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Invited review

Targeting multiple signal pathways by chemopreventive agents for cancer prevention and therapy[1]

Fazlul H SARKAR[2], Yi-wei LI

Department of Pathology, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA

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[2] Correspondence to Dr Fazlul H SARKAR.
Phn 1-313-576-8327.
Fax 1-313-576-8389.
E-mail fsarkar@med.wayne.edu

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Abstract

In recent years, growing interest has been focused on the field of cancer prevention. Cancer prevention by chemopreventive agents offers significant promise for reducing the incidence and mortality of cancer. Chemopreventive agents may exert their effects either by blocking or metabolizing carcinogens or by inhibiting tumor cell growth. Another important benefit of chemopreventive agents is their non-toxic nature. Therefore, chemopreventive agents have recently been used for cancer treatment in combination with chemotherapeutics or radiotherapy, uncovering a novel strategy for cancer therapy. This strategy opens a new avenue from cancer prevention to cancer treatment. *In vitro* and *in vivo* studies have demonstrated that chemopreventive agents could enhance the antitumor activity of chemotherapeutics, improving the treatment outcome. Growing evidence has shown that chemopreventive agents potentiate the efficacy of chemotherapy and radiotherapy through the regulation of multiple signaling pathways, including Akt, NF-κB, c-Myc, cyclooxygenase-2, apoptosis, and others, suggesting a multitargeted nature of chemopreventive agents. However, further in-depth mechanistic studies, *in vivo* animal experiments, and clinical trials are needed to investigate the effects of chemopreventive agents in combination treatment of cancer with conventional cancer therapies. More potent natural and synthetic chemopreventive agents are also needed to improve the efficacy of mechanism-based and targeted therapeutic strategies against cancer, which are likely to make a significant impact on saving lives. Here, we have briefly reviewed the role of chemopreventive agents in cancer prevention, but most importantly, we have reviewed how they could be useful for cancer therapy in combination with conventional therapies.

Introduction

Despite significant investments in capital, manpower, and intellectual innovations for the development of cancer therapies over the past several decades, cancer still remains the second leading cause of death in the United States[1]. Therefore, cancer prevention has become an important avenue through which the fight against cancer could be feasible. Cancer chemoprevention applies specific natural or synthetic chemical compounds to inhibit or reverse carcinogenesis and to suppress the development of cancer from premalignant lesions. The emerging field of cancer prevention by chemopreventive agents offers significant promise for reducing the incidence and mortality of cancer. Several studies have shown that the incidence of cancer could be decreased by chemoprevention[2–4]. Chemoprevention appears to be a promising avenue for reducing cancer incidence in both high-risk groups and the general population with a low risk of developing cancer. Because of its promising effects, chemoprevention has been increasingly recognized as a powerful tool by cancer investigators and the National Cancer Institute[5].

Chemopreventive agents may exert their effects either by blocking or metabolizing carcinogens or by inhibiting

tumor cell growth. Another important benefit of chemopreventive agents is their non-toxic nature. Therefore, chemopreventive agents have been recently used in cancer treatment in combination with conventional chemotherapeutics or radiotherapy. *In vitro* and *in vivo* preclinical studies have demonstrated that chemopreventive agents could enhance the antitumor activity of chemotherapeutics[6–11], improving treatment outcome. The combination treatment may also decrease the systemic toxicity caused by chemotherapies because lower doses of therapeutic agents could be used, and as such, no systemic toxicity has been found from chemopreventive agents. Growing *in vitro* and *in vivo* data have shown that chemopreventive agents enhance the efficacy of chemotherapy and radiotherapy in various cancers through the regulation of Akt, NF-κB, c-Myc, cyclooxygenase (COX)-2, apoptotic, and other pathways, suggesting a novel and multitargeted therapeutic strategy against cancer[6,7,11–14]. This strategy opens a new avenue from cancer prevention to cancer treatment.

