Review article
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Pleiotropic effect of genistein makes it a promising cancer protective compound

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Of all environmental factors known to influence cancer, diet appears to be one of the most significant. Worldwide geographical variation in cancer incidence has shown a relation with differences in the dietary habits of populations at high and low cancer risk. The interest in soy products has begun when scientists rose the question why the incidence of breast, colorectal and prostate cancer is much higher in the Western world compared to the countries in Asia. One of the reasons for much lower mortality rate from cancer in Asia than in America for example, they found in commonly consumption soy-foods in the Oriental civilizations. Genistein, main isoflavone in soy, interferes with many biochemical pathways and its mode of action in the live cell is complex and multidirectional. Genistein was shown to be a specific and potent inhibitor of PTK activity, as well as an inducer of cancer cell differentiation mainly through the inhibition of inositol phospholipid metabolism. This isoflavone, as a weak phytoestrogen, may exerts both estrogenic and antiestrogenic effects on metabolism, depending on many factors, including its concentration, the concentration of endogenous estrogens, and individual characteristics such as sex and menopausal status. Genistein antagonizes tumor cell growth through both cell cycle arrest and induction of apoptosis. By stabilizing DNA topo II to DNA, genistein produces dynamic changes in the chromatin structure and alternations in gene expression that favor the differentiated phenotype. Genistein was able to inhibit in vitro angiogenesis. All mentioned properties and its extremely low toxicity make genistein a strong candidate in cancer prevention, and probably one day even in cancer treatment.

KEY WORDS: *Genistein; Isoflavones; Neoplasms+prevention and control*

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INTRODUCTION

It has been estimated that more than 80% of all cancer risk is somewhat related to lifestyle, environment, tobacco smoke, and diet (1). Diet is related to slightly more cancers than tobacco is. Worldwide geographical variation in cancer incidence has shown a relation with differences in the dietary habits of populations at high and low cancer risk. Thus, it is not surprising why the role of diet has drawn not only scientific but also media attention. The interest in soy products has begun when scientists rose the question why the incidence of breast, colorectal and prostate cancer is much higher in the Western world compared to the countries in Asia. Epidemiologists noticed that cancer rates is increased in the second generation of families that migrate to the United States from these countries as their diet becomes Westernized (2). They found that commonly consumption of soy-foods in the Oriental civilizations was one of the reasons for much lower mortality in Asia than in America. In spite of the fact that modern soy products (bland in flavor and processed into chocolate, cooking oils, milk drinks, breakfast cereals, precooked meals, dessert) are present in more than half of Western supermarkets, the intake of isoflavones is still less than 5 mg daily compared with 20 to 80 mg for Asians (3). Soybeans are unique in that they are a rich dietary source of the isoflavones, among which is genistein as a strong candidate not only as natural cancer-protective compound (4) but also as a potential anticancer drug. Chemically, genistein is *5,7-Dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one*, (mol. Wt. 270.24). In 1995, the Division of Cancer Prevention and Control, National Cancer

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Institute recommended genistein for clinical development as a cancer chemopreventive agent (5).

Although more speculative, there is a rising number of data suggesting that genistein may have a role in cancer treatment. In addition to cancer, genistein might be useful in the treatment of menopause, postmenopausal bone lose and osteoporosis, and some cardiovascular diseases.

ANTICANCER PROPERTIES OF GENISTEIN

Numerous experiments that have been undertaken show that genistein interferes with many biochemical pathways and its mode of action in the live cell is complex and multidirectional (6-8). Various mechanisms of genistein action at the molecular and cellular level are listed in Table 1.

Table 1. Biological effects of genistein

Anticancer effects	
At molecular level	**At cellular level**
Inhibition of tyrosine kinase	Induction of apoptosis
Inhibition of topoisomerase II	Induction of cellular differentiation
Inhibition of phosphatidyl-inositol turnover	Alternation in cell cycle progression
Estrogenic/antiestrogenic activity	Inhibition of cell proliferation
Inhibition of multidrug resistance	Inhibition of angiogenesis

Biological properties of genistein at molecular level

Inhibition of tyrosine kinase. The most interesting and at the same time the most controversial, is the possibility of genistein to block protein tyrosine kinases (PTKs), a group of critical components of the biological networks that control cellular growth and differentiation. This broad class of enzymes due to tyrosine residue phosphorylation on their substrate proteins includes (9):

- Receptor protein tyrosine kinases (RTKs) which play a crucial role in the signal transduction from the outer cellular environment into the interior of the cell and include many peptide growth factor receptors. RTKs are transmembrane glycoproteins with a single membrane-spanning domain and a conserved cytoplasmic tyrosine kinase domain (6,10),
- Cytoplasmic and nuclear protein tyrosine kinases which play role in regulation of various biochemical processes in cellular life. In nonmalignant cells, PTKs activity is strongly regulated with finely tuned balance between tyrosine kinases and phosphatases. On the contrary, permanent increased level of tyrosine phosphorylation, which is implicated in many cancers, give cells a proliferative advantage.

