The Role of Genistein and Synthetic Derivatives of Isoflavone in Cancer Prevention and Therapy

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Abstract: Genistein, one of the predominant soy isoflavones, has been shown to compete with 17β-estradiol for estrogen receptor binding because of its structural similarity, resulting in agonistic or antagonistic activity. It causes inhibition of cell growth in breast and prostate cancers *in vivo* and *in vitro*. From gene expression profiles, genistein has been found to regulate the genes that are critical for the control of cell proliferation, cell cycle, apoptosis, oncogenesis, transcription regulation, and cell signal transduction pathways. It has been reported that genistein induces apoptosis and inhibits activation of NF-κB and Akt signaling pathways, both of which are known to maintain a balance between cell survival and apoptosis. Recently, we found that genistein sensitized cancer cells to apoptosis induced by chemotherapeutic agents including docetaxel, gemcitabine and cisplatin through inactivation of NF-κB in multiple cancer cell lines. To enhance the anti-cancer activity of genistein, we have synthesized structurally-modified derivatives of isoflavone based on the structural requirements for optimal anti-cancer effect. We found that these synthetic derivatives of isoflavone exerted higher anti-cancer activity with lower IC_{50}. These derivatives of isoflavone also induced more apoptosis compared to genistein. These results suggest that genistein and synthetic structurally-modified derivatives of isoflavone may be promising agents for cancer chemoprevention and therapy either alone or in combination with existing chemotherapeutic agents.

INTRODUCTION

In the last decade, soy isoflavones mainly derived from soybean have received much attention as dietary components having inhibitory effects on cancers. The lower risk of breast and prostate cancers in Asians, who consume 20-50 times more soy than Americans, has raised the question whether compounds in the soy diet act as a natural chemopreventive agent [1-4]. Indeed, a cross national study involving 59 countries identified soy products as having a highly significant effect against the development of prostate cancer [2]. Elevated levels of soy isoflavones in the micromolar range have been detected in the serum, urine, prostatic fluid, and prostate tissue in vegetarians and Asian men who consume a soy rich diet and have low levels of prostate cancer [5-10]. In contrast, serum concentration of genistein in Americans and Europeans is in the nanomolar range [6, 7, 10, 11].

Soy isoflavones include genistein, daidzein, glycitein, etc. However, genistein (4,5,7-trihydroxyisoflavone) is the principal isoflavone in soy that has been demonstrated to be responsible for reducing the incidence of hormone-related cancers. In laboratory *in vitro* experiments, genistein has been found to inhibit the growth of various cancer cell lines including prostate and breast cancer cells [12-14]. Moreover, the evidences from *in vitro* studies have demonstrated that genistein exerts its inhibitory effects on the development of cancers, cancer cell growth, and cancer progression [12]. Furthermore, we found that genistein can inhibit cancer cell invasion, metastasis, and angiogenesis [15, 16], suggesting that genistein may be a promising agent for cancer prevention and/or treatment.

The structure of isoflavone genistein is very similar to that of estrogen (Fig. **1**), thus isoflavones have been known as phytoestrogens. Because of its structural similarity with estrogen, genistein can bind to estrogen receptors (ERs). Kuiper *et al* reported that the binding affinity of genistein for ER-α was 4%, and for ER-β was 87%, compared with estradiol [17]. Through binding to estrogen, genistein exerts its effects on cell signal transduction system and regulates the molecules in the cell signaling pathways. From *in vitro* and *in vivo* studies, we and others have found some critical molecular mechanism(s) by which genistein inhibits cancer cell growth [13-16, 18-28]. Recently, we have also synthesized derivatives of isoflavone and tested their effects on cancer cells. Here, we summarize the molecular mechanisms of action of genistein and the effects of synthetic derivatives of isoflavone on cancer cell growth in order to provide a comprehensive view on the role of genistein and synthetic derivatives of isoflavone, especially in the inhibition of growth of prostate and breast cancer cells.

MOLECULAR STRUCTURE AND BIOLOGICAL PROPERTIES OF GENISTEIN

The basic structural feature of genistein is the flavone nucleus, which is composed of two benzene rings (A and B) linked through a heterocyclic pyrane ring (C) (Fig. **1**) [29]. Because of the structural similarity to estrogen, genistein can bind to estrogen receptors. By interaction with estrogen

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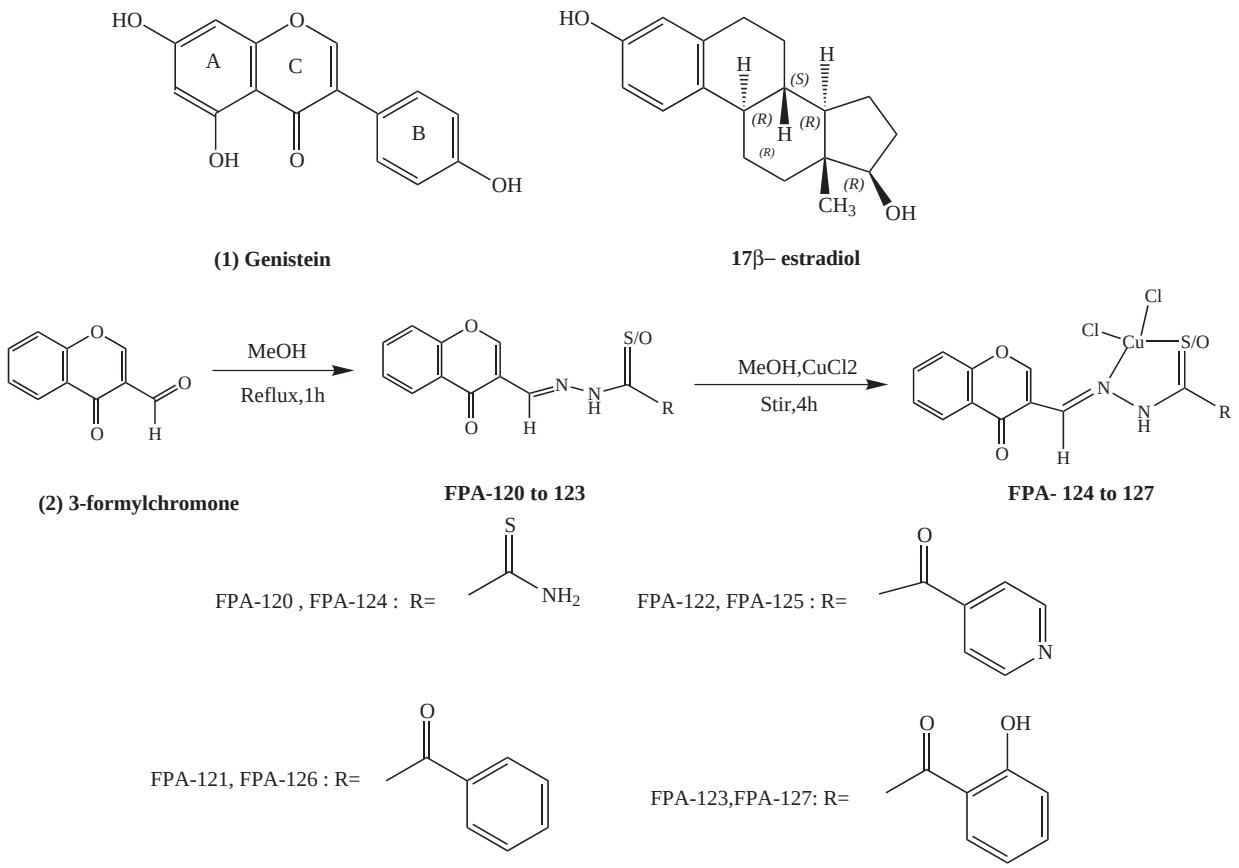

