Chanchal Cabrera MSc, MNIMH, AHG

Estrogen modulation as a treatment strategy in breast cancer

Chanchal Cabrera MSc, MNIMH. AHG

2005

1. How estrogen might cause breast cancer

2. Key therapeutic strategies

3. Botanical & nutritional intervention in breast cancer

4. Opportunities to inhibit estrogen formation

5. Opportunities to inhibit estrogen function

6. Opportunities to increase estrogen excretion and elimination

7. Other mechanisms of reducing breast tumorigenesis

ESTROGEN MODULATION AND TREATMENT OF BREAST CANCER

Evidence suggests that hormones play a major role in the etiology of breast cancer, with the risk of developing malignancies related to the cumulative exposure of the breast to estrogen and progesterone, which stimulate the growth of tumor cells (Murphy 1998, Yager 2000). Estrogen receptors are expressed in approximately 35-55% of all breast tumors but up to 80-90% of tumors from women older than 55 years (Kuerer et al 2001). Since 1896, when Sir George Beatson demonstrated that ovariectomy induced regression of mammary tumors in women, the aim of endocrine breast cancer therapy has been to selectively deprive the body of estrogen. Ovariectomy accomplished this by removing the gland that is the predominant source of estrogens in premenopausal women. Since the avoidance of such surgery is preferable, emphasis is devoted to the pharmacological inhibitors of estrogen production. The most recent area of clinical research has been in selective estrogen receptor modulators (SERMS) which are capable of behaving as both agonists and antagonists in different tissues at different times (NIH May 2002).

Substantial evidence supports the concept that estrogens cause breast cancer in animals and in women but the precise mechanism is unknown.

How estrogen might cause breast cancer

Endogenous estrogens stimulate proliferation of breast cells and thus statistically increase the chances for genetic mutations.

Estrogen metabolism generates oxygen-free radicals and quinones which produce both stable and unstable DNA adducts and result in genetic mutations which accumulate and could ultimately cause cancer.

In situ synthesis of estrogen due to the over-expression of intra-cellular aromatase (Santen 2001).

Estrogen is a steroid hormone, manufactured in the body from cholesterol by way of pregnenolone, progesterone, androstenedione and testosterone. The final step in the pathway is conducted in the ovaries, adrenal glands and skin. Exogenous estrogens may contribute to the total load although the extent to which this is significant is unproven (Nakagawa et al 2000, McDougal 1998).

Xeno-hormones may work bi-functionally, through genetic or hormonal paths, depending on the periods and extent of exposure. Xeno-hormones can modify DNA structure or function..

Two distinct mechanisms can influence the potential for aberrant cell growth: compounds can directly bind with endogenous hormone or growth factor receptors affecting cell proliferation or compounds can modify breast cell proliferation altering the formation of hormone metabolites that influence epithelial-stromal interaction and growth regulation. Beneficial xeno-hormones, such as indole-3-carbinol, genistein, and other bioflavonoids, may reduce aberrant breast cell proliferation, and influence the rate of DNA repair or apoptosis and thereby influence the genetic or hormonal micro-environments (Davis et al 1997, Osbourne 1999).

Estrogen is comprised of three main types: Estrone, Estradiol and Estriol, with Estrone conferring the least cell-proliferant activity and Estriol conferring the most. Estrogen manufactured and active in peripheral tissues and organs eventually ends up in the liver where it is metabolized for excretion. Hydroxylation reactions in phase I detoxification yields 16alpha hydroxyestrone (16alpha-OH), 4 hydroxyestrone (4-OH) or 2 hydroxyestrone (2-OH).

All three hydroxyestrones can be excreted via glucuronidation, methylation or sulfation and are eliminated in bile and urine. The 2-OH is the most readily excreted and hence is considered favorable in cancer prevention (Yue et al 1997, Muti et al 2000). 16alpha-OH may be reduced to form estriol..

In the presence of certain critical co-factors, (L-methionine, B12, B6, S-adenosyl methionine, S-adenosyl homocysteine, choline, folate, magnesium) 4-OH is cycled into 2-OH in the liver. In the absence of these co-factors, and in the absence of adequate levels of anti-oxidants, then 4-OH may oxidize into toxic quinines..

Unavailability or deficiency of glutathione-S-transferases, the family of iso-enzymes that act as intra-cellular anti-oxidants, permits the onwards formation quinones into toxic mercapturates (Maugard et al 1998).

The physiological effects of estrogens are mediated by ligand-activated nuclear transcription factors, the estrogen receptors (ERs). The ERs and other steroid hormone receptors are members of the nuclear receptor family of transcription factors that exhibit common structural domains. .

ERs are predominantly located in the nucleus, although they can move between the nucleus and the cytoplasm, where they are complexed with heat shock proteins (hsp56, hsp90 and possibly hsp70) until activated by an appropriate ligand (COT 2002). Bound ERs dissociate from hsp's and subsequently dimerise and undergo a conformational change. The activated dimers have high binding affinities for specific DNA-binding sites called oestrogen receptor response elements (ERE) which are situated in promoter regions upstream of estrogen-sensitive genes. Proteins known as coactivators or corepressors, are believed to be essential for ER action and influence the level of expression of oestrogen-responsive genes. .

Binding to the ERE results in the initiation or repression of target gene transcription and ultimately elicits a biological response (Clarke et al, 1996; Diel et al, 1999; Fitzpatrick, 1999; Gillesby & Zacharewski, 1998). Normal human mammary tissue has been shown to predominantly express ERb mRNA, whereas most ER-positive breast tumours appear to exhibit increased ratios of ERa:ERb (Leygue et al, 1998; Speirs et al, 1999). .

It has been shown that ERa and ERb signal in opposite ways from the activator protein 1 (AP1) site when complexed with estradiol. With ERa, estradiol has been found to activate transcription, whereas with ERb, estradiol inhibits transcription. Moreover, anti-estrogens such as tamoxifen and raloxifene are potent activators of transcription with ERb at an AP1 site. Thus the two ERs signal in different ways, depending on both ligand and response element (Paech et al, 1997). The estrogen binding site is flexible and can accommodate a wide variety of compounds with structural similarities to estradiol, such as the phytoestrogens. Estradiol binds with equal affinity to both ERa and b (Kuiper et al, 1997). However, the phytoestrogens, coumestrol, genistein and daidzein show greater selectivity towards binding to ERb (Kuiper et al, 1997).

The difference in the amino acid sequence between ERa and b accounts for this selectivity (Pike et al, 1999). It is unknown whether the lignans have selectivity for one receptor subtype (COT 2002). The estrogen receptor can also be activated in the absence of a ligand by phosphorylation. Epidermal growth factor, human epidermal growth factor receptor-2 (HER-2) and insulin are all capable of activating the ER through mechanisms involving the Ras signal transduction pathway. This pathway includes the mitogen-activated protein kinase (MAPK) family and is responsible for the phosphorylation of many proteins involved in cell signalling. .

Several studies have shown that phosphorylation by MAPK can bring about the activation of ER in the absence of ligand (Gillesby & Zacharewski, 1998). Thus, it is possible that interference with this pathway may produce oestrogen-like effects without direct ER-ligand interactions..

Key therapeutic strategies

A number of factors contribute to the formation, development and progression of breast cancer and many of them offer opportunity for intervention. .

