Molecular links between obesity and breast cancer

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Abstract

Breast cancer continues to be a major health problem for women in the USA and worldwide. There is a need to identify and take steps to alter modifiable breast cancer risks. Conditions of obesity and overweight are risk factors that have reached epidemic proportions. This article reviews the evidence in the literature that test mechanism-based hypotheses which attempt to provide a molecular basis for a causal link between obesity and breast cancer risk, particularly the effects of metabolic syndrome and insulin resistance, peripheral estrogen aromatization in adipose tissue, and direct effect of adipokines. Future areas for study and implications for therapy are discussed.

Endocrine-Related Cancer (2006) 13 279–292

Obesity and breast cancer

Over 40 000 women in the USA die each year of metastatic breast cancer, for which there are currently no permanent cures. In fact, about one-half of women with metastatic disease who undergo therapeutic surgery will experience a metastatic relapse within 5 years. The inability to effectively predict, prevent, and treat metastatic breast cancer is a major problem in breast cancer care. One factor that may impact survival outcome is obesity.

Obesity is a known risk factor for breast cancer. It is generally accepted that obese women have an increased risk for postmenopausal, but not premenopausal, breast cancer (Lahmann *et al.* 2004, van den Brandt *et al.* 2000). A recent study examined overweight, obesity, and mortality from cancer in 495 477 US women over a 16-year period, and found that obese women in the highest quintile of body mass index (BMI) had double the death rate (relative risk, 2.12) from breast cancer when compared with women in the lowest quintile of BMI (Calle *et al.* 2003). Obese breast cancer patients appear to have a higher risk for lymph node metastasis, large tumors, and death when compared

with non-obese breast cancer patients (Berclaz *et al.* 2004, Calle *et al.* 2003).

Compared with some other risk factors for breast cancer such as germline mutations in BRCA1 (relative risk, 2.00) or diagnosed carcinoma *in situ* (relative risk, ~16), the risk from obesity (relative risk, 1.1–2.5) is minor. However, there is some evidence to suggest that, in women with a family history of breast cancer, obesity significantly increases the risk of developing breast cancer compared with slimmer women with a positive family history (Carpenter *et al.* 2003). The physiological mechanism by which these two risk factors interact to promote tumorigenesis is not understood. However, the weight of the evidence in the literature supports the statement that in women with a positive family history of breast cancer the maintenance of a lean body mass could reduce the risk of developing postmenopausal breast cancer.

The prevalence of obesity in the USA and in the world has reached epidemic proportions. Overweight and obesity are defined by the World Health Organisation as a BMI of ≥ 25 and ≥ 30 kg/m², respectively. Normal values of fat mass are 9–18% of the body in males and 14–28% in females, but it may be as much as 60–70% in

morbidly obese individuals. Given the worldwide epidemic of overweight and obesity, it is critical to understand the physiological impact of obesity on cancer development and progression.

Current hypotheses for correlation between obesity and breast cancer

Several hypotheses have been proposed to explain the association of obesity with postmenopausal breast cancer. One hypothesis is that the biological cause of the association between obesity and postmenopausal breast cancer is the elevated circulating estrogens from peripheral aromatization of androgens in adipose tissue in obese postmenopausal women compared with slim postmenopausal women. A second hypothesis is that obesity, as associated with metabolic syndrome, results in an increase in circulating insulin and insulin-like growth factor (IGF), which act as mitogens. Part of their action is also mediated by the crosstalk of this pathway with that of the estrogen receptor pathway in breast cells.

A newer hypothesis places adipocytes and their autocrine, paracrine, and endocrine functions at center stage. This hypothesis sets forth that obesity should be considered an endocrine tumor (Dizdar & Alyamac 2004). Adipocytes, once thought of as exclusively energy-storing cells, are dynamic endocrine cells and secrete various cytokines, polypeptides, and hormone-like molecules. Adipocytes make up the bulk of the human breast, with epithelial cells accounting for only approximately 10% of human breast volume. In many human breast cancers there is reduced connective tissue separating adipocytes from tumor cells. Furthermore, invasive tumors break through the basement membrane and infiltrate fibrous tissue barriers, resulting in an immediate juxtaposition of adipocytes and breast cancer cells, thus allowing paracrine interactions between the two cell types. This review will examine all three hypotheses that offer biological explanations for the underlying mechanism that associates obesity with postmenopausal breast cancer.