Fig. (1). Schematic structures of 17β-estradiol, genistein (**1**), 3-formylchromone (**2**), its Schiff bases (**FPA-120 to FPA-123**), and their copper conjugates (**FPA-124 to FPA-127**).

receptor, genistein blocks the binding of more potent estrogens at the same time and affects estrogen metabolism, thereby exerting a potential role in the prevention of hormone related cancer. It has been reported that genistein might either induce cell proliferation by estrogenic agonistic properties (at concentrations ≤1 μM) or prevent hormone-dependent growth of cancer cells by potential estrogen-antagonistic activity (at concentrations ≥5 μM) dependent on its concentrations [29]. Moreover, we and others have found that genistein could also inhibit cell growth and induce apoptosis in estrogen receptor negative breast cancer cells [13, 30], suggesting that genistein may exert its effects through ER dependent or independent mechanisms.

Genistein has many important biological effects that are believed to be responsible for the health benefits, especially the anti-cancer effect of genistein. In addition to function as an estrogen agonist or antagonist, genistein is a known inhibitor of protein-tyrosine kinase, which may attenuate the growth of cancer cells by inhibiting protein-tyrosine kinase mediated signaling pathway [31-33]. Genistein also inhibits topoisomerase I and II, 5α-reductase, and protein histidine kinase activity [34-36], all of which may contribute to anti-proliferative or proapoptotic effects of genistein. It has been found that soy isoflavones including genistein have antioxidant effect against TNF-α induced NF-κB activation in humans both *in vitro* and *in vivo* [20, 37-39]. Soy isoflavone genistein also protects cells against reactive oxygen species by scavenging free radicals, inhibiting the expression of stress-response related genes, and reducing their contribution to the progression of carcinogenesis [37, 38]. In animal studies, soy isoflavones including genistein have been found to suppress spontaneously developed and chemical induced prostate and breast cancers [40, 41], suggesting their inhibitory effects on oncogenesis. Growing evidences have shown that genistein can inhibit cancer cell growth and induce apoptotic cell death in both hormone-related and hormone-independent cancer cells through regulation of cell signaling transduction pathways [13-15, 18, 19, 23, 25, 28, 37, 38, 42, 43]. Moreover, genistein has shown its inhibitory effects on angiogenesis and cancer metastasis [15, 16, 23], suggesting that genistein may be a potent agent for prevention and/or treatment of cancers.

MOLECULAR MECHANISMS OF ACTION OF GENISTEIN

1. Regulation of AR Pathway

Because genistein is known as phytoestrogen, its effect on androgen receptor (AR) has also been investigated. AR signaling pathway has been involved in the development and progression of prostate cancer through regulation of transcription of prostate specific antigen (PSA) [44, 45]. We have investigated the effects of genistein on the expression of PSA through androgen regulation [21, 22]. We have found that genistein at low concentration (<10 μM) transcriptionally down-regulated AR and decreased nuclear protein binding to ARE, inhibiting the transcription and protein expression of PSA in androgen-sensitive LNCaP

