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Phytoestrogens and Colon Cancer

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1. Introduction

Colorectal carcinoma (CRC) represents the most frequent malignancy of the gastrointestinal tract in the Western world in both genders. There is a wide variation of incidence rate for both colonic and rectal cancer among the populations of different countries: up to a 30-40-fold difference is seen between North America (Canada, Los Angeles, San Francisco), New-Zealand (non-Maori), Northern Italy (Trieste), Northern France (Haut- and Bas-Rhin) in which the rate of CRC is around 50/100,000 inhabitants, and India (Madras, Bangalore, Trivandrum, Barshi, Paranda, Bhum, Karunagappally), Algeria (Setif), and Mali (Bamako) in which the rate is around 3/100,000 [1]. It is estimated that approximately 6% of the United States population will eventually develop a CRC, and that 6 million of American citizens who are living will die of CRC [2].

The geographic differences in CRC incidence are due more to environment, life-style, and diet than to racial or ethnic factors. Demonstration of this fact is that migrants from low to high incidence areas have the same incidence as the host country within one generation, having assimilate western lifestyle and diet [3].

Colonoscopy to screen asymptomatic adults older than 50 years allows an estimation of the prevalence of adenomatous polyps or CRC: in North America CRC is found in 2%, and advanced adenoma (more than 1 cm in diameter) in 10% [4].

Population-based studies have investigated several environmental factors as contributors to the initiation of sporadic colorectal carcinogenesis. High-calorie diet, high red meat consumption, overcooked red meat consumption, high saturated fat consumption, excess alcohol consumption, cigarette smoking, sedentary lifestyle, and obesity are considered to increase the incidence of CRC, while consumption of fiber, fresh fruit and vegetables, and a high-calcium diet could have a protective effect [5]. A recent review [6] provided an over-

view of the epidemiological evidence supporting the roles of diet, lifestyle, and medication in reducing the risk of colorectal cancer. Similarly, many studies that implicate effects of dietary agents in various types of cancers are available and suggest that much of the suffering and death from cancer could be prevented by consuming a healthy diet, reducing tobacco use, performing regular physical activity, and maintaining an optimal body weight [7]. Even if several epidemiological and experimental studies support the role of these factors in the genesis of CRC, other well-designed prospective and randomized clinical trials conducted in recent years report conflicting evidence, in particular on the role of the diet component in the etiology of CRC [8, 9].

Meanwhile, the great majority of CRC are sporadic, with 2 to 6% of them related to a hereditary disease due to mutations of highly penetrant autosomic dominant genes. Mutations of *APC* tumor suppressor gene is responsible for familial adenomatous polyposis (FAP), and mutations of the mismatch repair (*MMR*) genes are related to hereditary non polypoid colorectal cancer (HNPCC or Lynch's syndrome). Mutations of *MLH1* and *MSH2* are responsible for more than 90% of the family affected by HNPCC. In these familial events, the onset of CRC is greatly anticipated in comparison to the sporadic counterpart which is usually diagnosed after 50 years of age. However, an increasing incidence rate of CRC not clearly related to the presence of inheritable or predisposing colonic diseases was observed in individuals less than 40 years of age in recent decades [10]. Furthermore, an enhanced risk for CRC and colonic adenomas is present in individuals whose first-degree relatives are affected by CRC, especially if the tumor occurs before the age of 60 [8]. Possible factors of this inherited susceptibility to CRC are polymorphisms of genes deputed to glutathione synthesis such as *GSTP1*, *GSTM1* and *GSTT1* genes [11].

Prognosis of CRC is in relationship to local and distant tumor progression. Deep penetration of carcinogenic cells in the colonic wall, invasion of adjacent organs, diffusion in lymph nodes or peritoneum, and distant metastases must be evaluated for staging of the disease and correct therapeutic planning. One third of all colorectal tumors are located in the rectum: prognosis of distally sited rectal cancer is worse than that of proximally sited rectal cancer or of colonic cancer. Despite great advances in population screening, early diagnosis, surgical interventions, and complementary therapies, long-term survival for CRC remains in the range of 50-60%.

Tumor formation in humans is a multistage process involving a series of events, and generally occurs over an extended period. During this process, several genetic and epigenetic alterations lead to the progressive transformation of a normal cell into a cancer cell. These cells acquire various abilities that transform them into malignant cells: they become resistant to growth inhibition, proliferate without dependence on growth factors, replicate without limit, evade apoptosis, and invade, metastasize, and support angiogenesis. Mechanisms by which cancer cells acquire these capabilities can vary considerably, but most of the physiological changes associated with these mechanisms involve alteration of signal transduction pathways [7].

