Cancer Chemopreventive Potential of Procyanidin

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Chemoprevention entails the use of synthetic agents or naturally occurring dietary phytochemicals to prevent cancer development and progression. One promising chemopreventive agent, procyanidin, is a naturally occurring polyphenol that exhibits beneficial health effects including anti-inflammatory, antiproliferative, and antitumor activities. Currently, many preclinical reports suggest procyanidin as a promising lead compound for cancer prevention and treatment. As a potential anticancer agent, procyanidin has been shown to inhibit the proliferation of various cancer cells in "*in vitro* and *in vivo*". Procyanidin has numerous targets, many of which are components of intracellular signaling pathways, including pro-inflammatory mediators, regulators of cell survival and apoptosis, and angiogenic and metastatic mediators, and modulates a set of upstream kinases, transcription factors, and their regulators. Although remarkable progress characterizing the molecular mechanisms and targets underlying the anticancer properties of procyanidin has been made in the past decade, the chemopreventive targets or biomarkers of procyanidin action have not been completely elucidated. This review focuses on the apoptosis and tumor inhibitory effects of procyanidin with respect to its bioavailability.

Key words: Chemoprevention, Procyanidin, Signaling pathway, Apoptosis, Biomarker, Transcription

INTRODUCTION

According to both clinical observations and experimental models, carcinogenesis consists of three steps: initiation, promotion, and progression. In most cases, full-fledged malignancy requires several years to develop (1). Even the most sophisticated treatment is highly unlikely to fully cure malignant tumors and save the lives of patients. Treatment cannot guarantee a 100% cure rate for even less advanced cancers, and imposes significant social and economic burden on patients. To reduce this burden, many studies have sought effective cancer prevention approaches and interventions to stop tumor progression before reaching full-fledged malignancy, and some approaches have been found to hold great potential in epidemiological and clinical trials worldwide. These attempts and approaches—a cancer prevention strategy using non-toxic chemical materials—are collectively known as chemoprevention (1).

For the last few decades, there have been considerable efforts to identify chemopreventive agents from dietary phytochemicals. Dietary phytochemicals are expected to have relatively few toxic effects, so they may be useful in preventing cancers requiring treatment or intervention against tumor progression (2-4). Among the many phytochemicals derived from dietary or medicinal plants that show substantial chemopreventive properties, a good example is the large class of polyphenolic compounds. Detailed research has been conducted on polyphenolic compounds such as resveratrol, hesperidin, genistein, catechin, and procyanidin to identify effective chemopreventive phytochemicals. Through research compilations that compared the chemopreventive potentials and probable mechanisms of polyphenolic compounds since 1995, procyanidin has received increasing interest for its phar-

macological properties and antioxidant effects (5-7). Recent cell culture studies have shown that treatment of human breast cancer MCF-7 (8) and MDA-MB-468 (9), human lung cancer A427 (10), human oral squamous cell cancers CAL27 and SCC25 (11), human prostate cancer DU145 (12,13) and LNCaP (14,15), human bladder cancer BIU87 (16), and human colorectal cancer HCT-8, HT29, LoVo, and Caco-2 cells (17-23) with procyanidin resulted in an inhibition of cell proliferation and/or an induction of cell apoptosis without an additional cytotoxic effect on normal cells (20,24-26). Procyanidin can alter gene expression in animal models, which indicates its potential as a chemopreventive agent. In this regard, a comprehensive identification of chemopreventive targets and biomarkers of procyanidin action will be

