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Inflammation and Increased Aromatase Expression Occur in the Breast Tissue of Obese Women with Breast Cancer

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Abstract

Obesity is a risk factor for the development of hormone receptor–positive breast cancer in postmenopausal women and has been associated with an increased risk of recurrence and reduced survival. In humans, obesity causes subclinical inflammation in visceral and subcutaneous adipose tissue, characterized by necrotic adipocytes surrounded by macrophages forming crown-like structures (CLS). Recently, we found increased numbers of CLS, activation of the NF- κ B transcription factor, and elevated aromatase levels and activity in the mammary glands of obese mice. These preclinical findings raised the possibility that the obesity→inflammation axis is important for the development and progression of breast cancer. Here, our main objective was to determine if the findings in mouse models of obesity translated to women. Breast tissue was obtained from 30 women who underwent breast surgery. CLS of the breast (CLS-B) was found in nearly 50% (14 of 30) of patient samples. The severity of breast inflammation, defined as the CLS-B index, correlated with both body mass index ($P < 0.001$) and adipocyte size ($P = 0.01$). Increased NF- κ B binding activity and elevated aromatase expression and activity were found in the inflamed breast tissue of overweight and obese women. Collectively, our results suggest that the obesity→inflammation→aromatase axis is present in the breast tissue of most overweight and obese women. The presence of CLS-B may be a biomarker of increased breast cancer risk or poor prognosis. *Cancer Prev Res*; 4(7); 1021–9. ©2011 AACR.

Introduction

Obesity is a risk factor for the development of hormone receptor (HR)-positive breast cancer in postmenopausal women (1, 2). Estrogen synthesis is catalyzed by aromatase, which is encoded by *CYP19*. Following menopause, peripheral aromatization of androgen precursors in adipose tissue is largely responsible for estrogen synthesis (3). The increased risk of developing HR-positive breast cancer in obese postmenopausal women has been attributed, in part, to increased levels of circulating estradiol related to both increased adipose tissue and elevated aromatase expression in subcutaneous adipose tissue (1, 2, 4, 5). In addition to its impact on breast cancer risk, obesity has been recognized as

a poor prognostic factor among breast cancer survivors (6–12). Obesity-related effects on hormones, adipokines, and proinflammatory mediators have been suggested to contribute to the worse prognosis of obese patients (13–16).

Obesity causes subclinical inflammation in adipose tissue (2, 17–19). In both mouse models of obesity and obese humans, macrophages infiltrate visceral and subcutaneous adipose tissue and form characteristic crown-like structures (CLS) around necrotic adipocytes (18–21). These macrophages produce a variety of proinflammatory mediators (19, 22–24). In obese women, increased levels of proinflammatory mediators are commonly found in the circulation and may contribute to breast cancer progression and mortality (25–27). Recently, we showed in both dietary and genetic models of obesity that CLS occurs in the adipose tissue of the mouse mammary gland in addition to visceral fat (28). Importantly, the presence of CLS was associated with increased levels of proinflammatory mediators, which were paralleled by elevated levels of aromatase expression and activity in both the mammary gland and visceral fat. We concluded that the obesity→inflammation→aromatase axis may contribute to the increased risk of HR-positive breast cancer in postmenopausal women and the generally worse prognosis of obese breast cancer patients. In addition, we suggested that the presence of CLS may be a biomarker of increased breast cancer risk or poor prognosis.

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Although CLS occurs in both visceral and subcutaneous fat in overweight and obese women, nothing is known about the existence of CLS of the breast (CLS-B) in humans. In the current study, we had 2 main objectives. Our goals were to determine whether obesity was associated with CLS-B in women and then to investigate whether obesity-related inflammation was associated with increased aromatase expression and activity in the breast. Here we show that being overweight or obese is associated with CLS-B. Both breast inflammation and obesity correlated with increased aromatase expression and activity. Given the link between chronic inflammation and carcinogenesis in other tissues (29), it is likely that obesity-related inflammation in the breast, as indicated by the presence of CLS-B, will contribute to both increased risk of developing breast cancer and worse prognosis in patients with breast cancer.

Materials and Methods

Materials

IgG and antibody to phospho-p65 were from Santa Cruz Biotechnology. Lowry protein assay kits, bovine serum albumin (BSA), glucose-6-phosphate, glycerol, pepstatin, leupeptin, T4 polynucleotide kinase, and glucose-6-phosphate dehydrogenase were from Sigma. Both 1β - ^3H androstenedione and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ were from Perkin-Elmer Life Science. MuLV reverse transcriptase, RNase inhibitor, oligo(dT)₁₆, and SYBR green PCR master mix were obtained from Applied Biosystems. Real-time PCR primers were synthesized by Sigma-Genosys.

