Nutritional modulation of the cell cycle and breast cancer

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Abstract

In the USA, breast cancer accounts for approximately 30% of all cancers diagnosed in women and is the second leading cause of cancer death in women. An understanding of the molecular genetic events governing breast cancer lead to both prevention and intervention strategies in an attempt to reduce mortality and morbidity from breast cancer. The last three decades of medical research examining the molecular pathogenesis of cancers have provided compelling evidence for the universal disruption of the cell cycle in human tumors. The importance of cell cycle control in human cancer was recognized by the recent award of the Nobel Prize to Drs Nurse and Hartwell for their discovery of the cyclins. More recent studies have demonstrated a critical interface between hormonal signaling and the cell cycle. In parallel, epidemiological studies have identified as being associated with breast cancer important dietary and environmental components that regulate hormonal signaling. This review describes the intersection of these two fields of study, which together imply a role for dietary prevention and intervention in human breast cancer perhaps through altering cell cycle components.

Introduction

Breast cancer accounts for approximately 30% of all cancers diagnosed in women in the USA. As the second leading cause of cancer death in women, breast cancer accounts for approximately 15% of all female cancer deaths (Zafonte et al. 2000). A comprehensive analysis of cancer mortality and morbidity over the last 25 years has demonstrated a reduction in breast cancer mortality in the 1990s. However, the benefit of reduction in death is greater in white women than in black women. The decline in mortality is likely due to improved screening and early detection, risk factor reduction, and perhaps improved treatment of early stage disease. Risk factors proposed for breast cancer include family history and lifestyle elements, such as diet and lack of exercise.

Over the last two decades, a compelling body of evidence has identified a disruption of cell cycle control mechanisms as a common pathway in human cancer. The importance of the cell cycle to human disease is evidenced by the recent award of the Nobel Prize to Drs Hartwell and Nurse for their discovery of the cyclins. These two parallel pathways function in such a manner that inactivation of one component of the pathway is sufficient for disruption of the pathway’s activity. In this regard, the p16INK4a, retinoblastoma protein (pRb), cyclin D1, cdk4 pathway is frequently inactivated in human breast cancer due to overexpression of cyclin D1. In melanoma, loss of p16INK4a is considered a key initiating event. Thus, distinct tumor types are thought to result from inactivation of distinct components of a common signaling pathway.

Breast cancer and the mammalian cell cycle

Cyclin-dependent kinases

The mammalian cell cycle has been divided into a series of sequential phases. The G1, S, G2, and M phases are sequentially transitioned in response to growth factor or oncogenic stimulation. The DNA synthetic (S phase) and mitotic (M phase) phases are preceded by gap phases (G1, G2). During the transition of the cell cycle, distinct
checkpoints are inactivated. These checkpoints provide mechanisms by which the intracellular compartment senses a favorable growth factor environment and continually transduces this information to ensure genetic integrity during cellular replication. The presence of DNA damage and the integrity of mitotic spindles are assessed during transition between these phases of the cell cycle. Cell cycle progression is orchestrated by the relative activity of a family of serine threonine kinases (Fig. 2). The cyclins encode co-regulatory subunits of a holoenzyme that phosphorylates and inactivates a number of substrates including pRb. Cyclin D1 encodes the labile regulatory subunit of the cyclin cdk4/6 holoenzyme. Phosphorylation of the pRb protein is thought to change the confirmation of pRb which, in turn, alters further upon phosphorylation by cyclin E/cdk2 complexes. It is considered that the phosphorylation of the pRb protein by the cyclin D1/cdk4/6 holoenzymes is required for transition through the G1/S phase of the cell cycle.

The human cyclin D1 gene was initially cloned at a breakpoint rearrangement within parathyroid adenoma. These arrangements brought the parathyroid hormone promoter upstream of the cyclin D1 gene. The gene identified at this breakpoint rearrangement, initially referred to as the PRADI gene, was proposed to encode a cyclin based on homology to the yeast CLN genes (Motokura et al. 1991). Subsequent studies have demonstrated that the cyclin D1 gene is sufficient for the development of parathyroid adenomas (Imanishi et al. 2001). Two decades of subsequent experimentation have confirmed the important role for cyclin D1 in a broad array of human cancers, including human breast cancer.

The cyclin D1 gene is shown to be overexpressed in 30–50% of human breast cancers. Overexpression of cyclin D1 correlates in several studies with poor prognosis. Indeed, the correlation of cyclin D1 with estrogen receptor (ER)-α-positive tumors is said to convert ERα-positive tumors from good to poor prognosis (Kenny et al. 1999). The cyclin D1 gene is shown to physically associate with the ER and induce ER signaling in a cdk-independent manner (Zwijnen et al. 1997). Immuno-neutralizing experiments have shown that cyclin D1 is required for estrogen-induced cellular proliferation (Lukas et al. 1996). Cyclin D1 enhances ERα signaling through functioning as a coactivator-like protein, analogous to the p160 steroid receptor coactivator (SRC) of the ERα, in cultured cells.

Subsequent studies have shown the cyclin D1 gene regulates a number of transcription factors in a cdk-independent manner (Table 1). Two key publications have provided strong evidence for a biological role for two of these molecular targets. In this regard, cyclin D1 was shown to inhibit the activity of two gene products involved in fat metabolism, the CCAAT/enhancer binding protein (CEBP)-β (Lamb et al. 2003) and peroxisome proliferator-activated receptor (PPAR)γ proteins (Wang et al. 2003b). CEBPβ and PPARγ function in a common molecular pathway of adipocyte differentiation. CEBPβ induces PPARγ2 and PPARγ1 in turn, co-ordinates the expression of a number of genes involved in adipocyte differentiation. In these recent studies, cyclin D1 was shown to inhibit the functional activity of either CEBPβ (Lamb et al. 2003) or PPARγ (Wang et al. 2003b).