Hormonal (estrogen) signaling may be modulated by regulating the formation, function and excretion of estrogens. This may be acheived, for example,.

by ingestion of dietary phyto-estrogens or by modulation of gut microflora and of hepatic metabolic processes with dietary items and nutritional supllements, as well as with targetted drugs..

Angio-genesis and consequent metastasis may be inhibited in many ways:

* Signal transduction pathways may be modulated by inhibiting a variety of growth factors (Fidler 2000, Goldman and Melo 2001, Earlbaum 2000), Epidermal Growth Factor (EGF), Fibroblast Growth Factors (FGF), Transforming Growth Factor (TGF), Platelet-Derived Growth Factor (PDGF), Insulin-Like Growth factors(ILGF), Protein Tyrosine Kinase (PTK), Protein Kinase C (PKC), tumor suppressor genes, Vascular Endothelial Growth Factor (VEGF) and reticular activating system (RAS). Protein kinases are essential enzymes for cellular growth and differentiation.

They allow cells to respond to external stimuli through signal transduction pathways by catalysing the phosphorylation of serine, threonine and tyrosine residues in proteins (Scott & Pawson, 2000). A number of studies have shown that genistein can inhibit tyrosine kinases in vitro (COT 2002). Akiyama et al (1987) demonstrated that genistein (150 mM) inhibited tyrosine kinase activity by binding to the catalytic domain of the enzyme. Genistein (100 mM) suppressed growth of MCF-7 breast cancer cells in response to insulin by inhibiting insulin induced tyrosine kinase activity (Pagliacci et al, 1994).

Dalu et al (1998) demonstrated that increasing concentrations of genistein in the diet (250-1000 mg/kg diet) inhibited tyrosine-phosphorylated proteins of 85 and 170 kD in rat prostate. Genistein (1000 mg/kg diet) repressed the expression of EGF (epidermal growth factor - a protein involved in cellular proliferation) and its phosphorylation product by 50% and inhibited expression of ERb2. (estrogen receptor beta 2).

However, kinase inhibition may be cell dependent and modulated by the concentrations of estradiol and other growth factors suggesting that genistein may not consistently inhibit tyrosine kinase (Panno et al, 1996; Peterson, 1995; Peterson, 1995; Peterson & Barnes, 1993; Peterson & Barnes 1996; Peterson et al, 1996; Twaddle et al, 1999). Alpha-tocopherol (vitamin E) supplementation has demonstrates an inhibitory effect on VEGF (WoOdson et al 2002)..

* Angio-genesis may also be inhibited by regulating the process of inflammation that the tumor uses to promote angiogenesis (Harris et al 1999, Watson 2001, Tang et al 1995, Dethelfsen et al 1994). This is achieved through several mechanisms including cyclo-oxygenase 2 (COX 2), 5, 12 & 15 lipoxygenase (LOX), platelet aggregation and Hydroxyl-3-methylgutaryl coenzyme A reductase (HMG CoA reductase) and by chelating copper from the system. Many compounds exert anti-inflammatory and chemo-protective effects, including Curcumin from Turmeric. (Levi et al 2001, Jaga and Duvvi 2001).

Urokinase type plasminogen activator (uPA) and matrix metalloproteinease (MMP) are two families of enzyme modulators that tumor cells use to alter the extra-cellular fluid matrix (ECF) such that they can spread more easily. Their expression is regulated by oncogenes, tumor promoters and growth factors. In health their expression is prevented by specific inhibitors in the matrix. (Heber et al 1999, Folkman 1995). Stabilizing the ECF, which is a form of connective tissue, can reduce cancer spread. The use of glycosaminoglycans (GAGS) as a nutritional supplementation has shown promise in treating cancer for this reason. The herbalist would turn to collagen strengthening agents (connective tissue tonics) such as Horsechestnut, Gotu kola or Plantain.

Cell adhesion molecules (CAM) are cell receptors that control intra-cellular and inter- cellular communication. CAMs regulate organ architecture, cell migration, cell differentiation, apoptosis (programmed cell death), mitosis, platelet aggregation and the activity of the immune system. There are 4 main classes of CAMs: cadherins, integrins, cell surface lectins and Immuno- globulin Super Family Cell Adhesion Molecules (ISCAMs). (Heber et al 1999). Activating CAMs may represent a novel approach to cancer therapy.

PHYTO-ESTROGENS

Isoflavones

(soybeans, lentils
and other legumes)

Genistein
Diadzein
Equol
Glycitiein
Biochanin A
Formononetin

Coumestans
(Legume sprouts)

Coumestrol

Prenylflavonoids
(Hops, beer)

8 Prenyharingenin
6 Prenyharingenin
Xanthohumol
Isoxanthohumo

Lignans
(cereal.Flax, fruits, vegetables)

Lariciresinol
Iso- Lariciresinol
Matairesinol
Enterolactone
Seco-isolariciresinol
Enterodiol

Botanical & nutritional intervention in breast cancer

Much recent research has focused on the influence of a variety of naturally occurring substances or their synthetic analogues. Phyto-estrogens, many of them flavonoids in nature, as well as other flavonoids, lignans, catechins, progestins and essential fatty acids have been investigated and many have shown significant promise in reducing the occurance and spread of breast cancer. An extensive literature review revealed thousands of supportive citations for the idea of supplementing the diet with specific botanicals and nutritional supplements and a few inconclusive or dismissive.

In many cases the results of research have demonstrated variable results according to dose or potency of test material. At low concentrations, genistein and coumestrol significantly enhanced E2-induced and tyrosine kinase-mediated DNA synthesis; at high concentrations, inhibition was observed. Differing effects are observed with other compounds (Wang and Kurzer 1998).

Research also suggests that the estrogenic effect of isoflavones may not be mediated via direct interaction with estrogen receptors but via stimulation of the central nervous system by hypothalamic GnRH and subsequent production of endogenous oestrogens mediated by gonadotrophins released from the pituitary (COT 2002).

Additionally, phytoestrogens have been shown to both stimulate and inhibit cell proliferation in estrogen dependent cell lines. These effects appear to be concentration dependent, as phytoestrogens stimulate proliferation in the 0.1-10 mM concentration range but inhibit proliferation at higher concentrations (> 10mM). It has been suggested that the proliferative effects of phytoestrogens may reflect direct receptor mediated responses, whereas the inhibitory effect are not directly mediated via the estrogen receptors. This proposal is supported by evidence that phytoestrogens do not stimulate proliferation of ER-negative cells, but are still inhibitory to their growth at high concentrations

(Pagliacci et al, 1994; Wang et al, 1996; Record et al, 1997; Wang & Kurzer MS, 1997; Zava & Duwe, 1997; Barnes, 1998; Miodini et al, 1999).

Estrogen receptors are expressed in the central nervous system (CNS) (Kuiper et al, 1998; Shughrue et al, 1997). Estrogens are known to be active in a number of areas of the brain and spinal cord, where they are thought to influence behaviour, movement, cognition, pain sensitivity and have a protective effect on the development of neurodegenerative diseases (McEwen, 1999). Therefore phytoestrogens may also exert similar effects in the CNS despite the fact that they do

not easily cross the blood brain barrier. In a study by Lund et al (2001), an increase in COX-2 was found in the brain tissue of adult male, but not female, rats fed an isoflavone supplemented diet (600 mg/kg) during gestation, lactation through to adulthood.