Increased estrogen in obese postmenopausal women

Estrogen biosynthesis is catalyzed by the enzyme aromatase (aromatase cytochrome P450), a product of the *CYP19* gene. Aromatase catalyzes the aromatization of the A ring of C19 androgens to the phenolic A ring of C18 estrogens. In obese-post

menopausal women, adipose tissue of the breast, abdomen, thighs, and buttocks are the main sites of estrogen biosynthesis, with levels of aromatase increasing with age and BMI (Grodin *et al.* 1973). In fact, local estrogen levels in breast tumors are as much as 10 times greater than in the circulation of postmenopausal women (van Landeghem *et al.* 1985). This is presumably due to tumor–adipocyte interactions that stimulate the increased production of aromatase (Bulun *et al.* 1994). Other factors, such as tumor necrosis factor α (TNF- α) and interleukin (IL)-6, are secreted by adipocytes and act in an autocrine or paracrine manner to stimulate production of aromatase (Purohit *et al.* 2002).

Estrogen has long been known to be essential for normal mammary development and ductal growth and plays a central role in the development and progression of human breast cancer. Exposure to estrogen and/or an increase in estrogen receptor (ER) expression in human mammary epithelial cells (HMECs) increases the risk of breast cancer. The most convincing, but indirect, evidence for a role for estrogen with obesity is that circulating levels of estrogen are strongly and linearly related to adiposity in postmenopausal women, although this relationship is not seen in obese premenopausal women. Obese postmenopausal women have an increased production of estrogens, and obesity-related breast cancers are more often ER-positive (Rose *et al.* 2004).

Obesity leads to insulin resistance and increased insulin concentrations

Hyperinsulinemia has been correlated with BMI, risk of recurrence, and mortality in breast cancer regardless of ER status (Goodwin *et al.* 2002). In insulin resistance and metabolic syndrome, there is an increase in insulin and glucose (in fasted and in fed states with certain kinds of carbohydrates), an increase in fasted and fed triglycerides and very-low-density lipoproteins, and a decrease in high-density lipoproteins (Grundy *et al.* 2004). Insulin resistance develops as a metabolic adaptation to increased levels of circulating non-esterified fatty acids released from adipose tissue, especially intra-abdominal adipose tissue. Increasing concentrations of non-esterified fatty acids force the liver, muscles, and other tissues to shift towards storage and oxidation of fats for energy. In metabolic syndrome, tissues are not able to absorb, store, and metabolize glucose efficiently. Therefore, to prevent elevated concentrations of glucose in the blood, the pancreas

secrete increasing amounts of insulin in both the fed and fasted states. Insulin signals via its receptors to activate tyrosine kinase signaling and a cascade of intracellular responses. Interestingly, type-2 diabetes in postmenopausal women is correlated with a slight increase in breast cancer risk (Michels *et al.* 2003, Mink *et al.* 2002). Direct evidence for this connection is provided by the finding that diabetic animal models show an increase in susceptibility to chemically induced mammary tumors (Cocca *et al.* 1998, Shafie & Grantham 1981).

Does a high concentration of circulating insulin correlate with incidence of breast cancer? It is well established that insulin stimulates DNA synthesis and is essential for cell growth *in vitro*. In premenopausal women undergoing biopsies for invasive breast cancer without lymph node metastasis or for benign non-proliferative disease, circulating insulin concentration correlated with breast cancer risk (Del Giudice *et al.* 1998). However, a positive effect of insulin concentration on breast cancer risk was seen only in the highest quintile of insulin levels. Hyperinsulinemia can also affect tumorigenesis indirectly by contributing to the synthesis and activity of IGF-I, a growth factor increasingly recognized as critical to breast cancer. IGF-I is a member of the IGF family, which consists of two polypeptide ligands, IGF-I and IGF-II, two membrane-bound tyrosine receptors, IGF-IR and IGF-IIR, and six insulin-like-binding proteins (IGFBPs), as well as IGFBP proteases. IGF-I and IGF-II can act in an endocrine, paracrine, or autocrine fashion to regulate cell growth, survival, transformation, and differentiation, and can synergize with other growth factors to produce enhanced mitogenic effects (Sachdev & Yee 2001, Zhang & Yee 2000). IGFBP-3 binds to and regulates the bioavailability of over 80% of circulating IGF-I. When bound to IGF-I, IGFBP-3 inhibits IGF-I signaling and promotes apoptosis through induction of caspases-7 and -8 (Kim *et al.* 2004). IGF-I can specifically target its receptors on human breast epithelial cells to induce mitogenic and antiapoptotic pathways. Binding of IGF-I to its receptor, IGF-IR, leads to dimerization of the receptor, activation of its tyrosine kinase activity and phosphorylation of key substrate proteins such as insulin receptor substrate-1 (IRS-1) and -2 (IRS-2) proteins and Src homology collagen (SHC). Then, the phosphorylated substrate proteins recruit different SH2-containing proteins and activate various intracellular signaling pathways, including phosphoinositide 3-kinase (PI 3-kinase) signaling pathways and mitogen-activated protein

kinase (MAPK) signaling pathways. Both the PI 3-kinase and MAPK pathways are important for IGF-I-stimulated proliferation of MCF-7 human breast cancer cells *in vitro* (Jackson *et al.* 1998).

