

Using Chemopreventive Agents to Enhance the Efficacy of Cancer Therapy

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Abstract

Emerging evidence suggests that cancer preventative agents might be combined with chemotherapy or radiotherapy for the more effective treatment of cancer. Recent studies suggest that genistein and other dietary compounds that prevent cancer may enhance the efficacy of cancer therapeutics by modifying the activity of key cell proliferation and survival pathways, such as those controlled by Akt, nuclear factor- κ B, and cyclooxygenase-2. In this article, we summarize the findings of recent investigations of chemopreventive agents in combination with cancer treatment regimens. (Cancer Res 2006; 66(7): 3347-50)

Background

Conventional cancer therapies, including surgery, chemotherapy, and radiotherapy, as single modalities have a limited but important role in the overall treatment of most solid tumors. Thus, the strategies of cancer treatment using combined therapies or combined agents with distinct molecular mechanisms are considered more promising for higher efficacy, resulting in better survival. In recent years, more dietary compounds [i.e., genistein, 3,3'-diindolylmethane, indole-3-carbinol (I3C), curcumin, (–)-epigallocatechin-3-gallate (EGCG), resveratrol, etc.] have been recognized as cancer chemopreventive agents because of their anticarcinogenic activity (1). Moreover, these compounds also exert the antitumor activities through regulation of different cell signaling pathways. Therefore, common cancer therapies combined with these dietary compounds may exert enhanced antitumor activity through synergic action or compensation of inverse properties. The combination treatment may also decrease the systemic toxicity caused by chemotherapies or radiotherapies because lower doses could be used. In this short article, we review current knowledge of the effects and the molecular mechanisms of the combination treatments published thus far, to give a brief view on the new and emerging field for optimal treatment of cancer patients with better survival.

Antitumor Activity of Common Cancer Therapies Are Potentiated by Chemopreventive Agents

Recently, there has been a growing interest in investigating the effects of genistein and other chemopreventive agents on the inhibition of cancer cell growth in combination with chemotherapeutics or other common therapies. The number of publications regarding potentiated antitumor effects of cancer therapies by chemopreventive agents has dramatically increased in 2005, suggesting that novel combination treatments with common cancer

therapies and chemopreventive agents are beginning to receive much attention in cancer research.

Potentiation of chemotherapeutic effects. The *in vitro* and *in vivo* studies from our laboratory and others have shown that the antitumor effects of chemotherapeutic agents could be enhanced by combination treatment with chemopreventive agents. We have reported that genistein *in vitro* potentiated growth inhibition and apoptotic cell death caused by cisplatin, docetaxel, doxorubicin, and gemcitabine in prostate, breast, pancreas, and lung cancers (2–4). We found that pretreatment of cancer cells with 15 to 30 μ mol/L genistein before the treatment with lower doses of chemotherapeutic agents caused a significantly greater degree of growth inhibition and apoptotic cell death, suggesting that increased antitumor activities of chemotherapeutic agents with lower toxicity to normal cells could be achieved by introducing genistein into the chemotherapeutic strategy. To investigate whether these phenomena that we observed *in vitro* could also exit *in vivo*, we conducted animal studies. We found that dietary genistein could potentiate the antitumor activities of gemcitabine and docetaxel in a tumor model, resulting in more tumor cell killing and apoptotic cell death (2, 3). By *in vitro* and *in vivo* studies, we also found that genistein could sensitize diffuse large cell lymphoma to cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) chemotherapy (5). These results suggest that genistein enhances antitumor activities of chemotherapeutic agents both *in vitro* and *in vivo* in multiple tumors.

Other investigators have reported similar observations showing that the antitumor effects of chemotherapeutics could be enhanced by genistein. Hwang et al. recently reported that the combination of genistein and 5-fluorouracil (5-FU) synergistically induced apoptosis in chemoresistant HT-29 colon cancer cells (6). Genistein was also shown to enhance necrotic-like cell death in HER-2 over-expressing breast cancer cells treated with Adriamycin (7). Tanos et al. found that 1 to 10 μ g/mL genistein inhibited the growth of dysplastic and malignant epithelial breast cancer cells *in vitro*, and that the addition of tamoxifen has a synergistic/additive inhibitory effect on breast cancer growth (8). These effects were not modulated by estrogen receptor. In addition, genistein and its isoflavone analogues have been found to decrease the side effects of tamoxifen through P450-mediated pathways (9). Thesis results support our findings and suggest the beneficial effects of genistein on cancer chemotherapy.