It is commonly agreed that the first step of colorectal tumorigenesis is the shift of the proliferative zone in the glandular crypts, accompanied by the development of aberrant

crypt foci, and followed by the formation of an adenomatous polyp. These pathological features are considered the precursor of the carcinoma in a temporal sequence that also can be completed in several years. However, CRC is not a homogenous disease: several histological types can be distinguished such as tubular or villous, mucinous, serrated, medullary, signet-ring, squamous cell, adenosquamous, small cell, and undifferentiated, and different molecular basis can also be recognized in histologically similar tumors. In recent years, the identification of the genetic mutations of hereditary forms of CRC has clarified two fundamental types of carcinogenesis. The first is similar to that described for the development of the FAP, and is characterized by a progressive accumulation of genetic changes starting from a biallelic inactivation of *APC*. Additional mutations either of oncogenes *KRAS* and *p53* or of oncosuppressor genes (*DCC* and *DPC4*) are necessary for the neoplastic progression and invasivity [12]. The genetic alterations are responsible for an increased mucosal proliferation and a reduced apoptosis, causing a clonal cellular expansion. The second, similar to the CRC arising in the HNPCC, is due to inactivation of *MLH1* or of other *MMR* genes. Repetitive sequences of DNA, sited in non-encoding microsatellite regions throughout the genome, are specifically found in this type of CRC, hence, the definition of micro satellite instability (MSI). The mechanism responsible for the carcinogenesis is epigenetic due to an extensive DNA methylation. Rarely in this type of CRC both proto-oncogenes (*KRAS*, *p53*) and oncosuppressor genes (*APC*, *TGFBR1*, *IGF2R*, *BAX*) are mutated or inactivated [13]. The former genetic mechanism explains the most frequent form of sporadic CRC characterized by the sequence adenoma-carcinoma and a long period for the formation of cancer; vice versa, the last mechanism is only present in 15% of sporadic CRC, and can have the character of an accelerated carcinogenesis.

Improved knowledge of the molecular mechanisms of colorectal carcinogenesis allows a rationale chemopreventive use in individuals who have an increased risk of developing colorectal adenomas or cancer. Both natural or synthetic agents have been employed to prevent or suppress the colorectal tumorigenesis. In particular, in experimental animals, cohort and clinical case-control studies have shown inverse association between the use of either anti-inflammatory non steroidal drugs (NSAIDs), estrogens or phytoestrogens, and incidence of both colonic adenomas and CRC. NSAID use appears to prevent the occurrence of carcinogen-induced animal colonic tumors [14] and to decrease the number and size of colo-rectal polyps in FAP (Familial Adenomatous Polyposis) patients [15]. Randomized placebo controller trials showed that aspirin reduced the risk of colorectal adenomas in populations with an intermediate risk of developing adenomas [16]. Furthermore, NSAIDs or selective COX-2 inhibitors reduce the in vitro growth of human colon cancer cell [17]. The effect of NSAIDs is mediated by cell cycle arrest due to inhibition of the Wnt-signaling pathway that favors the phosphorylation of beta-catenin and by induction of apoptosis [18, 19].

The fact that estrogens have an effect in decreasing the risk of colo-rectal cancer is shown by the following data:

1. Several epidemiologic studies show a smaller incidence of sporadic CRC in the female gender. Also the occurrence of CRC in HNPCC is lower in females than in males;
2. women who are multipare are a reduced risk of CRC in confront to nullipare;
3. epidemiologic studies of postmenopausal women show that users of HRT have a significant reduction of CRC development in respect to women who had never used HRT. The risk appears to be halved with 5-10 years of HRT use [20, 21];
4. use of non-contraceptive hormones for more than 5 years reduces by (OR = 0.47, 95 percent CI = 0.24-0.91) the risk of colon cancer [22].

2. Nutrition and colon cancer

It is now believed that 90–95% of all cancers are attributed to lifestyle, with the remaining 5–10% attributed to faulty genes [7]. Almost 30 years ago epidemiological research suggested that appropriate nutrition could prevent approximately 35% of cancer deaths, and up to 90% of certain cancers could be avoided by dietary enhancement [23, 24].

Colon cancer is a multifactorial disease that results from the interaction of different factors such as aging, family history, and dietary style. Identifying modifiable factors associated with colorectal cancer is of importance, the ultimate goal being primary prevention, and particularly the role of diet in the aetiology, initiation, and progression of colorectal cancer remains an area of important research. Moreover, several components of food can exert a potent activity also in the later stages of cancer. Several studies have indicated that inhibition of metastasis by genistein, one of the most important constituents of soy foods, represents an important mechanism by which it is possible to reduce mortality associated with solid organ cancer.