Overexpression of IGF-I is particularly effective in promoting tumor growth. Approximately one-half of primary breast tumors overexpress IGF-IR compared with normal tissue, suggesting that these carcinomas have enhanced responses to the mitogenic and antiapoptotic effects of IGF-I (Shimizu *et al.* 2004). Conversely, inactivation of IGF-IR results in reduced mammary tumor growth and metastasis *in vivo* (Le Roith 2003, Sachdev *et al.* 2004). Recently, a transgenic mouse model that overexpresses a CD8-IGF-IR fusion protein under the MMTV promoter was developed. These transgenic mice show abnormal mammary gland development and develop palpable mammary tumors within 8 weeks (Carboni *et al.* 2005). Taken together, these data suggest that IGF-IR may represent a potent target for anti-tumor therapy.

There is no simple, direct relationship between circulating concentrations of IGF-I and degree of adiposity. Several studies have shown a non-linear relationship between circulating concentrations of IGF-I and BMI, with the highest levels of IGF-I at a BMI of between 24 and 27 kg/m² (Allen *et al.* 2003, Lukanova *et al.* 2002). High circulating concentrations of IGF-I were positively correlated with breast cancer risk in premenopausal women, but not postmenopausal women (Muti *et al.* 2002). Likewise, increased IGF-I and decreased levels of IGFBP-3 are correlated with ductal carcinoma *in situ* (DCIS) in premenopausal women (Zhang & Yee 2002). Therefore, the relationship between IGF-I and IGF-I-IR and breast cancer risk in obese postmenopausal women may not be directly causal. However, the crosstalk between IGF pathways and estrogen-mediated signaling, both of which are greatly increased in obese postmenopausal women, may be an important physiological link between obesity and breast cancer risk.

Insulin and estrogen work together

The mechanisms through which estrogen stimulates cell proliferation are believed to be through the activation of ER transcriptional activity and possibly through the direct activation of intracellular signaling pathways such as the MAPK pathway. Insulin and IGFs can activate ER- α transcriptional activity in breast cancer cell lines (Moschos & Mantzoros 2002, Sachdev & Yee 2001), even in the

absence of estradiol (Jackson *et al.* 2001). Together, IGF-I and estradiol increase the transcriptional activation of ER to levels greater than observed with either ligand alone, suggesting synergistic capability (Yee & Lee 2000). Inhibition of MAPK or PI 3-kinase pathways with specific inhibitors abrogates the mitogenic effects of both IGF-I (Jackson *et al.* 1998) and estrogen in human breast cancer cells (Lobenhofer *et al.* 2000). Insulin increases proliferation of ER-positive breast cancer cell lines, but not ER-negative cell lines (Godden *et al.* 1992). In fact, ER- α function may be required to maintain IGF signaling pathways (Dupont & Le Roith 2001, Yee & Lee 2000). In ER-positive MCF-7 breast cancer cells, IGF-I can synergize with estrogen to increase tumorigenicity, while loss of ER- α in MCF-7 cells leads to a decrease in IGF signaling and failure to proliferate in response to IGF or estrogen (Clarke *et al.* 1997). Thus, the insulin/IGF-I pathway interacts with estrogen to synergistically induce mitogenic responses in breast epithelial cells.

Conversely, estrogen can increase the expression of IGF-I, IGF-IR, and IRS-1, resulting in enhanced tyrosine phosphorylation following IGF-I stimulation (Molloy *et al.* 2000, Yee & Lee 2000). Ligand-bound ER may activate the IGF-I pathway by direct binding and activation of IGF-IR (Kahlert *et al.* 2000) or by binding the p85 subunit of PI 3-kinase and the tyrosine kinase, Src, leading to activation of the PI 3-kinase and p21ras/MAPK pathways (Castoria *et al.* 1999).

Ultimately the mitogenic pathways stimulated by estrogen and insulin/IGF-I intersect at the G₁-S phase of cell-cycle progression. Estrogens induce mitogenic effects in breast epithelial cells by stimulating G₀/G₁ resting cells to re-enter the cell cycle and progress through the G₁-S transition and complete a round of cell division. Estrogen action in G₁ phase is mediated by the transcription factor c-Myc. Expression of c-Myc is rapidly induced following estrogen stimulation and downregulated following antiestrogen treatment (Carroll *et al.* 2002). c-Myc is a direct downstream effector of estrogen action; antisense oligonucleotides to c-Myc inhibit the mitogenic effects of estrogen (Watson *et al.* 1991). Estrogen and insulin cooperate through their differential regulation of c-Myc and cyclin D1 to promote cell-cycle progression (Mawson *et al.* 2005).