Lamb et al. (2003) overexpressed either cyclin D1 wildtype or a cyclin D1 that was defective in cdk binding in the MCF-7 breast cancer cell line. Although MCF-7 cells express the cyclin D1 gene, microarray analysis of the cyclin D1-transfected cells showed that cyclin D1 overexpression regulated a subset of genes, some of which were targets of CEBPβ. In turn, a subset of these genes was shown to be dysregulated in human breast cancer. In

![Figure 1 Model for dysregulation of cell cycle proteins (Weinberg 1995, Sherr & Roberts 1999). There are two parallel arms that include either the p16\textsuperscript{INK4a} (INK4a) pathway or the p19\textsuperscript{ARF} pathway, and inactivation of one arm can occur at any point in the respective pathway. Overexpression of cyclin D1 (D1), mutations in CDK4, or mutations in pRB (Rb), for example, will inactivate one arm of the signaling pathway and lead to unchecked cell cycle progression. Inactivation of both arms may therefore be one of the keys to tumorigenesis.](http://www.endocrinology-journals.org)
studies by Wang et al. (2003b), cyclin D1 was found to inhibit the PPARγ protein and its transcriptional activity. Mutagenesis of the cyclin D1 gene again demonstrated that the repression of PPARγ was cdk independent. Using cyclin D1 knockout mice, Wang et al. (2003b) have demonstrated that the biological function of cyclin D1 is to inhibit PPARγ function in vivo. Consistent with a model in which cyclin D1 repressed PPARγ, analysis of cyclin D1 knockout mice demonstrated the presence of hepatic steatosis, a hallmark of PPARγ overexpression. Furthermore, using cyclin D1 antisense-inducible transgenic mice, Wang et al. (2003b) showed that cyclin D1 regulated PPARγ-responsive genes in vivo. Together, these studies suggest that an important physiological function of cyclin D1 is to regulate cellular metabolism, in particular, fat metabolism through PPARγ. The relationship between cyclin D1 and cancer through the regulation of fat metabolism remains to be determined.

**The cyclin-dependent kinase inhibitors**

The cyclin-dependent kinases are inhibited by two families of inhibitors. The cyclin inhibitor protein/kinase inhibitor protein (CIP/KIP) family includes the p21CIF1/WAF1, the p27KIP1, and the p57KIP2 family. The INK4 inhibitor group includes p16 INK4a, p15 INK4b, p18 INK4c, and p19INK4d. The INK4 proteins inhibit the catalytic domains of cdk4 and cdk6. Several additional functions of the p16INK4a protein have been identified, including the regulation of RNA polymerase II (polII) carboxyl terminal kinase domain (CTD) (Nishiwaki et al. 2000). The RNA pol II CTD plays an important role in regulating expression of a number of genes suggesting that p16INK4a may play a broad role in co-ordinating gene expression. In addition, p16INK4a regulates cellular motility through an effect on cdk4 at the cell membrane (Fahraeus & Lane 1999). The CIP/KIP family of proteins inhibit the cyclin E/cdk2 kinase family. The role of the...
CIP/KIP proteins in cell cycle regulation appears to be dose dependent, inhibiting cyclin-dependent kinase activity in cell cycle progression at higher concentrations but serving as a chaperone or assembly protein enhancing cyclin-dependent kinase activities at lower concentrations (Sherr & Roberts 1999). The functions of the CIP/KIP family are, in turn, co-ordinated by additional proteins that regulate the subcellular distribution of the CIP/KIP proteins including jun-activation binding protein-1 (JAB1).

The p27KIP1 protein was characterized as a protein homologous to the p21CIP1 tumor suppressor. The abundance of p27KIP1 is regulated primarily at the post-translational level through an SCF (composed of SKIP, CUL, and F box proteins) complex. The substrate specificity of the ubiquitin ligase or SCF is determined by the F box protein which binds the substrate. The F box protein that binds p27KIP1 is called Skip2. The abundance of Skip2 may therefore contribute to the destruction of p27KIP1. Skip2 is frequently overexpressed in tumor cell lines and collaborates with Ras in cellular transformation.

### Hormonal control of the cell cycle

**Steroid hormones induce cellular proliferation in the breast**

A comprehensive analysis of cell cycle control proteins in this process has been reviewed (Pestell et al. 1999, Fu et al. 2002, Wang et al. 2003a). Estrogens are known to stimulate cell cycle progression, particularly in early G1, and the induction of cellular proliferation correlates with the induction of cyclin D1 expression (Lippman & Bolan 2002).

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**Table 1** Cyclin D1-associated proteins

