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Targeting Tumor Ubiquitin-Proteasome Pathway with Polyphenols for Chemosensitization

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Abstract

The development of tumor drug resistance is one of the biggest obstacles on the way to achieve a favorable outcome of chemotherapy. Among various strategies that have been explored to overcome drug resistance, the combination of current chemotherapy with plant polyphenols as a chemosensitizer has emerged as a promising one. Plant polyphenols are a group of phytochemicals characterized by the presence of more than one phenolic group. Mechanistic studies suggest that polyphenols have multiple intracellular targets, one of which is the proteasome complex. The proteasome is a proteolytic enzyme complex responsible for intracellular protein degradation and has been shown to play an important role in tumor growth and the development of drug resistance. Therefore, proteasome inhibition by plant polyphenols could be one of the mechanisms contributing to their chemosensitizing effect. Plant polyphenols that have been identified to possess proteasome-inhibitory activity include (–)-epigallocatechins-3-gallate (EGCG), genistein, luteolin, apigenin, chrysin, quercetin, curcumin and tannic acid. These polyphenols have exhibited an appreciable effect on overcoming resistance to various chemotherapeutic drugs as well as multidrug resistance in a broad spectrum of tumors ranging from carcinoma and sarcoma to hematological malignancies. The *in vitro* and *in vivo* studies on polyphenols with proteasome-inhibitory activity have built a solid foundation to support the idea that they could serve as a chemosensitizer for the treatment of cancer. In-depth mechanistic studies and identification of optimal regimen are needed in order to eventually translate this laboratory concept into clinical trials to actually benefit current chemotherapy.

Keywords

Polyphenols; flavonoids; EGCG; genistein; curcumin; structure-activity relationship; ubiquitin-proteasome pathway; proteasome inhibitors; bortezomib; chemotherapy; drug resistance; NF- κ B; Bcl-2; chemosensitization; clinical trials

1. INTRODUCTION

The development of tumor drug resistance is the major obstacle to the success of chemotherapy in cancer treatment. A growing amount of studies suggest that plant polyphenols are able to sensitize drug-resistant tumors to chemotherapy *via* various mechanisms [1]. One of the involved mechanisms is targeting tumor ubiquitin-proteasome

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pathway which plays a critical role in tumor growth and drug resistance development [2, 3]. In this article, we will review natural polyphenols and their synthetic analogs, and the relationships between their chemical structures and biological activity, especially the proteasome-inhibitory activity. We will then discuss the molecular mechanisms underlying the ability of plant polyphenols to reverse drug resistance and enhance chemosensitivity. Lastly, we will summarize preclinical and clinical studies that combine plant polyphenols with conventional chemotherapy in the treatment of various human cancers, and discuss the issue of potential interaction of plant polyphenols with the first therapeutic proteasome inhibitor bortezomib.

2. PLANT POLYPHENOLS

2.1. Subclasses, structures and diet sources

Polyphenols are secondary metabolites of plants, protecting plants from ultraviolet radiation and pathogen assault [4, 5]. They constitute an important component of our daily diet, and are found in fruits, vegetables and beverages. Plant polyphenols can be divided into three classes based on their chemical structure: phenolic acids, flavonoids, and other non-flavonoid polyphenols [6]. Of note, the physiological action of the plant polyphenols is strongly affected by their chemical structure. For example, many isoflavones have the pseudohormonal property due to their structural similarity to the estradiol molecule, and they are consequently classified as phytoestrogens [4, 5].

Phenolic acids are a class of substances containing one or more hydroxyl functions and a carboxylic acid function at the benzene ring. Two subclasses of phenolic acids can be distinguished: derivatives of benzoic acid, whose polymer forms hydrolysable tannins (either gallotannins or ellagitannins), and derivatives of cinnamic acid, whose polymer forms lignins [4]. Flavonoids comprise the largest class of polyphenols, which are non-hydrolysable/condensed tannins with a common structure of two aromatic rings linked through three carbons (C₆-C₃-C₆) [5]. Flavonoids can be further divided into six subclasses: flavones, flavonones, flavonols, flavanonols, flavanols and isoflavones [7]. For non-flavonoid polyphenols, two of the most well-known ones are curcumin and resveratrol [6].

The backbone structures, representative compounds and dietary sources of plant polyphenols in each class are summarized in Table 1.