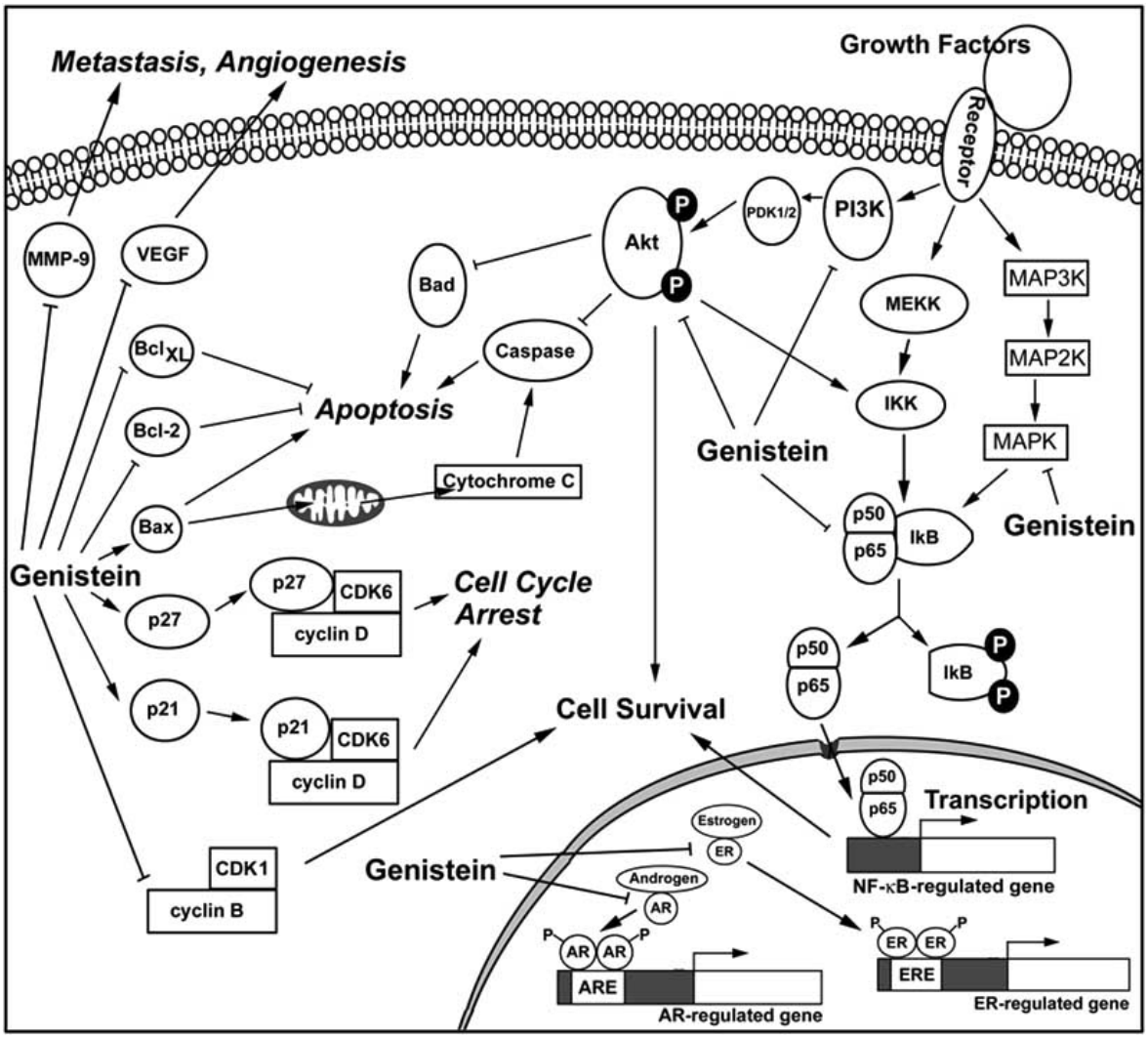

Fig. (2). The effects of genistein on cell cycle, apoptosis, ER, AR, NF-κB, Akt, and MAPK pathways.

cells (Fig. **2**). However, higher concentrations (10 to 50 μM) of genistein were needed to significantly inhibit PSA secretion in androgen-insensitive VeCaP cells. No alternation in the AR expression or ARE binding activity was observed in VeCaP cells. By transfection experiments, we found that genistein inhibited PSA synthesis in prostate cancer cells through both androgen-dependent and androgen-independent pathways. Other investigators also reported similar results, suggesting the inhibitory effects of genistein on AR and PSA [46].

2. Regulation of the Expression of Genes Related to Cell Cycle and Apoptosis

We and others have shown that the treatment of cells with different concentrations of genistein caused a dose-dependent decrease in the cyclin B_1 and cdc2 expression and the cdc2 kinase activity [19, 28, 42, 47], corresponding with the G2/M phase cell cycle arrest as observed by flow cytometry. Experimental data also showed a significant up-regulation of $p21^{WAF1}$ expression in genistein treated cancer cells compared to control cells [13, 15, 19, 23, 28, 28, 42, 48]. By microarray analysis, we further found that genistein inhibited cell growth through down-regulation of cell proliferation and cell cycle related genes (cyclin B, CDC25A, TGF-beta, ki67) [15]. These results suggest that down-regulation of cyclin B_1, cdc2, CDC25A, TGF-beta, and ki67, and up-regulation of $p21^{WAF1}$ could be one of the mechanisms by which genistein arrests cancer cells in G2/M phase and inhibits cancer cell growth (Fig. **2**), however, these molecules may not be involved during genistein induced apoptotic cell death.

To explore the molecular mechanisms by which genistein induces apoptosis, our laboratory has examined the expression of genes that are critically involved in the apoptotic pathways after genistein treatment. The results showed that genistein treatment reduced Bcl-2 protein expression and significantly increased expression of Bax in all cancer cells tested [13, 19, 23, 28]. Other investigators have also reported that soy isoflavone genistein could induce apoptosis in human hepatoma cells and breast cancer cells through caspase-3 activation and down-regulation of Bcl-2, Bcl-X_L, and HER-2/neu [49-52]. Kazi *et al* showed that genistein induced apoptosis by inhibition of proteasome and induction of $p27^{KIP1}$, IκB-α, and Bax [49]. These results suggest that caspase activation, inhibition of proteasome, up-regulation of Bax, and down-regulation of Bcl-2, Bcl-X_L,

and HER-2/neu may partly represent the molecular mechanisms by which genistein induces apoptosis (Fig. **2**) and many of these molecules may also be regulated directly or indirectly by the DNA-binding activity of NF-κB.

3. Regulation of NF-κB Pathway

Nuclear factor-κB (NF-κB) pathway plays important roles in the control of cell growth, differentiation, apoptosis, and stress response. Our laboratory examined NF-κB DNA-binding activity in genistein treated prostate and breast cancer cells by electrophoretic mobility shift assay (EMSA) [14, 20]. We found that 50 μM genistein significantly inhibited the NF-κB DNA-binding activity in prostate and breast cancer cells. NF-κB can be induced by known NF-κB inducers including H_2O_2 or TNF-α. However, when cancer cells were pre-treated with 50 μM genistein for 24 hours prior to stimulation with the inducing agents, genistein abrogated the induction of NF-κB DNA-binding activity induced by either H_2O_2 or TNF-α. By immunochemistry and confocal microscopic analysis, we also found that treatment of cells with TNF-α significantly increased nuclear staining of the p50 and p65 subunits; however, 24 hour pre-treatment with genistein prior to TNF-α stimulation blocked p50 and p65 nuclear translocation. By inhibition of NF-κB translocation to nucleus, genistein prevents NF-κB from binding to its target DNA and thereby inhibits the transcription of target genes (Fig. **2**) [20, 39].