The effect of coumestrol on oestrogen responsive receptors in the brain was examined in ERa knock out and wild type ovariectomised mice. The mice were fed coumestrol (200 mg/kg diet) for 10 days from PND 45 and some animals were also treated with physiological levels of estradiol. Coumestrol treatment had no effect on progesterone receptor expression in the brain but did reduce plasma LH levels. In contrast, estradiol treatment increased progesterone receptor expression and decreased plasma LH levels.

The increase in progesterone receptors seen with estradiol was attenuated when animals were co-administered with coumestrol and estradiol. The effects of coumestrol and estradiol were markedly reduced in ERa knock out animals suggesting the effects may be mediated by ERa (Jacob et al, 2001).

Estradiol is known to affect the development and organization of lymphoid tissues and the activity of various cellular vectors of immune function. In general terms, females display more vigorous immune responses than males (Ansar Ahmed et al, 1985; Grossman, 1984). Estrogen regulates the activation of macrophage production and can cause depression of cell-mediated immunity (Luster et al, 1984). Exogenous levels of estrogens administered to adult female mice increase activity of lymphoid target cells, as well as with non-lymphoid tissue, resulting in the release of soluble immuno-regulatory factors. There is evidence that estrogen receptor activation can influence lymphoid development.

In recent investigations by Erlandsson et al (2001), it was shown that in male mice ERa, but not ERb, is required for normal development of lymphoid tissue. Mice lacking ERa (ERKO mice) exhibited hypoplasia of both the thymus and spleen and had an increased number of immature (CD4+/CD8+) thymocytes. Several other in vivo animal studies have demonstrated marked anti-inflammatory and immune-stimulating effects of genistein and diadzein (Sadowska-Krowicka et al 1998, Zhang et al 1997, Guo et al 2001). These relationships of estrogen and immune function may hold promise for therapeutic intervention in immuno-compromised conditions such as cancer using phyto-estrogens, but much clinical research remains to be done in this area.

Opportunities to inhibit estrogen formation

Circulating steroid sulphates are thought to be the major source of estradiol in post-menopausal breast tumors and sulphation is a key step in the activation of some dietary pro-carcinogens (Kirk *et al* 2001). *Steroid sulfotransferase* catalyses the addition of sulfate to steroid-like compounds and *steroid sulfatase* catalyses the reverse reaction. The equilibrium between these opposing reactions usually lies towards the sulfated compounds and their concentrations are 10-30-fold higher than the unconjugated forms (Harris *et al,* 2000).

Steroid sulfatase activity is of particular importance to post-menopausal women as it produces biologically active estrogens from the less active estrone sulfate and dehydroepiandosterone (DHEA) sulfate in breast tissue. In many breast cancer tumours, estrogen sulfotransferase activity is lower than in normal tissue, and this may account for the increased sensitivity of breast tumours to estrogen (Qian *et al*, 1998). Harris et al (2000) reported that 4-p-ethylphenol, a metabolite of genistein can act as a sulfotransferase substrate. However it remains to be determined if dietary phytoestrogens can generate sufficient 4-p-ethylphenol to reduce the activity of these enzymes in target tissues. Wong & Keung (1997) demonstrated that daidzein sulfoconjugates are potent inhibitors of steroid sulfatase and sulfotransferases. The 17b-hydroxysteroid oxidoreductase (17b-HSOR) enzymes, occur as two isoforms (I and II), involved in the interconversion of estrone to the more active estradiol. Thus 17b-HSOR I converts estrone to estradiol while 17b-HSOR II converts estradiol to estrone.

Phytoestrogens have been found to inhibit both 17b-HSOR enzymes in vitro and have shown a selectivity which is structure dependent (Makela *et al,* 1995; Santti *et al*, 1998; Krazeisen *et al*, 2001; 2002). Inhibition of reductive and oxidative activities of 17beta-HSD is seen in the presence of many dietary compounds, especially zearalenone, coumestrol, quercetin and biochanin A. The position and number of hydroxyl groups on the isoflavone structure appear to be the principal determinant of activity.

Inhibitor potency increases with an increasing number of hydroxylations in the flavonoids molecule (Krazeisen *et al* 2001). Compounds with hydroxyl groups at the 5, 7 and 4' positions were the most potent inhibitors of 17b-HSOR I and compounds with hydroxyl groups at positions 3, 5 and 7, inhibited 17b-HSOR II. In addition, hydroxylation at position 3, or methylation of the hydroxyl group at position 4' reduced 17b-HSOR I activity while hydroxylation at position 3 reduced 17b-HSOR II activity. The IC_{50} values of these compounds for inhibition of 17b-HSOR I

and II are in the range 0.1-1 mM (Makela et al, 1998). Thus, phytoestrogens may alter the activities 17b-HSOR I and II to shift the equilibrium concentrations of estrone and estradiol. However, the direction of this equilibrium shift is dependent on phytoestrogen structure.

Inhibition of aromatase enzyme (CYP19 of the cytochrome P450 series) through use of lignans and flavonoids (Wang *et al* 1994, Campbell and Kurzur 1993).

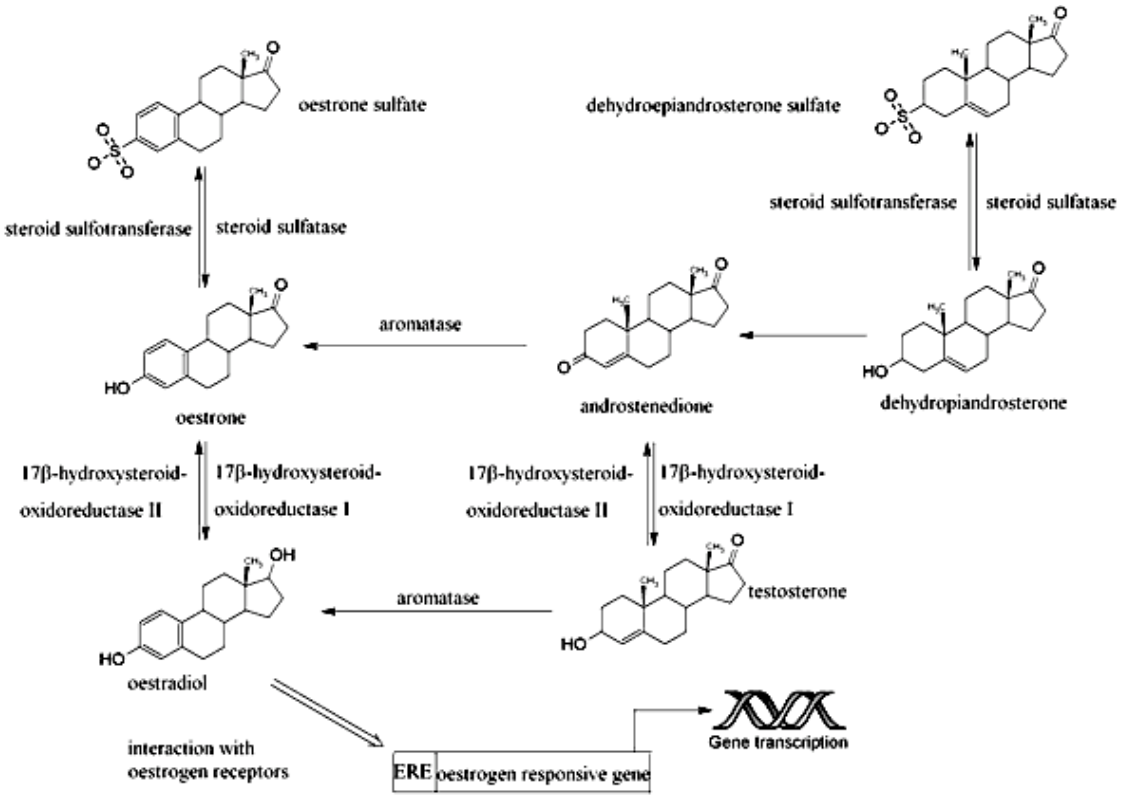
Wine has been shown to contain phytochemicals that are capable of suppressing aromatase. Red wine was shown to be much more effective than white wine in the suppression of aromatase activity (Eng *et al* 2001).

