Epidemiological studies have indicated a significant difference in the incidence of cancers among ethnic groups, who have different lifestyles and have been exposed to different environmental factors. It has been estimated that more than two-thirds of human cancers, which are contributed by mutations in multiple genes, could be prevented by modification of lifestyle including dietary modification. The consumption of fruits, soybean and vegetables has been associated with reduced risk of several types of cancers. The in vitro and in vivo studies have demonstrated that some dietary components such as isoflavones, indole-3-carbinol (I3C), 3,3′-diindolylmethane (DIM), curcumin, (−)-epigallocatechin-3-gallate (EGCG), apigenin, etc., have shown inhibitory effects on human and animal cancers, suggesting that they may serve as chemopreventive agents. Experimental studies have also revealed that these components regulate the molecules in the cell signal transduction pathways including NF-κB, Akt, MAPK, p53, AR, and ER pathways. By modulating cell signaling pathways, these components, among other mechanisms, activate cell death signals and induce apoptosis in precancerous or cancer cells, resulting in the inhibition of cancer development and/or progression. This article reviews current studies regarding the effects of natural chemopreventive agents on cancer-related cell signaling pathways and provides comprehensive knowledge of the biological and molecular roles of chemopreventive agents in cancer cells.

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Keywords: Signaling pathway; Cancer; Prevention

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1. Introduction

It has been known that most human cancers are induced by environmental factors including chemical, radioactive and biological factors that exist in our living environment. There are significant differences in the cancer incidence and mortality among ethnic groups, who have different lifestyles and have been exposed to different environmental factors [1]. It has been estimated that more than two-thirds of human cancers could be prevented by modification of lifestyle including dietary modification [2]. The consumption of fruits, soybean and vegetables has been associated with reduced risk of several types of cancers [3–5]. The experimental in vitro and in vivo studies have demonstrated that some dietary components such as isoflavones, indole-3-carbinol (I3C), 3,3′-diindolylmethane (DIM), curcumin, (−)-epigallocatechin-3-gallate (EGCG), apigenin, have inhibitory effects on human cancers [6–13] suggesting that they may serve as chemopreventive agents. The dietary components as chemopreventive agents have received much attention among the public and the medical community. Soy isoflavones including genistin, daidzein, and glycitein, mainly derived from soybean have been found to inhibit cancer growth in vivo and in vitro [14–16]. I3C and its in vivo dimeric product DIM, produced from naturally occurring glucosinolates in the family Cruciferae, have shown the inhibition of cancer cell growth through the modulation of genes that are related to the control of cell proliferation, cell cycle, apoptosis, signal transduction, oncogenesis, and transcription regulation [6,9]. Curcumin, a natural compound present in turmeric and possessing anti-inflammatory and antioxidant effects, has been studied as a chemopreventive agent in several cancer models [11,17]. Beneficial effects attributed to green tea, such as its anticancer and antioxidant properties, are believed to be mainly mediated by EGCG [4,18]. Apigenin, one of the flavonoids, is widely distributed in many fruits and vegetables, and has been shown to possess anti-inflammatory and anticancer effects [19,20]. It is becoming clear that these dietary components exert their pleiotropic effects on cancer cells, affecting cell survival and physiological behaviors. However, the precise molecular mechanisms of actions of these components have not been fully elucidated, although the data from published literature does indicate that these components regulate transcription, translation and activation of the molecules in the cell signal transduction pathways. Among the cell signaling pathways, NF-κB, Akt, MAPK, p53, AR, and ER pathways are more important signaling pathways related to cancer development and progression. This article reviews current studies regarding the effects of natural chemopreventive agents on these cancer-related cell signaling pathways and provides comprehensive knowledge of the biological and molecular roles of chemopreventive agents in cancer cells.

2. Effects of chemopreventive agents on NF-κB

It has been well accepted that nuclear factor-κB (NF-κB) signaling pathway plays important roles in the control of cell growth, apoptosis, inflammation, stress response, and many other physiological processes [21–25]. There are several important molecules such as NF-κB, IκB, IKK, within NF-κB signaling pathway (Fig. 1). However, NF-κB is the key protein
Akt, NF-κB, and MAPK signaling pathways and the effects of genistein, I3C, and DIM on the pathways.