Table 1. Molecular targets of procyanidin as a chemopreventive agent

Molecular targets	Experimental models	Structure
Ah receptor		
Anti HAT activity, ↓ p300 mediated AR acetylation	LNCaP, PC-3 cells (39)	B3
Enzyme		
Attenuation of DNMT activity	MDA-MB-231 cells (41)	B2
↑Expression of DNMT targeting gene (E-Cadherin, Maspin, BRCA1)		
Mediators for pro-inflammation		
↑Antineoplastic effect,↑PGI2 and 15HETE production, ↓Cas-3 activity		
↓NF-κB-DNA binding, ↓gene expression of (IL-6, TNF-α and RANTES	L-428, KM-H2, L-540, L-1236 and HDML-2 (35)	B2
↓Expression of COX-2, iNOS	A431 cells (44)	
↓COX-2 mRNA and protein expression	SW-480 cells (20)	
Molecules for cell cycle		
Increase of G1 population	BxPC-3 cells (45)	
Increase of sub G1/G0 population	OVCAR-3 cells (46)	
Increase of sub G1/G0 population	Caco-2 cells (23)	
↓Expression of p53, c-myc, ODC, Arrest in G1 phase	SCC 25 (11), OE-33 cells (48)	
↓Expression of cyclin D1, CDK4 and survivin , G1 arrest	BIU87 cells (16)	
↓Expression of cyclin D1, E, A, B_1, ↑Cip1/p21 expression	BxPC-3 cells (45)	
Arrest in G1 phase		
Molecules of the apoptotic signaling		
↓Bcl 2 level, ↑Cas-9 activation	MIA-Paca-2 cells (45)	
↑Activation of Cas-3	QAW42, OVCAR3 cells (51)	
↑Expression of Cas-2,8	CAL27, SCC25 cells (11)	
↑Expression of Cas-3	BIU87 cells (16)	
JNK activation of c-jun	OA cells (52)	
↓Bcl-2 protein expression	MIA-Paca-2 cells (53)	
↓Bcl-2 protein expression, ↓c-jun, c-fos protein	MDA-MB-231 cells (54)	
↓Bcl-2 protein activation, ↑Bax protein, ↑Bax/Bcl-2	OSCC cells (55)	
↓protein expression of Bcl-xL, Bcl-2, XIAP and cFLIP	L-428, KM-H2, L-540, L-1236 and HDML-2 (35)	B2
↑Cas-3 protein activation, ↑mitochondrial membrane potential	HT-29, SW480, LoVo cells (21)	
Upstream kinases and transcription factors		
↓Expression of MAPK protein, ↓MAPK protein phosphorylation	A431 cells (44)	
↓PI3K/Akt phosphorylation, ↓ expression of NF-κB/p65-targeted proteins		
↓NF-κB protein expression	SW-480 cells (20)	
↑mRNA expression of IGF-2R and PTEN, ↓Akt phosphorylation	A549 cells (61)	
↑Inhibition of NRF-2 activation, inhibition of proliferation	Nrf-2 overexpressed cells (62) (A549, LK-2,DU 145 cells)	
No inhibition of proliferation	Nrf-2 low activated cells (62) (LU-99, RERF-LC-MS cells)	
↓Protein expression and activity of NF-κB, AP1, Stat3	PC-3, 22Rv1,C4-2B cells (34)	B2 3,3-di-O-gallate
↑Cleaved PARP level, ↓expression of survivin		

Table 1. Continued

Molecular targets	Experimental models	Structure
Molecules of angiogenesis and metastatic progression		
↓Expression of pro-MMP2, ↓Expression of MMP2	QAW42, OVCAR3 cells (51)	
↓Expression of MMP 9	MIA-PaCa-2 cells (45), SW-480 cells (20)	
↓Expression of MMP2	BxPC-3 cells (45)	
↓Angiogenic VEGF, ↓MMP-9 activity	MDA-MB-231 cells (54)	
↓Activity of MMP-2, MMP-9, uPA	MDA-MB-231 cells (77)	
↓Expression of MMP2 and MMP9	OEC -M1 cells (55)	
↓Migration capability	HeLa cells (78)	B3
Chemotherapeutic potential		
↓Expression of MIR-19a-19b and MIR 17 Host Gene ↑mRNA and protein level of IGF-2R, PTEN	A549 xenocraft model (61)	
↓Tumor cell proliferation, ↓NF-κB activity, ↑apoptosis ↓Expression of cyclin D1 and PCNA	A431 xenocraft model (82)	
↓Neoplastic formation of JB6P+cell, ↓activation of AP-1 and NF-κB, Blocking MEK/ERK/p90RSK signaling pathway	JB6P+cell xenocraft model (79)	B2
↓expression of MVD and VEGF	H22 cell s.c. injected mice (80)	

helpful for the future development of cancer treatment.