Chemopreventive agents enhance antitumor activity of conventional cancer therapies

Most of the chemopreventive agents currently being studied are natural products or their derivatives. Many natural compounds, particularly plant products and dietary constituents, have been found to exhibit cancer chemopreventive activities both *in vitro* and *in vivo*[15,16]. Drug development from natural products is a rapidly emerging and highly promising strategy to identify novel anticancer agents. In recent years, novel combination treatments with conventional cancer therapies and chemopreventive agents have received much attention in cancer research. Experimental studies and clinical trials have demonstrated the beneficial effects of chemopreventive agents, including soy isoflavone, curcumin, epigallocatechin-3-gallate (EGCG), non-steroidal anti-inflammatory drugs (NSAIDs), resveratrol, indole-3-carbinol (I3C), and 3,3'-diindolyl-methane (DIM) in cancer prevention and treatment.

Evidence from epidemiologic and *in vivo* studies show a decreased risk of cancer associated with soy consumption[17]. Soy isoflavone genistein is believed to be responsible for the decreased risk of cancer. Isoflavone genistein has been found to inhibit the growth of various cancer cells *in vitro* and *in vivo*[17]. More importantly, the published studies have shown that isoflavone genistein could potentiate the antitumor effects of chemotherapeutic agents in various cancers *in vitro* and *in vivo* in preclinical studies. We have reported that *in vitro* genistein potentiated growth inhibition and apoptotic cell death caused by cisplatin, docetaxel, doxorubicin, gemcitabine, and CHOP (cyclophosphamidine, doxorubicin, vincristine, prednisone) in lymphoma and cancers of prostate, breast, pancreas, and lung[6,7,12,18]. We have also found that dietary genistein *in vivo* could enhance the antitumor activities of gemcitabine and docetaxel in a tumor model, resulting in apoptotic cell death and the inhibition of tumor growth[6,7]. Similar observations by other investigators have also showed that the antitumor effects of chemotherapeutics, including 5-fluorouracil (5-FU), adriamycin, and tamoxifen could be potentiated by genistein[13,19,20]. Genistein also enhanced the antitumor effect of bleomycin in HL-60 cells, but not in normal lymphocytes in an *in vitro* study[21]. The synergistic action of genistein and cisplatin or carmustine (BCNU) on the growth inhibition of glioblastoma and medulloblastoma cells has also been observed[22,23]. These reports suggest that isoflavone genistein is not just a chemopreventive agent, but could also be used as a potential therapeutic agent in combination with other chemotherapeutics for cancer treatment. In radiotherapy, experimental studies have demonstrated that the combination of genistein and radiation exerted enhanced inhibitory effects on DNA synthesis, cell growth, colony formation, and metastasis[24,25]. Genistein also enhanced radiosensitivity in human esophageal and cervical cancer cells[26,27], suggesting the beneficial effects of genistein in cancer radiotherapy.

To enhance the antitumor activity of isoflavone, several isoflavone derivatives have been synthesized and used in *in vitro* and *in vivo* experiments and in clinical trials. These compounds have shown a low IC_{50} in the inhibition of cancer cell growth *in vitro*. Moreover, at low concentrations, these compounds were able to enhance the antitumor activity of clinically available chemotherapeutic agents, suggesting their potent effects as therapeutic agents for combination treatment. Phenoxodiol is one such analog of isoflavone genistein and has shown a broad-spectrum, anticancer effect. In an animal study, phenoxodiol inhibited dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis in female Sprague-Dawley rats, suggesting that phenoxodiol is an effective chemopreventive agent against DMBA-induced oncogenesis[28]. In experimental studies and clinical trials, phenoxodiol has been used both as a monotherapy and in combination with standard chemotherapeutics. These studies have shown that in some cancers phenoxodiol appears to be strong enough to work on its own as a monotherapy. However, one of the major benefits of phenoxodiol is its ability to sensitize cancer cells to the antitumor effects of conventional chemotherapeutics[29]. It

has been found in cancer cells that are susceptible to the effects of standard chemotherapeutics that phenoxodiol increases their sensitivity to those agents. In cancer cells that have become resistant to the effects of conventional chemotherapeutics, phenoxodiol restores chemosensitivity[14,30]. By exposing chemoresistant cancer cells to phenoxodiol first, long-standing drug resistance is removed, making cancer cells susceptible once again to standard chemotherapeutics, such as cisplatin, carboplatin, taxanes, and gemcitabine. Phenoxodiol is currently undergoing clinical studies in the USA and Australia. So far, phase I/II clinical trials have shown some disease stabilization without severe toxicity[31].

