Abstract. In this review, we consider phytoestrogens and different epigenetic modifications in breast cancer. Epigenetic phenomena are mediated by several molecular mechanisms comprising histone modifications, small non-coding or anti-sense RNA and DNA methylation. These different modifications are closely interrelated. De-regulation of gene expression is a hallmark of cancer. Although genetic lesions have been the focus of cancer research for many years, it has become increasingly recognized that aberrant epigenetic modifications also play major roles in breast carcinogenesis. The incidence and mortality rates of breast cancer are high in the Western world compared with countries in Asia. There are also differences in the breast cancer incidence rates in different Western countries. This could be related to phytoestrogens.

Breast cancer remains the first cause of cancer-related mortality in women. This can be explained by the high histological and molecular heterogeneity of the disease, making it hard to choose a therapy adapted to each patient. Over this past year, several groups have evaluated the epigenetic component of breast cancer, as epigenetics appears to be important in carcinogenesis.

The progression through the multiple steps of breast cancer from epithelial hypertrophy to highly invasive breast carcinoma involves multiple coordinated changes in gene expression programming. Such coordinated changes are bound to be controlled by global mechanisms of gene expression programming. The genome is programmed by the epigenome, which consists of the chromatin structure, a pattern of modification of DNA by DNA methylation, and a profile of expression of non-coding RNAs, such as microRNA. The revolution of epigenetics has re-vitalized cancer research, shifting focus away from somatic mutation towards a more global perspective involving the dynamic states of chromatin. Disruption of chromatin organization can directly and indirectly precipitate genomic instability and transformation. Epigenetic changes are reversible and may lead to loss or gain of biological functions. More importantly, many of the elucidated epigenetic changes are linked to pathogenesis of human diseases, including cancer. Epigenetic aberrations arise early in carcinogenesis, before gene mutations in DNA supplies targets for early detection.

The incidence and mortality rates of breast cancer are high in the Western world compared with countries in Asia. There are also differences in regional cancer incidence rates in the Western countries. Several studies involving immigrants to Western countries suggest that lifestyle and diet are two of the main causes of these differences. In Eastern countries, the incidence of breast cancer is approximately one-third compared to that of Western countries, whilst the high dietary intake of phytoestrogens, mainly in the form of soy products, can produce circulating levels of phytoestrogens that are known experimentally to have estrogenic effects.

Phytoestrogens

Phytoestrogens are plant-derived xenoestrogens functioning like the primary female sex hormone; however, not generated within the endocrine system but consumed by eating phytoestrogenic plants. Also called ‘dietary estrogens’, they are a diverse group of naturally occurring non-steroidal plant compounds, that due to their structural similarity to estradiol (17-β-estradiol), have the ability to cause estrogenic and antiestrogenic effects. An increasing number of
epidemiological and experimental studies have suggested that the consumption of a phytoestrogen-rich diet may have protective effects on estrogen-related conditions, such as prostate and breast cancer, osteoporosis and cardiovascular diseases (1). However, concerns have been raised about the potential dangers of consuming high levels of these compounds (2). Consequently, phytoestrogens are currently under active investigation for their role in human health. Phytoestrogen sources can be grouped into three: i: the naturally-occurring steroidal and non-steroidal estrogens; ii: human or animal steroidal estrogens (17-β-estradiol, estrogen sulfate); and iii: estrogenically active compounds arising from fungal attack (3).

Phytoestrogens are non-steroidal compounds produced by many plants and contained in many natural dietary products, such as soybeans, wheat, barley, corn, alfalfa, and oats. Structurally, they are similar to endogenous estrogens and share a similar mechanism of action through their affinity for binding to estrogenic receptors. Although not steroids, phytoestrogens mimic or antagonize some of the actions of endogenous estrogens, but their potency is much lower than that of steroidal estrogens. Phytoestrogens have been ascribed certain putative health benefits against osteoporosis, heart disease, and some types of cancer (4).