Study population and samples

The study was approved by the Institutional Review Boards of Memorial Sloan-Kettering Cancer Center (MSKCC) and Weill Cornell Medical College. Women undergoing mastectomy at MSKCC were consented under a standard tissue acquisition protocol. Patients undergoing lumpectomy were excluded to ensure adequate tissue for planned analyses. A sample size of 30 women was recruited to ensure inclusion of women with a range of body mass indexes (BMI). For each patient, electronic medical records were reviewed to register menopausal status and *BRCA1/2* mutation status. In 29 of 30 women, height and weight were measured to calculate the BMI. In one subject, BMI was calculated on the basis of self-reported measurements of height and weight. Standard definitions of normal, overweight, and obese were used: normal, BMI 18.5 to 24.9; overweight, BMI 25 to 29.9; and obese, BMI 30 or more.

For each of the 30 study cases, paraffin blocks and snap-frozen samples were prepared. Frozen samples were stored in the presence or absence of RNAlater (Ambion). To ensure samples were representative of normal breast tissue, specimens were examined grossly and with hematoxylin and eosin (H&E) staining by a breast histopathologist (D.G.). For cases with ipsilateral invasive tumors, samples were obtained from quadrants other than the one involved by the tumor. After the first patient was enrolled, the protocol was amended so that the number of paraffin blocks

acquired per case was increased from 2 to 5 (each approximately $2 \times 1 \times 0.3 \text{ cm}^3$). Of the remaining 29 cases, all had 5 paraffin blocks, with the exception of the third case in whom there were 4 blocks. From each available tissue block, 2 sections (5- μm thick and approximately 2 cm in diameter) were stained by H&E and for a macrophage marker, CD68 (mouse monoclonal KP1 antibody; Dako; dilution 1:4,000), to identify CLS-B. All cases were reviewed by a breast histopathologist (D.G.). Light microscopy was used to assess for evidence of CLS-B. A CLS-B index (0–1.0) was defined using the following formula: (number of slides with evidence of CLS-B)/(number of slides examined).

Adipocyte diameter

Breast biopsies were photographed at 20 \times , utilizing an Olympus BX50 microscope and MicroFire digital camera (Optronics). Images were stored in the tagged image file format (TIFF) and adipocyte size determined utilizing the Linear Dimensional Tool (LDT) in Canvas 11 (ACS Systems of America, Inc.). The LDT was calibrated utilizing a 20 \times photomicrograph of a stage micrometer etched with 10- μm divisions. Each photomicrograph of breast adipose tissue was bisected by a vertical and horizontal line. The maximum diameter of adipocytes that fell on the vertical and horizontal lines was determined utilizing the LDT. In this manner, adipocytes were randomly selected for measurement. Two histologic slides were prepared for each biopsy. A total of 18 to 42 individual cells were measured for each patient. Photomicrographs and measurement of adipocytes were conducted blind to patient identity. To determine the reproducibility of these measurements, we photographed 2 regions from 9 biopsies and compared the average adipocyte diameters. The mean diameters of adipocytes measured in different fields from the same patient were highly correlated ($r = 0.95$; $P < 0.001$).

Aromatase activity

To determine aromatase activity, microsomes were prepared from tissue lysates by differential centrifugation. Aromatase activity was quantified by measurement of the tritiated water released from 1β - ^3H androstenedione (30). The reaction was also carried out in the presence of letrozole, a specific aromatase inhibitor, as a specificity control and without NADPH as a background control. Aromatase activity was normalized to protein concentration and expressed as femtomoles per microgram of protein per hour.

Aromatase expression

Total RNA was isolated from frozen breast tissue, using the RNeasy Mini Kit (Qiagen). Poly A RNA was prepared with an Oligotex mRNA mini kit (Qiagen). One hundred nanograms of poly(A) RNA was reverse transcribed using murine leukemia virus reverse transcriptase and oligo(dT)₁₆ primer. The resulting cDNA was then used for amplification. The volume of the PCR was 20 μL and contained 5 μL of cDNA with the following primers: for aromatase, the forward and reverse primers used were 5'-CACATCCTCAATACCAGGTCC-3' and 5'-CAGAGATC-

CAGACT CGCATG-3', and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), the forward and reverse primers used were 5'-TTCCTTTTGGCTCGCCAGCCGA-3' and 5'-GTGACCAGGCGCCCAATACGA-3'. Real-time PCR was conducted using 2× SYBR green PCR master mix on a 7500 HT real-time PCR system (Applied Biosystems), with expression determined using the $\Delta\Delta C_T$ analysis protocol.

Electrophoretic mobility shift assay

Nuclear extracts were prepared from frozen breast tissue, using an electrophoretic mobility shift assay (EMSA) kit (Promega). For binding studies, oligonucleotides containing NF- κ B sites (Active Motif) were used. The complementary oligonucleotides were annealed in 20 mmol/L Tris (pH 7.6), 50 mmol/L NaCl, 10 mmol/L MgCl₂, and 1 mmol/L dithiothreitol. The annealed oligonucleotide was phosphorylated at the 5'-end with [γ -³²P]ATP and T4 polynucleotide kinase. The binding reaction was conducted by incubating 10 μ g of nuclear protein in 20 mmol/L HEPES (pH 7.9), 10% glycerol, 300 μ g of BSA, and 1 μ g of poly(deoxyinosinic-deoxycytidylic acid) in a final volume of 10 μ L for 10 minutes at 25°C. The labeled oligonucleotides were added to the reaction mixture and allowed to incubate for an additional 20 minutes at 25°C. The samples were electrophoresed on a 4% nondenaturing polyacrylamide gel. The gel was then dried and subjected to autoradiography at -80°C.