The anticancer and anti-aging effects of procyanidin partly stem from its high antioxidant and pro-oxidant activity (27-29). A 2015 study systematically reviewed the efficacy of procyanidin against oxidative damage, with a special focus on enzymes (30). This research review, in contrast, focuses on the molecular targets and animal applications of procyanidin, mainly apoptosis signaling, that have been discovered in the last decade, rather than antioxidative effects (except the antioxidative effects related to pro-inflammation). Most experimental studies reviewed here discussed procyanidin while some discussed procyanidin B1 and B2, which will be indicated as such in Table 1.

CHEMOPREVENTIVE POTENTIAL OF PROCYANIDIN

Materials and polymerization. Procyanidin belongs to the proanthocyanidin (or condensed tannins) class of flavonoids, and exists as dimers, trimers, tetramers, and other oligomers of catechin and epicatechin molecules. Dimeric procyanidins are procyanidin A1, A2, B1, B2, B3, B4, B5, B6, B8, while trimeric procyanidins are C1, C2, and tetrameric proanthocyanidins are named as arecatannin A2 and cinnamtannin A2. Dietary phytochemicals in the form of procyanidin are dimeric, trimeric, tetrameric, and mostly oligomeric, and found in apple, cocoa, grapes, and berries at high concentrations. The concentration of condensed tannins expressed as mg catechin equivalents is colorimatically analyzed (31), and the concentrations of dimeric B1 or B2 are expressed as μM in most papers.

It has been known that the extent of cell viability reduction and apoptosis induction after exposure to flavan-3-ol oligomeric/polymeric fractions positively correlate with the degree of polymerization (DP). Oligomers and polymers of flavan-3-ol have a higher antiproliferative effect in cells than do monomers and dimers (32,33). Recently, synthesized procyanidin B2 (100 μM) was shown to significantly inhibit nuclear transcription factor kappa B (NF-κB), activator protein-1 (AP1) transcriptional activity, and nuclear translocation of signal transducer and activator of transcription 3 (Stat 3) in prostate cancer cells (34), whereas natural procyanidin B2 (50 μM) showed cytotoxicity without apoptosis in MCF-7 cells (8). Mackenzie *et al*. (35) reported that B2 produced a concentration-dependent (2.5~100 μM) decrease in NF-κB-DNA binding that reached a maximum effect at 25 μM (35~47% decrease) in all Hodgkin and Reed-Sternberg (H-RS) cell lines (L-428, KM-H2, L-540, L-1236, and HDML-2 cells). Treatment of those cells with B2 (25 μM) led to a inhibition of tumor necrosis factor (TNF)-α and RANTES secretion (35). Therefore, procyanidin B2 exerts cytotoxicity/apoptotic activity at higher concentrations, depending on the types of cell lines tested.

Ah receptor and enzyme. Androgen receptor (AR) acetylation is known to be critical for prostate cancer cell growth (36-38). Thus, the development of phytochemical HATi [HAT (histone acetyltransferase) inhibitors] has been a therapeutic goal. Procyanidin-B3 is reported to possess the highest anti-HAT activity among the catechin derivatives, and suppressed p300 mediated AR acetylation, thus inhibiting prostate cancer cell growth (39). There are some therapeutic interventions targeting enzymes, wherein inhibitors binding to a catalytic domain and inhibiting enzyme activity are essential mechanisms. Recently, DNA methyltransferases (DNMTs) have been reported as a potential tar-

get for anticancer drug development (40). It was demonstrated that procyanidin B2 attenuated DNMT activity at an IC_{50} of 6.88 ± 0.647 M, and subsequently enhanced the expression of DNMT target genes, E-cadherin, Maspin, and BRCA1 in MDA-MB-231 cells (41).