Fig. 1. Akt, NF-κB, and MAPK signaling pathways and the effects of genistein, I3C, and DIM on the pathways.

in the pathway, and has been described as a major culprit and a therapeutic target in cancer [26–29]. The data from experimental studies have demonstrated that genistein, I3C, curcumin, EGCG, and apigenin inhibit activation of NF-κB in different cancer cell lines, suggesting the inhibitory effects of these agents on cancer cells [8,20,30–33].

Our laboratory examined NF-κB DNA-binding activity in genistein treated PC3 and LNCaP prostate cancer cells by electrophoresis mobility shift assay (EMSA) [31]. The results showed that 50 μM genistein significantly inhibited the NF-κB DNA-binding activity in both cell lines. Furthermore, genistein pretreatment also abrogated the activation of NF-κB stimulated by H2O2 or TNF-α. Immunochemistry and confocal microscopic analysis also showed that TNF-α treatment significantly increased nuclear staining of the NF-κB p50 and p65 subunits, however, 24 h pretreatment of cells with genistein prior to TNF-α stimulation blocked p50 and p65 nuclear translocation. These results clearly demonstrate that genistein inhibits the translocation of NF-κB subunits to the nucleus, suggesting that genistein may reduce the NF-κB binding to its target DNA and thereby inhibit the transcription of target gene. Similar results in human lung epithelial cells and myeloid cells have been reported by other investigators [34,35]. It is important to note that the concentration of genistein used in experimental study is achievable in humans. The study reported by Busby et al. has shown that up to 27.46 ± 15.38 μM of genistein in human plasma can be achieved after receiving genistein supplement at a dose of 16.0 mg/kg [36], suggesting the bioavailability of genistein from supplement.

It has been found that oxidative stress activates NF-κB DNA binding activity [37,38]. Because soy isoflavones have been known as antioxidants, their inhibitory effects on oxidative stress may be mediated through inhibition of NF-κB DNA binding activity. We investigated whether soy isoflavone supplementation could inactivate NF-κB in vivo and reduce oxidative damage in lymphocytes in human volunteers [39]. We found that when human volunteers received 50 mg of soy isoflavone supplements NovasoyTM (Archer Daniels Midland Company, Decatur, IL, USA; containing genistein, daidzein, and glycitein at a 1.3:1:0.3 ratio) twice daily for 3 weeks, TNF-α failed to activate NF-κB activity in lymphocytes harvested from these volunteers, while lymphocytes from these volunteers collected prior to soy isoflavone intervention showed activation of NF-κB DNA binding activity upon TNF-α treatment in vitro. These results demonstrated that soy isoflavone supplementation had a protective effect against TNF-α induced NF-κB activation in humans. We further measured the levels of 5-OHmdU, a modified DNA base that represents endogenous status of cellular oxidative stress, in the peripheral blood lymphocytes of human volunteers before and after supplementation with NovasoyTM. The mean value of 5-OHmdU before supplementation was 156.7 ± 25.72 and it was decreased to 60.83 ± 12.61 (P < 0.01) after 3 weeks of soy supplementation. These results provide evidence showing that soy isoflavones function as antioxidants and inhibit NF-κB activation, suggesting that these effects of soy isoflavones may be responsible for its cancer chemopreventive activity.

It has been known that NF-κB could be activated by phosphorylation and degradation of IκB [40]. IκB could be phosphorylated by activated IκB kinase (IKK), and IKK could be phosphorylated and activated by mitogen activated kinase kinase 1 (MEKK1), one of the molecules in MAPK pathway [41–43]. We found
that genistein treatment reduced the amount of phosphorylated IκB, demonstrating that genistein inhibits the phosphorylation of IκB and ultimately prevents the translocation of NF-κB to the nucleus. Moreover, we found that genistein treatment did not alter the protein expression of MEKK1; however, it did inhibit MEKK1 kinase activity in prostate cancer cells. These results suggested that genistein could inhibit MEKK1 kinase activity, which could be responsible for the decreased phosphorylation of IκB and, thereby, result in the inactivation of NF-κB (Fig. 1).