Currently phytoestrogens include more than 100 molecules, divided according to their chemical structure into isoflavones (genistein, daidzein, biochanin A, formononetin) (Figure 1), lignans (matairesinol, secoisolariciresinol-diglucoside), and coumestans (coumestrol, 4-methoxycoumestrol), stilbenes (resveratrol).

Some of these substances (e.g. resveratrol) act as natural antioxidants and findings concerning their effects in humans, especially on the cardiovascular system, have been repeatedly reported in physiological research (4-6).

Mechanistically, phytoestrogens have been shown to bind to two types of estrogen receptors: estrogen receptor α (ERα), which was cloned in 1986, and estrogen receptor β (ERβ), cloned in both rats and humans. The two receptors differ in their tissue distribution and affinity for ligands, yet there is some overlap. In rats, ERα and ERβ both are clearly expressed in ovarian and uterine tissues. ERβ has been shown to have ligand specificity toward phytoestrogens and is distributed in humans in ovary, spleen, testis and thymus and in rats in bladder, brain, lung, ovary, prostate, testis and uterus. Phytoestrogens have a lower binding affinity than 17-β-estradiol and some exhibit a higher binding affinity for ERβ than for ERα, which may suggest different pathways for their actions and explain tissue-specific variability of phytoestrogenic action. The complexity of phytoestrogens and ERs appears to be further compounded because different transcriptional activities in vitro are activated depending on the ligands, as well as on the environment of the promoter region of specific genes for translated ERα and ERβ (7).

Isoflavones interact with sex steroids in multiple ways. Influence on the metabolism of sex hormones may be quite complex and may depend on several factors including species, sex, age, hormonal status, etc. Moreover, the dose and duration of isoflavone administration may not be linearly related to the treatment effect, which could add to the significant variability of research findings. Isoflavones were found to inhibit the activity of both 5α-reductase, which catalyzes the conversion of testosterone to 5α-dihydrotestosterone, and aromatase P450, which mediates the conversion of testosterone to 17-β-estradiol (6).

Isoflavones belong to the flavonoid group of compounds, the largest class of polyphenolic compounds. Isoflavones are found in a number of plants including soybeans, fava and beans. Several isoflavones have been investigated and indications are that they have antiangiogenic and anticancer properties. The three major isoflavones found in soybeans are genistin, daidzin, and glycitin. Their abundance in soy protein preparations varies widely and depends on the processing techniques used during production (8). Genistein, a phytoestrogen primarily found in soybeans, is perhaps the most studied of these bioactive compounds. This estrogen-like compound acts as a chemopreventive agent in several types of cancer (9). The affinity of genistein for ERβ is about 20-30 times higher than that for ERα and is comparable to the affinity of 17-β-estradiol (10). The affinity of other
isoflavones is approximately 100-500 times lower than that of 17-ß-estradiol. Isoflavones act as agonists of ERs, but their activity is lower than that of 17-ß-estradiol. At sufficiently high levels (over about 100 nmol/l for genistein), the effects of isoflavones may approach the effect of endogenous 17-ß-estradiol at its physiological level.

Genistein has estrogenic properties in receptor-binding assays, cell culture and uterine weight assays. Genistein inhibits topoisomerase II, platelet-activating factor and epidermal growth factor-induced expression of c-Fos, diacylglycerol synthesis and tyrosine kinases. It also inhibits microsomal lipid peroxidation and angiogenesis. Most of these mechanistic data were derived from in vitro studies (11).

Not only do phytoestrogens differ in their biological activity, but they also differ structurally because they come from diverse chemical classes, which may affect their influence on tissues and receptors. Due to the diversity of chemicals that exhibit estrogenic effects, it appears that estrogenic activity is often emphasized over chemical structure in defining phytoestrogens (7).

Phytoestrogens and Breast Cancer

The weak estrogenic action of soy isoflavones and other phytoestrogens suggested the possibility that they could lessen the deleterious effects of more potent endogenous estrogens on breast and endometrial cancer. This hypothesis came from the low incidence of breast and endometrial cancer in Asian countries where soy products are prevalent in the diet and from certain animal models of breast and endometrial cancer showing the benefit of soy isoflavones (8).