We have also investigated the role of NF-κB up-stream genes including MEKK and IκB [53]. We found that genistein treatment did not alter the protein expression of MEKK1; however, it did inhibit the MEKK1 kinase activity in prostate cancer cells. Cells treated with TNF-α or H_2O_2 showed increased MEKK1 kinase activity as expected, and genistein pre-treatment blocked MEKK1 kinase activity [54]. We also found that genistein treatment reduced the amount of phosphorylated IκB, one of the MEKK1 downstream genes. These results suggested that genistein inhibits MEKK1 kinase activity, resulting in the decrease in the phosphorylation of IκB, thereby inactivating the DNA binding activity of NF-κB.

Genistein has also been found to potentiate the anti-tumor activity of chemotherapeutic agents through regulation of NF-κB. It has been reported that some chemotherapeutic agents such as cisplatin and docetaxel induce the activation of NF-κB in cancer cells and this may be responsible for drug resistance in cancer cells [55-57]. By *in vitro* and *in vivo* studies, we have shown that pre-treatment with genistein followed by treatment with lower doses of docetaxel or cisplatin elicited significantly greater inhibition of cell growth and induction of apoptosis compared to either agent alone [25, 27, 58]. By EMSA, we found that NF-κB activity was significantly increased by docetaxel or cisplatin treatment, and the NF-κB inducing activity of these agents was completely abrogated in cells pre-treated with genistein. These *in vitro* results were also recapitulated in our *in vivo* studies [27, 59]. Our results clearly suggest that genistein pre-treatment, which inactivates NF-κB activity, together with other cellular effects of genistein, may contribute to increased cell growth inhibition and apoptosis with non-toxic doses of docetaxel or cisplatin. Hwang *et al* also reported that combination of genistein with 5-fluorouracil induced apoptosis synergistically in chemo-resistant colon cancer cells [60]. Other investigators also found that genistein in combination with EGCG or vitamin D could exert enhanced anti-tumor activity through synergic action or compensation of inverse properties [61, 62], suggesting that administration of combined agents with distinct molecular mechanisms could be a more effective strategy for cancer prevention and therapy.

4. Regulation of Akt Pathway

In addition to NF-κB, the Akt signaling pathway also plays important roles in cell growth and apoptosis. Activated Akt, which is phosphorylated by PDK, functions to promote cell survival by inhibiting apoptosis through inactivation of pro-apoptotic factors [63-65]. By immunoprecipitation and kinase assay, we found that genistein reduced the level of phosphorylated Akt protein and the Akt kinase activity under non-stimulated conditions [14]. Genistein also abrogated Akt activation stimulated by EGF, suggesting that genistein could inactivate Akt kinase under both non-stimulated and stimulated conditions.

Akt also regulates NF-κB pathway *via* phosphorylation and activation of molecules in NF-κB pathway. We have investigated the inhibitory mechanisms of genistein on Akt and NF-κB pathways by transfection experiments [14]. Akt expression construct (pLNCX-Akt) or dominant-negative Akt construct (pLNCX-K179M) was transiently co-transfected with NF-κB-Luc reporter construct into PC-3 prostate cancer cells. We found an increased luciferase activity in PC-3 cells co-transfected with pLNCX-Akt and NF-κB-Luc, and a decreased activity in cells co-transfected with pLNCX-K179M and NF-κB-Luc. However, genistein inhibited the luciferase activity in PC-3 cells co-transfected with pLNCX-Akt and NF-κB-Luc, and abrogated EGF induced luciferase activity. EMSA testing for NF-κB DNA-binding activity in transfected cells also showed similar results. Stoica *et al* also demonstrated that genistein could inhibit Akt activation induced by estradiol in MCF-7 cells [66]. These results demonstrate that genistein exerts its inhibitory effects on NF-κB pathway through Akt pathway. 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. **2**).

5. Regulation of MAPK Pathway

In recent years, the molecules in MAPK pathway have also received increasing attention as targets for cancer prevention and therapy. 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 and cell survival [67, 68]. 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 [69].

Genistein has been found to regulate the molecules in MAPK pathway. Huang *et al* recently reported that genistein inhibited p38 map kinase activation, matrix metalloproteinase type 2, and cell invasion in human prostate epithelial cells [70]. They found that p38 MAPK is necessary

for TGF-β–mediated increases in MMP-2 activity as well as cell invasion. When prostate cancer cells were treated with genistein, TGF-β–mediated activation of p38 MAPK was blocked, resulting in the subsequent inhibition of MMP-2 activation and cell invasion. It has been known that phosphorylation of both threonine and tyrosine on p38 MAPK is associated with activation of its kinase activity. Genistein is a well known inhibitor of tyrosine kinase. Therefore, it is possible that genistein may inhibit tyrosine kinase in upstream of p38 MAPK and subsequently inhibit the phosphorylation of tyrosine on p38 MAPK, leading to inactivation of MAPK pathway (Fig. **2**).

6. Regulation of the Expression of Genes Related to Angiogenesis and Metastasis

Genistein has been shown to reduce the angiogenic and metastatic potential of cancers [23, 71-73]. Our laboratory has examined the inhibitory effect of genistein on tumor cell invasion and metastasis of MDA-MB-435 breast cancer cells transfected with *c-erb*B-2, which has been shown to promote secretion of MMPs and subsequent metastasis in experimental models [23]. We found that the expression of *c-erb*B-2, MMP-2, and MMP-9 in MDA-MB-435 cells stably transfected with *c-erb*B-2, was much higher than that in parental MDA-MB-435 cells. However, the high expression of *c-erb*B-2, MMP-2, and MMP-9 in 435 transfectants was significantly down-regulated by genistein treatment. These results suggest that increased *c-erb*B-2 expression in 435 transfectants may result in increased secretion of MMPs, and that genistein may inhibit the expression of *c-erb*B-2 and subsequently decrease the secretion of MMPs in breast cancer cells.