Given that obese postmenopausal women have more estrogen and more IGF-I and insulin than slim women, it is logical to conclude that the above mechanisms for crosstalk between the IGF and

estrogen pathways would influence breast tumorigenesis to a greater extent in obese postmenopausal women. However, verification of these interactions as the causal link between obesity and postmenopausal breast cancer is still needed.

Hyperinsulinemia results in decreased concentrations of sex-hormone-binding globulin (SHBG)

As adipose tissue mass increases circulating concentrations of insulin and IGF-I, blood concentrations of SHBG begin to diminish (Pugeat *et al.* 1991). In one study, obese women (BMI >30 kg/m²) had an average SHBG concentration that was half that of women with a BMI of <22 kg/m² (McTiernan *et al.* 2003). SHBG binds testosterone and estradiol with high affinity. A decrease in SHBG in obesity results in an increase in the bioavailable fraction of circulating estradiol. In postmenopausal women, breast cancer risk has been shown to be directly related to concentrations of various sex hormones, including estrone, total estradiol, and bioavailable estradiol, while blood levels of SHBG are inversely correlated with breast cancer risk (Zeleniuch-Jacquotte *et al.* 2004). Reanalysis of data taken from prospective cohort studies on BMI and breast cancer risk concluded that the relationship between BMI and breast cancer risk was heavily, if not exclusively, dependent on total or bioavailable levels of estradiol (Key *et al.* 2002). No significant relationship has been seen between serum levels of SHBG, sex hormones, and premenopausal breast cancer risk (Zeleniuch-Jacquotte *et al.* 2004).

SHBG may act directly on breast cancer cells to inhibit estradiol induced cell proliferation. Binding of SHBG to its specific site on MCF-7 breast cancer cells induces the second messenger cAMP (Fortunati *et al.* 1999), and causes a complete inhibition of estradiol-induced MCF-7 cell proliferation (Fortunati *et al.* 1996). Pre-incubation of MCF-7 cells with SHBG before estradiol treatment abrogates the antiapoptotic effect of estradiol (Catalano *et al.* 2005). Thus, SHBG appears to be a regulator of estradiol action in breast cancer cells, acting as an anti-proliferative factor, loss of which in obese women could contribute to tumorigenesis.

Adipocytes secrete adipokines that affect tumorigenesis

Studies show that adipose tissue can directly influence tumor growth. *In vitro*, mature rat adipocytes

promoted the proliferation of breast carcinoma cells when co-cultured in a three-dimensional collagen matrix (Manabe *et al.* 2003). *In vivo*, mice injected subcutaneously or peritoneally with the murine mammary carcinoma cell line SP1 and adipose tissue developed mammary tumors and metastasis; however, no tumor growth or metastasis was seen with injection of SP1 cells distant from any fat pad (Elliott *et al.* 1992). Using a more global and direct approach to identifying genes that respond to factors secreted by adipocytes, MCF-7 breast cancer cells were treated with conditioned media from either murine fibroblasts or murine adipocytes (Iyengar *et al.* 2003). Microarray analysis revealed that the adipocyte-conditioned media upregulated genes involved in invasion, proliferation, and metastasis; while simultaneously downregulating p18 and BARD1, a cell-cycle checkpoint inhibitor and tumor suppressor, respectively. Adipocyte-secreted factors (from 3T3-L1 murine adipocytes) stimulated MCF-7 cell migration and proliferation *in vitro*, and stimulated angiogenesis when compared with fibroblast-secreted factors. *In vivo*, tumors formed from SUM-159PT human breast adenocarcinoma cells co-injected with murine adipocytes grew to more than three times the size of tumors formed from co-injection of SUM-159PT cells with murine fibroblasts (Iyengar *et al.* 2003). This study, for the first time, provided the most direct evidence for a role for adipocytes in promoting aggressive growth in tumors, and served to better define the contribution of different components of the stroma.

The role of preadipocytes in tumor growth is somewhat more controversial. Preadipocytes are defined as immature adipocytes that express the S-100 protein, a marker that is not found in fibroblasts or endothelial cells. Some studies suggest that preadipocytes support the growth of breast tumor cells (Chamras *et al.* 1998), while other studies show that preadipocytes are either inhibitory (Johnston *et al.* 1992) or have no effect (Manabe *et al.* 2003) on breast cancer cell growth. Further studies are needed to elucidate what role, if any, preadipocytes play in breast tumorigenesis.