In Western countries, breast cancer is the most common type of cancer affecting women. Historically, the risk of breast cancer was much higher in American women than in Asian women prior to the influence of the Western diet on Asian cultures (7). It is known that some types of tumors, such as breast, prostate and colon cancer, have a lower incidence in Asian countries compared to the population of Western countries (6).

At the beginning of the 1980s, it was suggested that lignans and isoflavonoids may prevent breast cancer. This idea led to numerous epidemiological, experimental, case-control, and prospective studies investigating this hypothesis (12). In epidemiological studies, associations varied between intake of soy foods and isoflavones and breast cancer incidence. Much of the epidemiology of breast cancer can be explained by reproductive and hormonal factors; in relation to diet, the only factors definitely related to breast cancer risk are obesity in post-menopausal women and alcohol consumption. Several large prospective studies have investigated whether high intakes of fruit and vegetables might be associated with a reduced risk of breast cancer, but overall the results are close to null. It seems unlikely that high intakes of fruit and vegetables in general have a significant protective effect, but it is still possible that specific vegetables rich in isoflavones, especially soya beans, might have a protective effect by reducing the estrogenic stimulation of breast cells (13).

Several studies have shown that in breast cancer cells treated with a low concentration of genistein, the promoter of the glutathione S-transferase Pi-1 (GSTP1) gene was demethylated. Moreover, genistein combined with DNA methylation inhibitors or other inhibitors of DNA methyltransferases (DNMTs) can enhance the reactivation of genes silenced by methylation (14). It was also found that genistein inhibits DNMT1, -3a -3b and inhibits the expression of telomerase reverse transcriptase (hTERT). Genistein also increases acetylation by enhancing histone acetyltransferase (HAT) activity. Furthermore, investigations have demonstrated that genistein-mediated hypomethylation and hyperacetylation reactivate the expression of tumor suppressor genes in prostate and breast cancer cells. Genistein and other isoflavones have also been found to regulate miRNA expression in several cancer cell lines (14, 15).

Animals fed with high doses of soybeans exhibited a lower incidence of breast and mammary gland cancer (16). In post-menopausal women, consumption of isoflavones was found to be associated with reduction of breast cancer incidence, mammary gland density and proliferative ability of mammary gland cells (17). These effects have been associated with the ability of isoflavones to increase the sex hormone-binding globulin (SHBG) concentration in serum, thereby reducing the bioavailability of sexual hormones in hormone-dependent tissues (17). Moreover, in peripheral tissues, isoflavones inhibit enzymes involved in the processes of cell proliferation (e.g. tyrosine kinase) and reduce estradiol availability through an inhibitory effect on aromatase P450 (6). On the other hand, it has been reported that high doses of genistein may activate cell proliferation in estrogen-dependent tumors (18).

However, despite the observation of inhibition of estradiol-forming enzymes in vitro, adding coumestrol or genistein to breast cancer cells in culture does not reduce or prevent estradiol formation (19). The conversion of estrogens to androgens in breast cells is thought to be important for the development of breast cancer. Genistein stimulates several antioxidative enzymes, such as catalase, superoxide dismutase, glutathione peroxidase and reductase, and it is also an inducer of tumor cell differentiation. Genistein down-regulates the epidermal growth factor receptor (EGFR) and (ERBB2/Neu) receptors in cancer cells, and may also inhibit tumor-cell invasion by inhibiting (MMP9) (92-kDa type IV collagenase), while up-regulating tissue inhibitor of metalloproteinases (TIMP1), and various trypsin inhibitors. Daidzein may also enhance immune function (12).
At the present time, there is a growing number of studies showing that a high soy intake during childhood is associated with reduced breast cancer risk. But there is no convincing evidence to suggest that soy or isoflavone consumption in Western countries during adult life is protective against breast cancer (3, 8, 12). Soy consumption before puberty may have the same risk-lowering effects as an early pregnancy. It is suggested that phytoestrogens promote cell differentiation in the mammary gland, resulting in enhancement of mammary gland maturation.