In another study, among several flavonoids tested, only 7-methoxyflavanone and 7,8-dihydroxyflavone at high concentrations (50 microM) possessed anti-estrogenic and anti-proliferative activities.

These results suggest that two hydroxyls (in positions 7 and 8) or 7-methoxy substitutes are essential for the anti-estrogenic activity of flavonoids. However, the authors emphasize that flavonoids at high concentrations appear to exert their anti-proliferative activity through other estrogen receptor-independent mechanisms as well (Le Bail *et al* 1998).

Enzymes involved in oestrogen biosynthesis and metabolism

Adapted from Kirk et al (2001)



Opportunities to inhibit estrogen function

Human estrogen receptors (ER) exist as two subtypes, ER alpha and ER beta, which differ in the C-terminal ligand-binding domain and in the N-terminal transactivation domain. Uterine tissue favors the alpha type while in breast tissue the predominant type is beta.

Malignant breast tumors usually demonstrate more alpha type receptors. Competition with estrogens at the receptor site of the target tissue can modulate estrogen activity at the functional level. Several lignans and the isoflavonoids daidzein and equol were found to compete with estradiol for binding to the rat uterine type II estrogen binding site (Adlercreutz 1992). Some phytoestrogens such as coumestrol, genistein, apigenin, naringenin, and kaempferol compete more strongly with E2 for binding to ER beta than with ER alpha (Kuiper et al 1998) and the effect of this on clinical outcomes has yet to be investigated. The compound Indole-3-carbinol

(I3C), extracted from cabbage, and its more metabolically available and active form, diindolyl-methane (DIM) have received much attention recently as potent mechanisms for influencing estrogen function at the receptor site.

DIM is a major in vivo product of I3C, and can inhibit the proliferation of both estrogen-dependent and -independent breast tumor cells. It is an antagonist of estrogen receptor function and a weak agonist of aryl hydrocarbon (Ah) receptor function (Chang et al 1999, Chen et al 1998, Jaga and Duvvi 2001).

Green tea extract inhibited protein kinase C activation by teleocidin, a tumor promoter, as did (-)-epigallocatechin gallate (EGCG), the main physiologically active polyphenol in green tea extract.

In addition, EGCG and green tea extract showed inhibitory effects on the growth of lung and mammary cancer cell lines with similar potencies. An experiment using the estrogen-dependent MCF-7 cell line showed the mechanisms of action of these compounds to be inhibiting the interaction of estrogen with its receptors. The authors suggest that EGCG and compounds in green tea extracts may block the interaction of tumor promoters, hormones and growth factors with their receptors (Komori et al 1993).

Several flavonoids, including kaempferide, apigenin, and flavone, are distinct, in that their anti-estrogenic activity does not appear to correlate with binding to ER, and therefore their suppression of estrogen-mediated gene trans-activation and proliferation may occur independent of direct antagonism of the receptor. It is suggested that receptor binding-independent anti-estrogenic chemicals may function through alternate signaling pathways as indirect ER modulators in a receptor- and cell type-specific manner (Collins-Burrow et al 2000).

Research with coumestrol, genistein, biochanin A, apigenin, luteolin, kaempferol, and enterolactone showed conflicting results. Induction of DNA synthesis in estrogen-dependent cell lines but not in estrogen-independent cell lines is consistent with an estrogenic effect of these compounds. Inhibition of estrogen-dependent and -independent breast cancer cells at high concentrations suggests additional mechanisms independent of the estrogen receptor (Wang and Kurzur 1997).

One interesting piece of research found that genistein and curcumin together exerted a significantly greater effect than the individual substances given in isolation (Verma et al 1997, Santibanaz et al 2000).

In women approximately 37% of circulating estradiol is bound to SHBG and 61% to serum albumin. Stimulation of SHBG synthesis by phyto-estrogens reduces the concentrations of free hormones (COT 2002). Isoflavones and lignans support SHBG formation and function (Adlercreutz et al 1992, Schottner et al 1997). Conversely, phyto-estrogens may inhabit binding sites on SHBG and displace endogenous hormones, this increasing the bio-activity, however concentrations of >100 mcg. of genistein, diadzein, and coumestrol were required to competitively inhibit estradiol and Dihydrotestosterone. It is unlikely that sufficient serum levels would be achieved through normal dietary intake (COT 2002).

Opportunities to increase estrogen excretion and elimination

Estrogen is metabolized along three pathways to form the 2-hydroxylated, the 4 hydroxylated and the 16alpha-hydroxylated metabolites. Based on proposed differences in biological activities, the ratio of 2-hydroxyestrogen:16alpha-hydroxyestrone (2:16alpha-OHE1), has been used as a biomarker for breast cancer risk. Women with an elevated 2:16alpha-OHE1 ratio are hypothesized to be at a decreased risk of breast cancer.

Flaxseed, the most significant source of plant lignans, and wheat bran, an excellent source of dietary fiber, have both been shown to have chemo-protective benefits.

In one study urinary excretion of 2-hydroxyestrogen and 16alpha-hydroxyestrone, as well as their ratio, 2:16alpha-OHE1, were measured by enzyme immunoassay. Flaxseed supplementation significantly increased the urinary 2:16alpha-OHE1 ratio ($P = 0.034$), but wheat bran had no effect (Haggans et al 2000).

In another study flaxseed supplementation significantly increased urinary 2-OHEstrogen excretion ($p < 0.0005$) and the urinary 2/16 alpha-OHE1 ratio ($p < 0.05$) in a linear, dose-response fashion. There were no significant differences in urinary 16 alpha-OHE1 excretion. These results suggest that flaxseed may have chemo-protective effects in postmenopausal women (Haggans et al 1999).

Soluble fiber such as is found in fruits, vegetables and certain grains including oats, undergoes metabolism in the small and large intestine and has an appreciable effect on modifying carcinogens in the colon. Insoluble fiber such as is found in wheat and rice bran, does not alter carcinogenic metabolites but does give bulk to the stool, thus diluting potential toxins and speeding the transit time and hence reducing toxin exposure overall (Weisburger et al 1993).

- Rat studies have demonstrated that administration of green tea stimulated liver microsomal glucuronidation of estrone and estradiol by as much as 37%. Enzyme kinetic analysis indicates that the inhibition of estrone glucuronidation by 10 microM (-)-epigallocatechin gallate was competitive while inhibition by 50 microM (-)-epigallocatechin gallate was noncompetitive. Similarly, several flavonoids (naringenin, hesperetin, kaempferol, quercetin, rutin, flavone, alpha-naphthoflavone and beta-naphthoflavone) also inhibited rat liver microsomal glucuronidation of estrone and estradiol to varying degrees. Naringenin and hesperetin displayed the strongest inhibitory effects.

These two hydroxylated flavonoids had a competitive mechanism of enzyme inhibition for estrone glucuronidation at a 10 microM inhibitor concentration and a predominantly noncompetitive mechanism of inhibition at a 50 microM inhibitor concentration (Zhu et al 1998, Nakachi et al 1998).