By gene expression profiling of genistein treated PC-3 prostate cancer cells and PC-3 bone tumor, we also found that genistein down-regulated the expression of MMP-9, MMP-2, protease M, uPAR, VEGF, neuropilin, TSP, BPGF, LPA, TGF-β2, TSP-1, and PAR-2, and up-regulated the expression of connective tissue growth factor and connective tissue activation peptide [16]. All of these genes are related to angiogenesis and metastasis (Fig. **2**). We also investigated the effect of dietary genistein on the growth of metastatic prostate cancer cells in a SCID-human experimental model of prostate cancer bone metastasis. Our results demonstrated that genistein inhibited prostate cancer cell growth in bone and down-regulated the transcription and translation of genes critically involved in the control of tumor cell invasion and metastasis *in vitro* and *in vivo*, suggesting the possible therapeutic role of genistein for metastatic prostate cancer [26]. Other investigators have also demonstrated similar results showing that isoflavones inhibited bone metastasis of human breast cancer cells in a nude mouse model and metastasis of androgen-sensitive human prostate tumors in mice [74, 75].

Taken together, it appears that genistein causes a pleiotropic effect on cancer cells; however, genistein by itself may not be an attractive agent for the treatment of human cancers. In order to alleviate this problem, synthetic derivatives of isoflavone with robust biological activity could be very attractive for the treatment of human cancers.

NOVEL SYNTHETIC DERIVATIVES OF ISOFLAVONE FOR INHIBITION OF CANCER

1. Synthesis and Molecular Characterization of Derivatives of Isoflavone

Many therapeutics used in the clinic today are actually derived from natural sources [76-78]. In recent years, growing interest has been shown for screening natural products as anti-tumor agents. Using high throughput technology for profiling genomics and proteomics, the molecular mechanisms by which natural products exert their inhibitory effects on cancer cells have been documented [15, 79]. The correlation between molecular structure and biological activity has revealed the fundamental impact of natural products on cancer prevention and treatment. Based on this information, natural product analogs or modified natural product-like small molecules can be developed and screened to provide more effective agents with greater efficacy for cancer prevention and therapy.

The molecular structure of an agent is tightly related with its biological activity. The changes in molecular structure have been shown to cause extensive changes in biological activity. Because of the structural similarity to estrogen, genistein is capable of influencing and modulating the action of estrogen. However, genistein also exerts its distinct biological effects different from estrogen. It has been shown that the anti-tumor properties of isoflavonoids are in part due to some structural motifs that include a benzopyran motif with a double bond between C2-C3 positions and a side chain containing a phenyl ring having metal chelating ability [80-82]. These structures can be easily built into a compound 3-formylchromone**,** which has anti-inflammatory and anti-cancer activities, by condensing it with various amines in alcoholic medium yielding corresponding Schiff bases. More importantly, these compounds are capable of forming metal conjugates with therapeutically important metal ions, among which copper is particularly effective in yielding moieties with potent radical scavenging properties [83].

To achieve success in the synthesis of derivatives of isoflavone, we designed an assembly process of an evolving soy isoflavone genistein motif and its possible variations and conjugating them with copper, providing the molecules capable of killing tumor cells efficiently in breast, prostate and pancreatic cancers. Specifically, the Schiff base ligands (**FPA-120 to FPA-123**) (Fig. **1**) were synthesized by condensing equimolar amounts of molecule (**2**) with various amines in methanolic solvent. The resulting compounds were recrystallized from DMF-methanol (1:1) solvent. The ligands were further interacted with copper chloride in the stoichiometric ratio to precipitate out the corresponding copper conjugates (**FPA-124 to FPA-127**), which were purified by the chromatographic work-ups. The amines we used for synthesis of (**FPA-120 to FPA-123**) are effective pharmacophores found in many therapeutic compounds currently used in the clinic, and they serve as spacers in the present design, keeping the cytotoxic metal conjugates away from the genistein moiety. Such a strategy is found to be useful for retaining pharmacological properties of both the carrier and cytotoxic moieties. Recent reports show that

other investigators are also interested in designing derivatives of isoflavone [84-88], which provide confidence in our recent synthetic derivatives of isoflavone for their anti-tumor activity.

2. Biological Activity of Synthetic Derivatives of Isoflavone in Human Cancer Cells

We have tested the effects of synthetic derivatives of isoflavone on the growth of hormone independent breast (BT20) and prostate (PC-3) cancer cells and K-ras positive (Colo357) and negative (BxPC-3) pancreatic cancer cells. We found that the synthetic derivatives of isoflavone inhibited cell proliferation in all cancer cell lines tested. The inhibition was dose-dependent in BT20 and PC-3 cells showing 50 % growth inhibition at 10 μM. This IC_{50} value is much lower than that of parent genistein (at 30-50 μM), suggesting that these derivatives of isoflavone are more potent for cancer cell growth inhibition than the parent molecule genistein. More importantly, we observed a higher degree of anti-proliferative activities of copper conjugated derivatives of isoflavone compared to their corresponding parent ligands. Significant lower IC_{50} values were obtained using copper conjugated derivatives of isoflavone, indicating therapeutically achievable efficacy of these conjugates.

By ELISA apoptosis assay, we found that these synthetic derivatives of isoflavone significantly induced apoptotic cell death in BT20 breast and PC-3 prostate cancer cells. More importantly, we compared apoptosis inducing activities between synthetic derivatives of isoflavone and parent genistein. We found that synthetic derivatives of isoflavone at lower doses induced more apoptosis compared to parent genistein, suggesting more potent activity of synthetic derivatives of isoflavone. Since all copper compounds were redox active metal conjugates, we believe that regulation of oxidative stress may be involved in the molecular mechanisms by which copper conjugated derivatives of isoflavone induce apoptotic cell death in breast and prostate cancers.

