with chemotherapeutic agents.

<table>
<thead>
<tr>
<th>Resveratrol in combination</th>
<th>Resveratrol alone</th>
<th>Mechanism of action</th>
<th>Phase modulating</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resveratrol (0-500 μM) + Doxorubicin (0.25-10 μM)</td>
<td>cyclin D1/D2-ckd4</td>
<td>G1 arresting</td>
<td>(Gatouillat et al., 2010)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol (0-300 μM)</td>
<td>p21/waf1, p27Kip1</td>
<td>(Venkatadri et al., 2016)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol (≥44 μM)</td>
<td>cyclin E/ckd2, p21/waf1, p27Kip1</td>
<td>G1 arresting</td>
<td>(Nakagawa et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Phosphorylation of pRb</td>
<td>(Ahmad et al., 2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol (10 mg/kg, wt) + Doxorubicin (5 mg/kg b.wt)</td>
<td>p21/waf1, p53</td>
<td>G1 arresting</td>
<td>(Rai et al., 2016)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol (50 μM or 200 μM) + Melphalan (25 μM, 50 μM, or 75 μM)</td>
<td>cyclinA/CDK2</td>
<td>S arresting</td>
<td>(Cassanova et al., 2012)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol (50 μM, 100 μM, 200 μM)</td>
<td>p21/waf1, p27Kip1</td>
<td>(Pozo-Gaisado et al., 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol analogues (10 μM)</td>
<td>cyclinB/CDK1, cyclinD1/CDK4</td>
<td>G2/M arresting</td>
<td>(Pozo-Gaisado et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol (20 μM) + Paclitaxel (10 nM)</td>
<td>cyclinB/CDK1, p21/waf1, p27Kip1, p53</td>
<td>G2/M progression</td>
<td>(Fukai et al., 2010)</td>
<td></td>
</tr>
</tbody>
</table>

#### 3.3.4.1. Fas pathway
Fas/CD95/APO-1/DR2 and TRAILR are related to the death receptor family of proteins that activate a death-signaling cascade after binding to the corresponding ligands (Fas et al., 2006).

In one study, it was reported that resveratrol triggered Fas L signaling-dependent apoptosis through CD95L over expression in T47D breast carcinoma cells and in HL60 leukemia cells. Notably, resveratrol treatment of normal human peripheral blood lymphocytes (PBLs) did not affect cell survival for up to 72 h. These data highlighted the absence of a significant change in the CD95–CD95L system expression on treated PBLs and specific involvement of this system in the chemotherapeutic potential of resveratrol (Clement et al., 1998; Bai et al., 2016).

Furthermore, another study demonstrated that in the CEM-C7H2 leukemia cell line blocking of either Fas or Fas ligand signaling with antagonistic antibodies or constitutive expression of
the phosphorylation of AKT and increased pro-caspase 9 (Sinha 2008). Likewise, in MCF-7 breast cancer cells resveratrol decreased caspase-3 leading to apoptosis in MDA-MB-231 cells (Nguyen et al., 2016). Resveratrol repressed Bcl-2 by activation of ERK1/2 and with Bcl-2 and Bcl-XL reduction, Bax over expression and caspase-9 exposure to resveratrol led to dose dependent apoptosis associated with p53 activation pathways (Venkatadri et al. 2016). MCF-7 cells and to trigger apoptosis in a caspase independent pathway, resveratrol increased the mitochondrial pathway which is located in the inner membrane of mitochondria and the plasma membrane of normal endothelial cells and in several cancer cell lines (Zheng and Ramirez, 1999; Dorrie et al., 2001). Resveratrol has been shown to improve mitochondrial function (Miltonprabu et al., 2016).

Multidrug Resistance reversion via induction of mitochondrial pathway has been reported by Venkatadri et al. (2016). MCF-7 cells exposed to resveratrol led to dose dependent apoptosis associated with Bcl-2 and Bcl-XL reduction, Bax over expression and caspase-9 activation. Resveratrol repressed Bcl-2 by activation of ERK1/2 and caspase-3 leading to apoptosis in MDA-MB-231 cells (Nguyen et al., 2008). Likewise, in MCF-7 breast cancer cells resveratrol decreased the phosphorylation of AKT and increased pro-caspase-9 (Sinha et al., 2016). In another report, Filomeni et al. (2007) presented evidence showing stilbene trans-resveratrol to consign human breast cancer MCF-7 cells to apoptosis at 6.25 μg/ml concentration, mainly due to the mitochondrial pathway.

Based on studies by several investigators, resveratrol has been shown to up-regulated Ca\(^{2+}\)-discharge and calpain activity in MCF-7 cells and to trigger apoptosis in a caspase independent pathway, whereas in MDA-MB-231 cells, resveratrol increased the mitochondrial membrane dissociation with the release of cytochrome c (Sareen et al., 2007; Izquierdo-Torres et al., 2017).