Leptin

One of many proteins important to obesity is leptin. Leptin, a product of the *Ob* gene, is a 167-amino acid protein secreted by adipocytes in proportion to adipocyte tissue mass (Considine *et al.* 1996). First identified through positional cloning, leptin acts upon the hypothalamus to regulate food intake

and increase activity of thermogenic components of sympathetic nervous system (Ahima *et al.* 1996). Leptin circulates while bound to a soluble form of its receptor and exerts its effects through binding to the leptin receptor (Ob-R), a member of the cytokine family of transmembrane receptors. There are five Ob-R isoforms; the best-characterized one is a long isoform called Ob-Rb, which activates the Janus kinase/signal transduction and activators of transcription (JAK/STAT) signal transduction pathway. A rise in circulating leptin was thought to prevent obesity by decreasing appetite and increasing thermogenesis. However, most cases of human obesity occur simultaneously with increased levels of leptin (Considine *et al.* 1996, Frederich *et al.* 1995, Maffei *et al.* 1995), indicating that obesity may result in resistance to the anorectic effects of the hormone. Although rare, mutations that result in a lack of functional leptin or Ob-R result in extreme obesity in humans (Montague *et al.* 1997).

Leptin gene expression occurs in normal mammary tissue, in breast cancer cell lines, and in solid tumors (Dieudonne *et al.* 2002, Laud *et al.* 2002). Mice deficient for leptin or leptin receptor have impaired mammary gland development (Hu *et al.* 2002). In a direct comparison, leptin receptors were not detectable in normal mammary epithelial cells by immunohistochemistry, whereas carcinoma cells showed positive staining for Ob-R in 83% of cases (Ishikawa *et al.* 2004). In fact, leptin was found to be overexpressed in as many as 92% of breast carcinomas examined, but in none of the cases of normal breast epithelium examined. Interestingly, distant metastasis was present in 21 (34%) cases of 61 Ob-R-positive tumors with leptin overexpression, but in none of the 15 cases where the tumors lacked Ob-R expression or leptin overexpression ($P < 0.05$; Ishikawa *et al.* 2004). Consequently, breast cancer patients with leptin receptor-positive tumors show significantly lower survival than those without leptin or leptin receptor overexpression or expression.

Leptin has been characterized as a growth factor for breast cancer. Leptin induces the proliferation of breast cancer cell lines T47D (Hu *et al.* 2002, Laud *et al.* 2002) and MCF-7 (Dieudonne *et al.* 2002, Okumura *et al.* 2002) by MAPK activation. Although exogenous leptin treatment stimulates proliferation of both transformed and normal breast cell lines, leptin-stimulated, anchorage-independent cell growth was enhanced only in the breast cancer cell line T47D (Hu *et al.* 2002). This suggests that besides having mitogenic effects,

leptin can impact transformed breast cancer cells to induce an alteration to a more aggressive phenotype.

In the obese Zucker fatty rat model, which has a mutation of the long isoform of the leptin receptor, injection of the chemical carcinogen *N*-methyl-*N*-nitrosourea (MNU) caused only 10% of the obese rats to develop mammary carcinoma, while about one-half of MNU-treated lean rats developed mammary carcinoma (Lee *et al.* 2001). Curiously, 60% of MNU-treated obese rats developed epidermal mammary cysts, compared with 0% of MNU-treated lean rats. These results contrast the findings of a recent study in which obese Zucker fatty rats treated with the carcinogen 7,12-dimethylbenz(*a*)anthracene (DMBA) had a mammary tumor incidence double that of the lean control group with shorter tumor latency and more invasive histology (Hakkak *et al.* 2005). Hakkak and colleagues (2005) argue that different mechanisms of action of MNU and DMBA are responsible for the discrepancy in mammary tumor incidences in obese rats.

MMTV-TGF- α /*Lepr^{db}Lepr^{db}* mice that are genetically deficient in the long isoform of the leptin receptor and overexpress the oncogene TGF- α do not develop oncogene-induced mammary tumors (Cleary *et al.* 2003, 2004). These results suggest that, although obesity is linked to shorter latency period and higher tumor incidence, an absence of leptin in obese mice reduces the risk of developing oncogene- and carcinogen-induced mammary tumors very significantly. Clearly, leptin is an important growth factor secreted by adipocytes that strongly influences mammary tumor development.