Many plant-derived dietary agents have multitargeting properties and are therefore called nutraceuticals. A nutraceutical (a term formed by combining the words “nutrition” and “pharmaceutical”) is simply any substance considered to be a food or part of a food that provides medical and health benefits. During the past decade, a number of nutraceuticals have been identified from natural sources. Nutraceuticals are chemically diverse and target various steps in tumor cell development [7].

Several epidemiological studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of human cancers at many sites. Wickia & Hagmann (2011) recently reported that many case-control and cohort studies are dealing with the effect of fruits and vegetables on cancer incidence [25]. Early data indicated a beneficial effect [26] and, as recently as 2008, Freedman et al. found a reduced occurrence of head and neck cancers with increased fruit and vegetable consumption [27].

The concept that a diet that is high in fiber, especially from fruits and vegetables, lowers risk of colorectal cancer has been in existence for more than 4 decades. The majority of case-control studies have shown an association between higher intake of fiber, vegetables, and possi-

bly fruits, and lower risk of colon cancer [28]. A meta-analysis of six case-control studies found that a high intake of vegetables or fiber was associated with an approximate 40%–50% reduction in risk for colon cancer [29]. Similarly, a pooled analysis of 13 case-control studies reported an approximately 50% lower risk of colon cancer associated with higher intake of fiber [30].

Increasing intake of fruits, vegetables, or fiber is unlikely to prevent a large proportion of colorectal cancers, particularly among the US population, which has a food supply already fortified with folate and other dietary factors that might protect against colorectal neoplasia. There is also little evidence that concentrated sources of one type of fiber are efficacious, although fiber-rich diets have health benefits for other gastrointestinal conditions, such as diverticular disease and constipation, and possibly other chronic diseases [6].

All evidence supporting the decreased risk include results from a few studies of adenomatous polyps (which may progress to colorectal carcinomas). Fruit and grain intake also appears to be inversely related to risk of colorectal cancer and polyps, although less consistently than vegetables. These potentially protective associations may result from the high levels of dietary fibres, antioxidants (e.g., beta-carotene, vitamin C), or other anticarcinogenic constituents (e.g., protease inhibitors, phytoestrogens) in these vegetables, fruits, and grains. However, the association of adenomatous polyps of the large bowel with intake of vegetables, fruits, and grains has not been studied to any great degree, and existing data on these associations are not entirely consistent. Because adenomatous polyps are precursors to colorectal cancer, studying polyps instead of cancer might allow one to measure the diet of relatively asymptomatic subjects closer to the time of the initial neoplastic process. [31].

A recent meta analysis and data review, conducted by Magalhães B. [32], substantiates that the risk of colon cancer was increased with patterns characterized by high intake of red and processed meat, and decreased with those labelled as 'healthy.'

There are many plausible mechanisms by which intake of vegetables, fruits and “healthy foods” may prevent carcinogenesis.

Plant foods contain a wide variety of anticancer phytochemicals with many potential bioactivities that may reduce cancer susceptibility [7,33, 34].

3. Soyfoods and colon cancer

Many epidemiologic studies evidence a lower rate of hormone-related cancers among Asian populations which are characterized by regular consumption of soy based foods. Soy is a major plant source of dietary protein for humans. A review of epidemiologic studies (most of which were case-control studies published before 2000) suggested an inverse association between high soy intake and colon cancer risk in humans [35]. Moreover, migration studies show that Japanese immigrants in the United States have incidence rates of colorectal can-

cers very near to the rates among the whites in the country [6]. Thus the protective effect of soy foods and isoflavones is a matter of interest in the etiology of colorectal cancer.

Soy and soy foods contain a wide variety of chemical compounds, biologically active, that may contribute, individually or synergistically, to the health benefits of this plant; in particular, polyphenols are considered to possess chemopreventive and therapeutic properties against cancer.

Among these compounds, certainly, there are isoflavones, the most important and abundant of which is genistein, which also have estrogenic properties. In fact, in recent decades, there have been several studies showing that isoflavones are promising candidates for cancer prevention [36, 37, 38, 39].

Data associating soybean consumption with reduced cancer rates have been used as evidence for a role of isoflavones in cancer prevention. However, soybeans are also a rich source of trypsin inhibitor, other proteins with health benefits, phosphatidyl inositol, saponins, and sphingolipids, all of which have potential health benefits. All of these soybean constituents demonstrate tumor preventive properties in animal models. Research by Birt et al. demonstrated that 20% by weight of dietary soy protein significantly reduced rat intestinal mucosa levels of polyamine, a biomarker of cellular proliferation for colorectal cancer risk [39].

Surely, soy foods are complex foods, and it is difficult to assume that associations which suggest protective properties of soy foods are due only to a single constituent. Because of the association between diets in Japan and China and lower rates of cancers, such as those of the breast, prostate, and colon, than in Europe and the United States, many investigators have assumed that this is due to soy food consumption in Japan and China.