Research into chrysin-induced UDP-glucuronosyltransferase (UGT) activity and expression in human intestinal cell lines, demonstrated that flavonoids may be important for the glucuronidation and detoxification of cells (Galijatovic 2001)

Potential exists to promote the preferential excretion of 2-OH through the use of several compounds, notably Indole-3-carbinol (IC3) or its more potent and bio-available metabolite, Di-indolyl-methane (DIM). Similar effects can be obtained with flax, kudzu and soya. Supplementation with L-methionine, S-adenosyl-methionine (SAM), vitamin B12, helps the inter-conversion of 2-OH and 4-OH in the liver.

4-OH may convert into quinones which are potentially carcinogenic and need lots of free radical quenching. Anti-oxidants can inhibit the formation of these compound.

Maintain or elevate plasma Sex Hormone Binding Globulin which has been correlated to increased urinary excretion of 16 alpha-hydroxyestrone and estriol (Adlercreutz 1992). High intakes of caffeinated coffee, green tea, and total caffeine were commonly correlated with increasing sex hormone-binding globulin after controlling for potential confounders. Although the effect of caffeine cannot be distinguished from effects of coffee and green tea, consumption of caffeine-containing

Unquenched quinoines become mercapturates. Formation and excretion of these products is mediated by glutathione-s-transferase (GST) which can be supported by supplementation with glutathione, selenium and N-acetyl-cysteine (NAC). Reduced glutathione and N-acetylcysteine can inhibit both apoptosis and necrosis of several cell types, suggesting a critical role for reactive oxygen species (ROS) in cell death (Gouaze et al 2001, Hamada et al 2001). However, research into the effects of NAC is equivocal and more investigation is called for (Lubet et al 1997).

Recent research has indicated that certain GST genotypes may have greater susceptibility to malignant changes than others (Mitrunen et al 2001) and in future this may be used as a clinical test to evaluate the need or potential benefit from supplementation with GST or its pre-cursors.

An enzyme in the bowel called glucuronidase can de-conjugate estrogen waste metabolites and allow them to be re-absorbed in an oxidized and highly reactive form (Gorbach and Goldin 1987, Goldin et al 1987).

The body of evidence suggests that supplementation of the diet with potent botanical and nutritional extracts provides positive health benefits.

Other mechanisms of reducing mammary tumorigenesis

As described above, many factors play into the formation, development and progression of breast cancer and many of them offer opportunity for intervention.

Reduction of Insulin-Like Growth Factor I (IGF1) levels which are associated with abnormal cell turnover. Reduced plasma levels of IGF1 are inversely related to urinary lignan excretion after supplementation with flaxseed (Rickard et al 2000). Both the oil and the seed have been investigated. Flaxseed, a rich source of lignan precursor secoisolariciresinol-diglycoside (SD) and alpha-linolenic acid (ALA), has been shown to be protective at the early promotion stage of carcinogenesis. Reduction in tumor size is due in part to the lignans.

The effect of flaxseed oil may also be related to its high ALA content. The SD in flaxseed appears

to be beneficial throughout the promotional phase of carcinogenesis whereas the oil component is more effective at the stage when tumors have already been established (Thompson et al 1996).

Involvement of non-hormonal mechanisms such as may be triggered by lignans. Research with rats into the lignan hydroxymatairesinol (HMR) from Norway Spruce, the most abundant single component of spruce lignans, has demonstrated that it is metabolized to enterolactone (ENL) as the major metabolite in rats after oral administration. HMR decreased the number of growing tumors and increased the proportion of regressing and stabilized tumors. HMR (50 mg/kg body wt) did not exert estrogenic or anti-estrogenic activity. Neither ENL nor enterodiol showed estrogenic or anti-estrogenic activity via a classical alpha- or beta-type estrogen receptor-mediated pathway. HMR was an effective antioxidant in vitro (Saarinen et al 2000).

Many flavonoids are strongly anti-oxidant and this may reduce tumorigenesis by inhibiting DNA damage and promoting DNA repair. Regulation of cell protein content and inhibition of protein, DNA and RNA synthesis has been demonstrated by quercitin (Rodgers and Grant 1998).

Increased intracellular reduced glutathione (GSH) content and hence quenching of free radicals and inhibition of oxidative damage has been demonstrated by quercitin and myricetin. (Rodgers EH, Grant MH, 1998).

Investigation into a number of naturally occurring chemo-preventive agents such as curcumin, quercetin, auraptene, 1'-acetoxychavicol acetate (ACA) and indole-3-carbinol showed generation of apoptosis as well as inhibition of cell proliferation (Mori et al 2001).

Progestins may also exert direct anti-estrogenic action by increasing the oxidative activity of 17 beta-hydroxy-steroid-dehydrogenase, thereby facilitating the conversion of estradiol (the more active form) to estrone (the less active form). Progestins may exert additional anti-estrogenic effects by suppressing estrogen receptor levels. They also cause estrogen deprivation indirectly through suppression of pituitary ACTH secretion, resulting in reduced production of adrenal androgen precursors.

Aromatase inhibition in pre-menopausal women interrupts estrogen biosynthesis; the reflex rise in FSH then stimulates production of new aromatase enzyme, and the LH increment results in enhanced ovarian steroidogenesis, counteracting the inhibitory action of aromatase-blocking drugs on the ovary (Schneider et al 1994).

Dihydrobenzofuran lignans (2-phenyl-dihydrobenzofuran derivatives) constitute a new group of anti-mitotic and potential anti-tumor agents that inhibit tubulin polymerization. A dimerization product of caffeic acid methyl ester, showed promising activity. It inhibited mitosis at micromolar concentrations in cell culture through a relatively weak interaction at the colchicine binding site of tubulin (Pieters at al 1999).

Reduction of the highly proliferative terminal end bud (TEB) structures in the developing mammary gland by differentiation to alveolar buds (ABs) and lobules has been suggested to be protective against mammary cancer and may be achieved through the ingestion of flaxseed and exposure to protective lignans. Research showed that flax seed also caused endocrine changes, as suggested by early puberty onset and lengthened cycles due to prolonged estrus. This increased exposure to endogenous estrogens and stimulated mammary gland differentiation, as indicated by fewer TEBs and more Abs (Tou and Thomson 1999).

Two citrus flavonoids, hesperetin and naringenin, found in oranges and grapefruit, respectively, and four non-citrus flavonoids, baicalein, galangin, genistein, and quercetin, showed inhibitory effects on proliferation and growth of a human breast carcinoma cell line. The most potent single flavonoids was baicalein and the addition of quercitin to any of the other flavonoids increased their potency. Although tumor incidence and tumor burden (grams of tumor/rat) were somewhat variable in the different groups, rats given orange juice had a smaller tumor burden than controls, and they grew better than any of the other groups (So et al 1996).

In vitro, anti-proliferative effects of different progesterone antagonists or anti-progestins (PAs) are observed, mainly in estrogen-stimulated growth of PR-positive tumor cell lines. In various experimental animal tumor models, different PAs showed a greater anti-tumor activity than tamoxifen Combination treatment of different PAs, or progesterone receptor modulators (PRMs) with different anti-estrogens or with an aromatase inhibitor showed greater antitumor efficacy than treatment with each single type of drug alone. In some studies, these effects were accompanied by additive effects on several cell biologic parameters (Klijn et al 2000).