Mediators for pro-inflammation. Procyanidin significantly increased prostacyclin and 15-hydroxy eicosatetraenoic acid (15-HETE) production in A549 cells. Procyanidin also increased prostacyclin synthase and caspase 3 inhibition, and the transfection of 15-LOX-2 siRNA abrogated procyanidin-induced apoptosis in A549 cells (42). It was also reported that supernatants from *ex vivo* procyanidin-treated baseline BAL cells significantly decreased malignancy formation. These recent research results require more in-depth studies on procyanidin's potential as a chemopreventive for lung cancer. Pro-inflammatory cytokines, such as IL-1, 6, 8, and TNF-α are known to be involved directly or indirectly in carcinogenesis (43). Dimeric procyanidin B2 (B2) inhibits cytokine formation by interacting with NF-κB proteins, causing concentration-dependent inhibition of NF-κB-DNA binding to a similar extent (41~48% inhibition at 25 mM B2) in all tested H-RS cell lines. Production of cytokines (IL-6, TNF-α, and RANTES) and expression of anti-apoptotic proteins (Bcl-xL, Bcl-2, XIAP, and cFLIP) were inhibited by procyanidin B2 (35). Procyanidin inhibited the expression of NF-κB/p65-targeted proteins, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), which are pro-inflammatory mediators in human squamous carcinoma A431 cells (44). Inhibition of COX-2 mRNA and protein expression by procyanidin was also recently reported in colon cancer SW-480 cells (20).

Effects on cell cycle. Procyanidin treatment increased the G1 cell population in BxPC-3 (45), OVCAR (46), oral squamous cell carcinoma 25 (SCC-25) (11), and Caco-2 cells (23), which is demonstrative of its apoptotic effects. Earlier studies suggest that the procyanidin-induced arrest of the cell cycle might be mediated by the induction of p21 expression (19,47). A more recent report showed that p21 expression knock-down with p21-specific si-RNA in human esophageal carcinoma OE-33 cells had no detectable effect on the induction of G0/G1 cell cycle arrest by procyanidin (48); thus, p21 is not responsible for the procyanidin-induced cell cycle arrest.

Procyanidin derived from grape seed extract decreased cell viability (−72%), and reduced the expression of p53 (−51%), Bax, and caspase-3 mRNA, without significantly altering total RNA in SCC25 cells (11). Procyanidin treatment produced G1 arrest and inhibited the expression of cyclin D1, CDK4, and survivin in BIU87 cells (16). Some reports indicate that the cyclinD1-CDK4 protein pathway plays a key role in the transition of G1-S phase in the cell cycle, and its regulation was correlated with each type of

cancer (49,50). Procyanidin arrested BxPC-3 cells in the G1 phase (45), which was also mediated by decreases of cyclin D1, E, A, and B1. It is considered that the effects of procyanidin in inducing cell cycle arrest and apoptosis may be due to its downregulation of cyclinD1, CDK4, and survivin.

Effects on apoptotic signaling. Procyanidin treatment induced DNA damage and caspase-3-mediated cell death in QAW42 and OVCAR 3 cells (51), and increased the expression of caspase-2, 8 in CAL27 and SCC25 cells (11). Recently, it was also reported that procyanidin induced G1 cell phase arrest, apoptosis and caspase-3 protein activation. in BIU87 cells, while the expression of cyclin D1, CDK4, and survivin was decreased. It is considered that caspase-3 activation by procyanidin ultimately lead to cell to apoptosis.

Chung *et al*. (45) reported that procyanidin induced MIA PaCa-2 cell apoptosis, which was mainly mediated by the suppression of Bcl-2 expression and activation of the caspase 9 pathway. Connor *et al*. (52) reported that procyanidin induced the activation of JNK and p38 and the phosphorylation and expression of c-Jun. They also reported that inhibition of JNK (c-Jun N-terminal kinase), prevented the procyanidin-induced phosphorylation and expression of c-Jun, and that knockdown of JNK1, JNK2, or JUN expression diminished procyanidin-induced effects on apoptosis (52). Based on these research findings, the authors concluded that JNK activation of c-Jun by procyanidin leads to the induction of apoptosis of OA cells (52). It was also observed that downregulation of the anti-apoptotic protein Bcl-2 increased apoptosis in MIA PaCa-2 cells (53). Procyanidin also decreases protein expression levels of c-Fos, c-Jun, and Bcl-2 in MDA-MB-231 cells (54). Apoptotic induction by procyanidin results from decreased Bcl-2 protein activation, increased Bax protein activation, and increased Bax/Bcl-2 ratios in OSCC cells (55). Similarly, the expression of anti-apoptotic proteins (Bcl-xL, Bcl-2, XIAP, and cFLIP) was inhibited by procyanidin B2 (35). Increases in cas-3 protein activation and mitochondrial membrane potential were observed in HT-29, SW-480, and LoVo cells (21).