In addition to isoflavone and its derivatives, other chemopreventive agents have shown their effects on the enhancement of the antitumor activities of chemotherapeutic agents. Curcumin has been found to inhibit the carcinogenic activity of azoxymethane or DMBA in the colon or orally in rats[32,33]. Curcumin also inhibited cancer cell growth and induced apoptotic cell death in various cancers[34]. Moreover, curcumin and celecoxib synergistically inhibited the growth of colorectal cancer cells[35]. Curcumin also potentiated the antitumor activities of cisplatin, doxorubicin, and Taxol in HA22T/VGH hepatic cancer cells, HeLa cells, or CAOV3 and SKOV3 ovarian cancer cells[8–10]. In addition, the combination treatment with curcumin and TRAIL increased the number of hypodiploid cells and induced DNA fragmentation in LNCaP cells[36]. More recently, curcumin was found to sensitize pancreatic cancer cells to gemcitabine-induced killing[37]. In cancer radiotherapy, curcumin at a low concentration also showed significant enhancement to radiation-induced clonogenic inhibition and apoptosis in PC-3 prostate cancer cells[38]. These results demonstrated that curcumin is a potent agent in cancer prevention and therapy.

Epidemiologic evidence showed that consumption of green tea, which contains EGCG, significantly decreased overall cancer incidence[39]. As an antioxidant and photoprotective agent, EGCG promotes cell cycle arrest and apoptosis in cancer cells through the modulations of cyclin/CDK and Bcl-2 family proteins[40]. EGCG *in vivo* also inhibits tumor promotion and metastasis in murine melanoma[41]. Importantly, it has been found that EGCG combined with tamoxifen significantly induced apoptosis and growth inhibition in MDA-MB-231 human breast cancer cells[42]. EGCG could also chemosensitize resistant tumor cells to doxorubicin in the human carcinoma xenograft model[43], suggesting its effects on cancer therapy in combination with chemotherapeutics.

COX-2 inhibitor NSAIDs have been shown to decrease the risk of various cancers, including colon and lung cancers[44,45]. NSAIDs exert apoptotic effects in a variety of cancer cells, including esophageal, liver, colon, lung, oral, and bladder cancer cells in a COX-2-dependent or COX-2-independent manner through the regulation of other molecules in cellular signaling pathways[46]. Celecoxib, the first selective COX-2 inhibitor approved for the chemoprevention of colon cancer in patients with familial adenomatous polyposis, has also been found to decrease the incidence of various cancers in various animal models with no associated toxicity[47]. Interestingly, the forced expression of COX-2 caused enhancement in multiple drug resistance (MDR)1 expression and functional activity, suggesting the existence of a causal link between COX-2 activity and MDR1 expression[48]. Therefore, the use of COX-2 inhibitors to decrease MDR1 function may enhance the accumulation of chemotherapy agents and decrease the resistance of tumors to chemotherapeutic drugs. Altorki *et al* found that celecoxib enhanced the response to paclitaxel and carboplatin in early-stage, non-small-cell lung cancer[49]. Moreover, selective COX-2 inhibitors were found to enhance tumor response to radiotherapy or radiochemotherapy, suggesting that these agents can improve the response of various cancers to conventional cancer therapies[50–52].

Resveratrol is a phytoalexin mainly found in grapes. It exhibits anticancer properties in a variety of cancer cells *in vitro*, including lymphoid, myeloid, breast, prostate, and colon cancers[53]. Importantly, resveratrol has been reported to sensitize non-Hodgkin's lymphoma and multiple myeloma cells to paclitaxel-mediated apoptosis[54]. The oral administration of proanthocyanidin, another compound from grapes, has also been found to decrease the tumor progression and the size of cutaneous carcinomas in an animal study[55]. Another murine study showed that the administration of grape seed extract significantly reduced metastatic melanoma pulmonary nodules[56]. Moreover, proanthocyanidin has been found to enhance doxorubicin-induced antitumor effects and reverse drug resistance in doxorubicin-resistant K562/DOX cells, breast cancer cells, and mouse tumor xenograft models[57,58].