It has been reported that copper chelators induce apoptosis in cancer cells through inhibition of NF-κB signaling cascade [89]. We have tested the effects of synthetic derivatives of isoflavone on Akt and NF-κB pathways, which play important roles in cell survival and apoptosis. By using fluorescence polarization-based assays for detection of Akt kinase activity, we found that all synthetic derivatives of isoflavone exhibited 50% inhibition of Akt kinase activity at a dose less than 15 μM whereas >70 μM of genistein was needed to show similar activity against Akt. The compound **FPA-124** showed the lowest IC_{50} value (0. 1 μM) compared to other copper conjugates in the inhibition of Akt kinase activity. We have also tested the effects of **FPA-124** on tumor growth and NF-κB activity in an orthotropic pancreatic tumor model using COLO 357 pancreatic cancer cells. Our results showed that **FPA-124** had no apparent animal toxicity, as indicated by no changes in the body weight of treated animals, but it caused a decrease in the tumor weight, suggesting the inhibitory effect of **FPA-124** on tumor growth *in vivo.* Moreover, we found that **FPA-124** significantly inhibited NF-κB DNA binding activity in animal tumor tissues. These results are similar to those observed by genistein treatment, suggesting that the growth inhibitory and apoptosis inducing effects of the synthetic derivatives of isoflavone are partly mediated by inactivation of Akt and NF-κB pathways. Our results are also supported by recent publications, suggesting that derivatives of isoflavone may be excellent new anti-cancer drugs with other health benefits and without any adverse effects [84-88].

SUMMARY AND PERSPECTIVE

The data from epidemiological studies, *in vivo* human and animal studies, and *in vitro* experiments clearly demonstrate that genistein exerts its inhibitory effects on carcinogenesis, cancer cell growth, and cancer progression. These effects of genistein have been known to be mediated by pleiotropic molecular mechanisms through regulation of cell cycle, apoptosis, cell signaling pathways, cellular oxidative stress, and cell physiological behaviors. The synthetic derivatives of isoflavone including copper conjugates at much lower doses, have shown stronger effects on cell growth inhibition, induction of apoptosis, and inactivation of Akt and NF-κB pathways compared to parent genistein. Therefore, these synthetic copper conjugated derivatives of isoflavone could be potent agents for prevention and/or treatment of various cancers either alone or in combination with existing chemotherapeutic agents. However, further in depth experimental investigations along with animal studies and clinical trials are needed to fully evaluate the value of these synthetic derivatives of isoflavone against human cancers in the future.

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REFERENCES

[1] Adlercreutz, C. H.; Goldin, B. R.; Gorbach, S. L.; Hockerstedt, K. A.; Watanabe, S.; Hamalainen, E. K.; Markkanen, M. H.; Makela, T. H.; Wahala, K. T.; Adlercreutz, T. *J. Nutr.*, **1995**, *125*, 757S.

[2] Hebert, J. R.; Hurley, T. G.; Olendzki, B. C.; Teas, J.; Ma, Y.; Hampl, J. S. *J. Natl. Cancer Inst.*, **1998**, *90*, 1637.

[3] Jacobsen, B. K.; Knutsen, S. F.; Fraser, G. E. *Cancer Causes Control*, **1998**, *9*, 553.

[4] Lee, H. P.; Gourley, L.; Duffy, S. W.; Esteve, J.; Lee, J.; Day, N. E. *Lancet*, **1991**, *337*, 1197.

[5] Adlercreutz, H.; Honjo, H.; Higashi, A.; Fotsis, T.; Hamalainen, E.; Hasegawa, T.; Okada, H. *Am. J. Clin. Nutr.*, **1991**, *54*, 1093.

[6] Adlercreutz, H.; Markkanen, H.; Watanabe, S. *Lancet*, **1993**, *342*, 1209.

[7] Adlercreutz, H.; Fotsis, T.; Lampe, J.; Wahala, K.; Makela, T.; Brunow, G.; Hase, T. *Scand. J. Clin. Lab. Invest. Suppl.*, **1993**, *215*, 5.

[8] King, R. A.; Bursill, D. B. *Am. J. Clin. Nutr.*, **1998**, *67*, 867.

[9] Morton, M. S.; Wilcox, G.; Wahlqvist, M. L.; Griffiths, K. *J. Endocrinol.*, **1994**, *142*, 251.

[10] Rannikko, A.; Petas, A.; Rannikko, S.; Adlercreutz, H. *Prostate*, **2005**.

[11] Adlercreutz, H.; Fotsis, T.; Watanabe, S.; Lampe, J.; Wahala, K.; Makela, T.; Hase, T. *Cancer Detect. Prev.*, **1994**, *18*, 259.