Our laboratory also investigated whether I3C treatment could modulate NF-κB DNA binding activity in PC3 prostate cancer cells by EMSA [8]. The results showed that 60 μM I3C significantly inhibited NF-κB DNA binding activity with induction of apoptosis in PC3 prostate cancer cells, suggesting that inhibition of NF-κB signaling pathway may be one of the molecular mechanisms by which I3C induces apoptosis in cancer cells (Fig. 1).

Other chemopreventive agents also show their inhibitory effects on NF-κB pathway. It has been reported that curcumin inhibited IKK, suppressed both constitutive and inducible NF-κB activation, and potentiated TNF-induced apoptosis [30]. Curcumin also showed strong antioxidant and anticancer properties through regulating the expression of genes that require the activation of activator protein 1 (AP1) and NF-κB [44]. It has been reported that EGCG treatment resulted in a significant dose- and time-dependent inhibition of activation and translocation of NF-κB to the nucleus by suppressing the degradation of IκBα in the cytoplasm [45,46]. EGCG also showed to inhibit activation of IKK and phosphorylation of IκBα [32,47]. It has been found that EGCG had a concurrent effect on two important transcription factors p53 (stabilization of p53) and NF-κB (negative regulation of NF-κB activity), causing a change in the ratio of Bax/Bcl-2 in a manner that favors apoptosis [13]. Apigenin treatment also resulted in down-modulation of the constitutive expression of NF-κB/p65 [33].

3. Effects of chemopreventive agents on Akt

Akt plays critical roles in mammalian cell survival signaling and has been shown to be activated in various cancers [48,49]. It has been known that Akt is activated by phospholipid binding and phosphorylation at Thr308 by PDK1 or at Ser473 by PDK2 [50]. Activated Akt functions to promote cell survival by inhibiting apoptosis through inactivation of several pro-apoptotic factors including Bad, Forkhead transcription factors, and caspase-9 [51–53]. Recent studies have also shown that Akt regulates the NF-κB pathway via phosphorylation and activation of molecules in the NF-κB pathway [54,55] (Fig. 1). Like NF-κB, Akt has also been believed to be an attractive target for cancer prevention or treatment [56].

The data from our laboratory showed that genistein inhibit both Akt and NF-κB pathways [57]. We found no alteration on the total Akt protein expression in genistein treated PC3 cells; however, decreases in the phosphorylated Akt protein at Ser473 and the Akt kinase activity were observed in genistein-treated PC3 cells, suggesting the inactivation of Akt after genistein treatment. We also found that genistein pretreatment abrogated the activation of Akt by EGF. To further explore the inhibitory mechanism of genistein on Akt and NF-κB pathways, Akt expression construct (pLNCX-Akt) was transiently co-transfected with NF-κB-Luc reporter construct into PC3 prostate cancer cells. Luciferase assay showed an increased luciferase activity in PC3 cells co-transfected with pLNCX-Akt and NF-κB-Luc, and abrogated the activation in PC3 cells co-transfected with pLNCX-Akt and NF-κB-Luc followed by EGF stimulation. These results were further confirmed by examining NF-κB DNA-binding activity in transfected cells using EMSA, which showed similar results to those of transfection and luciferase assay. We also observed similar results in MDA-MB-231 breast cancer cells [58]. These results suggest that genistein exerts its inhibitory effects on NF-κB pathway through Akt pathway, and that down-regulation of NF-κB and Akt signaling pathways by genistein may be one of the molecular mechanisms by which genistein inhibits cancer cell growth and induces apoptosis (Fig. 1).

To explore the effect of I3C on Akt pathway, we examined Akt status in PC3 cells treated with 30, 60, and 100 μM I3C by Western blot, immunoprecipitation, and kinase assays [59]. We found a decrease in the phosphorylated Akt protein at Ser473 and Thr308 in I3C treated PC3 cells, suggesting inactivation of Akt after I3C treatment. These results were confirmed by
Akt kinase assay, which showed a decrease in the Akt kinase activity in I3C treated PC3 cells. We also found that I3C pretreatment abrogated the activation of Akt by EGF. From the gene expression profiles of PC3 cells exposed to I3C, we found down-regulation of PI3K expression, consistent with our results showing inactivation of Akt kinase by I3C [9]. These data demonstrated that I3C inhibited Akt signaling pathway, which may result in the inhibition of survival signals and the induction of apoptotic signals (Fig. 1).