In addition to genistein, other dietary chemopreventive agents, including curcumin, EGCG, resveratrol, I3C, proanthocyanidin, and vitamin D, have been shown to enhance the antitumor activities of chemotherapeutic agents. A recent report by Lev-Ari et al. showed that curcumin and celecoxib synergistically inhibited the growth of colorectal cancer cells (10). Curcumin also enhanced the antitumor activities of cisplatin, doxorubicin, and Taxol in HA22T/VGH hepatic cancer cells, HeLa cells, or CAO3 and SKOV3 ovarian cancer cells (11–13). In addition, the combined curcumin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)

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treatment increased the number of hypodiploid cells and induced DNA fragmentation in LNCaP cells, suggesting a potential use of curcumin to sensitize prostate cancer cells for TRAIL-mediated immunotherapy (14). It has been reported that EGCG and tamoxifen synergistically induced apoptosis and growth inhibition in MDA-MB-231 human breast cancer cells (15). EGCG could also chemosensitize resistant tumor cells to doxorubicin through an increase in the accumulation of doxorubicin in the tumors of human carcinoma xenograft model (16). It has been found that resveratrol from grapes could sensitize non-Hodgkin's lymphoma and multiple myeloma cells to paclitaxel-mediated apoptosis (17). Proanthocyanidin, another compound from grapes, has been reported to enhance doxorubicin-induced antitumor effect and reverse drug resistance in doxorubicin-resistant K562/DOX cells, breast cancer cells, and mouse tumor xenograft models (18, 19). We and others have found that combinations of I3C and cisplatin or tamoxifen cooperate to inhibit the growth of PC-3 prostate and MCF-7 breast cancer cells more effectively than either agent alone (20, 21). In addition, analogues of vitamin D were also shown to potentiate the antiproliferative effect of doxorubicin, cisplatin, and genistein *in vitro* (22). These results clearly show that dietary cancer preventive agents can potentiate antitumor activities of common chemotherapeutics.

Potential of radiotherapy. We have investigated the effect of the combination of genistein and radiation on PC-3 prostate cancer cells. We found that the combination of genistein and radiation showed enhanced inhibitory effects on DNA synthesis, cell growth, and colony formation *in vitro* (23). Furthermore, we found that genistein combined with radiation led to a greater control of the growth of the primary tumor and metastasis to lymph nodes than genistein or radiation alone, suggesting that genistein enhanced the radiosensitivity of PC-3 prostate cancer cells (24). A similar report by other investigators showed that genistein enhanced the radiosensitivity of cervical cancer cells through increased apoptosis, prolonged cell cycle arrest, and impaired damage repair (25). Genistein was also shown to enhance radiosensitivity in human esophageal cancer cells *in vitro* (26), suggesting that the enhancement of radiosensitivity by genistein is not cell type dependent. Apart from genistein, another important chemopreventive agent, curcumin, at a low concentration in combination with radiation showed significant enhancement to radiation-induced clonogenic inhibition and apoptosis in PC-3 prostate cancer cells (27). These reports show that the radiotherapy combined with chemopreventive agents can cause more growth inhibition and apoptotic cell death of various cancers compared with monotherapy.

Molecular Mechanisms of Combination Treatment

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. The major culprits involved in the development of drug resistance are multidrug resistance gene, nuclear factor- κ B (NF- κ B), and Akt. We and other investigators have found that the enhanced antitumor effects by chemopreventive agents could be, in part, through the regulation of NF- κ B, Akt, and cyclooxygenase-2 (COX-2) pathways, which play important roles in cell survival (Fig. 1). Chemopreventive agents could also sensitize cancer cells to apoptosis by regulating several important molecules (i.e., Bcl-2,

Bcl-X_L, survivin, caspases, p21^{WAF1}, etc.) in the apoptotic pathway (Fig. 1).