Procyanidin induced MIA PaCa-2 cells to undergo apoptosis, which was primarily mediated by suppression of the level of Bcl-2 and activation of the caspase 9 pathway, and was associated with decreased MMP-9 levels (45). Pancreatic cancer (PCa) is one of the most aggressive cancers in developed countries. Depending on the cell lines of PCa studied, procyanidin showed different apoptotic mechanisms. Procyanidin arrested BxPC-3 cells in the G1 phase, which was mediated by decreases of cyclin D1, E, A, and B$_1$ and an increase in the level of Cip1/p21, and inhibited MMP-2 expression (45). Thus, procyanidin treatment exerted anti-proliferative and anti-invasive effects in PCa cell lines, suggesting a potent chemopreventive or therapeutic agent for PCa.

Effects on upstream kinases and transcription factors.
NF-κB regulates tumor promotion markers such as COX-2, iNOS, proliferating cell nuclear antigen (PCNA), and cyclin D1 (56), and NF-κB is regulated by the phosphoinositide 3-kinase/serine threonine kinase Akt (PI3K/Akt) signaling pathway (57). Therefore, both NF-κB and PI3K/Akt signaling pathways are important molecular targets in cancer prevention (58). Procyanidin inhibited PI3K/Akt phosphorylation and the expression of NF-κB /p65-targeted proteins in A431 cells (44). The genes for COX-2, iNOS, and cyclin D1 have been shown to be up-regulated in human cancers, suggesting that downregulation of NF-κB, and subsequent downregulation of those genes, may suppress cancer development. NF-κB protein expression is reduced by procyanidin (20). Katiyar's report suggested that treatment of A431 cells with procyanidin inhibited the expression of MAPK protein, MAPK protein phosphorylation, PI3K/Akt phosphorylation, and NF-κB-targeted proteins, such as COX-2, iNOS, and cyclin D1. This report suggested that the NF-κB and PI3K/Akt signaling pathways were the key molecular target of procyanidin (44).

The PI3K/Akt signaling pathway is responsible for promoting cellular proliferation and resistance to apoptosis (59,60). Procyanidin significantly downregulated the expression of miR-19a/b and its host gene, MIR17HG, which subsequently increased the mRNA expression of tumor suppressor genes, insulin-like growth factor II receptor (IGF-2R) and phosphatase and tensin homolog (PTEN), and their respective protein products, and decreased p-Akt in A549 cells, and in A549 cell-xenograft animal models (61). This study also indicated that apoptosis induction and antineoplastic properties of procyanidin are mediated, in part, through modulation of the oncomiRs miR-19a and 19b.

Nuclear-related factor 2 (Nrf2) is an important transcription factor playing a significant role in inducible expression of many cytoprotective genes. Interestingly, procyanidin treatment inhibits Nrf2 expression and cell proliferation in cancer cells over-expressing Nrf2 (A549, LK-2, DU145), but these phenomena were not seen in LU-99 and RERF-LC-MS cells with low Nrf2 expression (62). A549, LK-2, and DU145 cells are reported to have mutations of KEAP1 and Nrf2 (63-66). Two cancer cell lines (LU-99 and RERF-LC-MS) have not been investigated for those mutations. Therefore, the effect of procyanidin on Nrf2 suppression is different from basal expression levels of Nrf2 (62), and selective action of Nrf2 suppression by procyanidin is currently under study. These results support the conclusion that, in cancer, aberrant activation of Nrf2 by epigenetic alterations induces high expression of cytoprotective proteins, which can decrease the effect of anticancer drugs used for chemotherapy (67).

It has been recently shown that transcription factors, such as NF-κB, AP1, and (Stat3), are the major regulators of cellular survival, apoptotic machinery, and inflammation. The activity of these transcription factors is critical for the growth and progression of cancers, including PCa (68-71). In addition, activation of NF-κB, AP1, and Stat3 signaling induces survivin expression and confers resistance to apoptosis in cancer cells (72,73). Mechanistic studies reported that procyanidin B2 significantly inhibits NF-κB and AP1 transcriptional activity and nuclear translocation of Stat3 in prostate cancer cells, and also decreases survivin expression, which is regulated by NF-κB, AP1, and Stat3, and increased cleaved PARP level (34).