Similar to our results about the effect of genistein on Akt, Kumar et al. have reported that an analogue of curcumin, 4-hydroxy-3-methoxybenzoic acid methyl ester (HMBME), targeted the Akt signaling pathway, inhibited the proliferation of human and mouse prostate cancer cells and induced apoptosis [60]. Transfection experiment showed that overexpression of constitutively active Akt reversed the HMBME-induced growth inhibition and apoptosis, demonstrating the direct role of Akt signaling in HMBME-mediated growth inhibition and apoptosis. HMBME also decreased the level of phosphorylated Akt, inhibited Akt kinase activity, and reduced DNA-binding activity of NF-κB [60]. Several reports by other investigators also suggest that curcumin has molecular targets within the Akt signaling pathways, and the inhibition of Akt activity may facilitate inhibition of proliferation and induction of apoptosis in cancer cells [61,62].

Recent report has shown that EGCG from green tea inhibits VEGF-induced angiogenesis in vitro through suppression of VE-cadherin phosphorylation and inactivation of Akt molecule, suggesting inhibitory effect of EGCG on Akt signaling pathway [63]. Masuda et al. also found that treatment with EGCG inhibited the constitutive activation of the Akt, EGFR, and Stat3 in both YCU-H891 head and neck squamous cell carcinoma and MDA-MB-231 breast carcinoma cell lines [64].

4. Effects of chemopreventive agents on MAPK

In addition to NF-κB and Akt pathways, MAPK has received increasing attention as a target molecule for cancer prevention and therapy. It has been reported that activation of the MAPK pathways may cause the induction of phase II detoxifying enzymes, and inhibition of MAPK pathways may inhibit AP-1-mediated gene expression [65]. MAPK pathway consists of a three-tiered kinase core where a MAP3K activates a MAP2K that activates a MAPK (ERK, JNK, and p38), resulting in the activation of NF-κB, cell growth, and cell survival [66,67] (Fig. 1).

We have utilized the high-throughput gene chip, which contains 22,215 known genes, to determine the alternation of gene expression profiles of PC3 prostate cancer cells exposed to I3C or DIM [9]. From microarray data, we observed down-regulation in the expression of MAP2K3, MAP2K4, MAP4K3, and MAPK3 by I3C and DIM treatment, suggesting the inhibitory effects of I3C and DIM on MAPK pathway. The down-regulation of the important molecules in MAPK pathway may result in the inhibition of cancer cell survival (Fig. 1).

The ability of curcumin to modulate MAPK signaling pathway might contribute to the inhibition of inflammation by curcumin. Salt et al. reported that curcumin is able to attenuate experimental colitis through a reduction in the activity of p38 MAPK [68].

The reported effects of EGCG on MAPK pathway are controversial. EGCG showed strong inhibition of tyrosine kinase and MAPK activities in transformed NIH-pATM ras fibroblasts, without affecting the kinases in the normal cells [69]. Katiyar et al. reported that treatment of H₂O₂ resulted in phosphorylation of ERK1/2, JNK, and p38 in human epidermal keratinocytes [70]. When these cells were pretreated with EGCG, H₂O₂-induced phosphorylation of ERK1/2, JNK, and p38 was found to be significantly inhibited. These findings demonstrate that EGCG has the potential to inhibit oxidative stress-mediated phosphorylation of MAPK signaling pathways. Maeda-Yamamoto et al. also reported that EGCG inhibited the phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), and suppressed p38 MAPK activity in human fibrosarcoma HT1080 cells [71]. However, EGCG has also been found to activate all three MAPKs (ERK, JNK, and p38) in a dose- and time-dependent manner in human hepatoma HepG2-C8 cells [72]. In breast cancer cell line T47D, catechin (containing approximately 53% of EGCG) showed to phosphorylate JNK/SAPK and p38. The phosphorylated JNK/SAPK and p38 inhibited the phosphorylation of cdk2, and regulated the expression of cyclin A, cyclin B1, and cdk proteins, thereby causing G2 arrest [73]. It is possible that activation of MAPK by low concentration of EGCG re-
sults in induction of ARE-mediated gene expression, whereas higher concentration of EGCG causes activation of MAPKs such as JNK leading to apoptosis [72].