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Functional relationship with cyclin D1</th>
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<tr>
<td><strong>Cell cycle machinery</strong></td>
<td></td>
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<tr>
<td>CDK4/6</td>
<td>Cyclin D1 forms complex with CDK4/6 and facilitates CDK4</td>
</tr>
<tr>
<td>p21 (CIP1)</td>
<td>p21 represses cyclin D1/CDK4 kinase activity and promotes cyclin D1 nuclear accumulation</td>
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<tr>
<td>p27</td>
<td>p27 assembles cyclin D1/CDK4 kinase complex and represses cyclin D1/CDK4 kinase activity</td>
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<tr>
<td>p57 (Kip2)</td>
<td>p57 inhibits cyclin D1/CDK4 kinase activity</td>
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<tr>
<td>pRb</td>
<td>Cyclin D1/CDK4s phosphorylate pRb and release E2F from an inhibitory complex</td>
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<tr>
<td>PCNA</td>
<td>Forms multiple kinase complexes</td>
</tr>
<tr>
<td>Hsc70</td>
<td>Hsc70 promotes cyclin D1 and cyclin D1-dependent kinase maturation</td>
</tr>
<tr>
<td>Hsp90</td>
<td>Hsp90 promotes cyclin D1 nuclear accumulation</td>
</tr>
<tr>
<td>MCM3/7</td>
<td>Cyclin D1 promotes dissociation of inhibitory pRb/MCM7 complex</td>
</tr>
<tr>
<td>GSK-3β</td>
<td>Phosphorylates cyclin D1</td>
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<tr>
<td>CRM1</td>
<td>Promotes cyclin D1 nuclear export</td>
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<tr>
<td><strong>Acetylase/deacetylase</strong></td>
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<tr>
<td>P300/CBP</td>
<td>Cyclin D1 represses HAT activity</td>
</tr>
<tr>
<td>PCAF</td>
<td>Cyclin D1 represses HAT activity</td>
</tr>
<tr>
<td>SRC-1</td>
<td>Cyclin D1 recruits SRC-1 to ERα</td>
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<tr>
<td>HDAC1</td>
<td>Cyclin D1 recruits HDAC1 to AR</td>
</tr>
<tr>
<td>HDAC3</td>
<td>Cyclin D1 recruits HDAC3 to TR to form ternary complexes</td>
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<tr>
<td><strong>Transcriptional factor</strong></td>
<td></td>
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<tr>
<td>ERα</td>
<td>Cyclin D1 recruits SRC-1 to ERα and activates unliganded ERα</td>
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<tr>
<td>AR</td>
<td>Cyclin D1 represses ligand-bound AR activity</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Cyclin D1 represses PPARγ-mediated transcription and differentiation</td>
</tr>
<tr>
<td>TR</td>
<td>Cyclin D1 represses both the unliganded TR and liganded TR activity</td>
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<tr>
<td>Myb</td>
<td>Cyclin D1 antagonizes B-Myc activity</td>
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<tr>
<td>DMP1</td>
<td>Cyclin D1 antagonizes DMP1 transactivation and overrides DMP1-mediated growth arrest</td>
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<tr>
<td>MyoD</td>
<td>Cyclin D1 represses muscle differentiation and MyoD-mediated transcription</td>
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<tr>
<td>Stat3</td>
<td>Cyclin D1 represses STAT3 activation</td>
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<tr>
<td>Sp1</td>
<td>Cyclin D1 represses Sp1-mediated transactivation</td>
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<tr>
<td>β2/NeuroD</td>
<td>Cyclin D1 represses the bHLH transcription factor, β2/neuroD</td>
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<tr>
<td>bHLH</td>
<td>Cyclin D1 inhibits the activity of myogenic bHLH regulator</td>
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<tr>
<td><strong>Others</strong></td>
<td></td>
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<tr>
<td>TAF(II)250</td>
<td>Cyclin D1 represses Sp1-mediated transcription</td>
</tr>
<tr>
<td>DIP1</td>
<td>Repression</td>
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<tr>
<td>BRCA1</td>
<td>Cyclin D1 rescues BRCA1-mediated ERα repression</td>
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<tr>
<td>GCIP</td>
<td>Cyclin D1 inhibits cyclin D1/CDK4 activity</td>
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Estrogens also alter subcellular localization of the cyclin-dependent kinase inhibitors. The relative distribution of p21
\(^\text{CIP}\) within cyclin E/cdk2 complexes is regulated by estradiol (Planas-Silva & Weinberg 1997). Estrogens can also reduce the amount of p21
\(^\text{CIP}\) and p27
\(^\text{KIP}\) bound to cyclin E/cdk2 (Prall et al. 1997). Estrogen induces both cyclin E/cdk4 and cyclin E/cdk2 activity. As Myc is induced by estrogens and Myc is sufficient for the induction of cyclin E/cdk2 activity, c-Myc may contribute to the enhanced DNA synthesis observed with estrogen treatment. As Myc and cyclin D1 collaborate in several models of oncogenesis, the proliferative activity of estrogens in human breast cancer may be in part regulated by both cyclin D1 and c-Myc.

**Nuclear receptors in breast cancer**

**Estrogen receptor**

The important role for ER\(\alpha\) activity in breast cancer onset and progression is underscored by the efficacy of ER antagonists as adjuncts for breast cancer prevention in high risk individuals and the efficacy of adjuvant therapy for treatment of patients with ER\(\alpha\)-positive breast cancer (Jordan 1998). The ER status thus forms part of the stathmin (TMN) staging classification category 1 (Fitzgibbons et al. 1998). Estrogens influence normal proliferation, differentiation, and physiology of breast tissue and the development and progression of breast cancer (Hoskins & Weber 1994, Korach 1994, Eisen & Weber 1998, Gustafsson 1998, Jordan 1998). The presence of ER\(\alpha\) immuno-reactivity serves as an important prognostic indicator of high survival rates and lower relapse risk (Jordan & Morrow 1999, Brodie 2003). Curiously, approximately 50% of ER\(\alpha\)-positive tumors fail to respond to anti-estrogen therapy, suggesting that important additional components play a role in ER antagonist therapies.

**Androgen receptor**

Androgens induce the expression of endogenous estrogen-responsive target genes and are capable of activating an ER\(\alpha\) reporter gene in MCF-7 cells. Androgens induce proliferation of ER\(\alpha\)-positive cells (Pollin & Labrie 1986, Najid & Habrioux 1990, Bocuzzo et al. 1992, Birrell et al. 1995). A subset of cell lines is exquisitely sensitive to the proliferative effects of androgens, including the Shianogi line (Stanley et al. 1977). The mechanisms by which androgens induce breast cellular proliferation may include induction of ER\(\alpha\). Thus, the androgens dehydroepiandrosterone, 5α-androstene-3β,17β-diol, testosterone, and dihydrotestosterone activate ER\(\alpha\) reporter activity and induce breast cancer cell proliferation (Maggiolini et al. 1999). The effectiveness of aromatase inhibitors in blocking...
the growth of ERα-positive tumors may be in part due to their ability to block androgen induction of ERα activity.