High concentrations of resveratrol (50—150 μM) induced apoptosis in MCF-7 cells by activation of both intrinsic and extrinsic apoptotic pathways. Resveratrol down-regulated PI3K/AKT and up-regulated the Fas associated proteins, cyt.c, Bid, caspase-9, and caspase-3 (Chen and Chien, 2014).

In fact, resveratrol at low concentrations (10 μM) activated PI3K leading to cell proliferation by promoting entry into the S phase and cyclin D1 expression in MCF-7 cells (Castoria et al., 2002).

In support of the concept of apoptotic induction, Rai et al. (2016) selected a synergistic combination of doxorubicin (IC20) and resveratrol (IC30) based on the combination index value found in MCF-7 and MDA-MB-231 cell lines to study. This combination induced significant growth inhibition, remarkable wound healing, and a clonogenic potential reduction of breast cancer cells, leading to induction of apoptosis in the breast cancer cells. A combination of cyclophosphamide and resveratrol significantly increased the caspase-mediated cytotoxic activity of MCF-7 cells. These results suggest a combination chemotherapeutic regimen, including resveratrol, leading to improvement in the treatment of breast cancer may be possible (Singh et al., 2009).

3.3.4.3. Rb-E2F/DP pathway. The retinoblastoma gene (Rb) product plays an important role in the G1/S transition. By the end of G1 phase, hypo-phosphorylated Rb becomes hyper-phosphorylated and is inactivated by the CDK/cyclin complex (Zhang, 2015). Prolonged treatment (24—48 h) with low concentrations of resveratrol decreased the phosphorylation of Rb by up-regulation of p21 and the related checkpoint was arrested in the G1 phase in human carcinoma cells (Adhami et al., 2001).

3.3.4.4. p53 activation pathway. Mutation of p53, a tumor-suppressor gene, and/or loss of it protein function, is responsible for more than half of human cancers (Yeo et al., 2016). An increasing number of studies has reported the role of p53 in resveratrol-induced apoptosis. It is therefore not surprising that resveratrol induces expression of pro-apoptotic Bax, Bak, PUMA, Noxa, and Bim, and suppresses the expression of anti-apoptotic Bc12, Bcl-XL, and Mcl-1 (Shankar et al., 2006). This phytochemical has been
shown to sensitize tumor cells like MDA-MB 468 breast cancer cells to certain chemotherapeutic agents through modulation of the p53-dependent pathway (Laux et al., 2004). In MDA-MB-231 breast cancer cell line, resveratrol induced p53-dependent apoptosis due to suppression of p70S6K and the phosphorylation of p65RP (Alkhalf, 2007). Significant up-regulation (P = 0.001) of p53 and p53-regulated pro-apoptotic Bax was observed in combination therapy with resveratrol in the Singh et al. study (Singh et al., 2009). Of note, methylated trans-stilbene resveratrol analogues in both MCF-7 and HepG2 cell lines were associated with up regulation in the level of p53 expression and increased in mRNA levels of the apoptosis-related genes Bax/Bcl-xl in a dose-dependent manner (Androustrupoulos et al., 2011).

The regulation of p53 by resveratrol has been proposed to occur via activation of mitogen-activated protein kinases (MAPKs) (specifically ERKs) in human breast cancer cells. Resveratrol acted similarly in stimulating MAPKs, either when administered alone or in combination with 17β-estradiol. Additionally, apoptosis occurred only in ER+ cells and it did not involve receptor mediated gene transcription. Steroids and resveratrol modulate MAPKs and thus upstream and downstream transcriptional events (Zhang et al., 2004).

In an animal model, resveratrol reduced E2-induced breast tumor development and caused apoptosis and suppressed enhancement in DNA damage in mammary tissues of female August Copenhagen Irish (ACI) rats by up regulating the Nrf2-mediated pathway (Singh et al., 2014).

3.3.4.5. Adenylyl-cyclase pathway. The effects of resveratrol on the activity of two nucleotides in MCF-7 cells, cyclic GMP (cGMP) and cyclic AMP (cAMP), were examined. cAMP but not cGMP, elevation of growth inhibitory/pro-apoptotic ceramide. Moreover, ceramide accumulation induced by resveratrol can be drawn to the activation of serine palmitoyltransferase (SPT), the key enzyme of a de novo ceramide biosynthetic route, and neutral sphingomyelinase (SMnase), a basic enzyme of the sphingomyelin/ceramide pathway. The inhibition of focal adhesion kinase (FAK) and protein kinase B (PKB/Akt) by resveratrol is responsible for triggering apoptosis in ER+ breast cancer cells (Brownson et al., 2002). It is of particular interest in light of another report in which the apoptotic effect of resveratrol is via Akt phosphorylation following downstream targets, such as p70 S6K, S6 ribosomal and FOXO-3a (Pavan et al., 2016).