We and others have found that I3C from cruciferous vegetables combined with cisplatin or tamoxifen could inhibit the growth of PC-3 prostate and MCF-7 breast cancer cells more effectively than either agent alone[59,60]. Phenethyl-ITC (PEITC) is another compound from cruciferous vegetables and has generated a great deal of research interest due to its cancer chemopreventive activity. PEITC administration was shown to significantly inhibit carcinogen-induced oncogenesis in mouse models[61–63]. More importantly, PEITC has been found to inhibit angiogenesis *in vitro*

and *ex vivo*, suggesting that it is not just a chemopreventive agent, but could also be used for cancer therapy[64]. Recent reports showed that sulforaphane, another compound from cruciferous vegetables, exerted its antiproliferative activity in Akt-overexpressing ovarian cancer cells[65]. These findings suggest the beneficial effects of cruciferous vegetables in the fight against cancer.

Vitamin D obtained from the diet and synthesized in the skin in response to UVB exposure has been advocated as a potential preventive agent for prostate, colon, and lung cancers[66–68]. Analogs of vitamin D were also shown to potentiate the antiproliferative effect of doxorubicin, cisplatin, and genistein *in vitro*[69]. *N*-(4-hydroxyphenyl) retinamide (4HPR; fenretinide), a synthetic derivative of retinoic acid, has been found to exert potent pro-apoptotic effects on a variety of cancer cells[70]. A recent report showed that 4HPR combined with low doses of celecoxib more potently inhibited growth and induced the apoptosis of premalignant and tumorigenic bronchial epithelial cell lines[71].

These reports clearly demonstrate that chemopreventive agents (natural or synthetic agents, which inhibit the development of cancer) could be used in cancer treatment to further enhance the antitumor activities of conventional chemotherapeutics (which have been used in the treatment of cancer in clinics) and radiotherapy.

Targeting cellular signaling pathways by chemopreventive agents in cancer prevention and treatment

In this review, we present a succinct summary of the major signaling pathways that are regulated by chemopreventive agents. It has been known that chemopreventive agents exert their inhibitory effects on carcinogenesis through multiple signaling pathways, including Akt, NF-κB, mitogen-activated protein kinase (MAPK), p53, COX-2, Ras, and many other molecules that are known to regulate cell growth and apoptotic pathways. However, the molecular mechanisms by which chemopreventive agents potentiate the antitumor effects of cancer therapies have not been fully elucidated. It is known that chemotherapy and radiotherapy can induce drug resistance in cancer cells, resulting in treatment failure. Emerging evidence has demonstrated that MDR, NF-κB, Akt, and some molecules in the apoptotic pathway are involved in the development of drug resistance. Chemopreventive agents could sensitize cancer cells to cancer therapies through the regulation of Akt, c-Myc, NF-κB, COX-2, and apoptotic pathways, all of which are known to play important roles in the regulation of cell survival and cell growth (Figure 1).