**PPAR**

The PPARs are ligand-activated nuclear receptors including PPARα, PPARγ, and PPARδ. Their modular structure resembles other nuclear hormone receptors with an N-terminal activation and DNA binding domain and a C-terminal ligand binding domain (Rosen & Spiegelman 2001b). PPARγ was cloned as a transcription factor promoting fat cell differentiation. The PPARγ ligands include eicosanoids such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), and synthetic ligands including the thiazolidinedione (TZD) class. It has been estimated that approximately 2 million Americans take anti-diabetic agents which are PPARγ ligands. There is some evidence that breast cancer risk is reduced in individuals taking PPARγ ligands (Michels et al. 2003). These epidemiological data suggest that the mechanisms by which PPARγ ligands may function are of broad importance in cancer.

PPARγ is expressed in breast, prostate, and colon epithelium. Several lines of evidence have implicated PPARγ as a candidate tumor suppressor (Rosen & Spiegelman 2001a, Koehler 2003). Heterozygous mutations of PPARγ were identified in four of five patients with colon cancer (Sarraf et al. 1999). A translocation between paired box homeotic gene 8 (PAX-8) and PPARγ was also identified in follicular thyroid cancer which appeared to serve as a dominant inhibitor of endogenous PPARγ expression (Kroll et al. 2000). Addition of PPARγ ligands to cultured tumor cell lines derived from breast, prostate, or colonic cancer inhibits cellular proliferation (Brockman et al. 1998, Elstner et al. 1998a, Mueller et al. 1998, Ricote et al. 1998). The role of PPARγ ligands in tumor onset in vivo is unresolved. Studies of murine familial adenoma models of adenomatosis polyposis coli using Apcmin mice demonstrated that PPARγ ligands may either promote (Saez et al. 1998b) or inhibit colon polyp formation (Niho et al. 2003). As the same murine model of tumor formation was used in both studies, the explanation for these disparate findings warrants further analysis.

The relative importance of PPARγ signaling in breast cancer onset and progression is an area of substantial current interest. PPARγ expression has been observed in a subset of human breast cancers and benign breast disease (Wang et al. 2003b). In breast cancer cell lines, PPARγ activation by TZDs or 15d-PGJ2 induced cellular differentiation (Elstner et al. 1998a, Mueller et al. 1998). Both TZDs and 15d-PGJ2 inhibit cell cycle progression through direct repression of cyclin D1 gene expression (Wang et al. 2001). The relative importance of PPARγ agonists as chemopreventive agents in humans remains to be explored. The presence of β-catenin activation in the subset of breast cancers (Lin et al. 2000) and the failure of PPARγ ligands to impact tumor progression in the presence of mutant β-catenin in some studies on colon cancer (Saez et al. 1998b) suggest that PPARγ ligands may be important adjuncts to prevent rather than treat human breast cancer.

**Diet and the cell cycle**

Diet has been suggested to contribute to the etiology of 30–50% of newly diagnosed breast cancers (Willett 2001). Animal studies strongly support the ability of dietary components either to increase or reduce breast cancer risk. For example, n-6 polyunsaturated fatty acids (PUFAs) present in vegetable oils pre-initiate (i.e. increase susceptibility to carcinogens and other cancer-initiating factors) and promote carcinogen-induced mammary tumorigenesis (Welsch 1992, Hilakivi-Clarke et al. 1997). In contrast, n-3 PUFAs present in fish, flaxseed, and canola oil reduce the growth of ER-negative human breast cancer cells implanted in nude mice (Rose 1997). Other dietary components that have been suggested to reduce the risk of breast cancer include the phytoestrogen genistein (Bouker & Hilakivi-Clarke 2000), vitamins (such as A, D, and E), and other components in fruits and vegetables (Smith-Warner et al. 2001, Riboli & Norat 2003). Restricted energy intake has also been shown to reduce mammary tumorigenesis in animal models (Zhu et al. 2002). Despite the compelling evidence from experimental models, studies in human populations that have attempted to link a particular dietary factor to increased or reduced breast cancer risk have generated contradictory data (Willett 2001).

Most human studies have assessed dietary intakes at the time of breast cancer diagnosis or shortly before. As breast cancer is a multi-step process, and there is likely to be a considerable time-lapse between initiation and diagnosis of this disease, studies which assess diet only at or around the time of diagnosis are limited and may miss or wrongly identify dietary components involved in increasing breast cancer risk. For instance, dietary components that affect tumor cell proliferation (e.g. alcohol, phytoestrogens, some fats) might be identified as affecting cancer risk. Those that affect susceptibility to malignant transformation (e.g. anti-oxidant vitamins) may not be different between the cases and controls at the time of diagnosis, although when breast cancer was initiated the cases theoretically could have been consuming fewer vitamins than the controls. It is not uncommon that, as an adult, individuals adopt a healthier diet that includes more fruits and vegetables compared with childhood dietary preferences. Interestingly, the evidence
in humans linking diet to breast cancer is strongest for alcohol and perhaps phytoestrogens and fats, but weak for vitamins.

Dietary factors have several different mechanisms of action, depending on dose or timing of exposure, that could ultimately affect breast cancer risk. The phytoestrogen genistein, present in soy products, is an example. Genistein is a weak estrogen that binds to both ERα and ERβ, and at physiological concentrations (<1 μM) causes cell proliferation (Wang et al. 1996, Zava & Duwe 1997). This genistein-induced increase in proliferation has been observed in vitro in human breast cancer cells, in vivo in animals, and also in normal human breast epithelium (Bouker & Hilakivi-Clarke 2000). Conversely, pharmacological doses (> 10 μM) of genistein cause changes that are potentially protective, including inhibition of tyrosine kinase activity, angiogenesis, and induction of apoptosis (Kim et al. 1998, Messina 1999). It is unlikely that soy consumption produces genistein exposures at pharmacological levels, since Asians who, on average, consume high levels of soy compared with Caucasians have blood concentrations around 1–4 μM (Xu et al. 1994). It is not clear whether the concentrations of genistein in the breast tissue reflect those in the circulation, or are lower or higher than in the blood.