2.2. Bioavailability and bioactivity

Plant polyphenols are mainly absorbed in the small intestines or colon. Most plant polyphenols are present in the form of esters, glycosides, or polymers that cannot be absorbed directly. They need to be hydrolyzed by intestinal enzymes or colonic microflora before absorption [4, 8]. Once absorbed, polyphenols are subjected to intestinal and hepatic conjugations, mainly methylation, sulfation, and glucuronidation. *O*-methyltransferase (OMT) catalyzes the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to polyphenols that have an *O*-phenolic moiety. The methylation occurs predominantly in the 3' position of the polyphenols, with a minor proportion in the 4' position. Sulfotransferase (SULT) catalyzes the transfer of a sulfate moiety from 3'-phosphoadenosine-5'-phosphosulfate to a hydroxyl group on various substrates including polyphenols, while UDP-glucuronosyltransferase (UGT) catalyzes the transfer of a glucuronic acid from UDP-glucuronic acid to its substrates including polyphenols [4, 8].

Not only protecting plants from ultraviolet radiation and pathogen assault, plant polyphenols are also protective against a variety of human diseases, particularly cardiovascular disease and neoplastic disease. The recorded pharmacological effects include anti-oxidation, anti-inflammation, anti-bacteria, anti-fungus, anti-virus and anti-neoplasm [4, 8]. These versatile

bioactivities are achieved through modulating (either by activating or inhibiting) multiple intracellular targets and signaling pathways, including reactive oxygen species, nuclear receptors/transcription factors, NF- κ B pathway, PI₃K/Akt pathway, as well as the ubiquitin-proteasome pathway [1, 9]. In this review article, we will focus on polyphenols' proteasome-inhibitory effect.

3. THE UBIQUITIN-PROTEASOME PATHWAY

3.1. Composition of proteasome

Eukaryotic 26S proteasome is a 2.4 MDa large complex consisting of one 20S core proteasome and two 19S regulatory caps [10] (Fig. 1). The 20S core proteasome harbors seven different α -subunits and seven different β -subunits in its twofold symmetrical $\alpha_7\beta_7\beta_7\alpha_7$ stacked complex, among which mainly three sets of β -subunits, β_1 (caspase-like, or peptidyl-glutamyl peptide-hydrolyzing-like, PGPH-like), β_2 (trypsin-like), and β_5 (chymotrypsin-like) are proteolytically active [10] (Fig. 1). The 19S regulatory caps consist of the lid, which is responsible for recognition and docking of polyubiquitinated proteins into the 20S core proteasome, and the base, which contains ATPase activity required for the unfolding and linearization of large proteins [10] (Fig. 1).

Unlike common proteolytic enzymes, which contain a catalytic triad, proteasome catalytic subunits, namely β_1 , β_2 and β_5 , belong to a special group termed N-terminal nucleophile hydrolases, which utilizes the side chain of the N-terminal residue as the catalytic nucleophile [11–13]. A variety of observations indicate that all three catalytic β -subunits indeed react with peptide bonds of substrates as well as with electrophilic functional groups of inhibitors through their –OH group of the N-terminal threonine (Thr1) [11–13] (Fig. 2A).

3.2. Function of the ubiquitin-proteasome pathway

More than 90% of intracellular protein degradation is performed by the ubiquitin-proteasomal system. Proteins marked for degradation with polyubiquitin tags are hydrolyzed by the proteasome in an ATP-dependent manner. Differing from the lysosome, which is mainly responsible for degrading extracellular and transmembrane proteins, the proteasome is in charge of degrading intracellular proteins which are aberrantly folded or normally short-lived. A large and growing body of evidence indicates that the proteasome affects cell-cycle progression partially by regulating the turnover of cyclins and cyclin-dependent kinase inhibitors (CIP/KIP family). Inhibition of proteasome function could result in cell-cycle arrest [10]. In addition, the ubiquitin-proteasomal system can regulate the level of apoptotic activity by affecting the Bcl-2 family, NF- κ B family, p53-MDM2 complex and others [10, 14]. Therefore, the proteasome is crucial for cell survival and proliferation.

3.3. Modulation of proteasome activity

Proteasome activity could be either up-regulated or down-regulated under *in vivo* conditions. In many cancer cases, proteasome activity is up-regulated by cellular oncogenic factors. Enhanced proteasome activity in turn promotes the degradation of tumor suppressor proteins, resulting in cancer cell survival and proliferation as well as the development of drug resistance [10, 15]. On the other hand, proteasome activity could be suppressed by several endogenous inhibitors as well as various exogenous inhibitors, including some synthetic compounds such as bortezomib (the first therapeutic proteasome inhibitor launched in 2003) and many natural products such as plant polyphenols [10, 15] (Fig. 1). In the following section, we will elaborate on how plant polyphenols modulate proteasome activity in drug-sensitive and drug-resistant cancer cells.