Regulation of the Akt pathway It has been well known

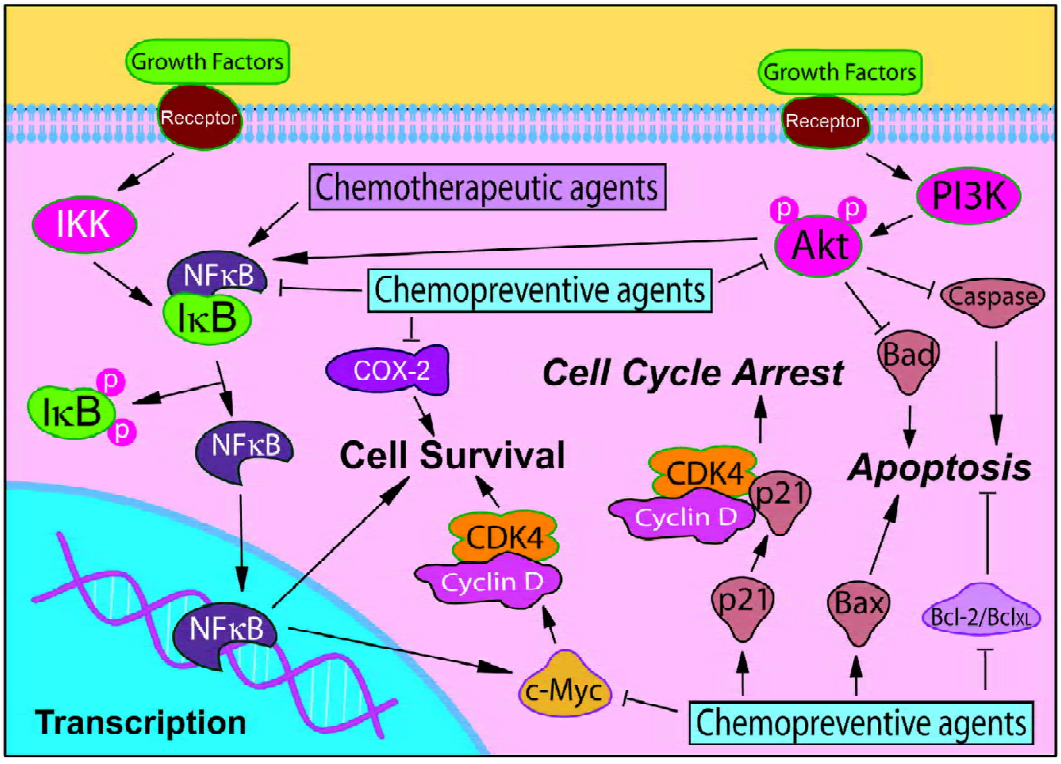

Figure 1. Chemopreventive agents enhance anti-tumor effects of chemotherapy through the regulation of Akt, NF-κB, c-Myc, COX-2, and apoptosis pathways.

that the Akt signaling pathway plays important roles in the control of cell survival. Many chemopreventive agents have been found to inhibit cancer cell growth and induce apoptosis through the inhibition of the Akt pathway[72]. A component of green tea, EGCG, promoted apoptosis in T24 human bladder cancer cells via the modulation of the PI3K/Akt pathway and Bcl-2 family proteins[73]. Indomethacin, a NSAID, has been found to induce apoptosis in renal cell carcinoma cells by activating Akt and MAPK signaling[46]. Other COX-2 inhibitors, including celecoxib and SC236, have also been found to inhibit cell growth and induce apop-tosis through the regulation of Akt and the COX-2 signaling pathway[70,74]. It has been known that the PEITC-mediated inhibition of the angiogenic features of human umbilical vein endothelial cells *in vitro* is associated with the inactivation of Akt, the suppression of vascular endothelial growth factor (VEGF) secretion, and the downregulation of VEGF receptor 2 protein levels[64]. In a study on sulforaphane from cruciferous vegetables, PI3K and both total Akt protein and active phosphorylated Akt (Ser473) were significantly decreased in sulforaphane-treated ovarian cancer cells, suggesting the inhibitory effect of sulforaphane on the Akt pathway[65]. Deguelin, a member of the flavonoid family with chemopreventive activities, has been found to decrease tumor incidence in animal models for lung, colon, mammary, and skin carcinogenesis through Akt inhibition[75,76]. We and others have found that isoflavone genistein could inhibit cancer cell growth and induce apoptosis through the downregulation of Akt. These results demonstrate that Akt is a target of chemopreventive agents in cancer prevention.

Growing evidence has also shown that activated Akt is critical for acquiring drug resistance[77–79], therefore the downregulation of Akt by chemopreventive agents could sensitize cancer cells to chemotherapeutics or radiotherapy. We and other investigators have found that activated Akt was inhibited by isoflavone genistein combined with gemcitabine or radiation in pancreatic, cervical, and esophageal cancer cells, suggesting that the enhancement of chemotherapeutic or radiation effects by isoflavone genistein may be partially mediated by the inhibition of Akt signaling[6,26,27]. It has been found that genistein also enhanced necrotic-like cell death with the significant inhibition of Akt activity in breast cancer cells treated with genis-tein and adriamycin, suggesting that the enhanced growth inhibition by combination treatment is through the inactivation of the Akt pathway[19]. Kamsteeg *et al* reported that phenoxodiol, one of the synthetic derivatives of genistein, could inhibit Akt signaling transduction and subsequently activate the caspase system, inhibiting X-linked inhibitor of apoptosis protein (XIAP) and in turn leading to increased chemosensitization[14]. It has been reported that curcumin downregulated the Taxol-induced phosphorylation of Akt, which interacts with NF-κB, suggesting that enhanced antitumor activity by curcumin is through the inactivation of the Akt and NF-κB pathways[9].