Genistein was shown to be a specific and potent inhibitor of PTK activity. PTK suppression is probably due to genistein binding with a common, highly conservative sequence at, or near to, the ATP-binding domain. Such sequence might be common target for genistein in most PTKs. Serine/threonine kinases, such as protein kinase C, and cAMP-dependent protein kinases, are not inhibited by genistein (10). Genistein penetration through cellular membrane is limited and in a whole cell system the inhibition of PTKs might requires relatively high concentration of genistein (11).

Inhibition of topoisomerase II. DNA topoisomerase (topo) II is involved in the processes of DNA replication, transcription and recombination which makes it one of the most important keys for DNA function as well as cell survival. Discovery of increased activity of topo II in various malignant cells, especially in S and G_2 phase of the cell cycle, classifies this nuclear enzyme in a row of the most important anticancer drug targets. Since that conclusion a rapid progress has been made in finding compounds which behave as topo II inhibitors. There is some evidence which point out genistein's capability of stabilizing the covalent enzyme-DNA complex by preventing the relegation step of the reaction. Because this effect is similar to that of a variety of topo II-targeting agents including etoposide (VP-16), and doxorubicin this isoflavone can be placed into the class of cytotoxic agents known as topo II-targeting agents or poisons (7).

Inhibition of phosphatidyl-inositol (PI) turnover. Two phosphorylated derivatives of PI, PI-phosphate and PI-bisphosphate (PIP_2), were found to be important in signal transduction pathways located mainly in the inner half of the plasma membrane lipid bilayer. In various types of cancer cells the cellular pathways of signal transduction involving Ca^{2+} were found markedly upregulated (8,12). Genistein blocks both PI and PIP kinase, thus reducing IP3 (inositol-1,4,5-triphosphate) concentration, second messenger that releases Ca^{2+} from intracellular stores. Genistein ability to induce cancer cell differentiation might be mainly attributed to the inhibition of inositol phospholipid metabolism.

Estrogenic/antiestrogenic activity. Genistein is weak phytoestrogen that may exert both estrogenic and antiestrogenic effects on metabolism, depending on many factors, including its concentration, the concentration of endogenous estrogens, and individual characteristics such as sex and menopausal status (13). Three distinctive types of biological response have been reported in genistein treated tumor cells. The first type is stimulated growth of mammary tumor cells by genistein at extremely low concentration (10 nanoM to 10 microM) (14). The second type are antiproliferative, cytostatic and cell differentiating effects, which have been reported at relatively low genistein concentrations (10-45 microM). At these physiologically relevant concentrations, genistein has been shown to cause alternations in reproductive hormones and induce cellular and mammary gland differentiation (15). Genistein, as a plant derived estrogen, competes with endogenous estrogens for binding to estrogen receptors. In doing so, it blocks the more potent endogenous estrogens from exerting their effects. The third type of biological response is the cytotoxic effect, reported in the range of 100-300 microM of genistein

(16). Since high blood levels of estrogen are an established risk factor for breast cancer, weak estrogen have been understood as being protective against this form of cancer. On the other side, research conducted at the University of Southern California found mixed results from adding soy to the diets of pre-menopausal women. Although soy decreased serum levels of hormones that might regulate breast cell proliferation, it is also increased levels of prolactin, which increases breast cell proliferation. These controversial results suggest that it is premature to advice immoderate consumption of soy to prevent breast cancer (17).

Inhibition of multidrug resistance. In many tumor cell lines with acquired multidrug resistance (MDR), drug resistance is associated with overexpression of a plasma membrane protein, P-glycoprotein (Pgp). Pgp functions as an energy-dependent drug efflux pump, which decreases free cellular drug concentrations, thus rendering cells resistant to cytotoxic agents. A number of drug-selected cell lines show the MDR phenotype (resistance to a wide range of cytostatic agents with no common structure or target) but without the expression of Pgp (so called non-Pgp MDR). Genistein has been shown to enhance the decreased drug accumulation in non-Pgp MDR cells, without affecting Pgp MDR cells, which restores drug accumulation in the resistant cells (8). Unfortunately, concentrations of genistein required for reversing MDR are always very high (200 microM), far above IC_{50} for the growth inhibition. Therefore, possible genistein application as a MDR modifier *in vivo* is rather out of the question (5).