Epigenetic Changes in Breast Cancer

The molecular mechanisms underlying the development and progression of breast cancer are far from understood. It is evident that the initiation of breast cancer, as well as its transition towards distinct breast cancer subtypes, is triggered by the accumulation of pathologically-altered gene functions. As in other types of cancer, an increasing number of deregulated genes subsequently affect virtually all important cellular networks, such as cell-cycle control, apoptosis, DNA repair, detoxification, inflammation, cell adhesion and migration. According to the somatic mutation theory, cancer has long been considered as a genetic disorder arising from migration. According to the somatic mutation theory, cancer progression of breast cancer are far from understood. It is evident that the initiation of breast cancer, as well as its transition towards distinct breast cancer subtypes, is triggered by the accumulation of pathologically-altered gene functions. As in other types of cancer, an increasing number of deregulated genes subsequently affect virtually all important cellular networks, such as cell-cycle control, apoptosis, DNA repair, detoxification, inflammation, cell adhesion and migration. According to the somatic mutation theory, cancer has long been considered as a genetic disorder arising from migration. According to the somatic mutation theory, cancer.

Post-translational histone tail modifications, another epigenetic mechanism that can modulate chromatin structure to regulate gene expression (24), are associated with DNA methylation. In addition, it has been shown that some regulators that control nucleosomal remodeling are also involved in the regulation of DNA methylation and histone modification (25, 26). An understanding of all these epigenetic changes and their contributions to breast tumorigenesis is very important for further progress in the field of diagnosis, prognosis and therapy of breast cancer.

DNA Methylation in Breast Cancer

DNA methylation patterns highlight significant differences in breast cancer between tumor tissues and corresponding normal tissues (27). A common paradox observed in carcinomas is that despite regional hypermethylation of tumor-suppressor genes, the global 5-methylcytosine content is drastically-reduced in the bulk of the tumor genome (26). Less frequent than regional DNA hypermethylation, regional DNA hypomethylation can also occur in cancer, resulting in the activation of potential oncogenes (27, 28).

DNA hypermethylation. DNA hypermethylation indicates which genes are turned-off in breast tumors and a unique pattern is observed in breast tumors. Interestingly, 40% loss of methylated cytosine is observed in breast tumors. Important genes in familial breast cancer are also epigenetically silenced. In sporadic tumors, BRCA1 expression has been shown to be suppressed by a combination of gene deletion and epigenetic silencing via DNA hypermethylation (29).

Ectopic cytosine hypermethylation is generally associated with transcriptional repression and ultimately tumor formation. Collaboration between genetic and epigenetic phenomena within breast cancer causes has been directly demonstrated by observations in which one tumor-suppressor allele is inactivated by mutation and the other allele is transcriptionally silenced because of hypermethylation (30). In addition hypermethylation of tumor-suppressor genes may be an early event in cancer development (31, 32), suggesting that epigenetic and mutational cancer causes may collaborate from an early time point in disease progression (32). The list of tumor-suppressor genes found transcriptionally inactivated by hypermethylation in cancer is long and steadily growing (33), and includes genes that are part of every cancer-related pathway, including important genes such as (CDKN2A), (pRB), (APC), (PTEN), (BRCA1), (VHL) and (CDH1). By extension, epigenetic silencing may underlie genetic cancer causes. Epigenetic induction of a classical mutator phenotype via transcriptional inactivation of the DNA mismatch repair gene (MLH1) has been proposed to account for microsatellite instability in colorectal cancer and silencing of the DNA repair gene coding for O-6-methylguanine-DNMT has been associated with specific mutations in (K-RAS) and p53 (34, 35).
DNA hypomethylation. Among solid tumor types, global DNA hypomethylation is most evident in breast cancer with up to 50% of cases showing reduced 5-methylcytosine content when compared with normal tissue counterparts (36). Hypomethylation in breast cancer mainly affects iterative DNA sequences and pericentromeric satellite DNA, which are normally heavily methylated in non-malignant cells (35).