Other factors in the Asian diet may be responsible, and it's important to evaluate the possible confounding dietetic factors in the studies.

Several studies suggest that soy foods, the predominant source of isoflavones, are associated with reductions in cancer rate, but they do not consistently appear to be the primary protective component of the Asian diet.

Wu et al. noted the difficulties in assessing the relationship between the level of intake and protection. Case control and prospective epidemiological investigations that have provided a suggestion of protection against cancer by soy foods have not provided adequate information on the bioactive constituents in the soy foods, the portion size, or other components that may be protective in the diets of people who eat soy foods [40].

Isoflavones and flavonoids may be rapidly and predominately glucuronidated in the GI mucosa, if genistein can be considered a model for all of these phenolic compounds [41]. Further, glucuronidation occurs in the liver. Genistein undergoes biliary excretion, with more than 70% of a dose recovered in bile within 4 hr after dosing in rats. Although genistein may be absorbed well initially, a maximum of 25% of an oral genistein dose would be eliminated in rat urine. About 20–25% of an oral dose of genistein (predominantly as its glucoside from soy foods) is recovered in human urine [42, 43].

The presence of hydroxylated and methylated genistein metabolites correlated positively with inhibition of cancer cell proliferation, but genistein sulfates were not associated with antiproliferative effects of genistein, suggesting that some types of metabolism of the isoflavones may be crucial for their action [44].

Witte, et al, showed that higher consumption of tofu (or soybeans) was inversely associated with polyps. Tofu (or soybeans) contain a number of potentially anticarcinogenic constituents, including isoflavones, saponins, genistein, and phytosterols. They were able to look at tofu (or soybeans) as a single food item (i.e., separate from legumes) because almost 15 percent of our multiethnic study population reported consuming tofu (or soybeans) at least once a week. The strongest association observed was for vegetables—including those high in carotenoids, cruciferae, and broccoli—as well as garlic and tofu (or soybeans), and these associations were found even after adjusting for dietary fiber, folate, beta-carotene, vitamin C, and other commonly measured antioxidants [31].

Men tend to have a slightly higher incidence of colorectal cancer than women of similar age (American Cancer Society, 2007), and oestrogen seems to be implicated for this decreased risk in women. Epidemiological studies and results of a Women's Health Initiative (WHI) clinical trial provide strong evidence that colorectal cancer is hormone sensitive because the cancer risk is reduced by post-menopause hormone therapy [35]

In effect, many epidemiological and experimental studies suggest a protective role of estrogens against colorectal cancer. The decrease in the number of deaths from large bowel carcinoma observed in the United States in the last 40 years was significantly higher in women (30%) as compared to men (7%). A link was observed between oral contraceptive use and a reduction of colorectal cancer, whereas there was a higher than expected frequency of colorectal tumors among non users [45].

Interestingly, as reported by Barone et al., although several experimental studies have confirmed a protective role of estrogens for CRC, few studies have been conducted, and with conflicting results, on the possible protective effect of estrogens against the development of adenomatous polyps in the colon, although it is well known that the development of adenocarcinoma mostly involves polyp formation [46].

Gender differences in the incidence and behavior of colorectal cancer (CRC), as well as epidemiologic data indicating a protective effect of hormone replacement therapy in women, have further supported the concept of hormonal influence on the development of CRC. It has been suggested that the protective effect of estrogens (or phytoestrogens) may be mediated through activation of ER β , which has been shown to be the predominant subtype of ER in the gastrointestinal tract [47].

ERs are nuclear receptors belonging to the steroid hormone receptor superfamily which have the characteristic of being activated upon binding of the ligand. If the ligand is not present, ERs bind to a shock protein. Otherwise, when the ligand is present, the ERs make a stable dimer and initiate the specific estrogenic response, with transcription of the target genes. Two main types of ER have been identified: alfa (ER α) and beta (ER β). They are the so-called ligand-activated transcriptional factors through which estrogens

exert their effects on various tissues and have a different tissue distribution. ER α is mainly present in the mammary glands and in the uterus; ER β is mainly present in endothelial cells, the urogenital tract, the central nervous system, and the colonic mucosa. Experimental data have demonstrated that CRC express an elevated number of estrogen receptors (ERs), but while ER α is detected in very low levels either in normal or pathological colonic mucosa (adenoma and carcinoma), ER β expression is high in the normal colonic mucosa, and progressively decreased in the pathological mucosa in relationship to the cellular differentiation and CRC stage.