Statistical analyses

The primary endpoints of the study included CLS-B positivity, defined as the presence or absence of CLS-B

in any of the sections stained for CD68, and CLS-B index was defined as the fraction of blocks with positive CD68 staining for each case. Baseline patient characteristics including age, BMI, menopausal status, and *BRCA* mutation status were recorded. Average adipocyte size, aromatase mRNA levels, and aromatase activity were also obtained for each case. The association between CLS-B positivity and each baseline patient characteristic including BMI (both as a continuous variable and a categorical variable) was examined using logistic regression and Fisher's exact test where appropriate. The association between CLS-B index and BMI and between CLS-B index and average adipocyte size was evaluated using logistic regression. Strength of correlation between BMI and adipocyte size, between CLS-B index and the levels of aromatase, and between BMI and aromatase were quantified using the Spearman's rank correlation coefficient. Correlation coefficients were tested against the null hypothesis that the correlation coefficients being 0. Results with *P* values less than 0.05 were considered statistically significant. Correlation was considered as strong, moderate, or weak if the correlation coefficient was 0.75 or more, 0.45 or more, and less than 0.75, or less than 0.45, respectively.

Results

CLS-B is commonly found in breast white adipose tissue

From April 2010 to December 2010, a total of 30 women of median age 50 years (range, 26–70) were enrolled. Twenty-eight of the 30 women had no evidence

Table 1. Baseline characteristics of patients

Characteristic	Overall (n = 30)	No evidence of CLS-B (n = 16)	CLS-B showed (n = 14)	P
Age, y				
Mean \pm SD	49.9 \pm 10.4	48.6 \pm 11.8	51.4 \pm 8.7	0.46
Median (range)	50 (26–70)	48.5 (26–70)	51.5 (38–68)	
Menopausal status, n (%)				
Pre	16 (53)	9	7	1.0
Post	14 (47)	7	7	
BMI				
Mean \pm SD		24.5 \pm 4.7	31.6 \pm 6.4	0.01
Median (range)		22.5 (19.3–35.7)	29.2 (22.1–45.6)	
<i>BRCA</i> status, n (%)				
Known mutation	7 (23)	5	2	1.0 ^a
No known mutation	9 (30)	6	3	
Unknown (not tested)	14 (47)	5	9	
Breast surgery				
Ipsilateral breast cancer	2 (7)	1	1	0.68
Contralateral breast cancer	14 (47)	8	6	
Carcinoma <i>in situ</i>	12 (40)	5	7	
No breast cancer history	2 (7)	2	0	

^aCompared among those with known *BRCA* status.

of invasive cancer, whereas 2 women had ipsilateral invasive duct cancer (Table 1). Fourteen patients had contralateral invasive breast cancer but no invasive cancer in the analyzed breast. Twelve had only carcinoma *in situ* in the breast subjected to analysis. One patient received preoperative systemic chemotherapy. Three other patients had a remote history of chemotherapy: 2 for breast cancer (16 and 106 months prior to surgery, respectively) and 1 for non-Hodgkin's lymphoma (28 years previously). Seven of the 30 patients who underwent surgery were known carriers of mutations in either the *BRCA1* or *BRCA2* genes.

On H&E examination of breast white adipose tissue, 7 (23%) patients had evidence of CLS-B (Fig. 1A). Immunohistochemical staining for CD68, a macrophage marker, revealed CLS-B in 14 of 30 (47%) cases (Fig. 1B). CLS-B was not identified in the patient who received preoperative chemotherapy. All tissue sections that were CLS-B positive by H&E staining were also CD68 positive. Given the higher sensitivity of detecting CLS-B by using CD68 immunohistochemistry compared with H&E, subsequent analyses were based on results obtained with CD68 immunohistochemistry. Notably, CLS-B was identified in both premenopausal and postmenopausal women as well as in 2 of the 7 *BRCA* mutation carriers.

Elevated BMI is associated with breast inflammation

To evaluate the association between BMI and CLS-B, we compared the percentage of cases positive for CLS-B among patients who were normal BMI, overweight, or obese. Increasing BMI was associated with increased likelihood of having CLS-B. Specifically, CLS-B was observed in 7 of 10 (70%) overweight and 6 of 8 (75%) obese patients whereas only 1 in 12 (8%) normal weight patients had evidence of CLS-B ($P = 0.003$; Fig. 2A). We also investigated whether the severity of inflammation, as determined by CLS-B index, varied according to BMI. Of the 14 CLS-B-positive cases, 7 patients had a CLS-B index of 0.2, 2 patients had a CLS-B index of 0.4, 2 patients had a CLS-B index of 0.6, 2 patients had a CLS-B index of 0.8, and 1 patient had a CLS-B index of 1.0. Importantly, increasing BMI was associated with increasing CLS-B index in logistic regression analysis ($P < 0.001$; Fig. 2B).