Inhibition of cyclin-dependent kinases has been demonstrated experimentally using Flavopiridol, a novel semisynthetic flavone analogue of rohitukine, a leading anticancer compound from an

Indian tree (Zand et al 2000).

Both genistein and equol interfere with signal transduction pathways but in one study genistein was 15-fold more growth-inhibitory than equol. At 100 mumol/l they both decreased c-fos levels, by 75 and 67%, respectively. Enterolactone and enterodiol had only a weak inhibitory effect. suggest that inhibition by genistein of epidermal-growth-factor (EGF)-induced c-fos mRNA transcription is probably related to its interruption of EGF receptor-linked protein tyrosine kinase, whereas genistein-induced growth arrest is not (Schultze-Mosgau 1998).

Aberrant hyperproliferation (AH) is a late occurring post-initiational event that precedes mammary tumorigenesis in vivo. Treatment of initiated cells with naturally occurring tumor inhibitors eicosapentaenoic acid (EPA), indole-3-carbinol (I3C), (epigallocatechin gallate (EGCG), squalene (SQE), and perillyl alcohol (PA) (analog of limonene) at non-toxic doses, resulted in a 70-99% inhibition of AH, depending on the initiator and the chemopreventive test compound.

Up-regulation of AH in initiated mammary epithelial cells in vitro prior to tumorigenesis in vivo, and persistent inhibition of AH by diverse naturally occurring tumor inhibitors, provides evidence for AH as a cellular surrogate endpoint for induction and modulation of mammary neoplastic transformation (Katdare et al 1997).

References

Adlercreutz H, Mousavi Y, Clark J, Hockerstedt K, Hamalainen E, Wahala K, Makela T, Hase T., 1992, Dietary phytoestrogens and cancer: in vitro and in vivo studies, J Steroid Biochem Mol Biol, Mar;41(3-8):331-337

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y., 1987, Genistein, a specific inhibitor of tyrosine-specific protein kinases. Journal of Biological Chemistry., 262:5592-5595.

Ansar Ahmed S, Penhale WJ, Talal N. 1985, Sex hormones, immune responses and autoimmune diseases. American Journal of Pathology, 121:531-539.

Barnes S. Phytoestrogens and breast cancer. 1998, Baillieres Clin Endocrinol Metabol. 1998, 12:559-579.

Campbell D, Kurzur M, 1993, Flavonoid inhibition of aromatase enzyme activity in human pre-adipocytes, J Steriod Biochem Mol Biol, Sep; 46 (3): 381-88

Chang YC, Riby J, Chang GH, Peng BC, Firestone G, Bjeldanes LF., 1999, Cytostatic and antiestrogenic effects of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane, a major in vivo product of dietary indole-3-carbinol, Biochemical Pharmacology, Sep 1;58(5):825-34

Chen I, McDougal A, Wang F, Safe S., 1998, Aryl hydrocarbon receptor-mediated antiestrogenic and antitumorigenic activity of diindolylmethane, Carcinogenesis, Sep;19(9):1631-9

Clarke R, Hilakivi-Clarke L, Cho E, James MR, Leonessa F. 1996, Estrogens, phytoestrogens, and breast cancer. Adv Exp Med Biol., 401:63-85.

Collins-Burow BM, Burow ME, Duong BN, McLachlan JA, 2000, Estrogenic and anti-estrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms, Nutr Cancer, 38(2):229-244

COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment), 2002, UK Ministry of Health, Working report available online at

Dalu A, Haskell JF, Coward L, Lamartiniere CA. 1998, Genistein, a component of soy, inhibits the expression of the EGF and ErbB2/Neu receptors in the rat dorsolateral prostate. Prostate, 37:36-43.

Davis DL, Telang NT, Osborne MP, Bradlow HL., 1997, Medical hypothesis: bifunctional genetic-hormonal pathways to breast cancer, Environmental Health Perspectives, Apr;105 Suppl 3:571-6

Dethlefsen S, Shepro D, D'Amore P, 1994, Arachidonic acid metabolites in bFGF, PDGF and serum-stimulated vascular cell growth, Experimantal Cellular Research, 212: 262-73

Diel P, Smolnikar K, Michna H. 1999, In vitro test systems for the evaluation of the estrogenic activity of natural products. Planta Medica., 65:197-203.

Earlbaum L Associates, 2000, Nutrition and Cancer, 37 (2) 119 -27

Eng ET, Williams D, Mandava U, Kirma N, Tekmal RR, Chen S, 2001, Suppression of aromatase (estrogen synthetase) by red wine phytochemicals, Breast Cancer Res Treat, May; 67(2):133-46

Erlandsson MC, Ohlsson C, Gustafsson JA, and Carlsten H., 2001, Role of oestrogen receptors alpha and beta in immune organ development and in oestrogen-mediated effects on thymus. Immunology, 103:17-25.

Fidler I, 2000, Regulation of neoplastic angio-genesis, Journal of the National. Cancer Institute. Monographs, No. 28 10-13

Fitzpatrick LA. 1999, Selective estrogen receptor modulators and phytoestrogens: new therapies for the postmenopausal women. Mayo Clinic Proc., 74:601-607.
flavonoids and citrus juices, Nutr Cancer, 26(2):167-181

Folkman J, 1995, The influence of angio-genesis research on management of patients with breast cancer, Breast Cancer Research and Treatment, 36, 109-118

Galijatovic A, Otake Y, Walle UK, Walle T., 2001, Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in Caco-2 cells--potential role in carcinogen bioinactivation, Pharm Res, Mar;18(3):374-9

Gillesby BE, Zacharewski TR. 1998, Xenoestrogens: mechanism of action and strategies for identification and assessment. Environ Toxicol Chem - Annual Review., 17:3-14.
Goldin BR, Adlercreutz H, Gorbach SL, 1982, Estrogen patterns and plasma levels in vegetarian and omnivorous women, New England Journal of Medicine, 307: 1542 - 1547

Goldman J, Melo J, 2001,Targeting the BCR-ABL tyrosine kinases in chronic myeloid leukemia, New England Journal of Medicine, 344: 1084 - 86

Gorbach SL, Goldin BR, 1987, Diet and the excretion and enterohepatic cycling or estrogens, Preventative Medicine, 16: 525-531

Gouaze V, Mirault ME, Carpentier S, Salvayre R, Levade T, Andrieu-Abadie N., 2001, Glutathione peroxidase-1 overexpression prevents ceramide production and partially inhibits apoptosis in doxorubicin-treated human breast carcinoma cells, Molecular Pharmacology, Sep;60(3):488-96

Grossman C. 1984, Regulation of the immune system by sex steroids. Endocrine Review., 5:435-454.

Guo TL, McCay JA, Zhang LX, Brown RD, You L, Karrow NA, Germolec DR and Ehite Jr KL. 2001, Genistein modulates immune responses and increases host resistance to B16F10 tumor in adult female B6C3F1 mice. Journal of Nutrition., 131:3251-3258.

Haggans CJ, Hutchins AM, Olson BA, Thomas W, Martini MC, Slavin JL., 1999, Effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women, Nutr Cancer, 33(2):188-195

Hamada J, Nakata D, Nakae D, Kobayashi Y, Akai H, Konishi Y, Okada F, Shibata T, Hosokawa M, Moriuchi T., Increased oxidative DNA damage in mammary tumor cells by continuous epidermal growth factor stimulation, J Natl Cancer Inst 2001 Feb 7;93(3):214-9

Harris R, Kasbari S, Farrar W, 1999, Prospective trial of non-steroidal anti-inflammatory drugs and breast cancer, Oncology Rep, 6, 71-73

Harris RM, Waring RH, Kirk CJ, Hughes PJ. 2000, Sulfation of oestrogenic alkylphenols and 17beta-oestradiol by human platelet phenol sulfotransferases. J Biol Chem, 275:159-166.