5. Effects of chemopreventive agents on p53

p53 is a tumor suppressor and transcription factor. It is a critical regulator in many cellular processes including cell signal transduction, cellular response to DNA-damage, genomic stability, cell cycle control, and apoptosis. As a tumor suppressor, functional p53 activates the transcription of downstream genes such as p21WAF1 and Bax to induce the apoptotic process, inhibiting the growth of DNA damaged cells or cancer cells [74–76]. The status of p53 is thought to be an important mediator in the cellular response to chemotherapy [77].

In order to investigate whether genistein inhibits proliferation and induces apoptosis through p53 pathway in non-small cell lung cancer (NSCLC) cells, we measured cell growth inhibition, apoptosis, and gene expression related to apoptosis in genistein treated H460 cells, which harbor wild type p53, and H322 cells that possess a mutation in the p53 gene (codon 248, CGG to CTG, Arg to Leu) [78,79]. Genistein was found to inhibit both H460 and H322 cell growth in a dose-dependent manner. DAPI staining, poly(ADP-ribose) polymerase (PARP) cleavage, and flow cytometric apoptosis analysis showed that genistein at 30 μM caused cell death via a typical apoptotic pathway in both cell lines. Western blot analysis revealed that the expression of Bax and p21WAF1 was up-regulated in both H460 and H322 cells treated with genistein. More importantly, significant up-regulation of p53 was detected in genistein-treated H460 cells, while no change in p53 expression was observed in H322 cells that underwent the same genistein treatment. Theses results suggest that genistein induces apoptosis in NSCLC cells through p53 independent pathway and, thus, may act as an anticancer agent regardless of the status of p53 in cancer cells.

Similar results have been observed in prostate cancer cells treated with EGCG. By using Western blot analysis, Gupta et al. found that EGCG treatment resulted in a dose-dependent increase of p53 in LNCaP cells (carrying wild-type p53), but not in DU145 cells (carrying mutant p53) [80]. They also found that EGCG induced stabilization of p53, which caused an up-regulation in its transcriptional activity, thereby resulting in the activation of its downstream targets such as p21WAF1 and Bax and the induction of apoptosis. In a human liver cancer cell line, EGCG also significantly increased the expression of p53 and p21WAF1 protein, and this contributed to cell cycle arrest [81].

Several studies examined the potential effects of I3C and DIM on the proliferation and induction of apoptosis in human prostate cancer cell lines with different p53 status. They found that induction of apoptosis by I3C was p53-independent [82]. Also, induction of p21WAF1 expression by DIM was independent of estrogen-receptor signaling and p53 [83].