In human subcutaneous and visceral fat, increased adipocyte size has been associated with adipocyte cell death and the presence of CLS (18). In fact, adipocyte hypertrophy has been associated with obesity-related adipocyte death. Given this background, the relationship between BMI and adipocyte diameter in the breast was determined. A positive correlation was observed between BMI and adipocyte size ($P < 0.001$; Fig. 3A). Notably, increasing adipocyte size was associated with a statistically significant increase in the CLS-B index ($P = 0.01$; Fig. 3B).

Breast inflammation is associated with increased aromatase expression and activity

Next, we determined if obesity-related breast inflammation was associated with increased levels of aromatase.

Levels of aromatase mRNA and activity were determined in breast tissue from each of the 30 subjects and correlated with BMI (Fig. 4A and B) and CLS-B index (Fig. 4C and D). Both elevated BMI and breast inflammation were associated with increased amounts of aromatase mRNA and activity. Levels of aromatase mRNA correlated with both BMI ($\rho = 0.42$, $P = 0.02$) and CLS-B index ($\rho = 0.75$, $P < 0.001$), but the correlation was stronger with CLS-B index. Similarly, aromatase activity correlated more strongly with CLS-B index ($\rho = 0.88$, $P < 0.001$) than BMI ($\rho = 0.5$, $P = 0.02$).

Activation of NF- κ B, a transcription factor implicated in obesity-related inflammation (19, 28), stimulates the

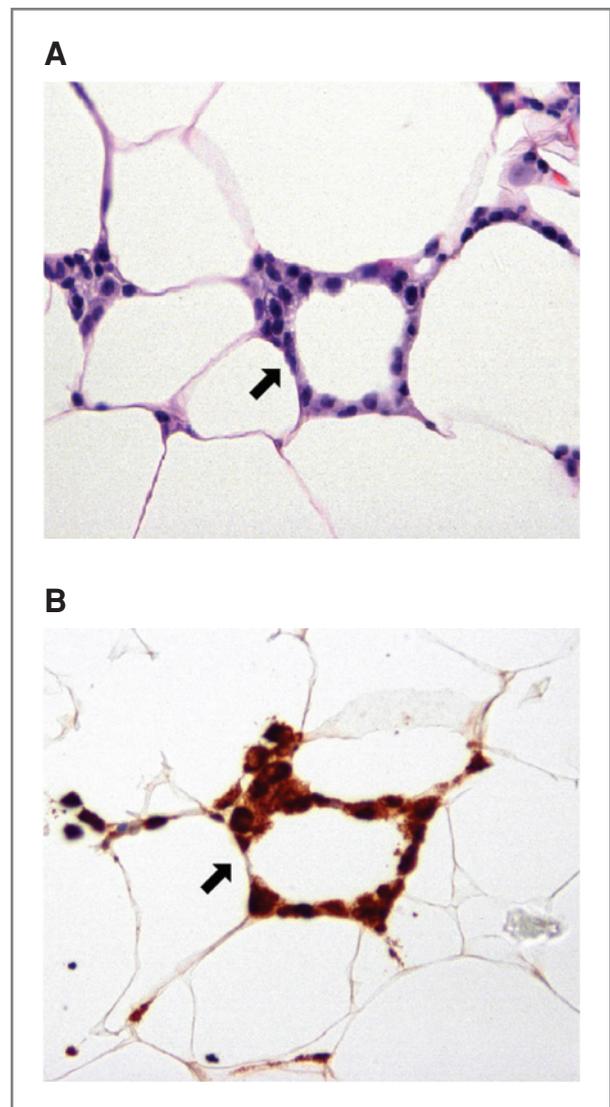


Figure 1. CLS-B occurs in humans. A, H&E-stained slide showing an inflammatory focus containing macrophages that surround an adipocyte (200 \times). B, immunohistochemical staining with CD68 of same lesion taken approximately 30 μ m from section shown in A confirms that the cells constituting CLS-B are macrophages (200 \times). In A and B, arrows indicate CLS-B.