What is the mechanism of leptin-mediated promotion of breast tumor growth? Its effects appear to be mediated primarily through ER action. Leptin upregulates the transcription of aromatase through enhanced binding of AP-1 to specific DNA sites in the promoter region (Catalano *et al.* 2003). Treatment of leptin also upregulates estradiol/ER- α signaling in MCF-7 cells exposed to aromatizable androgen, and this signal is down-regulated by the aromatase inhibitor letrozole. There is evidence that leptin treatment results in the direct activation of ER- α in MCF-7 breast cancer cells in the absence of its natural ligand. Leptin treatment caused the nuclear compartmentalization of ER- α and the upregulation of a classic estrogen-dependent gene *pS2* (Catalano *et al.* 2004). Leptin also interferes with the effects of anti-estrogen ICI 182,780 on ER- α in breast cancer cells. The exposure of cells to 10 nmol/l ICI 182,780 blocks cell proliferation, induces rapid ER- α degradation,

inhibits nuclear ER- α expression, and reduces ER- α -dependent transcription from estrogen response element-containing promoters. All of these effects of ICI 182,780 are significantly attenuated by simultaneous treatment of cells with 100 ng/ml leptin (Garofalo *et al.* 2004). Furthermore, estrogen stimulates leptin secretion both *in vitro* and *in vivo* (Machinal *et al.* 1999). Thus, high leptin levels in obese breast cancer patients might contribute to the development of antiestrogen resistance.

If leptin expression is common in breast cancer, it could potentially serve as a tumor marker. Here again, there have been conflicting reports as to the level of plasma leptin in breast cancer patients and its utility as a marker for breast cancer. Some studies show that breast cancer patients have higher circulating levels of leptin and greater leptin mRNA expression than control subjects (Tessitore *et al.* 2004), while other studies find no correlation in plasma leptin levels and postmenopausal or premenopausal breast cancer (Sauter *et al.* 2004, Stattin *et al.* 2004). In another study, leptin was shown to interfere with insulin signaling (Fischer *et al.* 2002), and plasma leptin levels directly correlated with degree of insulin resistance in patients with type-2 diabetes (Fischer *et al.* 2002, Wauters *et al.* 2003). In summary, the available evidence can be summarized to state that leptin expression is upregulated in obesity and promotes breast tumor growth by signaling through its receptor and by direct effects on the ER pathway, although its utility as a breast cancer marker is not clear.

Adiponectin

A novel complement-related hormone secreted exclusively by adipocytes, adiponectin (ApN; 30 kDa) has many described functions (Kadowaki & Yamauchi 2005). ApN inhibits the proliferation of several cell types (Arita *et al.* 2002, Brakenhielm *et al.* 2004, Yokota *et al.* 2000), is a negative regulator of angiogenesis (Brakenhielm *et al.* 2004), and promotes insulin sensitization (Yamauchi *et al.* 2002, 2003). ApN acts through its two receptors, AdipoR1 (42 kDa) and AdipoR2 (35 kDa). AdipoR1 is expressed widely in various tissues, including breast tissue (Lorincz and Sukumar, unpublished observations), with the highest level of expression in skeletal muscle, while AdipoR2 is most abundantly expressed in the liver (Yamauchi *et al.* 2003).

Binding of full-length or globular ApN to AdipoR1 or AdipoR2 activates AMP-activated

protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR)- α metabolic pathways, leading to an increase in fatty acid oxidation, glucose uptake, and a decreased rate of gluconeogenesis, thus enhancing insulin sensitivity (Yamauchi *et al.* 2002). ApN gene expression is reduced by TNF- α (Kappes & Loffler 2000), and IL-6 (Fasshauer *et al.* 2003), whereas thiazolidinedione agonists of PPAR- γ increase ApN expression and plasma levels (Combs *et al.* 2002).

Interestingly, low serum levels of ApN are associated with several metabolic diseases, including obesity and insulin resistance in type-2 diabetes (Kadowaki & Yamauchi 2005). Serum levels of ApN are strongly inversely correlated with waist circumference and estimated visceral fat, even more so than BMI (Steffes *et al.* 2004). Studies confirm a significant inverse correlation between serum ApN levels and poor-prognosis breast cancer (Mantzoros *et al.* 2004, Miyoshi *et al.* 2003). Brakenhielm *et al.* (2004) found that treatment of physiologic levels of ApN inhibited mouse fibrosarcoma tumor neovascularization. Thus, obese individuals with low levels of serum ApN could be at a higher risk of developing tumors with aggressive angiogenic support.

Little is known regarding the potential of ApN to directly affect breast epithelial cell growth, proliferation, and differentiation. It has been shown that ligand binding to AdipoR1 and AdipoR2 activates the PPAR- α pathway (Kadowaki & Yamauchi 2005). PPARs activate the transcription of diverse genes that are involved in the regulation of such processes as cell proliferation and differentiation, including adipocyte differentiation. Treatments with PPAR agonists have been useful in treating insulin resistance, although they have also been linked to body-weight gain, a problem that may be ameliorated by the use of partial PPAR agonists. Furthermore, PPAR ligands inhibit the proliferation and promote the differentiation of liposarcoma, colon, breast, and prostate cancer cells by lowering the threshold for apoptosis and inducing arrest in the G₁ phase of the cell cycle. These cells also show a more differentiated and less malignant phenotype. Previous reports have demonstrated the importance of PPARs in the pathogenesis of breast cancer. Treatment of MCF-7 cells *in vitro* with PPAR- γ agonist for 16 h significantly increased nuclear levels of the tumor suppressor BRCA1 (Pignatelli *et al.* 2003). Therefore, one plausible, yet unsubstantiated, explanation for an association between low levels of ApN and breast cancer risk is that downregulation

of ApN ligand in obesity may result in decreased PPAR signaling and low nuclear levels of BRCA1, and thereby lower levels of DNA repair.