[12] Barnes, S. *J. Nutr.*, **1995**, *125*, 777S.
[13] Li, Y.; Upadhyay, S.; Bhuiyan, M.; Sarkar, F. H. *Oncogene*, **1999**, *18*, 3166.
[14] Li, Y.; Sarkar, F. H. *Clin. Cancer Res.*, **2002**, *8*, 2369.
[15] Li, Y.; Sarkar, F. H. *J. Nutr.*, **2002**, *132*, 3623.
[16] Li, Y.; Sarkar, F. H. *Cancer Lett*, **2002**, *186*, 157.
[17] Kuiper, G. G.; Lemmen, J. G.; Carlsson, B.; Corton, J. C.; Safe, S. H.; van der Saag, P. T.; van der, B. B.; Gustafsson, J. A. *Endocrinology*, **1998**, *139*, 4252.
[18] Alhasan, S. A.; Ensley, J. F.; Sarkar, F. H. *Int. J. Oncol.*, **2000**, *16*, 333.
[19] Davis, J. N.; Singh, B.; Bhuiyan, M.; Sarkar, F. H. *Nutr. Cancer*, **1998**, *32*, 123.
[20] Davis, J. N.; Kucuk, O.; Sarkar, F. H. *Nutr. Cancer*, **1999**, *35*, 167.
[21] Davis, J. N.; Muqim, N.; Bhuiyan, M.; Kucuk, O.; Pienta, K. J.; Sarkar, F. H. *Int. J. Oncol.*, **2000**, *16*, 1091.
[22] Davis, J. N.; Kucuk, O.; Sarkar, F. H. *Mol. Carcinog.*, **2002**, *34*, 91.
[23] Li, Y.; Bhuiyan, M.; Sarkar, F. H. *Int. J. Oncol.*, **1999**, *15*, 525.
[24] Li, Y.; Sarkar, F. H. *Cancer Lett.*, **2002**, *186*, 157.
[25] Li, Y.; Ellis, K. L.; Ali, S.; El Rayes, B. F.; Nedeljkovic-Kurepa, A.; Kucuk, O.; Philip, P. A.; Sarkar, F. H. *Pancreas*, **2004**, *28*, e90-e95.
[26] Li, Y.; Che, M.; Bhagat, S.; Ellis, K. L.; Kucuk, O.; Doerge, D. R.; Abrams, J.; Cher, M. L.; Sarkar, F. H. *Neoplasia.*, **2004**, *6*, 354.
[27] Li, Y.; Ahmed, F.; Ali, S.; Philip, P. A.; Kucuk, O.; Sarkar, F. H. *Cancer Res.*, **2005**, *65*, 6934.
[28] Lian, F.; Bhuiyan, M.; Li, Y. W.; Wall, N.; Kraut, M.; Sarkar, F. H. *Nutr. Cancer*, **1998**, *31*, 184.
[29] Martin, P. M.; Horwitz, K. B.; Ryan, D. S.; McGuire, W. L. *Endocrinology*, **1978**, *103*, 1860.
[30] Fioravanti, L.; Cappelletti, V.; Miodini, P.; Ronchi, E.; Brivio, M.; Di, F. G. *Cancer Lett.*, **1998**, *130*, 143.
[31] Akiyama, T.; Ishida, J.; Nakagawa, S.; Ogawara, H.; Watanabe, S.; Itoh, N.; Shibuya, M.; Fukami, Y. *J. Biol. Chem.*, **1987**, *262*, 5592.
[32] Hunter, T. *Cell*, **1987**, *50*, 823.
[33] Ullrich, A.; Schlessinger, J. *Cell*, **1990**, *61*, 203.
[34] Okura, A.; Arakawa, H.; Oka, H.; Yoshinari, T.; Monden, Y. *Biochem. Biophys. Res. Commun.*, **1988**, *157*, 183.
[35] Evans, B. A.; Griffiths, K.; Morton, M. S. *J. Endocrinol.*, **1995**, *147*, 295.
[36] Huang, J.; Nasr, M.; Kim, Y.; Matthews, H. R. *J. Biol. Chem.*, **1992**, *267*, 15511.
[37] Ruiz-Larrea, M. B.; Mohan, A. R.; Paganga, G.; Miller, N. J.; Bolwell, G. P.; Rice-Evans, C. A. *Free Radic. Res.*, **1997**, *26*, 63.
[38] Zhou, Y.; Lee, A. S. *J. Natl. Cancer Inst.*, **1998**, *90*, 381.
[39] Davis, J. N.; Kucuk, O.; Djuric, Z.; Sarkar, F. H. *Free Radic. Biol. Med.*, **2001**, *30*, 1293.
[40] Barnes, S. *Breast Cancer Res. Treat.*, **1997**, *46*, 169.
[41] Wang, J.; Eltoum, I. E.; Lamartiniere, C. A. *Cancer Lett.*, **2002**, *186*, 11.
[42] Alhasan, S. A.; Pietrasczkiwicz, H.; Alonso, M. D.; Ensley, J.; Sarkar, F. H. *Nutr. Cancer*, **1999**, *34*, 12.
[43] Lian, F.; Li, Y.; Bhuiyan, M.; Sarkar, F. H. *Nutr. Cancer*, **1999**, *33*, 125.
[44] Kupelian, P.; Katcher, J.; Levin, H.; Zippe, C.; Klein, E. *Urology*, **1996**, *48*, 249.
[45] Sato, N.; Gleave, M. E.; Bruchovsky, N.; Rennie, P. S.; Goldenberg, S. L.; Lange, P. H.; Sullivan, L. D. *J. Steroid Biochem. Mol. Biol.*, **1996**, *58*, 139.
[46] Fritz, W. A.; Wang, J.; Eltoum, I. E.; Lamartiniere, C. A. *Mol. Cell Endocrinol.*, **2002**, *186*, 89.
[47] Frey, R. S.; Li, J.; Singletary, K. W. *Biochem. Pharmacol.*, **2001**, *61*, 979.
[48] Upadhyay, S.; Li, G.; Liu, H.; Chen, Y. Q.; Sarkar, F. H.; Kim, H. R. *Cancer Res.*, **1995**, *55*, 4520.
[49] Kazi, A.; Daniel, K. G.; Smith, D. M.; Kumar, N. B.; Dou, Q. P. *Biochem. Pharmacol.*, **2003**, *66*, 965.
[50] Katdare, M.; Osborne, M.; Telang, N. T. *Int. J. Oncol.*, **2002**, *21*, 809.
[51] Spinozzi, F.; Pagliacci, M. C.; Migliorati, G.; Moraca, R.; Grignani, F.; Riccardi, C.; Nicoletti, I. *Leuk. Res.*, **1994**, *18*, 431.
[52] Su, S. J.; Chow, N. H.; Kung, M. L.; Hung, T. C.; Chang, K. L. *Nutr. Cancer*, **2003**, *45*, 113.