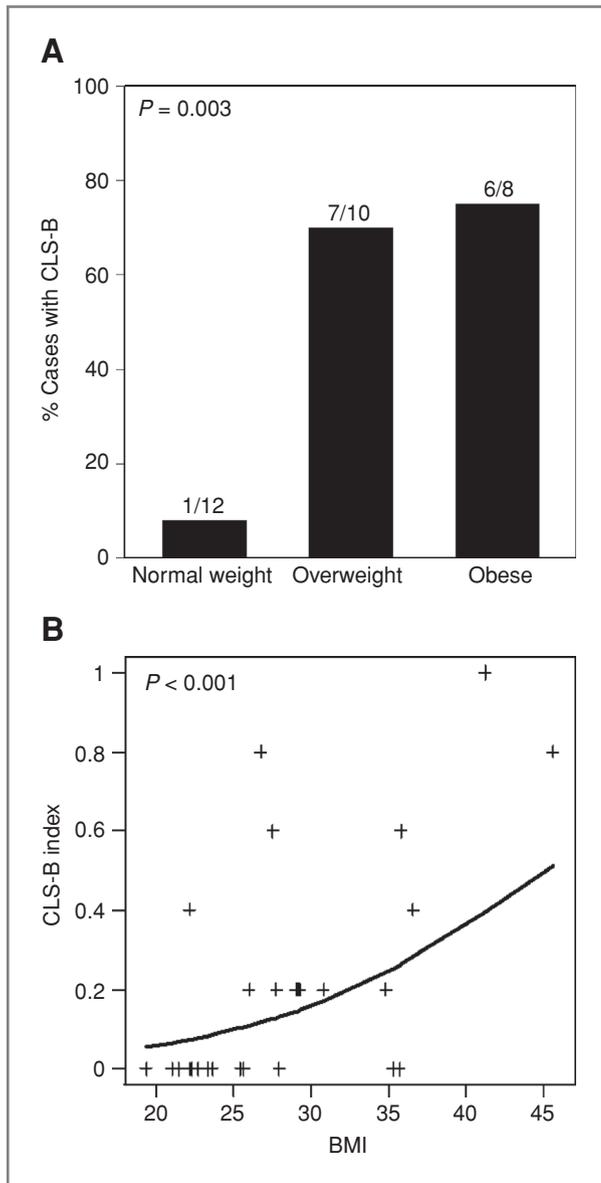


Figure 2. Breast inflammation is more common in obese women than in lean women. A, overweight and obesity are associated with the presence of CLS-B. Normal, BMI 18.5 to 24.9 ($n = 12$); overweight, BMI 25 to 29.9 ($n = 10$); obese, BMI 30 or more ($n = 8$). B, increasing BMI is associated with increased CLS-B index (measured as the proportion of slides positive for CLS-B), using logistic regression ($P < 0.001$). $n = 30$.

production of several proinflammatory mediators that can induce aromatase. Hence, EMSA was carried out to determine NF- κ B binding activity, using nuclear protein from breast samples with or without CLS-B. Breast samples containing CLS-B were from overweight or obese women. As shown in Figure 5A, NF- κ B binding activity was higher in samples containing CLS-B. Supershift assays indicated that p65 was present in the binding complex (Fig. 5B).

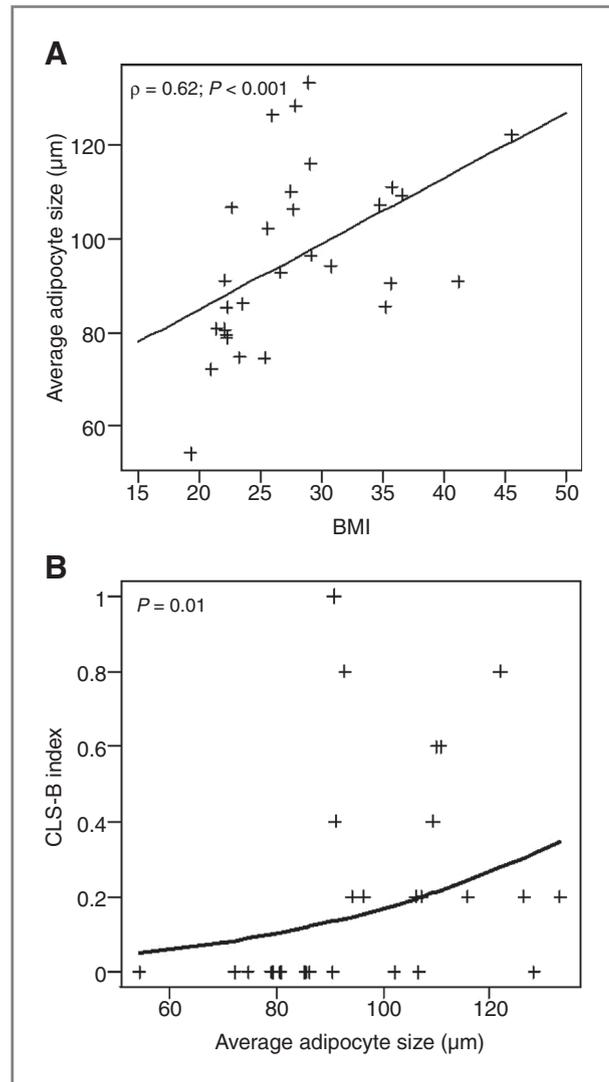


Figure 3. Adipocyte size correlates with both BMI and CLS-B index. A, a moderate correlation was observed between the average breast adipocyte size and BMI ($P < 0.001$). B, CLS-B index relative to average adipocyte size for each study subject ($n = 29$). Increasing adipocyte size was associated with a statistically significant increase in the CLS-B index ($P = 0.01$).