4. TARGETING TUMOR PROTEASOME BY PLANT POLYPHENOLS

4.1. EGCG

Tea is the second most popular beverage in the world after water. Green tea and, to a less extent, black tea are abundant in polyphenols. The major catechins in green tea are (–)-epigallocatechin-3-gallate [(–)-EGCG], (–)-epigallocatechin [(–)-EGC], (–)-epicatechin-3-gallate [(–)-ECG], and (–)-epicatechin [(–)-EC] [16] (Table 1). Among them, EGCG is the most abundant and active one, and has been extensively studied for its biological activities and cellular targets. One potentially important cellular target of EGCG is the proteasome [16]. Both naturally-occurring (–)-EGCG and its synthetic enantiomer (+)-EGCG are able to potently, specifically and irreversibly inhibit the chymotrypsin-like activity of proteasome $\beta 5$ subunit *in vitro* (IC_{50} = 86–194 nM) and *in vivo* (1–10 μ M) [17, 18] (Table 2). Of note, EGCG is able to interact with not only the $\beta 5$ subunit in constitutive proteasome but also the $\beta 5i$ subunit in interferon- γ inducible immunoproteasome (referring to as BrAAP activity) with even higher affinity [19].

Similar to another proteasome inhibitor β -lactone, molecular docking and structure-activity relationship (SAR) analysis suggest that the ester carbon linking the C and D rings of EGCG is important for mediating its proteasome-inhibitory activity, which could be nucleophilically attacked by the –OH group of the catalytic Thr1 residue in $\beta 5$ subunit [17] (Fig. 2B). In addition, the A ring mimics the Tyr residue and binds to the hydrophobic S1 pocket of the $\beta 5$ subunit [20]. The B and D rings are oriented towards the water phase, and the number of –OH groups on them is positively correlated with the proteasome-inhibitory activity [21].

However, the vicinal trihydroxy groups of EGCG not only contribute to its proteasome-inhibitory activity, but also cause its susceptibility to autooxidation even under physiological conditions [22]. In an effort to increase the stability of EGCG, a series of analogs with different replacements on benzene rings were synthesized and examined. Among them, the peracetate-protected prodrug of EGCG (with all –OH groups being peracetylated) was found to have improved stability, bioavailability, and intracellular proteasome-inhibitory activity [23, 24]. More recently, a para-amino substituent at 4'' position and a fluoro-substituent at 3'' and 4'' positions are found to have increased stability as well and are worthy to be further investigated [25, 26].

Interestingly, among all oxidative metabolites of EGCG, one metabolite resulted from the oxidative decarboxylation of the A ring and the hydrolysis of the D ring (the gallate group) was recently identified to possess a similar ability to EGCG in modulating proteasome functionality and apoptotic pathways. However, differing from EGCG, which has covalent interaction with the catalytic Thr1, this metabolite is most likely to have non-covalent interaction with Thr1, resulting in the hindering of Thr1 [27].

Besides the autooxidation issue, methylation is another issue impeding the application of EGCG. As mentioned previously, once absorbed, EGCG is readily methylated by catechol-*O*-methyltransferase (COMT) at the 4' and 4'' positions [28]. COMT is a mammalian OMT mainly involved in the inactivation of the catecholamine neurotransmitters. Methylation of EGCG significantly affects its binding to the proteasome $\beta 5$ subunit; as the number of methyl groups increases, the inhibitory potency decreases accordingly [29]. Thus, cancer cells with lower COMT activity are more vulnerable to EGCG-mediated proteasome inhibition and apoptosis induction than those with higher COMT activity [30]. These findings raise the exciting possibility of a combinational treatment of cancer with EGCG and a COMT inhibitor [31].

Regarding the application of EGCG, one fact that needs to be kept in mind is that EGCG is found to have the potential to negate the therapeutic efficacy of bortezomib and other boronic acid-based proteasome inhibitors [32]. This neutralizing effect is due mainly to the direct reaction between the pyrocatechol moieties on EGCG and the boronic acid moiety on bortezomib, which confers its proteasome-inhibitory activity [32] (Fig. 2C). However, this issue is still disputable. Wang *et al.* reported that EGCG-induced apoptosis was potentiated by bortezomib in KM3 multiple myeloma cells [33]. More recently, a group from Millennium Pharmaceuticals, Inc. reported that no antagonism of bortezomib was seen where EGCG plasma concentrations were commensurate with dietary or supplemental intake in their preclinical study using CWR22 human prostate cancer cell xenograft mouse model [34].