Although a relatively rare event, DNA hypomethylation can also affect individual genes. In breast cancer, this is the case for the melanoma-associated cancer/testis antigens MAGE. The MAGE gene family encodes for (HLA)-restricted tumor-associated rejection antigens recognized by cytotoxic T-lymphocytes. Some of these target antigens may be potentially useful for cancer-specific immunotherapy. The expression of MAGE genes has been reported not only in melanoma but also in various other malignant tumors, such as hepatocellular carcinoma and germ cell tumors. Genes for MAGE antigens are methylated and silenced in adult tissues, but hypomethylated and expressed in several tumors and breast cancer cells (37). Other hypomethylated genes in breast tumors include the gene encoding the plasminogen activator uPA (PLAU), the breast cancer-specific protein 1/synuclein-γ gene (SNCG), and more recently reported, the multidrug-resistance 1 gene (MDR1) (38, 39).

Global DNA hypomethylation is a hallmark in human cancer, but its functional consequences are unclear (39). The mechanism of global hypomethylation is a long-standing question in cancer epigenetics (40).

Histone Modifications in Breast Cancer

A number of studies have investigated the use of histone modifications as biomarkers in tumors. High relative levels of global histone acetylation and methylation were associated with a favorable prognosis, and were detected almost exclusively in luminal-like breast tumors (93%). Clustering analysis identified three groups of histone status patterns which correlate with clinical outcome (41).

Histone acetylation. Histone acetylation is a dynamic process directed by HATs and histone deacetylases (HDACs). Normally, transcription factors recruit co-activators with HAT activity to regulatory DNA sites, whereas transcriptional repressors recruit co-repressors with HDAC activity.

Many HATs have also been shown to be involved in breast cancer. Among them, p300/CREB-binding protein (CBP) and nuclear receptor coactivator family (NCOAs) are the most important and well-characterized HAT proteins associated with breast cancer (42).

p300 and its close homolog CBP are often referred to as a single-entity. A role for p300 in tumor suppression has been proposed by the fact that disturbance of p300 function by viral oncoproteins is essential for the transformation of rodent primary cells and, consistent with this hypothesis, mutations of p300 have been identified in certain types of human cancer, including breast carcinoma (43). Both the localization of p300 and the recruitment to aggresomes differ between breast cancer and normal mammary glands. The expression level of p300 in breast cancer epithelia is higher than that in normal mammary gland (44).

Cytoplasmic localization of p300 was also observed in tumor epithelia whereas nuclear localization was found in normal mammary glands in both animal models and in non-malignant adjacent areas of human breast cancer specimens. Proteasomal inhibition induced p300 re-distribution to aggresomes in tumor but not in normal mammary gland-derived cells (45). The regulation of gene expression by nuclear receptors (NRs) controls the phenotypic properties and diverse biologies of target cells. In breast cancer cells, ERα is a master regulator of transcriptional stimulation and repression (46).

Upon 17-[β]-estradiol treatment, gene transcription is widely impacted, creating highly complex regulatory networks whose ultimate goal is the stimulation or suppression of specific biological processes. p300/CBP can function as a transcriptional co-factor of ERs and of other nuclear hormone receptors (47).