The observation that the level of ER β protein is lower in malignant tumors than in normal tissue of the same organ has fostered the hypothesis that ER β may function as a tumor suppressor, protecting cells against malignant transformation and uncontrolled proliferation.

ER β is present in various isoforms: studying different types of colonic tumoral cells, isoform 1 of ER β is found in the Lo-Vo, HCT8, HCT116, DLD-1 and isoform 2,3,4 and 5 only in the HCT8 and HCT116. It has not been well investigated whether the function of the various isoforms of ER β , but loss of the expression of isoform 1 of ER β , is accompanied by undifferentiated proliferation, mucinous histological type, and tumor progression [48]. It is accepted that the binding of estrogens to the ER β blocks the activity of AP-1 on the genes involved in the cellular proliferation and provokes an activation of p53. Conversely, SERM, such as tamoxifene and raloxifene, induce an antiproliferative effect in human colorectal cell lines by a citostatic or cytotoxic effect [49]. Several observations on the CRC cellular cultures and on the experimental mouse with germinal mutation of APC have clarified the role of the ER and estrogens for colorectal cancerogenesis: 17 β estradiol decreases the proliferation in vitro of the HCT116, Lo-Vo and DLD1 cells, but increases the proliferation of the HCT8 cells. However, the effect on the last type of cells is completely changed by increasing the level of ER β by transfection with ER β . The overexpression of ER β can have an inhibitory effect on the proliferation. In the transfected HCT8 cells the levels of CD4 and CP21, which are oncosuppressor genes, are significantly increased, and the level of cyclinE, which have oncogenic activity, significantly decreased, in respect to normal HCT8 [50].

ER β is lower in the adenomatous polyps of FAP patients and in the intestinal adenomas which develop in APC Min $^{+/-}$ mouse than in the colonic normal mucosa. The restoration of normal levels of ER β obtained with dietary phytoestrogens is accompanied by regression or disappearance of the polyps in the experimental animal. Patients with sporadic adenomas in the colon show an increase of apoptotic activity, and ER β expression of the colonic mucosa, if their diet is supplemented by phytoestrogens [45]. These data strongly support a pivotal role of ER β in a protective action against the initiation and progression of colorectal cancerogenesis.

Many epidemiologic studies evidence a lower rate of hormone-related cancers among Asian populations which are characterized by regular consumption of soy based foods. Soy is a major plant source of dietary protein for humans. Among other components, soy contains large amounts of phytoestrogens.

As proposed for estrogens, genomic and non-genomic mechanisms have also been suggested for phytoestrogens to explain their biological activities

As reported by several authors in the past, genomic pathways are mediated through the ability of phytoestrogens to interact with enzymes and receptors, and cross the plasma membrane. In this way, they bind ERs and induce the transcription of estrogen-responsive genes, stimulate cell growth in the breast, and modify ER transcription itself. However, some of their effects are not due to interaction with ERs, and are therefore denominated non-genomic effects. For example: inhibition of tyrosine kinase and DNA topoisomerase, suppression of angiogenesis, and antioxidant effects [33, 36, 46].

The bioavailability of phytoestrogens (determined by: absorption, distribution, metabolism (bioconversion in the gut and biotransformation in the liver) and excretion) and their activity is highly variable and changes with respect to several factors, such as administration routes, dosage, metabolism and interaction with other pharmacological substances. Moreover, their biological effect is influenced by the type of target tissue, the number and type of ERs expressed in the tissue, their serum concentration, and sex steroid hormone concentration [51, 52].

Phytoestrogens, present in soy and soy-based food, may act through hormonal mechanisms to reduce cancer risk by binding to estrogen receptors (ER) or interacting with enzymes involved in sex steroid biosynthesis and metabolism [53].

Although cancer incidence in women is much lower than in men in both countries, there is also a difference when the 2 countries are compared. Japanese men as well as women have a lower colorectal cancer incidence than their American counterparts, although mortality is quite similar when related to specific incidence data. In hormone-dependent cancers such as those of the breast and prostate, incidence is exceedingly low in Japan (and was even lower in earlier decades) compared with that in the United States. Mortality, again in proportion to incidence, is rather similar. Numerous reports have suggested that this difference in tumor incidence is probably due to consumption of soy as a staple food in Asian countries in contrast to Western industrialized countries. These substances, through their potential to act as selective estrogen receptor modulators, may affect vitamin D-related inhibition of tumor growth by upregulating extrarenal synthesis of 1,25- D₃. Genistein, the most prominent phytoestrogen in soy, is known to regulate other P450 enzymes, such as 5-reductase and 17-hydroxysteroid dehydrogenase, which are essential for metabolism of sex hormones [54].