6. Effects of chemopreventive agents on AR

It has been found that androgen receptor (AR) signaling pathway plays important roles in the carcinogenesis and cancer progression through regulation of transcription of androgen-responsive genes [84] (Fig. 2). Several chemopreventive agents including genistein, I3C, DIM, and curcumin, have been found...
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to regulate the molecules in AR signaling pathway when they were used to inhibit growth of cancer cells. Prostate specific antigen (PSA), one of the androgen-responsive genes, is a clinically important marker used to monitor diagnosis, progression, and prognosis of patients with prostate cancer. By using Western, Northern blots and EMSA analysis, we found that genistein at low concentration (<10 μM) transcriptionally down-regulated AR, decreased nuclear protein binding to androgen-responsive element (ARE) and, thereby, inhibited the transcription and protein expression of PSA in androgen-sensitive LNCaP cells [85,86] (Fig. 2). However, higher concentrations (10–50 μM) of genistein were needed to significantly inhibit PSA secretion in androgen-insensitive VCaP cells without alterations in the AR expression and ARE binding activity. These results suggest that genistein may be a powerful agent in the inhibition of PSA. We further transfected a PSA promoter-reporter construct into LNCaP and VCaP cells followed by treatment with or without genistein (0.5–50 μM) in the presence of media with or without R1881, a synthetic androgen. We found that genistein inhibited PSA synthesis in prostate cancer cells through both androgen-dependent and androgen-independent pathway, suggesting that genistein may act as a chemopreventive and/or therapeutic agent for prostate cancer irrespective of androgen responsiveness. Fritz et al. found that dietary genistein down-regulated expression of AR in the rat prostate at concentrations comparable to those found in humans on a soy diet [87]. Down-regulated AR expression may be responsible for the lower incidence of prostate cancer in populations on a diet containing high levels of phytoestrogens. Another chemopreventive agent EGCG also showed a dose-dependent inhibition of cell growth in both androgen-insensitive DU145 and androgen-sensitive LNCaP cells [80].

Similar to our results about the effects of genistein on AR pathway in prostate cancer cells, Le et al. also reported that DIM inhibited cell proliferation, endogenous PSA transcription, and intracellular and secreted PSA protein expression induced by dihydrotestosterone (DHT) in LNCaP cells [88]. They found that DIM inhibited androgen-induced androgen receptor (AR) translocation into the nucleus. Results of receptor binding assays indicated that DIM was a strong competitive inhibitor of DHT binding to the AR, suggesting that DIM is a strong androgen antagonist in human prostate cancer cells. Gupta et al. also reported that epigallocatechin treatment resulted in a significant decrease in AR protein expression along with a decrease in intracellular and secreted forms of PSA in LNCaP cells [33]. The effects of curcumin on cell growth, activation of signal transduction, and transforming activities in both androgen-dependent and -independent cell lines have been evaluated. Nakamura et al. have found that curcumin down-regulates transactivation and expression of AR and AR-related cofactors (AP-1 and NF-κB), and reduces colony forming ability in soft agar [88]. A number of curcumin analogues was evaluated as potential androgen receptor antagonists against two human prostate cancer cell lines, PC-3 and DU-145, in the presence of androgen receptor (AR) and androgen receptor coactivator, ARA70 [90]. The results showed that some curcumin analogues possessed potent anti-androgenic activities and were superior to hydroxyflutamide, which is the currently available anti-androgen for the treatment of prostate cancer. Structure–activity relationship (SAR) studies demonstrated that some moieties seem to be important factors related to the anti-androgenic activity. These results suggest that these compounds may serve as a new class of anti-androgen agents to control androgen receptor-mediated prostate cancer growth.

7. Effects of chemopreventive agents on ER

Many environmental chemicals have been found to be estrogenic and have been shown to stimulate the growth of ER-positive human breast cancer cells [91,92]. Because it is difficult to avoid human exposure to environmental estrogens, it is important to develop dietary strategies to prevent the stimulated growth of breast tumors by environmental estrogens. Isoflavone has a close similarity in structure to estrogen, and has been known as phytoestrogen. Because of the structural similarity to estrogen, isoflavonoids have been believed to exert their effects through ER signaling pathway. However, experimental study has found that isoflavonoids at different concentration may exhibit different effects [93]. Genistein at concentrations ≤1 μM may induce breast cancer cell proliferation by estrogen agonistic properties, while genistein at concentrations ≥5 μM may prevent hormone-dependent growth of breast can-
cer cells by potential estrogen-antagonistic activity. Fritz et al. found that dietary genistein down-regulated expression of ER-α and -β in rat at concentrations comparable to those found in humans on a soy diet [87]. Recent studies from Chen et al. [94] showed that genistein at 50 and 100 μM significantly arrested the growth of MCF-7 cells at G2/M phase and down-regulated mRNA expression of ERLs, suggesting that the inhibitory action of genistein on human breast cancer cells appears to be partially mediated by the alteration of estrogen receptor-dependent pathways. However, experimental studies also showed that isoflavones exert their inhibitory effects on ER-negative MDA-MB-231 breast cancer cells [95] and hormone-independent cancer cells [78, 79, 96–99]. These results suggest that isoflavones may exert their effects through ER-dependent (Fig. 2) or independent pathway.