TNF- α

TNF- α was the first inflammatory cytokine to be identified as being secreted by adipocytes. TNF- α expression in white adipose tissue was initially demonstrated in rodents, and found to be markedly increased in obese models (Hotamisligil *et al.* 1993). It has been proposed that TNF- α plays a role in the development of insulin resistance through multiple effects, including the inhibition of the insulin receptor signaling pathway (Hotamisligil 2003). The extent to which TNF- α produced in adipose tissue is released into the circulation is unknown, although a correlation between the TNF- α system (including the soluble receptors) and indices of obesity has been reported (Bullo *et al.* 2003).

TNF- α in adipose tissue acts in both an autocrine and a paracrine manner to influence a range of processes, including apoptosis and the synthesis of several cytokines and other adipokines (Coppack 2001). Recent studies have shown that TNF- α is a key regulator of the synthesis of IL-6 (do Nascimento *et al.* 2004), and the biosynthesis of estrogen by stimulating aromatase expression in adipose tissue *in vivo* (Purohit & Reed 2002). Therefore, obese individuals with an overall increase in fat tissue mass could have an increase in circulating TNF- α that could lead to breast tumorigenesis by contributing to insulin resistance and regulating the synthesis of IL-6, a hypothesis that remains to be verified.

IL-6

IL-6 is expressed in, and secreted by, adipocytes and acts both locally and through circulation. Both plasma levels of IL-6 and expression in adipose tissue are elevated in obesity and insulin resistance (Vozarova *et al.* 2001, You *et al.* 2005). IL-6 receptors are found in the hypothalamus in mice, and therefore it is possible that IL-6 could act directly on the central nervous system (Wallenius *et al.* 2002). It has been proposed that IL-6, along with leptin, could be responsible for conveying information from adipocytes to the hypothalamus in the regulation of energy balance. Whereas IL-6 has been found to act as an inhibitory factor in early-stage breast cancer, high serum levels of IL-6 are associated with poor outcome in advanced metastatic breast cancer (Bachelot *et al.* 2003). IL-6 promotes

cell migration by activating the MAPK pathway, and acts as an antiapoptotic factor by inhibiting the activation of proteases involved in apoptosis. Finally, IL-6 promotes osteoclast formation and inhibits dendritic cell differentiation, thus facilitating metastatic growth (Grano *et al.* 2000). IL-6 is also known to stimulate aromatase expression in adipose tissue *in vitro* and *in vivo*, thereby stimulating estrogen biosynthesis (Purohit & Reed 2002), and possibly directly contributing to breast cancer progression.

Future directions

The influence of individual adipokines on breast carcinogenesis is beginning to be elucidated. The field is complex, with multiple adipokines contributing to adiposity. But it is clear that in the presence of obesity the levels of certain critical factors, like leptin secreted by adipocytes in the breast, will significantly influence the outcome. Even in obese animals, lack of leptin prevents tumors from developing in TGF- α transgenic mice. Delineating the function of each of the adipokines is important to continue to identify key molecules important for breast cancer development.

From the current body of literature it seems clear that, in humans, expression of adipokines varies with degree of adiposity. Micro-environmental cues depending on the degree of adiposity may direct the expression and secretion of adipokines from adipocytes. Murine 3T3-L1 adipocytes are a useful and widely used model system; however, 3T3-L1 adipocytes may not secrete the same array of adipokines in the same concentrations as human adipocytes from an obese individual. Human adipocyte cell lines from obese and slim individuals, very easily established in culture from liposuction samples, could provide a useful alternative to murine adipocyte cell lines. Another approach would be to utilize the *in vivo* model developed by Weinberg and colleagues. In this mouse model, the mammary fat pad of an immunodeficient mouse is cleared and reconstituted with desired experimental stromal and epithelial components (Kuperwasser *et al.* 2004). Reconstitution of the mammary fat pad with epithelial cells and human adipose tissue from obese or slim individuals may provide more precise information as to the function of adipocytes in obesity.

Implications for therapy and treatment

Currently there are at least three methods of intervention for disease in regard to obesity and breast cancer. The first method is preventive screening by

use of clinical breast examinations and mammograms. The second is weight loss as a preventive measure. The third is the use of pharmacologic drugs that target obesity-related pathways.