4.2. Other dietary flavonoids: genistein, luteolin, apigenin, chrysin, and quercetin

After the discovery of the proteasome-inhibitory activity of EGCG, several other dietary flavonoids, including genistein [35], luteolin [36], apigenin [36–38], chrysin [36] and quercetin [38], were subsequently found to possess proteasome-inhibitory activity too. Table 2 listed the median inhibitory concentration (IC_{50}) of these flavonoids for the inhibition of the chymotrypsin-like activity of both purified 20S proteasome and intracellular 26S proteasome. Docking studies suggested that the carbonyl carbon on the C ring might be the site of nucleophilic attack by the –OH group of Thr1 in proteasome $\beta 5$ subunit [38]. In addition, SAR analysis found that flavonoids with a hydroxylated B ring and/or unsaturated C ring tend to be potent proteasome inhibitors and tumor cell apoptosis inducers [36]. Flavonoids with a pyrocatechol structure such as quercetin could also be methylated by COMT, but less efficiently than EGCG [39, 40]. Although methylated flavonoids have been suggested to be metabolically more stable than unmethylated ones, their proteasome-inhibitory activity and cytotoxicity against tumor cells are largely compromised [41]. Furthermore, similar to EGCG, those flavonoids with a vicinal diol structure could also antagonize the anticancer effect of bortezomib. Theoretically, all flavonoids with pyrocatechol or pyrogallol structure are potentially able to chemically react with the boric acid group in bortezomib and other boronic acid-based proteasome inhibitors [42].

4.3. Curcumin

Curcumin is extracted from turmeric, which is commonly used as spice. It falls into the non-flavonoid polyphenol group. Due to the observation that the curcumin-induced cell death is very similar to that of the proteasome inhibitors, the proteasome-inhibitory property of curcumin was proposed and verified in proliferating neuroblastoma cells [43]. Proteasome inhibition, as one of the mechanisms responsible for the curcumin-induced cell death, was further supported by local and metastatic colon cancer models both *in vitro* and *in vivo* [44]. Consistent with previous findings that the ester bond carbon of β -lactone and tea polyphenols as well as the carbonyl carbon of flavonoids confer their proteasome-inhibitory potencies, two carbonyl carbons on curcumin were found to be susceptible to nucleophilic attack by the –OH group of Thr1 in proteasome $\beta 5$ subunit [44]. Although most polyphenols tend to be water-soluble, curcumin is an exception. Its poor solubility in water leads to its poor bioavailability in tissues remote from the gastrointestinal tract, which largely compromises its anticancer effect [45]. Among different analogs synthesized to improve the water solubility, amino acid conjugates of curcumin were found to be soluble in water and showed a potent antiproliferative effect in cell models, indicating its potential as a curcumin substituent [45]. Of note, in contrast to above-discussed flavonoids that contains pyrocatechol or pyrogallol structure, curcumin was reported to potentiate the effect of bortezomib against human multiple myeloma in nude mice model [46].

4.4. Tannic acid

Tannic acid (TA) refers to hydrolysable tannins, composed of either gallotannins or ellagitannins. Upon hydrolysis, gallotannin yields a D-glucose and 6 to 9 gallate groups which link to each other or the glucose core through ester bonds. Since ester bond-containing tea polyphenols are potent proteasome inhibitors, the ability of TA to inhibit the proteasome activity was tested and verified in purified 20S proteasome [47] and cellular 26S proteasome in different cell types [47, 48] as well as in tumor-bearing mouse models [48]. Inhibition of the proteasome function by TA resulted in increased p27 and Bax expression, and impaired cell cycle progression [47].

5. DRUG RESISTANCE OF CANCER

5.1. Mechanisms of tumor drug resistance

Development of tumor drug resistance is one of the major limiting factors of current chemotherapy. Drug resistance can occur through different mechanisms, some of which are intrinsic while others are acquired after chemotherapy. Acquired resistance is particularly critical, as tumors not only become resistant to the drugs originally used to treat them, but may also become cross-resistant to other drugs with different mechanisms of action [49]. The molecular mechanisms involved in drug resistance include evasion of apoptosis, increase in drug efflux, increase in drug detoxification, alterations in drug targets, loss of dependence on drug targets, as well as enhanced processing of drug-induced damage [49]. It has been well documented that the ubiquitin-proteasome pathway extensively participates in the development of drug resistance, as discussed in details below.

5.2. Involvement of the ubiquitin-proteasome pathway in drug resistance

A large amount of chemotherapeutic drugs exert antitumor effect *via* induction of apoptosis in tumor cells. Drug-resistant tumor cells evade apoptosis by enhancing survival signals and/or suppressing apoptotic signals. Up-regulated proteasome activity in tumor cells helps them evade apoptosis on at least two levels. First, increased proteasome activity could facilitate NF- κ B survival pathway in tumor cells by accelerating I- κ B turnover. Consistently, proteasome inhibitors were reported to enhance tumoricidal response to conventional chemotherapy in colon cancer model and others [50]. Increased proteasome activity in cancer cells could also interfere with the balance between pro- and anti-apoptotic Bcl-2 family members by accelerating Bax degradation and increasing Bcl-2 expression (which is a target gene of the transcription factor NF- κ B). Therefore, proteasome inhibition not only stabilizes/activates Bax, but also down-regulates Bcl-2 expression [51–53].