Although a tremendous amount of literatures suggests that resveratrol induced apoptosis paralles cell cycle arrest, an increasing number of studies have reported that the resveratrol-induced cell cycle is blocked without causing apoptosis. Hence, the question: is the resveratrol induced cell cycle arrest reversible or is it first step of an irreversible apoptotic program (Ragione et al., 1998; Haider et al., 2003).

3.3.5. Drug transporters.

It is clear that alterations in the drug uptake or efflux could be responsible for the acquisition of chemo-resistance because the antitumor agents must enter into the cancer cells in a concentration adequate to perform its effect. Drug efflux from cells is mediated by transporter proteins called ATP dependent multidrug transporters (Hee Choi and Yu, 2014). Most multidrug resistance proteins belong to the ABC family. P-glycoprotein (P-gp), multidrug resistance associated proteins (MRPs) and breast cancer resistance protein (BCRP) are members of the ABC transporter family (Nourbakhsh et al., 2015). BCRP and ABC proteins are commonly over expressed in breast cancer. Recent evidence suggests that these proteins have the ability to transport a broad range of chemotherapeutic agents involved in breast cancer treatment (Videira et al., 2014).

An attractive hypothesis is that resveratrol, like other flavonoids, is able to dominate drug resistance of tumor cells that express MRP (Fig. 3). Resveratrol enhances both growth inhibition and cytotoxicity activities of several chemotherapeutic agents, without affecting normal cells (Danz et al., 2009). Notably, resveratrol may interact with the BCRP by competing with other substrates for the BCRP, causing BCRP dysfunction and the drug to accumulate inside the cell (Cooray et al., 2004). Resveratrol also was reported to reduce the activity of BCRP by modulating both its transport and ATPase function in the MCF-7 cell line (Sjostedt et al., 2016). Furthermore, resveratrol induced accumulation of daunorubicin in human multidrug resistant carcinoma cells in a concentration dependent manner with a decrease in P-gp level. This combination therapy with resveratrol rendered a synergistic effect and reversed the multidrug resistant phenotype in these tumor cells (Pozo-Guisado et al., 2002; Nabekura et al., 2005).

Recent evidence has shown that resveratrol reversed the MDR of the MCF-7/DOX cells by down regulation of the MDR-1 gene and P-glycoprotein expression levels and at the same time increasing the concentration of doxorubicin in the MCF-7/DOX cells. Reversing MDR, via the MDR-1 expression, was concluded to be a mechanism of resveratrol, which showed the unique antitumor function of this molecule (Huang et al., 2014).
In another study, it was reported that polyphenols like resveratrol, quercetin or ferulic acid were able to inhibit human breast cancer doxorubicin resistant (MCF-7/DOX) cell proliferation; additionally, resveratrol more efficiently inhibited cancer cell proliferation than either quercetin or fumaric acid (Huang et al., 2009).

Additional evidence has shown that the capability of resveratrol to elevate the cytotoxicity of anticancer agents by enhancing their intracellular concentrations and preventing MDR-1 expression in selected solid tumor cell lines, like the MCF-7 cells. Moreover, a combination treatment of resveratrol and doxorubicin significantly down regulated the expression of MDR1 and MRPI. Moreover, in vivo experiments in a xenograft mouse model reported an inhibitory effect of this combination on tumor volume by 60%, compared with the control group (Al-Abd et al., 2011; Osman et al., 2013; Kim et al., 2014; Margina et al., 2015). Rai et al. (2016) have reported that a combined dosage of resveratrol and doxorubicin (10 mg/kg +5 mg/kg b.wt) inhibited tumor volume with an increased life span (139%, p value < 0.05) in an Ehrlich ascitic carcinoma (EAC) mouse model. Therefore, increasing the sensitivity and dose reduction of doxorubicin via a resveratrol combination therapy may be promising for improving the clinical management of breast malignancies and decreasing the side effects of doxorubicin.

4. Conclusions and future prospects

Phytochemicals like phytoestrogens have received much attention as potential preventive and therapeutic strategies against breast cancer. Resveratrol, a phytoestrogen, is such a chemical, which has been shown to be chemo-preventive with chemotherapeutic potential against breast cancer. This compound has been shown to reverse drug resistance in a variety of in vitro cell systems by sensitizing tumor cells to drug mediated effects in combination with other chemotherapeutic agents. In laboratory models and in vitro human cell systems, resveratrol has shown encouraging results; nevertheless, clinical studies are lacking. Additional research, particularly in better model systems and in humans, needs to be undertaken. None-the-less, resveratrol has shown promise as part of combination therapy, particularly in breast cancer.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors appreciate the Iran National Science Foundation and Mashhad University of Medical Sciences for partial financial support.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2017.03.024.

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