In vitro studies of DLD1 colon adenocarcinoma cells have linked the effects of soy with estrogen receptor beta. Experiments conducted on this cell line, with or without ER- β gene silencing by RNA interference (RNAi), have shown that soy isoflavones decreased the expression of proliferating cell nuclear antigen (PCNA), extracellular signal-regulated kinase (ERK)-1/2, AKT, and nuclear factor (NF)- κ B. Soy isoflavones dose-dependently caused G2/M cell cycle arrest and downregulated the expression of cyclin A. This was associated with inhibition of cyclin dependent kinase (CDK)-4 and upregulation of its inhibitor p21 expressions. ER- β gene silencing lowered soy isoflavone-mediated suppression of cell viability

and proliferation. ERK-1/2 and AKT expressions were unaltered and NF- κ B was modestly upregulated by soy isoflavones after transient knockdown of ER- β expression.

Soy isoflavone-mediated arrest of cells at G2/M phase and upregulation of p21 expression were not observed when ER- β gene was silenced. These findings suggest that maintaining the expression of ER- β is crucial in mediating the growth-suppressive effects of soy isoflavones against colon tumors. Thus, upregulation of ER- β status by specific foodborne ER-ligands such as soy isoflavones could potentially be a dietary prevention or therapeutic strategy for colon cancer [55].

4. Genistein and isoflavones: Other mechanisms of action

In addition to estrogenic/antiestrogenic activity, some mechanisms of action have been identified for isoflavone/flavone prevention of cancer: antiproliferation, induction of cell cycle arrest and apoptosis, prevention of oxidation, induction of detoxification enzymes, regulation of host immune system, and changes in cellular signaling [39, 56, 57]. It is expected that also combinations of these mechanisms may contribute to cancer prevention.

Gene silencing due to the promoter methylation provides an opportunity for clinical intervention, as gene-re-expression can be induced by a variety of DNA demethylating agents.

Recent studies show that genistein may affect DNA methylation, serves as a natural demethylation agent, and that it is specifically effective on colon cancer cells from early-stage colon cancer [58]. WNT family members are highly conserved, secreted signaling molecules that play important roles in both tumorigenesis and normal development and differentiation. Study of Hibi *et al.* evidences that genistein treatment affected the DNA methylation of *WNT5a*, and that *WNT5a* downregulation is correlated with hypermethylation of its promoter in human colon cancer patients [60, 59].

Moreover, genistein may inhibit cancer progression by inducing apoptosis or inhibiting proliferation, and the mechanisms by which genistein exerts its anti-tumor effects has been the subject of considerable interest [61, 62, 63].

Genistein has been shown to induce epigenetic changes in several cancer cell lines and in the *in vivo* animal models. [64].

The presence of hydroxylated and methylated genistein metabolites correlated positively with inhibition of cancer cell proliferation, but genistein sulfates were not associated with antiproliferative effects of genistein, suggesting that some types of metabolism of the isoflavones may be crucial for their action.

Genistein is a known inhibitor of protein-tyrosine kinase (PTK), which may attenuate the growth of cancer cells by inhibiting PTK-mediated signaling mechanisms [65]. Sakla *et al.* (2007) recently reported that genistein inhibits the protooncogene HER-2 protein tyrosine phosphorylation in breast cancer cells as well as delaying tumor onset in transgenic mice that overexpress the HER-2 gene. These data support its potential anti-cancer role in chemo-

therapy of breast cancer. However, effects independent of this activity have also been demonstrated [66, 67].

Soy isoflavone supplemented diets also prevented the development of adenocarcinomas in the prostate and seminal vesicles in a rat carcinogenesis model [68].

Phytoestrogens, present in soy based food, may act through hormonal mechanisms to reduce cancer risk by binding to estrogen receptors (ER) or interacting with enzymes involved in sex steroid biosynthesis and metabolism [53]. Moreover, genistein may inhibit cancer progression by inducing apoptosis or inhibiting proliferation, and the mechanisms by which genistein exerts its anti-tumor effects have been the subject of considerable interest [61, 62, 63].

Studies demonstrate that ER β is highly expressed in superficial and crypt epithelium of the normal colon in both genders. ER β expression was highly correlated among all cell types in both genders, and the strongest correlation was observed between surface and crypt ER β expression. This finding suggests that there may be an intersubject difference in ER β expression that is manifested in all cell types. ER β expression was significantly lower in colon cancer cells compared with normal colonic epithelium, and there was a progressive decline in ER β expression that paralleled the loss of cancer cell differentiation. The present findings are consonant with previous results reported by Foley and colleagues [69], who also detected a loss of ER β protein expression in malignant colon tissue by western immunoblotting. Another immunohistochemical study of ER β in 55 patients with colorectal adenocarcinomas showed that 32% of all tumors in both genders were ER β -negative; the 10% cut-off threshold was used to distinguish ER β -positive from negative tumors [70].