Timing of exposure

Timing of hormonal and dietary exposures has important ramifications for breast cancer risk. There are three periods during a female’s life-time when her breast epithelium undergoes extensive growth and is highly sensitive to estrogens: fetal life, puberty, and pregnancy. In rodents, maternal exposure during pregnancy to estrogenic compounds, including dietary compounds, increases breast cancer risk among female offspring (Walker 1984, 1990, Hilakivi-Clarke et al. 1997, 1999). Thus, some breast cancers may be pre-initiated during fetal life by an exposure to high levels of estrogens that may imprint the mammary gland in a manner that increases later susceptibility to breast cancer (Trichopoulos 1990, Hilakivi-Clarke et al. 2002a). Persistent changes in the mammary glands of rodents exposed to a high estrogenic environment in utero include increased number of targets for malignant transformation (Hilakivi-Clarke et al. 1997) and altered expression of ERα and tumor suppressor genes BRCA1 and p53 (Yu et al., unpublished data).

Prepubertal estrogenic exposures paradoxically reduce breast cancer risk (Cabanes et al. 2004), perhaps through differentiation of the mammary gland structures (terminal end buds; TEBs) that are known to be the sites for malignant transformation. The gland of an animal exposed to estradiol or genistein during prepuberty has been found to contain fewer TEBs and more lobulo-alveolar units (Cabanes et al. 2004). These changes are accompanied by long-lasting alterations in the expression of ERα and ERβ (Cabanes et al. 2004).

Prepubertal exposure to estradiol or genistein leads to a long-lasting up-regulation of BRCA1 mRNA in the rat mammary gland (Cabanes et al. 2004), suggesting an increase in DNA repair capacity. The tumor suppressor BRCA1 interacts with estrogens and the ER, at least in normal mouse mammary gland and in human breast cancer cell lines (Gudas et al. 1995, Marquis et al. 1995, Spillman & Bowcock 1996, Fan et al. 1999, 2000, 2001). Loss of wildtype BRCA1 is linked to inherited breast cancers (Garland et al. 1998, Satagopan et al. 2001), probably because this gene participates in DNA damage repair and recombination processes related to maintenance of genomic integrity, control of cell proliferation, and regulation of gene transcription (Rosen et al. 2001, Welch et al. 2002). BRCA1 regulates the expression of several genes implicated in breast cancer, including cyclin D1, c-myc, and components of the Jak-Stat pathway (Welch et al. 2002). In addition, BRCA1 inhibits the signaling of the ligand-activated ERα (Fan et al. 1999).

Pregnancy is closely linked to changes in breast cancer risk with early first pregnancy reducing and later first pregnancy increasing the risk (MacMahon et al. 1970). Higher estrogen levels during pregnancy increase the mother’s breast cancer risk (Richardson et al. 1998, Peck et al. 2002). The proposed mechanism is that the breast tissue of older first time mothers are more likely to have acquired malignant cells that are stimulated by high pregnancy hormonal environment. Collectively, these findings indicate that although estrogens stimulate the proliferation of ER-positive human breast cancer cells, their effect on the normal breast depends on the timing of exposure.

Dietary components that modify the cell cycle

Several dietary factors have been demonstrated to alter the normal cell cycle of non-malignant and malignant breast cells. Herein, we address the role of body mass index (BMI), energy restriction, dietary fat, soy, and vitamin A in affecting breast cancer risk and cell cycle-related end points.

Body weight

Excess weight and obesity are major problems in the Western world because of their link to several serious health problems, including cardiovascular diseases, diabetes, and cancer. According to the 1999 National Health
and Nutrition Examination Survey, it was estimated that 61% of Americans are overweight (BMI 25–30 kg/m²) and 25% are obese (BMI > 30 kg/m²) (Flegal et al. 2002). BMI affects breast cancer risk, but the effects depend on the developmental stage of an individual. Further, multiple pathways have been identified that could mediate the effects of obesity on breast cancer risk.

Adrenal androgens are aromatized to estrogens in adipose tissue. Most circulating estrogens present in postmenopausal women originate from adipose cells, and approximately one-third of estrogens in premenopausal women come from these cells. A high fat and/or a high total caloric intake increases the levels of circulating free estrogens (Goldin et al. 1982, Rose et al. 1987, 1993, Bennett & Ingram 1990) and Wu et al. (1999) have reported that women who reduce their total fat consumption lower their circulating estradiol levels. In postmenopausal women, BMI correlates with elevated expression of cyclin D1 and bel-2 mRNA in the mammary tissue (Suga et al. 2001), indicating that high BMI may increase breast cancer risk by increasing serum estrogens, modulating cell cycle, and inhibiting apoptosis.

Besides estrogens, other hormones and growth factors that are altered by BMI include leptin, adiponectin, insulin, and IGFs. Further, PPARγ has a key role in adipocyte differentiation and in mediating high fat-induced obesity (Kadowaki et al. 2003). Leptin is expressed by the adipose cells as well as epithelial cells: the levels of this hormone are closely linked to BMI, regardless of a woman’s age or reproductive status (Huang & Li 2000). Leptin has been linked to increased breast cancer risk (Marttunen et al. 2000, Tessitore et al. 2000, Ozet et al. 2001, Hu et al. 2002). Leptin also alters the rate of cell proliferation, and modifies the expression of genes linked to the cell cycle, such as cyclin D1, PPARγ, and ERα and ERβ (Okumura et al. 2002). Adiponectin is secreted exclusively by adipose tissue and it is present at high levels in the serum. However, it is reversely associated with BMI (Arita et al. 1999, Stefan et al. 2002). Reduced adiponectin levels have recently been linked to increased breast cancer risk (Miyoshi et al. 2003), and thus this hormone might act as a tumor suppressor. Extensive literature links IGF-I to breast cancer. In addition to being linked to obesity, all these hormones and growth factors interact with each other.