Hawkins E, Phyto-medial Research Conference, Zurich, HerbalGram, 50: p71

Heber D, Blackburn G, Liang V, Go W, 1999, Invasion and Metastases, Cancer Research Foundation, Academic Press

Jacob DA, Temple JL, Patisaul HB, Young LJ, Rissman EF, 2001, Coumestrol antagonizes neuroendocrine actions of estrogen via the estrogen receptor alpha. Exp Biol Med, 226:301-306.

Jaga K, Duvvi H., 2001, Risk reduction for DDT toxicity and carcinogenesis through dietary modification., J R Soc Health , Jun;121(2):107-13

Katdare M, Singhal H, Newmark H, Osborne MP, Telang N, 1997, Prevention of mammary preneoplastic transformation by naturally-occurring tumor inhibitors, Cancer Letters, Jan

1;111(1-2):141-7

Kirk CJ, Harris RM, Wood DM, Waring RH, Hughes PJ, 2001, Do dietary phyto-estrogens influence susceptibility to hormone-dependent cancer by disrupting the metabolism of endogenous estrogens?.Biochem Soc Trans, May; 29 (Pt 2):209-216

Klijn JG, Setyono-Han B, Foekens JA, 2000, Progesterone antagonists and progesterone receptor modulators in the treatment of breast cancer, Steroids, Oct;65(10 -11):825-830

Komori A, Yatsunami J, Okabe S, Abe S, Hara K, Suganuma M, Kim SJ, Fujiki H., 1993, Anticarcinogenic activity of green tea polyphenols, Japanese Journal of Clinical Oncology, Jun;23(3):186-90

Krazeisen A, Breitling R, Moller G, Adamski J., 2002, Human 17beta-hydroxysteroid dehydrogenase type 5 is inhibited by dietary flavonoids. Adv Exp Med Biol., 505:151-161.

Krazeisen A, Breitling R, Moller G, Adamski, 2001, Phytoestrogens inhibit human 17beta-hydroxysteroid dehydrogenase type 5, Journal of. Mo lCell Endocrinol , Jan 22;171(1-2):151-162

Kuerer HM, Buzdar AU, Singletary SE ,2001, Biologic basis and evolving role of aromatase inhibitors in the management of invasive carcinoma of the breast, Journal of Surgical Oncology June; 77(2):139-147

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA. 1997, Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology., 138:863-870.

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA., 1998, Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology, 139:4252-4263.

Le Bail JC, Varnat F, Nicolas JC, Habrioux G., 1998, Estrogenic and antiproliferative activities on MCF-7 human breast cancer cells by flavonoids, Cancer Letters, Aug 14;130(1-2):209-216

Levi MS, Borne RF, Williamson JS., 2001, A review of cancer chemopreventive agents Current Medical Chemistry, Sep;8(11):1349-62

Leygue E, Dotzlaw H, Watson PH, Murphy LC. 1998, Altered estrogen receptor alpha and beta messenger RNA expression during human breast tumorigenesis. Cancer Research., 58:3197-3201.

Lubet RA, Steele VE, Eto I, Juliana MM, Kelloff GJ, Grubbs CJ., 1997, Chemopreventive efficacy of anethole trithione, N-acetyl-L-cysteine, miconazole and phenethylisothiocyanate in the DMBA-induced rat mammary

Lund TD, Rhees RW, Setchell KDR, Lephart ED., 2001, Altered sexually dimorphic nucleus of the preoptic area (SDN-POA) volume in adult Long-Evans rats by dietary soy phytoestrogens. Brain Research, 914:92-99.

Luster MI, Hayes HT, Korach K, Tucker AN, Dean JH, Greenlee WF, Booran GA., 1984, Estrogen immunosuppression is regulated through estrogenic responses in the thymus. Journal of Immunology, 133:110-116.

Makela S, Poutanen M, Lehtimaki J, Kostian ML, Santti R, Vihko R, 1995, Oestrogen-specific 17□-hydroxysteroid oxidoreductase type 1 (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens. Proc Soc Exp Biol Med., 208:51-59.

Maugard CM, Charrier J, Bignon YJ., 1998, Allelic deletion at glutathione S-transferase M1 locus and its association with breast cancer susceptibility, Chem Biol Interact Apr 24; 111-112:365-75

McEwen BS., 1999, Clinical review 108: The molecular and neuroanatomical basis for estrogen effects in the central nervous system. J Clin Endocrinol Metab., 84:1790-1797.

Miodini P, Fioravanti L, Di Fronzo G, Cappelletti V., 1999, The two phytoestrogens genestein and quercetin exert different effects on oestrogen receptor function. British Journal of Cancer., 80:1150-1155.

Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, Vainio H, Uusitupa M, Hirvonen A., 2001, Glutathione S-transferase M1, M3, P1, and T1 genetic polymorphisms and susceptibility to breast cancer, Cancer Epidemiol Biomarkers Prev Mar;10(3):229-36

Mori H, Niwa K, Zheng Q, Yamada Y, Sakata K, Yoshimi N., 2001, Cell proliferation in cancer

prevention; effects of preventive agents on estrogen-related endometrial carcinogenesis model and on an in vitro model in human colorectal cells, Mutat Res, Sep 1;480-481:201-7

Murphy MJ Jr, 1998, Oncologist, 3(2):129-130

Muti P et al, 2000, Estrogen metabolism affects breast cancer risk in pre-menopausal women, Epidemiology, November

Nagata C, Kabuto M, Shimizu H., 1998, Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women, Nutr Cancer 30(1):21-4

Nakachi, K., et al., 1998. "Influence of drinking green tea on breast cancer malignancy among Japanese patients." Japanese Journal of Cancer Research 89: 254-61.

Nakagawa Y, Suzuki T, Tayama S., 2000, Metabolism and toxicity of benzophenone in isolated rat hepatocytes and estrogenic activity of its metabolites in MCF-7 cells, Toxicology, Dec 7;156(1):27-36

Osborne MP, 1999, Chemoprevention of breast cancer, Surg Clin North Am, Oct; 79(5):1207-21

Paech K, Webb P, Kuiper GGJM, Nilsson S, Gustafsson JA, Kushner PJ, Scalan TS. 1997, Differential ligand activation of estrogen receptors ER□ and ER□ at AP1 sites. Science, 277:1508-1510.

Pagliacci MC, Smacchia M, Migliorati G, Grignani F, Riccardi C, Nicoletti I., 1994, Growth Inhibitory Effects of the Natural Phyto-Oestrogen Genistein in MCF-7 Human Breast Cancer Cells. European Journal of Cancer., 30:1675-1682.

Panno ML, Salerno M, Pezzi V, Sisci D, Maggiolini M, Mauro L, Morrone EG, Ando S., 1996, Effect of oestradiol and insulin on the proliferative pattern and on oestrogen and progesterone receptor contents in MCF-7 cells. J Cancer Res Clin Oncol., 122:745-749.

Peterson G, Barnes S. 1993, Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. Prostate., 22:335-345.