Biological effects of genistein at cellular level

Induction of apoptosis: Apoptosis (programmed cell death) is a significant event in the physiological and pathological situations that control the development, differentiation and regression of tumor cells. Although the mechanism of genistein action has been studied extensively, the sequence of the molecular events involved in the apoptotic response is still subject to speculation. On the basis of *in vitro* results, it has been proposed that genistein antagonizes tumor cell growth through both cell cycle arrest and induction of apoptosis (16). These phenomena can be ascribed to the following events in cellular life induced by this compound:

- Inhibition of topo II and causing DNA strand brakes (7),
- Cell cycle derangement (16),
- Inhibition of protein tyrosine kinase since these enzymes protect cells against apoptosis,
- Interfering with PI cascade of the signal transduction (5).

Induction of cellular differentiation. Major strategy currently being used in the treatment of cancer might be the use of noncytotoxic concentrations of agents that promote terminal differentiation of tumor cells. Constantinou et al. (7) evaluated three human tumor cell lines for their ability to undergo differentiation in response to genistein. HL-60 cells (human promyelocytic leukemia) acquired granulocytic or monocytic markers. K562 terminal cells (erythroid leukemia) differentiation was obvious by the increase in percentage of hemoglobin-producing cells. Melanoma cells showed formation of dendrite-like structures and increase in melanin content and activity of tyrosinase, changes that represent maturation. Genistein reduced the proliferation of these cells in a time- and dose-dependent manner (0-7 days, 1-25 microg/ml). Higher concentrations or incubations longer than 7 days were cytotoxic (7). In another experiment, same authors report that genistein induces a mature phenotype in breast cancer cell lines as determined by the detection of milk components (casein and lipid droplets) in the cytoplasm, expression of the adhesion protein ICAM-1 in the cell membrane, and morphologic alternations (15). By stabilizing DNA topo II to DNA, genistein produces dynamic changes in the chromatin structure and alternations in gene expression that favor the differentiated phenotype. Inhibition of PTKs initiates differentiation pathway through alternations in protein phosphorylation.

Alternations in the cell cycle progression. Cell cycle is a sequence of events leading to cell division. Genistein, either directly or indirectly, can affect this process by controlling variety of enzymes. There are data pointing out that genistein inhibitory effect on cell proliferation is associated with a specific G_2/M arrest, induction of $p21^{WAF1/CIP1}$ and downregulation of bcl-2 (16,18,19). Genistein-induced blockage depends on several factors such as: the concentration of genistein, the time of exposure, the type of the cells, and the experimental condition (5).

Inhibition of cell proliferation. By the attack of different signal transduction enzymes, genistein mostly provokes cell cycle derangement, stimulation of apoptosis or differentiation, which result in the decreased rate of cell proliferation. A number of tumor cell lines *in vitro* stops proliferating after genistein administration (19,20). Whereas prolonged exposure of K562 human leukemic cells to genistein induced time- and dose-dependent growth inhibition at high concentration (unpublished data), in estrogen receptor (ER) positive cancer cell lines, such as MCF-7, genistein demonstrates biphasic grow response. At low concentrations (10 nM -10 microM) genistein stimulates proliferation while at higher doses (>10 microM) it behave as the growth suppressor. There are at least two mechanisms for such opposite genistein influence on cell proliferation. The stimulatory effect at low concentration is probably mediated through the ERs, while antiproliferative at high concentrations is likely mediated via inhibition of tyrosine phosphorylation or via some other cellular mechanism (5).

Inhibition of angiogenesis: Angiogenesis, the generation of new capillaries from preexisting vessels, is virtually absent in the healthy adult organism in which it is restricted to a few conditions including wound healing and the formation of corpus luteum, endometrium, and placenta. However, in certain pathological conditions, angiogenesis is dramatically enhanced and loses its self-limiting capacity. From a clinical perspective, probably the most important manifestation of pathological angiogenesis is that induced by solid tumors. Well-vascular tumors expand both locally and by metastasis, whereas avascular tumors do not grow beyond a diameter of 1-2 mm. Thus, it is clear why antiangiogenetic compounds attract so much attention as new anticancer agents. It has been demonstrated that genistein is the most potent amongst several plant derived inhibitors in preventing angiogenesis. Fotsis et al. have found that genistein was able to inhibit the cell proliferation and *in vitro* angiogenesis (6).

CONCLUSION

Although there is no firm conclusion that can be reached at this time because of the still controversial results, the potential role of genistein in cancer prevention, and probably one day even in cancer treatment, is encouraging. The pleiotropic properties of genistein are likely reason for difficulty to decipher its precise mechanism of anticancer activity. The future definition of genistein functions must be achieved through the study of its biochemistry, as well as its integrated actions in the cells. Multidirectional action in the live cell and extremely low toxicity, might be the main advantages of genistein as a potential drug.

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