The NCOA family, also named as p160 or steroid receptor co-activator, contains three homologous members: NCOA1 (SRC-1), NCOA2 (SRC-2, GRIP1 or TIF2) and NCOA3 (SRC-3, p/CIP, RAC3, ACTR, AIB1 or TRAM-1). These three members have an overall sequence similarity of 50-55% and sequence identity of 43-48%. As well as being NRs, NCOAs also serve as co-activators for many other transcription factors associated with breast cancer, such as (HIF1), (NF-κB), (E2F1), p53, RB and (MRTFs) (48, 49). By regulating a broad range of gene expression controlled by NRs and non-NR transcription factors, NCOAs regulate diverse events in the development of breast cancer. NCOA1 is overexpressed in 19% to 29% of breast cancer cases and plays important roles in cell proliferation, lymph node metastasis, disease recurrence and poor disease-free survival (DFS) (50). Therefore, elevated expression of NCOA1 has been regarded as an independent predictor of breast cancer recurrence following therapy (51). Although the evidence is not conclusive, NCOA2 overexpression might also promote proliferation and invasion of breast cancer cells. In addition, NCOAs play important roles in the chemotherapy resistance of breast cancer. Increased expression levels of the ER-NCOA3 complex were found in tamoxifen-resistant cells, and such overexpression of NCOA3 could enhance the agonist activity of tamoxifen and, therefore, reduce its antitumor activity in patients with breast cancer (52).

The 18 HDACs identified so far can be categorized into four classes: class I (HDAC 1-3, HDAC 8), class II (HDAC 4-7, 9-10), class III (Sirtuin 1-7) and class IV (HDAC 11). Class I, II and IV HDACs share homology in both sequence
and structure and all require a zinc ion for catalytic activity (53). HDACs remove the acetyl groups from histone lysine tails and are thought to facilitate transcriptional repression by reducing the level of histone acetylation. Like HATs, HDACs also have non-histone targets. Several HDACs have been found to be involved in breast cancer. In ER-positive MCF-7 breast cancer cells, expression of HDAC6 was increased after treatment by estradiol, and elevated expression of HDAC6 increased de-acetylation of alpha-tubulin and increased cell motility (54).

In vivo assays showed that patients with high levels of HDAC6 mRNA tended to be more responsive to endocrine treatment than those with low levels, indicating that the level of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prognostic indicator of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prognostic indicator of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prognostic indicator of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prognostic indicator of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prognostic indicator of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prognostic indicator of HDAC6 expression might be used both as a marker of endocrine responsiveness and also as a prog

**Histone methylation.** Histones can be mono-, di-, or tri-methylated at lysine or arginine residues by histone methyltransferases (HMTs). Many HMTs, including both lysine-specific HMTs (e.g. SMYD3) and arginine-specific HMTs (e.g. PRMT1 and CARM1), have been shown to act as ER coactivators and be involved in breast cancer.

Many histone lysine methyltransferase (HKMTs) have been isolated and characterized. Except for (Dot1), all HKMTs contains a conserved Su(var), enhancer of zeste, trithorax (SET) domain that is responsible for catalysis and binding of co-factor S-adenosyl methionine, and many have been shown to play roles in breast cancer (58). Other methyltransferase attack mechanisms of breast cancer is arginine methyltransferase (HRMT). The protein arginine methyltransferase (PRMT) family is the major family of HRMTs to date. The PRMTs are classified into four groups depending on the type of methylarginine they generate: Type I (PRMT1, PRMT2, PRMT3, PRMT4, PRMT6 and PRMT8), type II (PRMT5, PRMT7 and PRMT9), type III (remained unclear) and type IV (only found in Saccharomyces cerevisiae as yet) (59).

Compared to HKMTs, the evidence for the involvement of HRMTs in human cancer is not forthcoming. However, underexpression of PRMT1 has been observed in breast cancer (60). PRMT4, also known as co-activator-associated arginine methyltransferase-1 (CARM1), is a co-activator for nuclear receptors and is overexpressed in prostate and breast cancer (61). PRMT4 plays an important role in estrogen-induced cell-cycle progression in the MCF-7 breast cancer cell line. Upon estrogen stimulation, the E2F1 promoter is subject to PRMT4-dependent dimethylation on H3R17, and this recruitment of PRMT4 by ERα is dependent on the presence of NCOA3 (62).