Peterson G, Barnes S. 1996, Genistein inhibits both estrogen and growth factor-stimulated proliferation of human breast cancer cells. Cell Growth Differ., 7:1345-1351.

Peterson G. 1995, Evaluation of the biochemical targets of genistein in tumor cells. Journal of Nutrition, 125:784S-789S.

Peterson TG, Coward L, Kirk M, Falany CN, Barnes S. 1996, The role of metabolism in mammary epithelial cell growth inhibition by the isoflavones genistein and
biochanin A. Carcinogenesis, 17:1861-1869.

Pieters L, Van Dyck S, Gao M, Bai R, Hamel E, Vlietinck A, Lemiere G., 1999, Synthesis and biological evaluation of dihydrobenzofuran lignans and related compounds as potential antitumor agents that inhibit tubulin polymerization, J Med Chem, Dec 30;42(26):5475-5481

Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljunggren J, Gustafsson JA, Carlquist M. 1999, Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. EMBO J., 18:4608-4618.

Qian Y, Deng C, Song WC. 1998, Expression of oestrogen sulphotransferase in MCF-7 cells by cDNA transfection suppresses the oestrogen response: potential role of the enzyme in regulating oestrogen-dependent growth of breast epithelial cells. J Pharmacol Exp Ther, 286:555-560.

Record IR, Broadbent JL, King RA, Dreosti IE, Head RJ, Tonkin AL., 1997, Genistein inhibits growth of B16 melanoma cells in vivo and in vitro and promotes differentiation in vitro. International Journal of Cancer., 72:860-864.

Rickard SE, Yuan YV, Thompson LU., 2000, Plasma insulin-like growth factor I levels in rats are reduced by dietary supplementation of flaxseed or its lignan secoisolariciresinol diglycoside, Cancer Letters, Dec 8;161(1):47-55

Rodgers EH, Grant MH, 1998, The effect of the flavonoids, quercetin, myricetin and epicatechin on the growth and enzyme activities of MCF7 human breast cancer cells,
Chem Biol Interact, Nov 27; 116(3):213-228

Saarinen NM, Warri A, Makela SI, Eckerman C, Reunanen M, Ahotupa M, Salmi SM, Franke AA, Kangas L, Santti R., 2000, Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (Picea abies, Nutr Cancer, 36(2):207-216

Sadowska-Krowicka H, Mannick EE, Oliver PD, Sandoval M, Zhang XJ, Eloby-Childess S, Clark DA, Miller MJ 1998, Genistein and gut inflammation: role of nitric oxide. Proc Soc Exp Biol Med., 217:351-357.

Santen RJ, 2001, Potential for using aromatase inhibitors for preventing breast cancer, Vopr Onkology; 47(2):187-194

Santibanez, J.F.; Quintanilla, M, ; Martinez, J. 2000, Nutrition & Cancer - An International Journal, v37, n, p49-54

Santti R, Makela S, Korkman J, Kostain ML. 1998, Phytoestrogens: potential endocrine disruptors in males. Toxicol Environ Health., 14:223-237.

Schneider PG, Jackisch C, Brandt B, 1994, Endocrine management of breast cancer, Int J Fertil Menopausal Stud, 39 Review:115-127

Schottner M, Gansser D, Spiteller G, 1997, Lignans from the roots of Urtica dioica (Nettles) and their metabolites bind to human sex hormone binding globulin, Planta Medica, 63: 529-532

Schultze-Mosgau MH, Dale IL, Gant TW, Chipman JK, Kerr DJ, Gescher A, 1998, Regulation of c-fos transcription by chemopreventive isoflavonoids and lignans in MDA-MB-468 breast cancer cells, European Journal of Cancer, Aug;34(9):1425-1431

Scott JD, Pawson T. 2000, Cell communication: the inside story. Scientific American., 282:72-79.

Shughrue PJ, Lane MV, Merchenthaler I, 997, Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system. J Comp Neurol, 388:507-525.

So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK., 1996, Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by

Speirs V, Parkes AT, Kerin MJ, Walton DS, Carleton PJ, Fox JN, Atkin SL., 1999, Coexpression of estrogen receptor alpha and beta: poor prognostic factors in human breast cancer? Cancer Research., 59:525-528.

Tang D, Renaud C, Stojakovic S, Diglio C, Porter A et al, 1995, 12 (S)HETE is a mitogenic factor for micro-vascular endothelial cells: its potential role in angio-genesis, Biochemical Biophysical Research Communication, 211: 462-68

Thompson LU, Rickard SE, Orcheson LJ, Seidl MM., 1996, Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis, Carcinogenesis, Jun;17(6):1373-1376

Tou JC, Thompson LU., 1999, Exposure to flaxseed or its lignan component during different developmental stages influences rat mammary gland structures, Carcinogenesis Sep;20(9):1831-1835

Twaddle GM, Turbov J, Liu N, Murthy S. 1999, Tyrosine kinase inhibitors as antiproliferative agents against an oestrogen-dependent breast cancer cell line in vitro. Journal of Surgical Oncology, 70:83-90.

Verma SP, Salamone E, Goldin B., 1997, Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides, Biochemical Biophysical Research Communication, Apr 28;233(3):692-6

Wang C, Kurzer MS. 1998, Effects of phytoestrogens on DNA synthesis in MCF-7 cells in the presence of estradiol or growth factors, Nutr Cance, 31(2):90-100

Wang C, Kurzer MS., 1997, Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells, Nutr Cancer, ;28(3):236-247

Wang TTY, Sathyamoorthy N, Phang JM. 1996, Molecular effects of genistein on estrogen receptor mediated pathways. Carcinogenesis, 17:271-275.

Watson R, 2001,Vegetables, Fruits and Herbs in Health Promotion, CRC Press, Florida

Weisburger J, Reddy B, Rose D, Cohen L, Kendall M, Wynder E, 1993, Protective mechanisms of dietary fibers in nutritional carcinogenesis, Basic Life Sciences 61; 45-63

Wong CK, Keung WM. 1997, Daidzein sulfoconjugates are potent inhibitors of sterol sulfatase (EC 3.1.6.2). Biochem Biophys Res Commun., 233:579-583.

Woodson K, Triantos S, Hartman T et al, 2002, Long-term alpha-tocopherol supplementation is associated with lower vascular endothelial growrth factor, Anti-Cancer Research, 22: 375

Yager J, 2000, Endogenous estrogens as carcinogens through metabolic activation, Journal of the National Cancer Institute, MonographNo. 27

Yue T, Wang X, Louden C et al 1997, 2-methoxyestradiol, an endogenous estrogen metabolite, induces apoptosis in endothelial cells and inhibits angiogenesis; possible role for stress-activated protein kinase signaling pathway and Fas expression, Molecular Pharmacology, June; 51(6): 951-62

Zand RS, Jenkins DJ, Diamandis EP, 2000, Steroid hormone activity of flavonoids and related compounds, Breast Cancer Res Treat, Jul; 62(1):35-49

Zava DT, Duwe G., 1997, Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. Nutrition and Cancer., 27:31-40.

Zhang R, Li Y, and Wang W. 1997, Enhancement of immune function in mice fed high doses of soy daidzein. Nutr Cancer, 29:24-28.

Zhu BT, Taneja N, Loder DP, Balentine DA, Conney AH., 1998, Effects of tea polyphenols and flavonoids on liver microsomal glucuronidation of estradiol and estrone, J Steroid Biochem Mol Biol, Feb;64(3-4):207-15