There have been several studies that suggest that obese women are less likely to seek regular preventive screenings, such as clinical breast examinations or mammograms (Meisinger *et al.* 2004, Wee *et al.* 2004). This is significant because preventive screening is thought to be one of the most effective evidence-based tools for the control of breast cancer by early diagnosis. The psychological or sociological reasons for avoiding clinical examinations could be determined and ameliorated to ensure that breast cancer in obese women is detected as early as possible.

The effect of weight loss and maintenance of a lean body mass on breast cancer risk has been studied with moderate to significant results. In three studies, weight loss occurring over a prolonged interval was associated with only a slightly lower risk that did not reach significance (Ballard-Barbash *et al.* 1990, Brinton & Swanson 1992, Trentham-Dietz *et al.* 1997). Other studies show that overweight postmenopausal women who intentionally lose 20 lb or more have cancer rates similar to lean women (Parker & Folsom 2003), and that losing $\geq 10\%$ body weight significantly reduces the concentrations of estrone, total and bioavailable estradiol, serum leptin, blood lipids, and insulin (Jen *et al.* 2004). These studies suggest that weight loss through calorie deficit and/or exercise improves breast cancer risk. Obesity is therefore a modifiable breast cancer risk.

Obese postmenopausal women have an increase in circulating estrogen and aromatase compared with slimmer women. Antiestrogens are widely used in the management of hormonally responsive breast cancer in both adjuvant and palliative settings, and are currently being evaluated as chemopreventive agents. The classical mechanism of action of these drugs involves inhibition of estrogen-stimulated neoplastic cell proliferation by blockade of estrogen receptors present on breast cancer cells. The commonly used antiestrogen tamoxifen also acts to reduce serum IGF-I levels, which are increased in obese women. Aromatase inhibitors are a newer class of drug that bind reversibly or irreversibly to the aromatase enzyme in peripheral fat and also in the breast, leading to undetectable plasma levels of estrogens in postmenopausal women. These agents have been shown to be more effective than tamoxifen in first-line treatment of estrogen receptor-positive advanced and metastatic

breast cancer. Additionally, aromatase inhibitors have been demonstrated to be superior as both adjuvant and neoadjuvant treatment and show reduced uterine growth side effects when compared with tamoxifen (Morales *et al.* 2005). Therefore, antiestrogen therapy and aromatase inhibitors may show increased efficacy in obese breast cancer patients and may be used as a chemopreventive in obese postmenopausal women.

Examination of pathways that are altered in obesity and metabolic syndrome may offer new targets for breast cancer therapy. Drugs that target IGF-IR may prove effective in reducing insulin-mediated growth and proliferation of tumors cells. Adiponectin receptor agonists may provide novel therapies for ameliorating insulin resistance and possibly in directly inhibiting mammary epithelial

cell proliferation. Many more studies are needed to determine the feasibility of the latter.

Summary

Breast cancer continues to be a major health problem for women in the USA and worldwide. Maintenance of a lean body mass offers a way in which women can modestly to significantly reduce their relative breast cancer risk. There are several explanations as to the molecular mechanisms that physiologically link obesity with postmenopausal breast cancer risk. It may be that each of these ligand/growth factor receptor pathways crosstalk with each other to synergistically affect tumorigenesis (Fig. 1). It should be noted that it remains a puzzle as to why the proposed mechanisms only relate to postmenopausal breast

Figure 1 Molecular mechanisms supporting a causal link between obesity and breast cancer. This scheme integrates the three prevailing hypotheses on pathways important to the association of obesity with breast cancer. In obesity, levels of insulin and IGF-I are elevated, while the adipocytes serve as both a peripheral site of estrogen aromatization and as a paracrine/endocrine source of multiple secretory adipokines. The net result of the actions of all these factors is to support and promote tumorigenesis and tumor progression. E₂, estradiol; E₁, estrone; T, testosterone; Δ4A, Δ4A-androstenedione; ApN, adiponectin; PI3K, phosphoinositide 3-kinase.

cancer and not to premenopausal breast cancer, as there is no obvious reason why obesity would not activate the same mechanisms in obese premenopausal women. Nevertheless, examination of pathways that are altered in obesity may offer new targets for breast cancer therapy.

Acknowledgements

We thank Hexin Chen and Liangfeng Han for their thoughtful review of the manuscript. We are supported by Department of Defense Center of Excellence grant no. COE BCO30054 and NCI SPORE in Breast Cancer to SS and by a Department of Defense Predoctoral Traineeship Award BC051603 to A M L. The authors declare that they have no competing interests that would prejudice the impartiality of this work.

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