Molecules for angiogenesis and metastatic progression.
Taparia *et al*. (46) reported that procyanidin is cytotoxic to QAW42 and OVCAR3 epithelial ovarian cancer cells via several mechanisms, inducing apoptosis with DNA damage and caspase-3 mediation. Downregulation of pro-MMP-2 and reduction in active MMP-2 levels imply a decreased invasive potential of the cells (51). Regarding the inhibition of invasion or metastasis steps by procyanidin, many reports show that procyanidin inhibits the levels or activity of MMP-9 and MMP-2 (44,74-76). Chung's group also found that procyanidin treatment inhibits the level of MMP-9 in MIA PaCa-2 cells and the level of MMP-2 in BxPC-3 cells, indicating the inhibition of invasion by procyanidin in these two PCa cells (45).

Procyanidin caused a 10-fold reduction in MMP-9 activity. It reduced the expression levels of vascular endothelial growth factor (VEGF), and induced apoptosis in MDA-MB-231 cells (54). In conclusion, these findings collectively show that procyanidin inhibits cell viability by increasing apoptosis and decreases cell invasiveness by decreasing angiogenesis (54). Low concentrations of procyanidin decrease cell migration, invasion, and metastatic process, as well as the activity of urokinase-type plasminogen activators (uPA), MMP-2, and MMP-9 in MDA-MB-231 cells (77). Procyanidin also inhibits the migration and invasion of OEC-MI cells and SCC-25 cells, which is associated with the suppression of MMP-2 and MMP-9 (55). Procyanidin B3 from *Pinus massoniana bark* showed no significant effects on the adhesion capability of HeLa cells, but strongly inhibited their migration. This suggests that procyanidin B3 can be a potential therapeutic agent for the treatment of metastatic cancer (78).

Chemotherapeutic potential. Procyanidin increased the mRNA expression of tumor suppressor genes IGF-2R and PTEN, as well as their respective protein products, and decreased p-Akt in a A549 cell xenograft animal model (61). Therefore, procyanidin has been nominated as an antineoplastic and chemopreventive agent for lung cancer. Procyanidin treatment via oral gavage (50 or 100 mg/kg body weight/mouse) reduced the growth of A431-xenografts in mice and inhibited tumor cell proliferation in xenografts. These effects were indicated by the inhibition of mRNA

expression of PCNA and cyclin D1, and of NF-κB activity (44). These findings suggest that procyanidin can also be effective in the treatment of skin cancers.

Procyanidin B2 inhibited tumor promoter-induced neoplastic transformation of JB6 P+ cells. This inhibition was mediated by the blocking of the MEK/ERK/p90RSK signaling pathway, and subsequent suppression of AP-1 and NF-κB activities. Procyanidin B2 also inhibited MEK1 activity through binding with MEK1. The researchers suggested that MEK1 is a potent molecular target for the suppression of neoplastic transformation by procyanidin B2 (79). Procyanidin treatment decreased the expression of vascular endothelial growth factor (VEGF) and micro vessel density (MVD) in H22 cells subcutaneously injected into mice. This study suggests that procyanidin suppresses tumor growth, possibly by inhibiting tumor angiogenesis (80).

Chemo-sensitizing effects. P-glycoprotein (P-gp), a product of the multi drug resistant (MDR)-1 gene, has been considered as a main player in development of chemo-resistance (81). In most cases, P-gp causes drug efflux from cells and reduces intratumoral concentrations of chemotherapeutic drugs and hence, lowers their efficacy. Ling *et al.* (82) reported that the combination of 80 mg/kg procyanidin with 2 mg/kg adriamycin significantly increased days of survival, with an increase in life span of 76%. These findings suggest that procyanidin is a potent inhibitor of P-gp in the blood brain barrier, and markedly improves the therapeutic effects of adriamycin in nude mice transplanted with human cerebroma.