Regulation of the c-Myc/cyclin D/CDK pathway It is known that activated Akt can upregulate c-Myc through the activation of IkappaB kinase complex (IKK)/NF-κB and the inhibition of cyclin D1 and c-Myc proteolysis[80,81]. Almost all types of human cancers show high frequencies of c-Myc amplification or overexpression of its protein product, c-Myc. c-Myc can induce cyclin D1 which interacts with CDK4 and CDK6 to promote cell cycle progression[82,83]. It has been found that curcumin inhibited the expression of c-Myc and tumorigenesis[84,85]. Other chemopreventive agents, including EGCG, also showed their ability to downregulate c-Myc[86,87]. It has been reported that chemotherapeutics, including cisplatin, doxorubicin, paclitaxel, and 5-FU can induce c-Myc expression[88]. Interestingly, the surviving cancer cells from cisplatin treatment display a significant elevation in c-Myc expression[89] and the enhanced antitumor activity of chemotherapeutics can be achieved by the combination treatment with low-dose c-Myc antisense oligonucleotides[90], suggesting that c-Myc could cause chemoresistance of cancer cells to chemotherapeutics. Experimental studies have also shown that the overexpression of cyclin D1 contributed to the chemoresistance of pancreatic cancer cells because of the dual roles of cyclin D1 in promoting cell proliferation and inhibiting drug-induced apoptosis[91]. Therefore, the chemopreventive agent, which downregulates c-Myc, cyclin D, and CDK, could be used in combination with chemotherapeutics to improve the treatment outcome in cancer therapy. It has been reported that the combination of I3C and tamoxifen caused a more pronounced decrease in CDK2-specific enzymatic activity, CDK6 expression, and the level of phosphorylated retinoblastoma protein, leading to the more effective inhibition of the growth of human MCF-7 breast cancer cells compared to either agent alone[59].

Regulation of the NF-κB pathway NF-κB is an inducible and ubiquitously expressed transcription factor which regulates cell survival, inflammation, and differentiation[92]. It is becoming increasingly clear that NF-κB signaling plays critical roles in cancer development and progression. A large portion of cancer cells, especially poorly differentiated cancer cells, shows activated NF-κB in the nucleus, suggesting that activated NF-κB regulates its downstream genes to promote cancer cell growth. Therefore, NF-κB has long

been believed to be a target for the prevention and treatment of cancer. Several natural and synthetic chemopreventive agents that are able to inhibit the activation of NF-κB have been shown to either prevent cancer or to inhibit cell growth in animal models[93]. It has been reported that curcumin inhibited IKK, suppressed both constitutive and inducible NF-κB activation, and potentiated tumor necrosis factor (TNF)-induced apoptosis[94]. EGCG was also shown to inhibit the activation of IKK, the phosphorylation of IκBα, and the activation of NF-κB[95]. It has been found that deguelin, a member of the flavonoid family, also exerted its chemopreventive effects through the inhibition of NF-κB activity, even in the presence of inflammatory stimuli, such as TNF-α[96]. Other preventive agents, such as *Ganoderma lucidum* from an oriental medical mushroom and silibinin from the seeds of milk thistle, also inhibited cell growth and induced apoptosis through the inhibition of the NF-κB pathway[97,98]. We and others have reported that isoflavone genistein significantly inhibited cancer cell growth and induced apoptosis through the downregulation of NF-κB activity[99,100]. We also found that DIM from cruciferous vegetables inhibited NF-κB and its downstream genes VEGF, urokinase-plasminogen activator (uPA), and matrix metalloproteinase (MMP)-9, leading to the inhibition of angiogenesis, invasion, and metastasis in prostate cancer cells[101]. These results demonstrate that NF-κB is a target of chemopreventive agents in cancer prevention.