Studies conducted with ER subtype-specific ligands and those performed with estrogen receptor b-knockout mice (ER β KOs) have illustrated the involvement of ER β in cellular anti-inflammatory pathways and tissue homeostasis in the colon. These results suggest that ER β -specific ligands may be promising targets in the pharmaceutical and therapeutical treatment of inflammatory bowel disease and the prevention of CRC. ER β KOs suggest that ER β -specific agonists and ER β -selective phytoestrogens like genistein (GEN) and coumestrol may serve as potential regulators of intestinal tissue homeostasis [71, 72, 73].

Schleipen et al. investigate the influence of ER α and ER β -specific agonists, and of genestein on cell proliferation and apoptosis of the small intestine and the colon. Recent data indicate that ER β -specific agonists and GEN inhibit epithelial proliferation of the prostate and mammary gland, and can even impede prostate cancer development [74, 76, 75]. It can therefore be assumed that ER β -specific agonists may also inhibit the proliferation of the intestinal epithelium. To prove this hypothesis in the study, ovariectomized rats were treated with 17 β -Estradiol (E2), the phytoestrogen GEN and ER subtype-specific agonists for ER α and ER β for 3 weeks.

Genistein has been shown to induce epigenetic changes in several cancer cell lines and in *in vivo* animal models [64]. Recent studies show that genistein may affect DNA methylation, serves as a natural demethylation agent, and is specifically effective on colon cancer cells from early-stage colon cancer [58].

WNT family members are highly conserved, secreted signaling molecules that play important roles in both tumorigenesis and normal development and differentiation. Study of Hibi *et al.* evidence that genistein treatment affected the DNA methylation of *WNT5a*, and that *WNT5a* down-regulation is correlated with hypermethylation of its promoter in human colon cancer patients [59, 60]. Aberrant WNT signaling is considered one of the most correlated factors in over 90% of both benign and malignant colorectal tumors [77].

Many epigenetic silencing and activating events have been discovered in the WNT pathway that are also related to aberrant WNT signaling, including aberrant expression of *sFRP1*, *DKK1*, and *APC* [78, 79]. Therefore, Wang and Chen investigate the effect of genistein on WNT pathway regulation in colon cancer development [58]. This study showed that: genistein treatment selectively induced *WNT5a* expression in specific colon cancer cell lines; *WNT5a* showed the lowest expression compared to other more advanced tumor cell lines; and the novel finding that *WNT5a* mRNA expression was upregulated by genistein in this early-stage colon cancer cell line.

These results support the notion that genistein serves as a natural demethylation agent and that it is specifically effective on colon cancer cells from early-stage colon cancer. Genistein treatment affected the DNA methylation of *WNT5a*. It has been shown that *WNT5a* downregulation is correlated with hypermethylation of its promoter in human colon cancer patients [59, 60].

Wang and Chen studies showed that the time dependent induction of *WNT5a* by genistein in colon cancer cell line SW 1116 was correlated with decreased methylation of a CpG island within its promoter, as determined by bisulfate sequencing [58].

Demethylation of CpGs inhibition of Dnmt and MBD2 activity, and activation of the histones by acetylation and demethylation at the BTG3 promoter followed by genistein treatment, were observed in renal cancer cells [80]. Using the mouse differential methylation hybridization array, alteration of DNA methylation in specific genes in mice was observed following feeding of a diet containing genistein compared to that in mice fed a control casein diet [81].

Other direct evidence that genistein affected DNA methylation was that maternal exposure to dietary genistein altered the epigenome of offspring in viable yellow agouti (*Avy/a*) mice. Overall, the potential of genistein as an effective epigenome modifier, which may greatly impact CRC metastasis, highlights the potential ability of dietary genistein to improve CRC prognosis [82].

Downregulation by promoter hypermethylation occurs in cell lines from earlier stages of colon cancer but not in cell lines from later stages.

These findings suggest that maintaining the expression of ER- β is crucial in mediating the growth-suppressive effects of soy isoflavones against colon tumors. Upregulation of ER- β by specific foodborne ER-ligands, such as soy isoflavones, could potentially be a dietary prevention strategy for colon cancer. [55].