Birth weight

Body weight at birth may alter later risk of developing breast cancer. Women who had a high birth weight have an elevated breast cancer risk in most (Michels et al. 1996, Sanderson et al. 1996) (but not all studies, Potischman & Troisi 1999), particularly for premenopausal breast cancer (Michels et al. 1996, Sanderson et al. 1996). The increase in breast cancer risk by high birth weight is profound in twins (Sparano & Winer 2001), suggesting genetic modifiers of high birth weight on breast cancer risk.

Birth weight has been linked to an elevated estrogenic environment during pregnancy (Gerhard et al. 1987). In human populations, size at birth correlates with increased cellular proliferation, as mammary epithelial area and mammographic density are increased (Eckbom et al. 1995). In animal studies (Hilakivi-Clarke et al. 1997), the results indicate that maternal exposures to a high fat diet or estradiol during pregnancy increased the density of the offspring’s mammary epithelium. Thus, one mechanism by which high birth weight increases later breast cancer risk could be through an increase in epithelial cell proliferation.

Prepubertal BMI

High body mass during childhood is associated with a reduction in breast cancer risk (Le Marchand et al. 1988, Magnusson et al. 1998, Berkey et al. 1999, Hilakivi-Clarke et al. 2001, Swerdlow et al. 2002). This is puzzling in light of the fact that overweight and obese girls reach puberty earlier than lean girls, and early puberty is associated with increased breast cancer risk. A monozygotic twin that reached puberty first has a fivefold higher breast cancer risk than her twin sister with later puberty onset (Hamilton & Mack 2003), suggesting environmental modifiers affecting puberty onset and breast cancer risk. Since in animal studies in utero exposures to estrogenic compounds accelerate puberty onset and increase susceptibility to carcinogen-induced mammary tumors (Hilakivi-Clarke et al. 1997), early puberty may be a marker of high in utero estrogen exposure rather than directly increasing breast cancer risk.

High body mass during childhood may be linked to increased levels of circulating estrogens. Girls with elevated low-density lipoprotein cholesterol levels exhibited a reduction in serum estradiol levels after their dietary fat intake was reduced and fiber intake increased (Dorgan et al. 2003). In heifers, elevated energy consumption during the prepubertal period reduced the growth of mammary parenchyma (Hull & Harvey 2002) and increased breast adipose tissue mass, a predictor of reduced breast cancer risk (Oza & Boyd 1993, Boyd et al. 2001). Similarly, women who had high BMI at puberty have a persistently lower mammographic density (McCormack et al. 2003).

BMI during reproductive years and postmenopause

Postmenopausal breast cancer risk is elevated in individuals who were obese either during the pre- or postmenopausal years, or both (Kabuto et al. 2000).

High BMI during the postmenopausal years correlates with increased serum estrogens (Toniolo et al. 1995, Hankinson et al. 1998), although this may not be true for premenopausal women (Trichopoulos et al. 1983). In premenopausal women, estrogens derived from an excessive amount of adipose tissue are likely to inhibit the pituitary–gonadal axis, potentially causing a reduction in estrogens to be released from the ovaries. However, the total estrogen levels are not altered, because the loss of ovarian estrogen production is compensated for by adipose-derived estrogens.

Energy restriction

Dietary caloric restriction in monkeys and rodents extends lifespan, slows the aging process (Roth et al. 2000), and reduces mammary tumor risk in rodents (Thompson et al. 1999, Jiang et al. 2003). Energy restriction reduces oxidative tissue damage and mitochondrial free radical generation in rodents (Merry 2002). Energy restriction reduces mammary tumor cell proliferation via G1 cell cycle arrest, possibly through a reduced expression of cyclin D1, Cdk4 and increased expression of p21 and p27 (Jiang et al. 2003) perhaps due to increased adrenocortical steroid secretion (Zhu et al. 2003).

It is not known whether the health benefits of energy restriction extend to humans. In Norway (Robsahm & Tretli 2002) and The Netherlands (Dirx et al. 1999), reduction in energy intake in adolescents and women during World War II (1940–44) and the Hunger Winter (1944–45), increased breast cancer risk later in life (Robsahm & Tretli 2002). Consistent with recent data are the findings that low childhood BMI increased breast cancer risk (Le Marchand et al. 1988, Magnusson et al. 1998, Luo & Miller 2000, Hilakivi-Clarke et al. 2001, Swerdlow et al. 2002).

No studies have directly addressed the role of energy restriction during adult life in affecting breast cancer risk. However, women with anorexia nervosa are at a 20% reduced risk of developing a cancer in general (Mellemkjaer et al. 2001). Studies that have assessed longevity in thin individuals have generated conflicting findings (Lee et al. 2001), possibly because many severe illnesses cause weight loss.

Polyunsaturated fatty acids

High dietary fat intake has been linked to the promotion of breast cancer in animal models (Freedman et al. 1990, Welsch 1992), most case-control studies (Howe et al. 1990, Richardson et al. 1990, Van’t Veer et al. 1990), but not in prospective cohort studies (Welsch 1987, Willett et al. 1992, van den Brandt et al. 1993, Willett & Hunter 1994), with few exceptions (Cho et al. 2003a).

Diets are composed of several types of dietary fats, including mono-unsaturated fatty acids (olive and canola oils), PUFAs (vegetable oils, n-6 PUFA and fish, n-3 PUFA), and saturated fats (dairy products and meat). Saturated fats may be associated with increased breast cancer risk in women (Willett 1997, Cho et al. 2003a), but animal studies have generally been inconclusive (Rose 1997). Diets high in n-6 PUFAs are associated with elevated breast cancer risk in animal studies (Freedman et al. 1990, Welsch 1992), but not humans (Willett 2001, Cho et al. 2003a). n-3 PUFAs are protective in some (Eid & Berry 1988, Caygill et al. 1996, Klein et al. 2001), but not all studies (Holmes et al. 2003, Stripp et al. 2003). In nude mice, dietary n-3 PUFA reduces growth and metastasis of human breast cancer xenografts (Rose et al. 1995) and spontaneous or carcinogen-induced rodent mammary tumors (Karmali et al. 1984, Hirose et al. 1990, Fay et al. 1997).