More importantly, it has been well known that many chemotherapeutic agents induce the activity of NF-κB, which causes drug resistance in cancer cells[102]. Therefore, targeting NF-κB by chemopreventive agents could be a promising strategy to enhance the antitumor activity of chemotherapeutics in cancer treatment. From *in vitro* and *in vivo* experimental studies, we observed that NF-κB activity was significantly increased by cisplatin, docetaxel, gemcitabine, and radiation treatment and that the NF-κB-inducing activity of these agents was completely abrogated by isoflavone genistein treatment in prostate, breast, lung, and pancreatic cancer cells, suggesting that isoflavone genistein pretreatment inactivates NF-κB and thus contributes to increased growth inhibition and apoptosis induced by these agents[6,7,12,24,103]. We also found that isoflavone genistein enhanced the antitumor activity of CHOP by the inhibition of NF-κB in lymphoma cells[18], suggesting that the inhibition of NF-κB is the most important event for chemosensitization. Studies also showed that increased cell death by genistein and radiation occurred via the inhibition of NF-κB, leading to the altered expression of regulatory cell cycle proteins, cyclin B and $p21^{WAF1/Cip1}$, thus promoting G_2/M arrest and increased radiosensitivity[103]. Similarly, curcumin has been found to inhibit the activity of NF-κB, resulting in the sensitization of cancer cells to cisplatin or Taxol-induced apoptosis[9,104], suggesting its beneficial effects in cancer treatment.

Regulation of the COX-2 pathway COX plays an important role in the biosynthesis of prostanoids. COX-1 is constitutively expressed in many tissues and is involved in the housekeeping function of prostanoids, while COX-2, the inducible isoform, accounts for the elevated production of prostaglandins in response to various inflammatory stimuli, hormones, and growth factors. COX-2 has received more attention than COX-1 in cancer research because COX-2 expression is associated with cell growth regulation, tissue remodeling, and carcinogenesis. In recent years, COX-2 inhibitors and NSAIDs have been shown to decrease the risk of various cancers, including colon and lung cancers, suggesting that the downregulation of COX-2 could be one of the molecular mechanisms by which chemopreventive agents prevent and inhibit tumor growth. It has been found that curcumin or EGCG inhibited the expression of COX-2 along with the growth of colorectal or prostate cancer cells[35,105]. Experimental studies have also shown that curcumin and EGCG could downregulate COX-2 expression without any change in the expression of COX-1 at both the mRNA and protein levels in colorectal or prostate cancer cells, suggesting that a combination of chemopreventive agent, such as curcumin or EGCG, with chemotherapeutic agents could be an improved strategy for the treatment of colorectal or prostate cancer[35,105]. In support of this suggestion, the synergistic growth inhibitory effect of curcumin and celecoxib has been demonstrated in colorectal cancer cells through the inhibition of the COX-2 pathway[35]. The combination of 5-FU and isoflavone genistein also enhanced therapeutic effects in colon cancer through the COX-2 pathway[13], although the inhibition of tumor growth by some COX-2 inhibitors could also be mediated through the COX-2-independent pathway. Studies have shown that celecoxib at clinically feasible concentrations (≤5.6 μmol/L) markedly suppresses the biosynthesis of prostaglandin E2 (PGE_2) in COX-2-expressing lung cancer cells[106]. However, much higher doses of celecoxib (≥25 μmol/L) are required for growth inhibition and apoptosis induction in cell culture systems, suggesting its COX-2-independent activity[107]. A recent report also showed that even at a low concentration, celecoxib combined with 4HPR inhibited cell growth and induced apoptosis though COX-2-independent mechanisms[70], and as such, suggest that further studies are needed to fully elucidate the mechanism of action of COX-2

inhibitors toward cancer prevention and therapy.