Another important cause of drug resistance is the increased drug efflux. Drug efflux is mediated by some ATP-binding cassette (ABC) transporters, including ABCB1 (MDR1/P-glycoprotein/P-gp), ABCC subfamily members (MRPs) and ABCG2 (BCRP) [54, 55]. Newly synthesized P-gp is immature (only core-glycosylated, associated with chaperones) and trapped in endoplasmic reticulum (ER) [56]. Proteasome is believed to be involved in its maturation (full-glycosylation) as proteasome inhibition significantly suppressed P-gp maturation and exportation from ER to plasma membrane [56]. Proteasome inhibitors successfully reversed multidrug resistance phenotype in P-gp-overexpressing cancer cells through down-regulating P-gp expression [52, 57].

In addition to the involvement in the above-mentioned two major mechanisms of drug resistance, increased proteasome activity also facilitates the elimination of oxidatively damaged proteins that are caused by cytotoxic drugs so that relieves the drug-induced oxidative stress [58]. Proteasome inhibition led to considerable accumulation of oxidative proteins [58] and hypersensitized tumor cells to oxidative stress inducer [59].

Also, a crucial role of the ubiquitin-proteasomal system in regulating DNA repair has been unveiled. Among different DNA repair pathways, the ubiquitin-proteasomal system is involved in at least four pathways [60], namely nucleotide excision repair [61], post-replication repair [62], homologous recombination [63] and the fanconi anemia pathway [64]. Proteasome inhibitors significantly potentiated the cytotoxicity of cisplatin, a DNA damage agent that causes the formation of cisplatin-DNA adducts, by preventing nucleotide excision repair [65].

6. REVERSING DRUG RESISTANCE BY PLANT POLYPHENOLS

6.1. EGCG and overcoming drug resistance

Many studies have demonstrated the chemosensitizing effect of EGCG and other catechins *in vitro* and *in vivo*. Most of them found that EGCG reversed drug resistance by inhibiting the drug efflux transporter P-gp. For example, Kitagawa *et al* reported that catechins increased the cellular accumulation of P-gp substrates in the order of EGCG > ECG > EGC, while EC did not have any effect on substrates accumulation in P-gp-overexpressing KB-C2 human cervical epidermal carcinoma cells [66]. The chemosensitization by EGCG through the inhibition of P-gp expression/activity has also been validated in Caco-2 human intestinal adenocarcinoma cells (which are often used as a model for intestinal transport studies mediated by P-gp) [67] and tamoxifen-resistant MCF-7 human breast cancer cells [68], as well as in the xenograft mouse models using doxorubicin-resistant KB-A1 cells [69, 70] or doxorubicin-resistant BEL-7404/DOX hepatocarcinoma cells [71]. The study with tamoxifen-resistant MCF-7 cells also found that EGCG was able to inhibit the activity of the BCRP transporter [68]. EGCG was also reported to enhance the sensitivity of glioblastoma to temozolomide [72], of prostate carcinoma to doxorubicin [73], and of breast carcinoma to paclitaxel [74] in corresponding ectopic or orthotopic xenograft mouse models. Furthermore, the peracetate-protected prodrug of EGCG also exhibited a chemosensitizing effect in the treatment of leukemia cells by augmenting the efficacy of conventional chemotherapy daunorubicin and cytosine arabinoside [75]. A very recent study reported that polymer-based nanoparticle of polyphenols EGCG and theaflavin retained biological effectiveness with over 20-fold dose advantage than EGCG/theaflavin in exerting anti-cancer effects and also enhanced the potential of cisplatin in several different tumor cell types [76].