A diet high in n-6 PUFA corn oil, or a low or high fat n-3 PUFA menhaden oil increases serum estradiol levels in rats and mice when compared with a low fat n-6 PUFA standard AIN93 laboratory diet (Hilakivi-Clarke et al. 1996a,b, 1997, 2002b). Consumption of a high fat diet may increase the levels of adipose tissue, and thereby increase aromatization of estrogens. n-6 PUFA consumption may also directly enhance aromatization (Richards & Brueggemeier 2003). It is not clear why n-3 PUFA diet increases serum estrogen levels. PPARγ is either activated (Rubin et al. 2000) or inhibited by n-3 PUFAs (Thoennes et al. 2000), potentially reducing aromatase activity. However, PPARγ agonists can increase arachidonic acid release from the liver (Levine 2001), which could stimulate aromatization.

n-3 and n-6 fatty acids impact cell proliferation and differentiation; however, some studies report inconsistent data (Finstad et al. 1994, Rudolph et al. 2001). The effects of these fatty acids on the cell cycle suggest that n-6 PUFAs increase cyclin D1 mRNA in T47D breast cancer cells (Razanamahefa et al. 2000), the n-3 PUFA docosahexaenoic acid reduces cyclin D1-, E-, and A-associated kinase activity in HT-29 colon cancer cells (Chen & Istfan 2001), and the n-3 PUFA, eicosapentaenoic acid, reduces cyclin D1 and cyclin E in NIH 3T3 cells (Palakurthi et al. 2000).
Prepubertal exposure to PUFAs and breast cancer risk

Maternal exposure to a diet containing high levels of n-3 PUFAs reduces an offspring's risk of mammary tumorigenesis (Hilakivi-Clarke et al. 2002b). In contrast, prepubertal exposure to a high fat n-3 PUFA diet increases carcinogen-induced mammary tumorigenesis, while a prepubertal low fat n-3 PUFA exposure provides a protection against breast cancer (Olivo et al., unpublished data).

Identifying the pathways and timing of exposure by which in utero or prepubertal exposures to n-3 PUFAs may decrease breast cancer risk is complicated, because multiple potential mechanisms exist (Welsch 1987). We have found that changes induced by having been exposed to a low fat n-3 PUFA diet during prepuberty include an increase in lipid peroxidation, but also an increase in the expression of oxidative damage repair genes, apoptosis and cyclo-oxygenase 2. Animals fed a high fat n-3 PUFA diet prepubertally which increases their breast cancer risk also exhibit increased lipid peroxidation, but this is accompanied by an increase in DNA damage, cell proliferation, cyclin D1 expression, and phosphorylated Akt, and reduced apoptosis and BRCA1 expression (Olivo et al. unpublished data). Thus, high levels of n-3 PUFAs fed in a high fat context before the onset of puberty can cause multiple changes in the mammary gland, including disruption of the cell cycle. In contrast, a prepubertal exposure to a low fat n-3 PUFA diet may be protective because of increased oxidative damage repair and apoptosis.

Genistein

Soybeans contain large amounts of the isoflavones daidzein and genistein (Barnes et al. 1994, Adlercreutz 1995). Genistein or equol, a metabolite of daidzein, may play significant roles in the bioactivity of soy (Lampe 2003). It has been proposed that high soy intake contributes to low breast cancer incidence among Asian women (Adlercreutz et al. 1996), and our recent meta-analysis showed that a high soy intake reduces risk of developing premenopausal breast cancer (Trock et al. 2001).


In vitro, genistein has mitogenic effects at low, physiological doses (0.01–1 μM) and anti-proliferative effects at higher, pharmacological doses (>10 μM) (Wang et al. 1996, Hsieh et al. 1998). However, when estradiol is present, genistein may not induce cell proliferation (Trock et al. 2001). It is not clear whether genistein inhibits estrogens actions in humans, since exposure to soy induces breast proliferation in premenopausal, but not postmenopausal women (Petrakis et al. 1996, McMichael-Phillips et al. 1998).

Animal studies have investigated whether an exposure to genistein either in utero or during prepuberty alters the expression of nuclear receptors in the mammary gland or other tissues. At the time of genistein administration, ERα expression is reduced (Cotroneo et al. 2002), a result that is in accordance with findings obtained in vitro in cells treated with estradiol (Saceda et al. 1988). Both fetal and prepubertal genistein administration causes an increase in ERα expression in estrogen-responsive tissues (Jefferson et al. 2002), and prepubertal genistein exposure also up-regulates ERβ in the mammary gland (Y Zhu, R Clarke, S de Assis, J S Miller & L Hilakivi-Clarke, unpublished data). It is plausible that genistein’s ability to bind preferentially to ERβ (Kuiper et al. 1997) might be more important than its effect on ERα expression in affecting breast cancer risk.

Genistein can directly bind to PPARγ to either inhibit it at physiological doses (<1 μM) or stimulate at pharmacological (>5 μM) doses in mesenchymal progenitor cells (Dang et al. 2003), in adipose cells (Dang et al. 2003), and in murine macrophages (Mezei et al. 2003).

Physiological doses of genistein and another phytoestrogen, zearalenone, increased the expression of cyclin D1 and Cdk2 in MCF-7 cells in vitro (Dees et al. 1997) and in vivo (Ju et al. 2002), but higher doses (100–200 μM) inhibited cyclin D1 and cyclin E (Agarwal 2000). The effects of genistein on cyclin D1 are consistent with genistein’s reported ability to both stimulate and inhibit cell proliferation, depending upon the dose.