Regulation of the Akt pathway. Akt pathway is an important cell signaling pathway involved in drug resistance. It has been found that genistein enhanced necrotic-like cell death with the significant inhibition of Akt activity in breast cancer cells treated with genistein and Adriamycin, suggesting that the enhanced growth inhibition of combination is through the inactivation of the Akt pathway (7). Reports from our laboratory and others also showed that activated Akt was inhibited by genistein combined with gemcitabine or radiation in pancreatic, cervical, and esophageal cancer cells, suggesting that enhancement of chemotherapeutic or radiation effects by genistein may be partially mediated by the Akt pathway (3, 25, 26). Bava et al. recently reported that curcumin down-regulated Taxol-induced phosphorylation of Akt, which interacts with NF- κ B, suggesting that enhanced antitumor activity by curcumin is through the Akt and NF- κ B pathways (12).

Regulation of NF- κ B pathway. It has been known that many chemotherapeutic agents induce activity of NF- κ B, which causes drug resistance in cancer cells (28). By *in vitro* and *in vivo* studies, we found that NF- κ B activity was significantly increased by cisplatin, docetaxel, gemcitabine, and radiation treatment, and that the NF- κ B inducing activity of these agents was completely abrogated by genistein pretreatment in prostate, breast, lung, and pancreatic cancer cells, suggesting that genistein pretreatment inactivates NF- κ B and may contribute to increased growth inhibition and apoptosis induced by these agents (2-4, 23). We also found that genistein potentiated the antitumor activity of CHOP by inhibition of NF- κ B in lymphoma cells (5). Similarly, curcumin has been found to inhibit the activity of NF- κ B and sensitize cancer cells to cisplatin or Taxol-induced apoptosis (12, 29).

Regulation of apoptosis pathways. It has been reported that curcumin combined with cisplatin decreased the expression of several apoptosis-related genes, including *c-myc*, *Bcl-X_L*, *c-IAP-2*, *NAIP*, and *XIAP* (11). The combination of curcumin and TRAIL also induced cleavage of procaspase-3, procaspase-8, and procaspase-9; truncation of Bid; and release of cytochrome *c* from the mitochondria in prostate cancer cells, indicating that the apoptotic pathway is triggered in prostate cancer cells treated with combination of curcumin and TRAIL (14). We and others also found that genistein combined with docetaxel or gemcitabine significantly inhibited Bcl-2, Bcl-X_L, and survivin and induced p21^{WAF1}, suggesting that combination treatment regulates the important molecules in the apoptotic pathway (2, 3).

Regulation of other pathways. It has been found that the combination of 5-FU and genistein enhanced therapeutic effects in colon cancers through the COX-2 pathway (6). A recent report showed that curcumin or EGCG could down-regulate COX-2 expression without any change of COX-1 expression at both the mRNA and protein levels in colorectal or prostate cancer cells, suggesting that a combination of curcumin or EGCG with chemotherapeutic agents could be an improved strategy for the treatment of colorectal or prostate cancer (10, 30). Indeed, the synergistic growth inhibitory effect of curcumin and celecoxib was found in colorectal cancer cells through inhibition of the COX-2 pathway (10). Apart from the COX-2 pathway, the molecules in cell cycle regulation may also be involved in mechanisms of combination treatment. It has been reported that combination of I3C and tamoxifen caused a more pronounced decrease in cyclin-dependent kinase 2 (CDK2)-specific enzymatic activity, CDK6 expression, and