I3C has been known to be a negative regulator of estrogen. When cells were treated with I3C and genistein, a synergistic effect of I3C and genistein was observed on the increase in GADD (growth arrest and DNA damage) expression, the induction of apoptosis, and the decrease in gene expression driven by ERLs in MCF-7 breast cancer cells [100]. I3C significantly repressed the transcriptional activity of ERLs, the estradiol-activated ERLs signaling, and the expression of the estrogen-responsive genes, pS2 and cathepsin-D [101]. These results suggest that anti-tumor activities of I3C are associated not only with its regulation of estrogen activity and metabolism, but also its modulation of ER transcription activity. However, I3C has also been found to inhibit the expression of CDK6 and induces a G1 cell cycle arrest of human breast cancer cells, independent of estrogen-receptor signaling [102].

The effects of curcumin on ER signaling pathway have been investigated in ER-positive human breast cancer line MCF-7 and ER-negative human breast cancer line MDA-MB-231 [103]. The results showed that curcumin inhibited the proliferation of both ER-positive and ER-negative cells. The antiproliferative effect of curcumin was estrogen dependent in ER-positive MCF-7 cells. It has been reported that curcumin inhibited the expression of ER downstream genes including pS2 and TGF-α in ER-positive MCF-7 cells, and this inhibition was dependent on the presence of estrogen [17]. However, curcumin also exerted strong anti-invasive effects in vitro in ER-negative MDA-MB-231 breast cancer cells, and this effect was not estrogen dependent [17]. These results suggest that curcumin may exert its chemopreventive effects through ER-dependent or -independent pathway.

Because both genistein and curcumin showed inhibitory effects on ER-positive and -negative breast cancer cells, the inhibitory effects of a combination of curcumin and genistein were studied in ER-positive human breast cancer cells (MCF-7 and T47D) and ER-negative MDA-MB-231 cells [103]. The results showed that combination of curcumin and genistein significantly inhibited the growth of ER-positive cells. For ER-negative MDA-MB-231 cells, the IC50 for curcumin was 17 μM, which was reduced to 11 μM in the presence of 25 μM genistein. Curcumin and genistein also induced drastic changes in the morphological shape of both ER-positive and -negative cells. These results suggest that combination of natural plant compounds may have stronger preventive and therapeutic effects against the growth of breast cancers.

EGCG has been found to bind to ERs and ERβ, and elicit ER-mediated gene expression in vitro. It has been found that EGCG at higher dose is anti-estrogenic for ERs, however, it is estrogenic for ERs and also for ERβ at lower doses [104]. The in vitro and in vivo studies have demonstrated that polyphenolic catechins (EGCG and ECG) from green tea bind to ERs and ERβ, and inhibited breast cancer cell proliferation and tumor growth, but only EGCG elicited ER-mediated gene expression [105], suggesting that polyphenolic catechins may exert their chemopreventive effects through ER-dependent or independent pathway.

8. Summary and perspectives

The data from in vivo human and animal studies and in vitro experiments clearly indicate that natural chemopreventive agents exert their inhibitory effects on carcinogenesis and tumor progression. These effects have been believed to be mediated through the regulation of cell signaling pathways including NF-κB, Akt, MAPK, p53, AR, and ER pathways. As we discussed earlier, there are cross-talks between these pathways. Natural chemopreventive agents could exert their effects on these pathways separately or sequentially. By modulating cell signaling pathways, chemopreventive agents activate cell death signals and induce apoptosis in precancerous or cancer cells, resulting in the inhibi-
tion of cancer development or progression. It appears that the effects of these chemopreventive agents are not affected by the endogenous molecular status such as p53 mutations and ER status of cancer cells. However, the regulation of cell signaling pathways by natural chemopreventive agents is important event in the prevention of cancers. More in depth in vitro and in vivo experiments are needed to fully elucidate the molecular mechanisms of action of chemopreventive agents in future studies.

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