6.2. Genistein and overcoming drug resistance

Genistein has been widely studied as a promising agent for chemoprevention, chemotherapy, and chemosensitization. Genistein enhances the efficacy of chemotherapy mainly by inhibiting survival signals and/or enhancing apoptotic signals. Dr. Sarkar's group reported that genistein pretreatment inactivated NF- κ B and contributed to increased growth inhibition and apoptosis induced by cisplatin, docetaxel, doxorubicin or gemcitabine in various cancer cells including prostate, breast, lung, pancreatic and ovarian cancer cells [77–79]. Inactivation of Akt/NF- κ B by genistein not only potentiated the proliferation-inhibiting and apoptosis-inducing effect of arsenic trioxide on human hepatocellular carcinoma cells *in vitro*, but also dramatically augmented its suppressive effect on tumor growth and angiogenesis in tumor-bearing nude mice [80]. Genistein also restored the sensitivity of platinum-resistant epithelial ovarian cancer cells to cisplatin, taxotere or gemcitabine *via* the inhibition of NF- κ B and its target genes (c-IAP1, Bcl-2, Bcl-xL and survivin) [78]. Similarly, genistein restored the sensitivity to oxaliplatin in gemcitabine-resistant pancreatic cancer cells both *in vitro* and *in vivo* [81]. In addition to the effect in carcinoma cells, the combination of gemcitabine and genistein also showed enhanced antitumor efficacy in osteosarcoma cells through the same mechanism (abrogating Akt/NF- κ B pathway) [82]. Genistein also enhanced the cytotoxicity of cisplatin and, to a lesser extent, vincristine in

medulloblastoma cells [83]. Moreover, in acute myeloid leukemia cells, genistein exhibited synergistic anti-leukemia effect with cytosine arabinoside in both *in vitro* and *in vivo* models *via* affecting MAPK signaling pathway [84].

6.3. Curcumin and overcoming drug resistance

Extensive research has unveiled that curcumin can sensitize tumors to different chemotherapeutic drugs including doxorubicin, 5-FU, paclitaxel, vincristine, vinorelbine, melphalan, butyrate, cisplatin, oxaliplatin, celecoxib, gemcitabine, etoposide, sulfinosine and thalidomide [85]. Chemosensitization by curcumin has been observed in solid tumors of the lung, liver, pancreas, stomach, colon, head and neck, brain, breast, prostate, bladder, cervix, and ovary, as well as in hematological tumors of multiple myeloma, leukemia, and lymphoma [85]. Curcumin remarkably increased sensitivity to vinblastine/vincristine and reversed multidrug-resistance *via* modulation of P-gp expression and function in vincristine-selected P-gp overexpressing KB-V1 cervical epidermal carcinoma cells [86] and SGC7901/VCR gastric carcinoma cells [87]. Curcumin also sensitized the BRCP-expressing human breast cancer cells to different chemotherapeutic drugs including mitoxantrone, topotecan, SN-38 and doxorubicin by down-regulating the function of the BRCP transporter [88], which was further confirmed in a transgenic mice model [89]. Not only curcumin but also its major metabolite tetrahydrocurcumin was able to inhibit the efflux function of P-gp, MRP1 and BRCP and thereby reverse multidrug resistance [90]. Furthermore, curcumin was able to improve the sensitivity of drug resistant cancer cells by suppressing NF- κ B signal. Dhandapani *et al* reported that curcumin sensitized glioma cells to clinically used chemotherapeutic agents (cisplatin, etoposide, camptothecin and doxorubicin) *via* inhibition of AP-1 and NF- κ B, which correlated with reduced expression of Bcl-2 and IAP family members and some DNA repair enzymes [91]. Blockage of the NF- κ B pathway by curcumin also potentiated the effect of paclitaxel in breast cancer xenograft [92] and the effect of cisplatin in head and neck squamous cell carcinoma xenograft [93]. Whether inhibition of the proteasome activity by curcumin is responsible for or contributes to the blockage of NF- κ B pathway needs to be studied. Also, in a recent study, Yoon *et al* showed for the first time that curcumin effectively induced paraptosis, a new type of nonapoptotic cell death, in malignant breast cancer cell lines. Proteasomal dysfunction and superoxide anion contributed to those paraptotic changes characterized by vacuolation that begins with physical enlargement of mitochondria and ER. These findings suggest that curcumin could be beneficial to the treatment of apoptosis-resistant tumors [94].

7. CLINICAL TRIALS USING POLYPHENOLS AND CHEMOTHERAPY

7.1. EGCG in clinical trials

A few studies were conducted using green tea extract (GTE) as monotherapy in cancer patients. Although the oral consumption of GTE was well tolerated in these studies, its anticancer activity was unsatisfactory. For example, a phase I trial in patients with advanced lung cancer suggested that while relatively nontoxic at a dose of 3 g/m² per day, GTE likely has limited activity as a cytotoxic agent [95]; a phase II trial of green tea suggested that oral administration of green tea powder 6 g/d carried limited antineoplastic activity, as defined by a decline in PSA level, among patients with androgen independent prostate cancer [96].