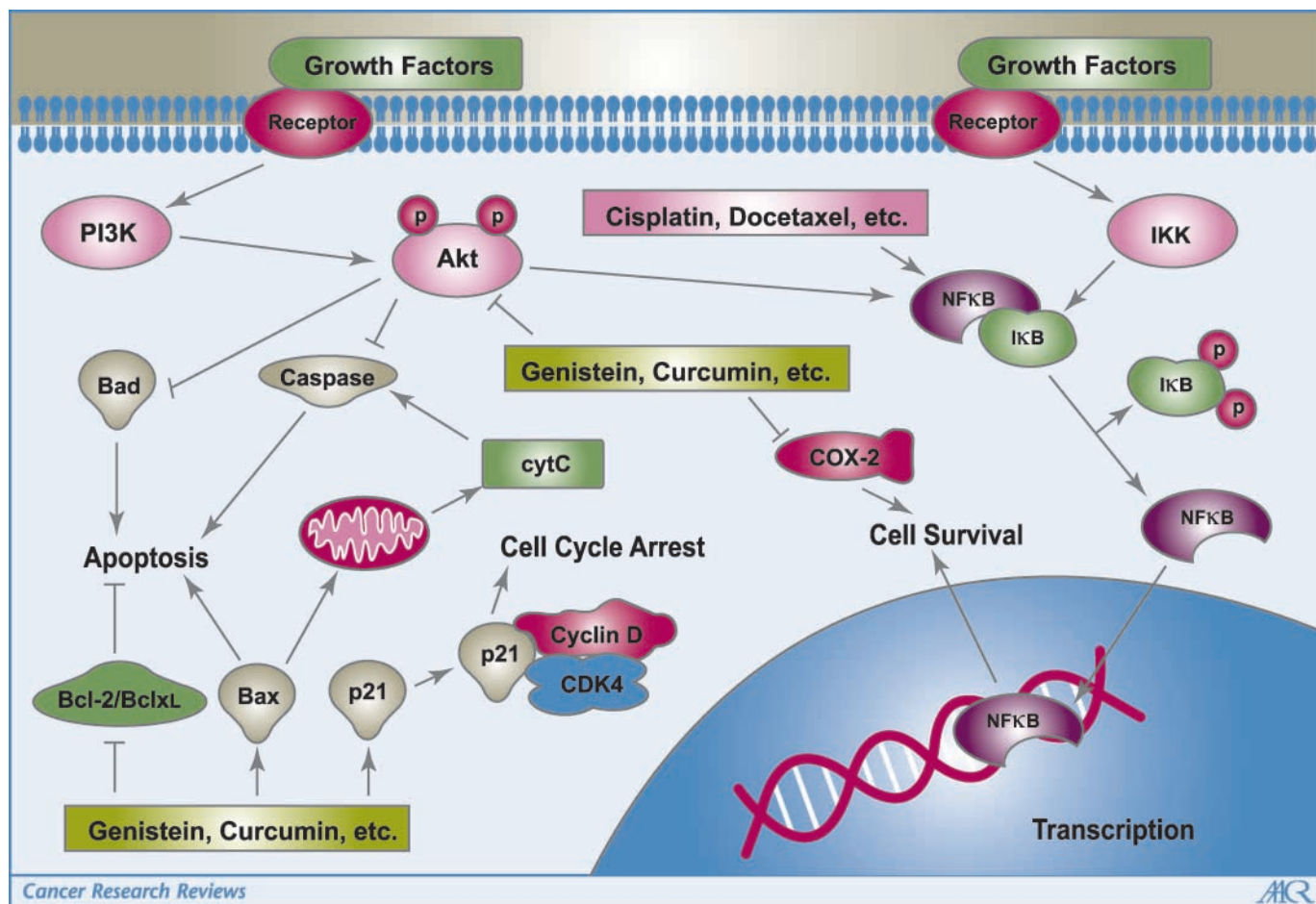


Figure 1. Chemopreventive agents enhance antitumor effects of chemotherapies and radiotherapies through the regulation of Akt, NF- κ B, COX-2, and apoptosis pathways. *cytC*, cytochrome *c*.

the level of phosphorylated retinoblastoma protein (20). The enhanced effects of chemotherapy by chemopreventive agents may also be related to immunopotentiating activities through reduction of interleukin-6 (IL-6; ref. 13) and enhancements of lymphocyte proliferation, natural killer cell cytotoxicity, CD4⁺/CD8⁺ ratio, IL-2, and IFN- γ productions (18). In addition, genistein and its isoflavone analogues showed the potential to decrease side effects of tamoxifen through metabolic interactions that inhibit the formation of α -hydroxytamoxifen via inhibition of CYP1A2 (9), suggesting the beneficial effects of genistein in combination with tamoxifen.

Conclusion and Perspective

The *in vitro* and *in vivo* studies reviewed above all suggest that dietary chemopreventive agents may serve as potent agents for

enhancing the therapeutic effects of chemotherapy, radiotherapy, or other standard therapeutics for the treatment of human cancers. However, further in-depth mechanistic studies, *in vivo* animal experiments, and clinical trials are needed to bring this concept into practice to fully appreciate the value of chemopreventive agents in combination therapy of human cancers.

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