Regulation of the apoptotic pathway It has been well known that Akt, c-Myc, NF-κB, and COX-2 signaling could mediate apoptotic processes through the regulation of molecules in the apoptotic pathway. Therefore, the regulation of these signaling molecules by chemopreventive agents could lead to alterations in the levels of important molecules in the apoptotic pathway. EGCG, which inhibits Akt and NF-κB signaling, has been found to promote apoptosis in T24 human bladder cancer cells via the modulation of proteins in the Bcl-2 family[73]. It has also been reported that the genistein derivative phenoxodiol can bind to the tumor-associated NOX (tNOX) receptor, block its function, and subsequently inhibit the anti-apoptotic proteins XIAP and FADD-like ICE (FLICE) inhibitory protein, eventually inducing apoptotic cell death[14]. We and others have also found that isoflavone genistein combined with docetaxel or gemcitabine significantly inhibited Bcl-2, Bcl-X_L, and survivin, and induced $p21^{WAF1}$, suggesting that the enhanced antitumor effect in combination treatment is through the regulation of these important molecules in the apoptotic pathway[6,7]. It has been found that curcumin combined with cisplatin decreased the expression of several apoptosis-related genes, including c-Myc, Bcl-X_L, c-IAP-2, neuronal apoptosiainhibitory protein (NAIP), and XIAP[8]. The combination of curcumin and TRAIL also induced the cleavage of procaspase-3, procaspase-8, and procaspase-9, the truncation of Bid, and the release of cytochrome c from the mitochondria in prostate cancer cells, indicating that the apoptotic pathway is triggered in prostate cancer cells treated with a combination of curcumin and TRAIL[36]. These findings suggest that chemopreventive agents also regulate the apoptotic pathway during cancer prevention and treatment.

Regulation of other pathways We have reported that the antitumor and antimetastatic activities of docetaxel are enhanced by isoflavone genistein through the regulation of osteoprotegerin/receptor activator of NF-κB (RANK)/RANK ligand/MMP-9 signaling in prostate cancer, suggesting that isoflavone genistein could be a promising non-toxic agent to improve the treatment outcome of metastatic prostate cancer with docetaxel[11]. Soy isoflavone also enhanced prostate cancer radiotherapy through the downregulation of apurinic/apyrimidinic endonuclease 1/redox factor-1 expression[108]. In addition, isoflavone genistein and its isoflavone analogs also showed the potential to decrease the side-effects of tamoxifen through metabolic interactions that inhibit the formation of α-hydroxytamoxifen via the inhibition of CYP1A2[109], resulting in the beneficial effects of isoflavone genistein in combination with tamoxifen.

It has been found that phenoxodiol, another analog of isoflavone, exerts its inhibitory effects on cancer through pleiotropic molecular mechanisms. In addition to the regulation of Akt and the caspase-dependent apoptotic pathway, phenoxodiol also induced G_1 arrest by specific loss in cyclin-dependent kinase 2 activity through the p53-independent induction of $p21^{WAF1/CIP1}$ [110]. In addition, phenoxodiol also inhibits the catalytic activity of topo II in a dose-dependent manner and stabilizes the topo II-mediated cleavable complex[111], demonstrating the pleiotropic effects of this agent in cancer prevention and treatment. The enhanced effects of chemotherapy by chemopreventive agents may also be related to immunopotentiating activities through the reduction of interleukin (IL)-6[10] and the enhancements of lymphocyte proliferation, NK cell cytotoxicity, the CD4+/CD8+ ratio, IL-2, and interferon (IFN)-γ productions[57]. These results clearly suggest that chemopreventive agents are pleiotropic and thus could be considered as multitarget agents that are likely to revolutionize our approach for the prevention and treatment of cancer.

Conclusion and perspective

The emerging evidence from *in vitro* and *in vivo* studies reviewed above demonstrate that chemopreventive agents could inhibit the development and progression of cancer by targeting multiple cell signaling pathways for cancer prevention and treatment. It is important to note that one chemopreventive agent could target several cell signaling pathways which crosstalk in a complex cellular signal transduction network that are responsible for the development and progression of cancer. Further in-depth mechanistic studies, *in vivo* animal experiments, and novel clinical trials are needed to investigate the effects of chemopreventive agents in the combination treatment of cancer with conventional cancer therapies. Moreover, further development of potent natural and synthetic chemopreventive agents are also needed to improve the efficacy of mechanism-based and targeted therapeutic strategies to win the battle against cancer.

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