Genistein has been shown to inhibit cancer metastasis through its ability to regulate nearly every step of the metastatic cascade, including cell adhesion, migration invasion, and angiogenesis. The effect of genistein on the metastatic cascade involves many metastasis suppressor or related signaling pathways, such as NF- κ B. Genistein can affect both of these processes, as well as modulate key regulatory protein such as Akt and nuclear factor κ B (NF- κ B). In general, low-to-mid micro molar concentrations of genistein are required for these effects in cell-culture-based models, although, interestingly, effects in animal models have been observed at lower concentrations. Genistein inhibits critical pathways in cancer invasion and can specifically target MEK4. This inhibition results in inactivation of the MEK4 pathway, decreased MMP-2 production, and decreased cell invasion. Genistein also activates Smad1, which is activated by the endoglin signaling pathway, and causes decreased cell invasion. Additionally, genistein inhibits FAK activation, resulting in increased cell adhesion. At this time, it is unclear whether the activation of Smad1 and FAK are due to genistein's inhibition of MEK4 or via a different signaling mechanism [83].

Several reports have demonstrated that genistein can induce cell cycle arrest and that it can therapeutically modulate key regulator cell cycle proteins at concentrations ranging from 5 to 200 μ M [84]. It is important to note that these concentrations are greater than the blood levels that are observed with dietary consumption, indicating that this is likely not the primary mechanism by which genistein inhibits metastasis. However, it is theoretically possible to achieve these levels in humans, and various animal studies have also demonstrated that genistein can reduce the primary tumor size in certain contexts.

Studies by Wentao et al. show that genistein inhibits EGF induced loss of FOXO3 activity by targeting the PI3K/ Akt pathway. Downstream, genistein inhibits EGF induced FOXO3 disassociation from p53(mut), which further promotes FOXO3 activity and leads to increased expression of the p27kip1 cell cycle inhibitor, which inhibits proliferation in colon cancer cells. The author demonstrated that one of the anti-proliferative mechanisms of genistein in colon cancer cells is to promote FOXO3 activity by inhibiting EGF-induced FOXO3 phosphorylation (inactivation) via the PI3K/Akt pathway. Active FOXO3 negatively regulates proliferation of colon cancer cells and shows that its inactivation is an essential step in EGF-mediated proliferation [85, 86].

5. Conclusion

Several studies shown that consumption of fiber, fresh fruit and vegetables, a high-calcium diet could have a protective effect on the increased risk of colorectal cancer, and suggest that much of the suffering and death from cancer could be prevented by consuming a healthy diet, reducing tobacco use, performing regular physical activity, and maintaining an optimal body weight [5].

Soy is one of the most consumed foods worldwide. Soy foods contain larger amounts of phytoestrogens of which the isoflavon genistein is surely the biologically most important.

This compound, in recent years, has received much attention in the field of oncology research, as it exerts a wide range of biological effects of direct relevance to cancer.

Phytoestrogens and in particular genistein, have shown to be an important tool for the inhibition of cancer metastasis, exerting effects on both the initial steps of primary tumor growth as well as the later steps of the metastatic cascade.

The international literature suggests that phytoestrogens have potentially a high clinical impact and the expansion of knowledge on soy, soy foods, and soy products will lead to novel future developments in the field of cancer treatment.

Phytoestrogens in soy foods	
Foods	Total isoflavons (mg/100 g)
Miso	41.45
Natto	82.29
Roasted soybeans	148.50
Soy beans	154.53
Soy cheese american	17.95
Soy flour (textured)	172.55
Soy milk	10-200
Soy milk curd, dried	83.30
Soy milk fortified or unfortified	10.73
Soy milk skin or film (Foo jook or yuba), cooked	44.67
Soy milk skin or film (Foo jook or yuba), raw	196.05
Soy protein concentrate	94.65
Soy protein drink	81.65
Soy protein isolate	91.05
Soy yogurth	33.17
Tempeh	60.61
Tofu (dried frozen)	83.20
Tofu raw regular with calcium and sulphate	22.73
Tofu yogurt	16.30

Table 1. Isoflavone Content of Selected Soy Foods (USDA Database 2008)

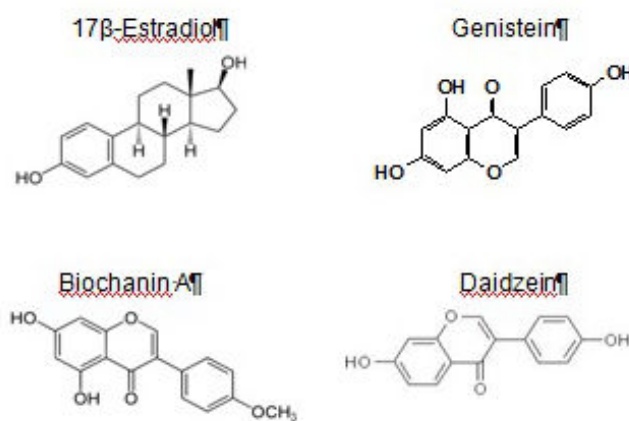


Figure 1. Chemical structures of soy phytoestrogens are similar to 17 beta estradiol

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