Therefore, a prospective clinical trial using green tea as a complementary/alternative medicine (CAM) was elicited in patients with hormone refractory prostate cancer. However, the results suggested that green tea, as CAM therapy, was found to have minimal clinical activity against hormone refractory prostate cancer [97]. In view of the greater efficacy of the prodrugs of EGCG (peracetate-protected or fluoro-substituted) than EGCG itself in xenograft mouse models of both estrogen-independent human breast cancer and androgen-

independent human prostate cancer [98–100], our future goal is to translate these EGCG prodrugs into clinical studies.

Despite the excellent safety records of EGCG, in 2006, the US Food and Drug Administration (FDA) temporarily suspended all human trials of EGCG for additional review of toxicity data, and later allowed trials of Polyphenon E to resume. In a phase I trial in patients with asymptomatic Rai stage 0 to II chronic lymphocytic leukemia, daily oral consumption of Polyphenon E was well tolerated, and declines in absolute lymphocyte count and /or lymphadenopathy were observed in the majority of patients. Based on the encouraging phase I result, a phase II trial using 2 g Polyphenon E twice a day to evaluate the efficacy started in 2007 [101].

Furthermore, two clinical studies using Polyphenon E in combination with erlotinib are currently ongoing in patients with advanced non-small cell lung cancer and premalignant lesions of the head and neck, respectively (Table 3).

7.2. Genistein in clinical trials

Genistein has been investigated as a sensitizer to gemcitabine and erlotinib in patients with breast cancer or pancreatic cancer (Table 3). In a phase II study in US, 20 patients with locally advanced or metastatic pancreatic cancer received gemcitabine and erlotinib-based chemotherapy, along with soy isoflavones (Novasoy[®], 0.531 g twice daily, genistein is the major component). According to the RECIST criteria, 1 patient had a partial response (PR) and 6 had a stable disease (SD); the 6-month survival rate was 50% and the median survival time was 5.2 months. Since these results were comparable to the outcome observed in gemcitabine/erlotinib bitherapy without genistein, the investigators concluded that the antitumor activity of gemcitabine and erlotinib was not improved by soy isoflavones [102].

Although the result from prototype genistein is unfavorable, phenoxodiol, a synthetic derivative of the genistein, has been granted ‘fast track’ status by the US FDA for being developed as a chemosensitizer for platinum and taxane drugs used in the treatment of recurrent ovarian cancer [103]. A phase III study treating recurrent platinum-resistant ovarian cancer patients with phenoxodiol (400 mg/8h) and carboplatin (weekly) is currently ongoing (Table 3). Of note, phenoxodiol could be administered not only orally but also *via* intravenous infusion which would greatly enhance the bioavailability of the drug. In a phase II study, patients with platinum/taxane-refractory/resistant ovarian cancer received phenoxodiol (intravenously 3 mg/kg on days 1 and 2) and cisplatin or paclitaxel (on day 2) each cycle (7 days per cycle). Among 16 platinum-resistant patients, 3 patients had PR and 9 had SD. Among 15 taxane-resistant patients, one patient had a complete response, 2 had PR and 8 had SD. These results, particularly the result from the cisplatin-phenoxodiol combination, are quite encouraging and warrant further investigation [104].

7.3. Curcumin in clinical trials

Clinical studies using the combination of chemotherapy and curcumin have been conducted around the world. In a phase I/II trial in Japan, 21 patients with gemcitabine-resistant pancreatic cancer received curcumin (8 g/d) in a combination with gemcitabine-based chemotherapy (gemcitabine/S-1 bitherapy or gemcitabine monotherapy). No dose-limiting toxicity was observed in the Phase I study and oral curcumin 8 g/d was selected for the Phase II study. Among 18 evaluable patients, no patient had a PR and 5 had SD. These results warranted further investigation into the efficacy of curcumin in combined with gemcitabine-based chemotherapy [105].

In a phase I trial in France, 14 patients with advanced and metastatic breast cancer were treated with docetaxel, along with curcumin (does started from 0.5 g/d for 7 consecutive

days and escalated until a dose-limiting toxicity occurred). The maximal tolerated dose of curcumin ended up as 8 g/d, and a dose of 6 g/d in combination with docetaxel was recommended. Among 8 evaluable patients, 5 patients had PR and 3 had SD. Based on the encouraging efficacy results, a comparative Phase II trial is ongoing [106].

Furthermore, a combined regimen of gemcitabine, Celebrex and curcumin in the treatment of colon cancer and pancreatic cancer has entered phase III trial in Israel (Table 3).

8. CONCLUSION AND PERSPECTIVES

The *in vitro* and *in vivo* studies reviewed above all suggest that plant polyphenols with proteasome-inhibitory activity (especially EGCG, genistein and curcumin) may serve as powerful agents for reversing tumor drug resistance and enhancing the efficacy of chemotherapy. Further in-depth mechanistic studies and high-throughput screening could help find the best combination to achieve the most favorable outcome. Most importantly, clinical trials are urgently needed to eventually translate this laboratory concept into clinical benefits.