Procyanidin is cytotoxic to ovarian cancer cells, OAW42 and OVCAR3 cells, and sensitizes them to doxorubicin. Chemosensitization was accompanied with decreased P-gp levels, and an increased cell population in the hypodiploid sub-G0 phase after treatment with procyanidin (46). Regarding the approaches to overcome chemo-resistance conferred by P-gp, Zhao *et al.* (83) reported that procyanidin reverses MDR in A2780/T cells by inhibiting the function and expression of P-gp in A2780/T cells. Their study discovered that procyanidin reversed MDR by inhibiting the function and expression of p-gp via inhibition of NF-κB and YB-1 translocation into the nucleus, mediated by dephosphorylation of AKT and ERK1/2, respectively (83). The study authors also suggested that procyanidin may be used as a supplementary drug, along with conventional P-gp substrate chemotherapeutic drugs to overcome MDR in ovarian cancer patients.

Recently, suppression of over-expressed Nrf2 was proposed as a new therapeutic approach against lung cancers, based on the report that lung cancer cells overexpressing Nrf2 exhibit increased resistance to chemotherapy. Ohuma *et al.* (84) found that procyanidin has an ability to suppress Nrf2-regulated enzyme activity and Nrf2 expression in human lung cancer A549 cells. This study also demonstrated

that procyanidin significantly enhances the chemosensitivity of A549 cells. Based on these results, procyanidin might be an effective agent to reduce anticancer drug resistance derived from Nrf2 overexpression.

CONCLUSIONS

Currently, we are witnessing a growth in the development of dietary phytochemicals as potential chemopreventive agents. Due to the diversity of cancer cells, it is difficult to identify specific molecular targets for cancer prevention or treatment. Thus, an ideal cancer preventive or therapeutic agent should target multiple biochemical pathways leading to carcinogenesis. As discussed in previous sections of this paper, procyanidin has been reported to target diverse molecular switches in carcinogen metabolism (inflammation, cell proliferation, cell cycle, apoptosis, angiogenesis).

There have been several studies on animal ADME (absorption, distribution, metabolism and excretion) which provide meaningful implications for therapeutic use of procyanidin. It has been known since the 1990s that procyanidin activity is highly dependent on the pH value of gastric juice, and some authors have reported that procyanidin is unstable under alkaline conditions (85-87). When procyanidin was subjected to the *in vitro* digestion model, high concentrations of the monomers catechin and epicatechin, and mainly dimer and trimer oligomeric flavanols were observed in the gastric fractions, demonstrating stability under acidic conditions (88). Similarly, dimer and trimer procyanidins are absorbable *in vivo*, reaching maximum concentrations in the plasma of rats as early as one hour after procyanidin ingestion (88,89). It was also reported that the maximum blood concentration of monomers rarely reaches 5 μM/L, and they are usually present as a combination of methylated, sulfated, and glucuronidated metabolites (90-92). Bioavailability of procyanidin B2 was 8~11% in blood after oral administration, while 63% was excreted via urine within 4 days. These data suggest that procyanidin administered orally is degraded by gut microflora before absorption (93). More recent studies showed almost the same results, that is, procyanidin polymers and oligomers with DP > 4 are not directly absorbed by the intestine *in vivo*, and methylated and glucuronidated procyanidin dimers and monomers are the main metabolites of procyanidin in plasma (94).

Despite substantial progress in the preclinical study of procyanidin, there have been few clinical studies validating positive preclinical results. Phase I clinical trials demonstrate that dimeric procyanidins are detected in human plasma as early as 30 min after the consumption of flavanol-rich food such as cocoa (0.375 g/kg) (95), and 4-week treatment with 75 mg of grape procyanidin in heavy smokers attenuates low density lipoprotein (LDL) concentrations with no adverse effects (96). These preliminary pharmacokinetic data suggest that the bioavailability of procyanidin is

relatively low. Results of these phase I trials will provide important background information useful in designing follow-up clinical trials for the chemopreventive or chemotherapeutic potential of procyanidin. Further studies are necessary to enhance the bioavailability of procyanidin by devising appropriate formulations and identifying possible interactions with other dietary components. Building on these existing preclinical and mechanistic data, next-phase clinical studies may prove procyanidin is a potential source of molecular target-based cancer prevention and adjuvant therapy.

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