**Histone phosphorylation.** Phosphorylation of H3 on S10 and S28 is important not only during mitotic chromosome condensation but also in transcriptional activation of immediate early genes and growth factors stimulating the (RAS)/(MAPK). Increasing H3 pS10 at transcriptionally active loci may contribute to aberrant gene expression and breast cancer progression (63).

**Other histone modifications in breast cancer.** Besides acetylation, methylation and phosphorylation, there are some other modifications which occur on histones. These epigenetic changes include ubiquitination/sumoylation, ADP-ribosylation, de-amination, and proline isomerization. Although the functions and mechanisms have not been demonstrated, some studies have shown that these modifications are also associated with breast cancer and other types of human cancer.

Recent studies revealed that E3 ubiquitin ligases play important roles in breast carcinogenesis. Ubiquitin-mediated protein degradation plays an important role in many cancer-related cellular processes. E3 ubiquitin ligases play critical roles because they control substrate specificity of histone. Accumulating evidence suggests that genetic and expression alteration of E3 ubiquitin ligases contributes to breast carcinogenesis (64).

**miRNAs in Breast Cancer**

miRNAs have been shown to play a critical role in the regulation of a wide range of biological and pathological processes. Recent large-scale profiling approaches have revealed that miRNAs are globally down-regulated in several cancer types, including breast cancer. Moreover, panels of miRNAs in breast carcinomas, characteristic for the HER2/neu or ER status of the analyzed tumors have been detected (65). There is now increasing evidence that signatures of miRNA expression may not only be used in the future as tumor biomarkers for diagnosis and patient risk stratification, but since hypermethylation was identified as an important mechanism of miRNA silencing, de-regulated miRNAs may also represent novel targets for an anticancer therapy. One study describing differential expression patterns of miRNAs in breast cancer also investigated their expression changes in relation to chromosomal localization. Interestingly, the authors found several miRNA candidates that reside in chromosomal regions which are either frequently deleted or amplified in breast cancer, e.g. down-regulation of miR-125b in the frequently deleted region 11q23-24, or overexpression of miR-21 in 17q23, which is commonly amplified in breast cancer (66). Another mechanism by which miRNA profiles may be altered in tumors lies within abnormalities in the miRNA-processing machinery. It was the same study that observed significant changes in expression of Dicer and (AGO1), both being involved in miRNA maturation processes (67).
Decreased Dicer expression was recently observed in breast cancer, where loss of expression represented an independent prognostic factor in metastatic disease, and reduced expression of Dicer was associated with the highly aggressive mesenchymal phenotype (68). Whether genetic lesions such as (TARBP2) mutations in colorectal cancer also account for impaired miRNA processing in breast cancer remains to be determined in future studies. It is, however, conclusive that besides DNA hypermethylation of miRNA genes, structural genetic alterations also contribute to the observed dramatic changes of miRNA expression profiles in human cancer.

Conclusion

Phytoestrogens seem to protect against breast cancer if consumed throughout life, particularly before and during adolescence, and a low plasma enterolactone concentration is known to increase the risk of breast cancer. Whether phytoestrogens are actually responsible for the protection is not known, however, it is more likely that the soybean products or grain-fiber complexes are protective.

Research in phytoestrogens has increased dramatically in recent years, as can be seen by the numerous publications. However, many questions remain. Research is still needed to evaluate the safety of phytoestrogens on human systems, beneficial and harmful doses, gender differences in response to phytoestrogens, differences in the chemical classes of phytoestrogens and the effects phytoestrogens may have with other drugs or dietary products. Due to the functional and structural differences of phytoestrogens, their biological activities are also highly variable and there may be other effects that have not yet been studied.

In summary, epigenetic modifications provide crucial regulatory functions in the process of gene transcription, and they play very important roles in the proliferation, metastasis, chemotherapy and other aspects of breast cancer, as well as in many other types of human cancer. An understanding of all these epigenetic changes and their contribution to breast cancer might allow great progress in the field of diagnosis, prognosis and therapy of breast cancer. We may thus hope that many of the open questions about the impact of phytoestrogen and epigenetics will be answered in the near future.

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