[53] Sarkar, F. H.; Li, Y. *Cancer Invest*, **2003**, *21*, 744.
[54] Sarkar, F. H.; Li, Y. *Cancer Invest*, **2003**, *21*, 744.
[55] Bian, X.; McAllister-Lucas, L. M.; Shao, F.; Schumacher, K. R.; Feng, Z.; Porter, A. G.; Castle, V. P.; Opipari, A. W., Jr. *J. Biol. Chem.*, **2001**, *276*, 48921.
[56] Chuang, S. E.; Yeh, P. Y.; Lu, Y. S.; Lai, G. M.; Liao, C. M.; Gao, M.; Cheng, A. L. *Biochem. Pharmacol.*, **2002**, *63*, 1709.
[57] Yeh, P. Y.; Chuang, S. E.; Yeh, K. H.; Song, Y. C.; Ea, C. K.; Cheng, A. L. *Biochem. Pharmacol.*, **2002**, *63*, 1423.
[58] Banerjee, S.; Zhang, Y.; Ali, S.; Bhuiyan, M.; Wang, Z.; Chiao, P. J.; Philip, P. A.; Abbruzzese, J.; Sarkar, F. H. *Cancer Res.*, **2005**, *65*, 9064.
[59] Banerjee, S.; Zhang, Y.; Ali, S.; Bhuiyan, M.; Wang, Z.; Chiao, P. J.; Philip, P. A.; Abbruzzese, J.; Sarkar, F. H. *Cancer Res.*, **2005**, *65*, 9064.
[60] Hwang, J. T.; Ha, J.; Park, O. J. *Biochem. Biophys. Res. Commun.*, **2005**, *332*, 433.
[61] Ohigashi, H.; Murakami, A. *Biofactors*, **2004**, *22*, 49.
[62] Swami, S.; Krishnan, A. V.; Peehl, D. M.; Feldman, D. *Mol. Cell Endocrinol.*, **2005**, *241*, 49.
[63] Cardone, M. H.; Roy, N.; Stennicke, H. R.; Salvesen, G. S.; Franke, T. F.; Stanbridge, E.; Frisch, S.; Reed, J. C. *Science*, **1998**, *282*, 1318.
[64] Brunet, A.; Bonni, A.; Zigmond, M. J.; Lin, M. Z.; Juo, P.; Hu, L. S.; Anderson, M. J.; Arden, K. C.; Blenis, J.; Greenberg, M. E. *Cell*, **1999**, *96*, 857.
[65] Rommel, C.; Clarke, B. A.; Zimmermann, S.; Nunez, L.; Rossman, R.; Reid, K.; Moelling, K.; Yancopoulos, G. D.; Glass, D. J. *Science*, **1999**, *286*, 1738.
[66] Stoica, G. E.; Franke, T. F.; Wellstein, A.; Czubayko, F.; List, H. J.; Reiter, R.; Morgan, E.; Martin, M. B.; Stoica, A. *Mol. Endocrinol.*, **2003**, *17*, 818.
[67] Sebolt-Leopold, J. S. *Oncogene*, **2000**, *19*, 6594.
[68] Seger, R.; Krebs, E. G. *FASEB J.*, **1995**, *9*, 726.
[69] Kong, A. N.; Yu, R.; Hebbar, V.; Chen, C.; Owuor, E.; Hu, R.; Ee, R.; Mandlekar, S. *Mutat. Res.*, **2001**, *480-481*, 231.
[70] Huang, X.; Chen, S.; Xu, L.; Liu, Y.; Deb, D. K.; Platanias, L. C.; Bergan, R. C. *Cancer Res.*, **2005**, *65*, 3470.
[71] Tan, M.; Yao, J.; Yu, D. *Cancer Res.*, **1997**, *57*, 1199.
[72] Fotsis, T.; Pepper, M.; Adlercreutz, H.; Hase, T.; Montesano, R.; Schweigerer, L. *J. Nutr.*, **1995**, *125*, 790S.
[73] Li, D.; Yee, J. A.; McGuire, M. H.; Murphy, P. A.; Yan, L. *J. Nutr.*, **1999**, *129*, 1075.
[74] Iwasaki, T.; Mukai, M.; Tsujimura, T.; Tatsuta, M.; Nakamura, H.; Terada, N.; Akedo, H. *Int. J. Cancer*, **2002**, *100*, 381.
[75] Zhou, J. R.; Yu, L.; Zhong, Y.; Nassr, R. L.; Franke, A. A.; Gaston, S. M.; Blackburn, G. L. *Prostate*, **2002**, *53*, 143.
[76] Cragg, G. M.; Newman, D. J.; Snader, K. M. *J Nat. Prod.*, **1997**, *60*, 52.
[77] Shu, Y. Z. *J Nat. Prod.*, **1998**, *61*, 1053.
[78] Baker, M. E. *Proc. Soc. Exp. Biol. Med.*, **1995**, *208*, 131.
[79] Li, Y.; Li, X.; Sarkar, F. H. *J. Nutr.*, **2003**, *133*, 1011.
[80] Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol.*, **1990**, *186*, 343.
[81] Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radic. Biol. Med.*, **1996**, *20*, 933.
[82] Jovanovic, S. V.; Steenken, S.; Hara, Y.; Simic, M. G. *J. Chem. Soc.Perk. Trans. 2*, **1996**, 2497.
[83] Patole, J.; Dutta, S.; Padhye, S.; Sinn, E. *Inorg. Chim. Acta*, **2001**, *318*, 207.
[84] Cabot, M. C.; Yu, J. Y.; Kelly, G. E.; Brown, D. M.; Lucas, K. M.; Tanabe, K.; Allen, J. D. *J. Clin. Oncol.*, **2005**, *23*, 153S.
[85] Kamsteeg, M.; Rutherford, T.; Sapi, E.; Hanczaruk, B.; Shahabi, S.; Flick, M.; Brown, D.; Mor, G. *Oncogene*, **2003**, *22*, 2611.
[86] Ullmann, U.; Metzner, J.; Frank, T.; Cohn, W.; Riegger, C. *Adv. Ther.*, **2005**, *22*, 65.
[87] Lania-Pietrzak, B.; Michalak, K.; Hendrich, A. B.; Mosiadz, D.; Grynkiewicz, G.; Motohashi, N.; Shirataki, Y. *Life Sci.*, **2005**, *77*, 1879.
[88] Ullmann, U.; Bendik, I.; Fluhmann, B. *J. Physiol. Pharmacol.*, **2005**, *56 Suppl 1*, 79.
[89] Liu, G. Y.; Frank, N.; Bartsch, H.; Lin, J. K. *Mol. Carcinog.*, **1998**, *22*, 235.

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