Vitamin A and retinoids

Since high dietary intake of fruits and vegetables is consistently linked to reduced breast cancer risk (Smith-Warner et al. 2001, Riboli & Norat 2003), it seems reasonable that the vitamins present at high levels in these foods contribute to this effect. These vitamins, including A, C, and E, have several potentially beneficial effects on biological pathways related to preventing oxidative
damage, inhibiting cell proliferation, and inducing differentiation. Surprisingly, the evidence linking each of these vitamins to reduced breast cancer risk is weak.

Retinoid receptor function
Retinoid receptors are members of the nuclear steroid/thyroid hormone receptor family that play a key role in the control of cell growth and differentiation (reviewed in Mangelsdorf et al. 1993, Fontana & Rishi 2002, Fu et al. 2002). Two distinct classes of retinoid receptors exist: the retinoic acid receptors (RARs α, β, γ) and the retinoid X receptors (RXRs α, β, γ). Like other nuclear receptors, these molecules homo- and heterodimerize in response to ligand binding and function as transcription factors at specific promoter elements or indirectly via other transcriptional regulators. In addition, RXRs can heterodimerize with vitamin D and thyroid hormone receptors as well as PPARγ (Dawson et al. 2000).

Activation of retinoid receptors has been shown to be growth inhibitory in breast cancer cells as well as other cell types (see Amos & Lotan 1990, Seewaldt et al. 1997, 1999), and consequently retinoids have been explored as potential therapeutic and chemopreventive agents. Retinoid receptor activation inhibits cell cycle progression, delaying the transition from G0/G1 and S phases (Seewaldt et al. 1997, 1999). RARs and RXRs interact with many different downstream effectors, resulting in cell type-specific mechanisms of growth inhibition and apoptosis. In breast cancer cells, the transcriptional regulator CBP/p300 is up-regulated by trans retinoic acid, functions as a coactivator for RARs, and is required for sensitivity to retinoid-mediated growth arrest (Dietze et al. 2003). Other transcriptional regulators implicated in retinoid responses include the Drosophila hairy and enhancer of split homologue transcriptional repressor and SOX-9 (Muller et al. 2002). The anti-apoptotic factor Bcl-2 is down-regulated by retinoic acid in MCF-7 cells, leading to enhanced caspase activation (Pratt et al. 2003). IGF signaling also appears to be altered in response to retinoids. Retinoic acid induces down-regulation of insulin receptor substrate-1 and Akt, resulting in growth arrest and apoptosis (del Rincon et al. 2003).

Heterodimerization of RXR and PPARγ may contribute to retinoid anti-proliferative signaling in breast cancer. Simultaneous activation of both receptors inhibits breast cancer cell proliferation in vitro and in vivo (Mukherjee et al. 1997, Elstner et al. 1998b). Inhibition of cyclin D1 expression by both RXR and PPARγ stimulation has been demonstrated in a pancreatic cancer cell line (Suh et al. 1999). RXR/PPARγ induce apoptosis by inhibiting nuclear factor-κB-dependent survival pathways (Dubuquoy et al. 2002) and repression of the P450 aromatase gene, thereby decreasing estrogen synthesis in some (Rubin et al. 2002) but not all studies (Mu et al. 2001).

Dietary intake of vitamin A and breast cancer
Most studies, however, have failed to identify a clear association between breast cancer and dietary vitamin A or carotenoid intake (Kushi et al. 1996, Jarvinen et al. 1997, Verhoveen et al. 1997, Michels et al. 2001) (reviewed in Fairfield & Fletcher 2002). Recent studies suggest that increased intake of vitamin A or carotenoids may prevent breast cancer (Bohlke et al. 1999, Zhang et al. 1999, Toniolo et al. 2001), particularly in premenopausal but not postmenopausal women, women who consume alcohol or women who are at high inherited risk for this disease. Vitamin A may be protective for premenopausal women who smoke, but not in non-smokers (Cho et al. 2003b).

In rats, vitamin A-deficient and -supplemented rats develop more carcinogen-induced mammary tumors than animals fed vitamin A-adequate diets (Metz et al. 2002).

Confounding epidemiological variables include the failure of serum levels of retinoids and carotenoids to correlate with consumption (reviewed in Cheung et al. 2003). There are some 600 carotenoid plant pigments, 50 of which may be converted into vitamin A; whether it is strictly vitamin A or other carotenoid metabolites that function to reduce breast cancer risk has not been determined. Similarly, women who consume high levels of carotenoids and vitamin A are also likely to obtain additional nutrients from other fruits and vegetables, and it may be that these contribute significantly to any observed reduction in breast cancer risk (Fairfield & Fletcher 2002).

Conclusions
Several dietary factors modify the cell cycle. Dietary factors that up-regulate cyclin D1 and ERα and down-regulate PPARγ and BRCA1 might play a role in increasing breast cancer risk (Fig. 3). Dietary factors that have opposing effects on these genes could reduce breast cancer risk. In utero exposures to genistein and perhaps n-6 PUFA mediate their effects on the breast partly by affecting these genes and increase breast cancer susceptibility (Ju et al. 2002, Dang et al. 2003). Prepubertal exposure to genistein or a low fat n-3 PUFA diet up-regulates BRCA1 (Cabanes et al. 2004, S Olivo, L Hilakivi-Clarke, Y Zhu, R G Lee, A Cabanes, G Khan, A Zwart, Y Wang & R Clarke, unpublished data) and other genes that lead to increased ability to repair DNA damage, or cause apoptosis and differentiation. Further studies will determine whether the timing of exposure to vitamins, such as vitamin A and E which
induce differentiation and have anti-oxidant properties, modifies later breast cancer risk, and whether changes in susceptibility to malignant transformation are related to diet-induced changes in the cell cycle.

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Figure 3 Examples of dietary factors that increase breast cancer risk, perhaps by up-regulating cyclin D1 and down-regulating PPARγ, and dietary factors that reduce breast cancer risk by having the opposite effects on the expression of these two genes.


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