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Fig. 1.

A schematic diagram of the 26S proteasome and its catalytic subunits inhibitable by various proteasome inhibitors.

Fig. 2. Proteasome-inhibitory mechanisms of bortezomib and EGCG, as well as their direct interaction

(A) Reversible inhibitory mechanism of bortezomib. (B) Irreversible inhibitory mechanism of EGCG. (C) Postulated interaction between bortezomib and EGCG.

Table 1

Classes and subclasses of plant polyphenols.

Class	Backbone	Representative compounds	Dietary sources
Phenolic acid
	Gallic acid	Blackberry
	Cinnamic acid	Cinnamon
Flavonoids			
Flavones		Luteolin ($R'_1=R'_2=OH$, $R'_3=H$) Apigenin ($R'_1=R'_3=H$, $R'_2=OH$) Chrysin ($R'_1=R'_2=R'_3=H$)	Parsley
Flavonones		Eriodictyol ($R'_1=R'_2=OH$, $R'_3=H$) Naringenin ($R'_1=R'_3=H$, $R'_2=OH$)	Orange juice, grapefruit juice
Flavonols		Quercetin ($R'_1=R'_2=OH$, $R'_3=H$) Kaempferol ($R'_1=R'_3=H$, $R'_2=OH$) Myricetin ($R'_1=R'_2=R'_3=OH$)	Black and green tea, red onion

Class	Backbone	Representative compounds	Dietary sources
Flavanonols
Flavanols		EC ($R'_1=R'_2=OH$, $R'_3=H$; $R_1=H$) ECG ($R'_1=R'_2=OH$, $R'_3=H$; $R_1=gallate$) EGC ($R'_1=R'_2=R'_3=OH$; $R_1=H$) EGCG ($R'_1=R'_2=R'_3=OH$; $R_1=gallate$)	Green tea
Isoflavones		Genistein ($R_2=OH$) Daidzin ($R_2=H$)	Soybeans
Non-flavonoid polyphenols
	Curcumin	Turmeric
	Resveratrol	Red wine, grapes

Table 2

Median inhibitory concentration (IC₅₀) for inhibition of the chymotrypsin-like activity of purified 20S proteasome and cellular 26S proteasome by plant polyphenols.

Polyphenols	IC ₅₀ in 20S (μM)	IC ₅₀ in 26S (μM)	References
(-)-EGCG	0.086 ~ 0.194	1~10 *	[17]
Luteolin	1.5 ± 0.10	1.3 ± 0.11 *	[36]
Apigenin	2.3 ± 0.20	1.9 ± 0.17 *	[36]
Chrysin	4.9 ± 0.15	6.1 ± 0.44 *	[36]
Quercetin	3.5 ± 0.05	2 ± 0.09 *	[38]
Genistein	9.26 ± 1.2	N/A	[107]
Curcumin	1.85	10~20 [#]	[44]

* data from a leukemia cell line Jurkat cells;

[#] data from two colon cancer cell lines, HCT-116 and SW480.

Table 3

Combination of polyphenols and chemotherapy in clinical trials^{*}

Polyphenols	Drugs	Tumor Type	Phase Stage	Country	NCT ID
EGCG (Polyphenon E)	Erlotinib	Advanced Non-Small Cell Lung Cancer	I/II	US	NCT00707252
	Erlotinib	Premalignant Lesions of the Head and Neck	I	US	NCT01116336
Genistein	Gencitabine	Stage IV Breast Cancer	II	US	NCT00244933
	Erlotinib/Gencitabine	Locally Advanced or Metastatic Pancreatic Cancer	II	US	NCT00376948
	Gencitabine	Pancreatic Cancer	I/II	SE	NCT01182246
Genistein derivative (Phenoxodiol)	Docetaxel	Recurrent advanced ovarian epithelial cancer, fallopian tube cancer, or peritoneal cavity cancer	I/II	US	NCT00303888
	Carboplatin	Recurrent platinum-resistant ovarian epithelial cancer, fallopian tube cancer, or peritoneal cavity cancer	III	US	NCT00382811
Curcumin	Gencitabine	Pancreatic Cancer	II	IL	NCT00192842
	Gencitabine and Celebrex	Metastatic Colon Cancer	III	IL	NCT00295035
	Gencitabine and Celebrex	Advance or Inoperable Pancreatic Cancer	III	IL	NCT00486460

^{*} data from www.clinicaltrials.gov.