Discussion

Although the connection between obesity, systemic inflammation, and breast cancer has been suggested, little is known about inflammation in the breast tissue itself. This is particularly important because chronic inflammation of other tissues including the colon, liver, pancreas, and stomach seems to increase the risk of site-specific cancers (29). Subclinical breast inflammation has not been previously described, nor associated with cancer risk. We recently identified inflammatory lesions (CLS) in mouse mammary glands in both dietary and genetic models of obesity (28). In mice, the presence of CLS was associated with activation of NF- κ B and increased levels of proinflammatory mediators,

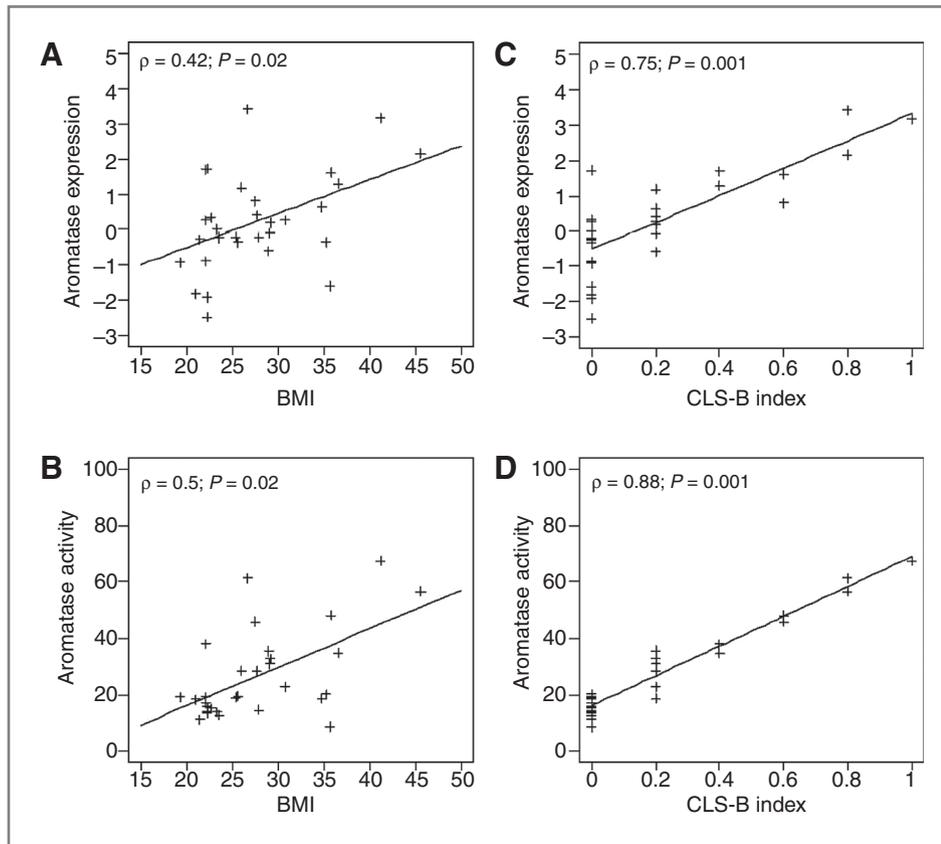


Figure 4. Increased levels of aromatase mRNA and activity are found in the breast tissue of obese women. Weak to moderate correlation was observed between BMI and aromatase expression (A) and activity (B). Strong correlation was observed between CLS-B index and aromatase mRNA (C) and activity levels (D).

which were paralleled by elevated levels of aromatase. The discovery of the obesity→inflammation axis and its association with CLS in mice provided insight into potential mechanisms underlying the increased risk of breast cancer in obese postmenopausal women. Hence, it was important to determine whether similar effects occur in women.

CLS-B has not been described in past studies. In the absence of evidence of a potential role for CLS-B in carcinogenesis, little attention may have been given to these inflammatory lesions. In addition, routine H&E staining seems to be inadequate for easy identification of these lesions. Indeed, special stains for CD68, a macrophage marker, led to the detection of numerous CLS-B-positive tissue sections that were missed on routine H&E staining. Therefore, future studies of CLS-B should not rely exclusively on H&E staining.

In the current study, nearly 50% of women had CLS-B. Consistent with our findings in mice, the presence and severity of CLS-B correlated with being overweight or obese. However, CLS-B was not found in all overweight and obese women and was observed in the breast tissue of 1 lean woman. Possibly, women with CLS-B will be at greater risk for developing breast cancer than women of similar body mass without CLS-B. The underlying mechanisms that account for why women of similar BMI differ in their sensitivity to developing CLS-B are unknown. Previously, avoiding obesity during adolescence was suggested to delay the onset of breast cancer in carriers of *BRCA1* or *BRCA2* muta-

tions (31). We found CLS-B in overweight women who are carriers of *BRCA1* or *BRCA2* mutations. Perhaps, avoiding obesity will prevent CLS-B formation and thereby suppress breast carcinogenesis in high-risk women and others. A related question concerns the possibility that adipocyte hypertrophy might promote obesity-associated adipocyte death, leading, in turn, to CLS-B formation. Consistent with previous reports for visceral and subcutaneous fat (18), we found that obesity was associated with adipocyte hypertrophy in the breast. Moreover, a correlation between adipocyte hypertrophy and CLS-B was observed. A potential limitation of the current study is the inclusion of patients with cancer. Although there is no evidence that CLS-B is a consequence of cancer, similar studies should be conducted in cancer-free individuals. This is particularly important if CLS-B is to be evaluated as a biomarker of cancer risk.

The increased risk of HR-positive breast cancer in obese postmenopausal women has been attributed, in part, to elevated levels of circulating estradiol related to both increased adipose mass and elevated aromatase expression in subcutaneous adipose tissue (1, 2, 4, 5). Here we show, for the first time, that levels of aromatase are increased in breast tissue obtained from obese compared with lean women. This finding is consistent with the results of previous studies that found higher levels of aromatase in thigh and abdominal adipose tissues from obese versus lean individuals (32). Several proinflammatory mediators induce aromatase (33, 34). Therefore, our

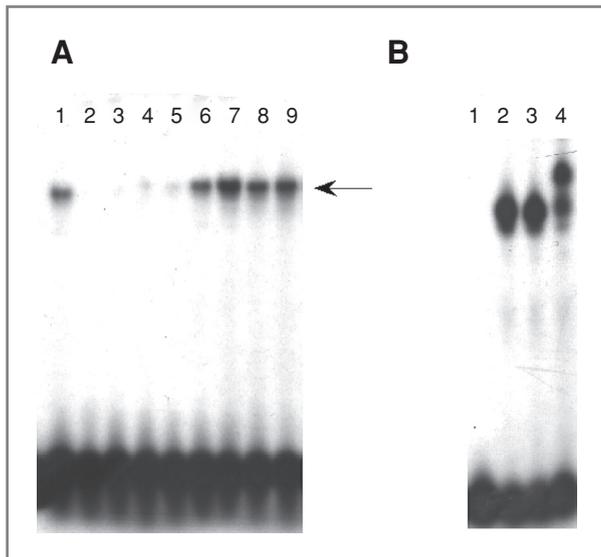


Figure 5. NF- κ B is activated in the breast tissue of obese women. Ten micrograms of nuclear protein isolated from human breast tissue was incubated with a 32 P-labeled oligonucleotide containing NF- κ B binding sites. A, lane 1, standard; lanes 2 to 5, binding of nuclear protein from breast tissue of 4 women with CLS-B index of 0; lanes 6 to 9, binding of nuclear protein from breast tissue of 4 women with CLS-B index ranging from 0.2 to 1.0. B, lane 1, nuclear protein from a breast tissue sample with a CLS-B index of 1.0 incubated with a 32 P-labeled oligonucleotide containing NF- κ B binding sites and a 50 \times excess of cold probe; lanes 2 to 4, nuclear protein from the same sample incubated with normal IgG (lane 2), 1 μ L (lane 3), or 2 μ L (lane 4) of phospho-p65 antibody. In A and B, the protein-DNA complexes that formed were separated on a 4% polyacrylamide gel.

finding that inflammation commonly occurs in breast tissue of overweight and obese women provided a rationale for determining whether the severity of breast inflammation (CLS-B index) correlated with levels of aromatase mRNA and activity. Levels of both aromatase mRNA and activity correlated with the severity of breast inflammation. The correlation was stronger with the severity of breast inflammation (CLS-B index) than with BMI. This difference in the strength of the correlation can be explained at least, in part, by evidence that not all overweight or obese women have evidence of breast inflammation in the form of CLS-B. In preclinical studies, we showed that activation of NF- κ B induced the transcription of several proinflammatory mediators (COX-2, TNF- α , and IL-1 β) that acted, in turn, to induce aromatase expression and activity (28). Consistent with these findings, elevated NF- κ B binding activity was found in the inflamed breast tissue of overweight and obese women. On the basis of these results, additional studies are warranted to determine whether levels of proinflammatory mediators correlate with both the CLS-B index and aromatase levels. Collectively, these findings suggest that the obesity \rightarrow inflammation \rightarrow aromatase axis is present in the human breast. Given the recognized importance of aromatase activity in both the development and progression of HR-positive breast cancer, it is highly likely that

obesity-mediated inflammation contributes to breast carcinogenesis. Furthermore, CLS-B may prove to be an early biomarker of breast cancer risk or prognosis (35).

Recently, obese women with breast cancer were found to derive less benefit from aromatase inhibitors than lean women (36). One potential explanation for this finding is that the dose of aromatase inhibitor required to maximally suppress estrogen production needs to be higher in obese women than in lean women. Our finding that aromatase levels and activity are higher in the breast tissue of obese women than that in lean women supports this possibility. Here we show a strong positive correlation between CLS-B index and aromatase expression and activity. Therefore, future studies should determine the utility of the CLS-B index in addition to BMI as a predictor of the dose of aromatase inhibitor required to maximally inhibit estrogen production. In the overweight and obese, if the CLS-B index predicts the dose of aromatase inhibitor required to maximize clinical benefit, then measurements of the CLS-B index could allow for the dose of aromatase inhibitor to be personalized.

In addition to being a risk factor for the development of postmenopausal breast cancer, obesity is recognized to be a poor prognostic factor among breast cancer survivors (10, 13–16). Additional data are needed to determine if CLS-B can be used as a prognostic biomarker in patients with a diagnosis of breast cancer. In breast cancers, an inverse relationship exists between the number of tumor-associated macrophages and prognosis (37, 38). Whether the same will be true for CLS-B, which are enriched in macrophages, remains to be evaluated.

Although our studies are the first to suggest that obesity-mediated breast inflammation may drive the estrogen synthesis that increases the risk of postmenopausal HR-positive breast cancer, chronic inflammation increases the risk of numerous other malignancies (39). Multiple mechanisms have been identified that can explain the link between chronic inflammation and cancer risk (39). Recently, obesity was suggested to increase the risk of triple-negative breast cancer in addition to HR-positive breast cancer (40). Perhaps, chronic breast inflammation contributes to this process by a yet to be defined mechanism. The discovery of the obesity \rightarrow inflammation \rightarrow aromatase connection provides a platform for developing risk reduction strategies. Studies are underway with the goal of developing lifestyle, dietary, or pharmacologic strategies to disrupt the inflammatory process and thereby reduce the risk of developing breast cancer or improve outcomes.

Disclosure of Potential Conflicts of Interest

A.J. Dannenberg is a member of the Scientific Advisory Board of Tragara Pharmaceuticals, Inc., a company that is developing a selective COX-2 inhibitor. The other authors disclosed no potential conflicts of interest.

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References

- Cleary MP, Grossmann ME. Minireview: obesity and breast cancer: the estrogen connection. *Endocrinology* 2009;150:2537-42.
- van Kruijsdijk RC, Van Der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. *Cancer Epidemiol Biomarkers Prev* 2009;18:2569-78.
- Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. *Endocr Relat Cancer* 2006;13:279-92.
- Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, et al. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. *J Natl Cancer Inst* 2003;95:1218-26.
- Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S, et al. Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. *Clin Endocrinol (Oxf)* 2007;66:440-6.
- Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003;348:1625-38.
- Whiteman MK, Hillis SD, Curtis KM, McDonald JA, Wingo PA, Marchbanks PA. Body mass and mortality after breast cancer diagnosis. *Cancer Epidemiol Biomarkers Prev* 2005;14:2009-14.
- Majed B, Moreau T, Senouci K, Salmon RJ, Fourquet A, Asselain B. Is obesity an independent prognosis factor in woman breast cancer? *Breast Cancer Res Treat* 2008;111:329-42.
- Petrelli JM, Calle EE, Rodriguez C, Thun MJ. Body mass index, height, and postmenopausal breast cancer mortality in a prospective cohort of US women. *Cancer Causes Control* 2002;13:325-32.
- Ewertz M, Jensen MB, Gunnarsdottir KA, Hojris I, Jakobsen EH, Nielsen D, et al. Effect of obesity on prognosis after early-stage breast cancer. *J Clin Oncol* 2011;29:25-31.
- Rose DP, Vona-Davis L. Influence of obesity on breast cancer receptor status and prognosis. *Expert Rev Anticancer Ther* 2009;9:1091-101.
- Protani M, Coory M, Martin JH. Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis. *Breast Cancer Res Treat* 2010;123:627-35.
- Sinicropo FA, Dannenberg AJ. Obesity and breast cancer prognosis: weight of the evidence. *J Clin Oncol* 2011;29:4-7.
- McTiernan A, Irwin M, Vongruenigen V. Weight, physical activity, diet, and prognosis in breast and gynecologic cancers. *J Clin Oncol* 2010;28:4074-80.
- Duggan C, Irwin ML, Xiao L, Henderson KD, Smith AW, Baumgartner RN, et al. Associations of insulin resistance and adiponectin with mortality in women with breast cancer. *J Clin Oncol* 2011;29:32-9.
- Goodwin PJ, Ennis M, Pritchard KI, Trudeau ME, Koo J, Madarnas Y, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol* 2002;20:42-51.
- Cancello R, Henegar C, Viguier N, Taleb S, Poitou C, Rouault C, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 2005;54:2277-86.
- Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* 2005;46:2347-55.
- Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 2010;72:219-46.
- Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, et al. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *J Lipid Res* 2008;49:1562-8.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796-808.
- Suganami T, Ogawa Y. Adipose tissue macrophages: their role in adipose tissue remodeling. *J Leukoc Biol* 2010;88:33-9.
- Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003;112:1821-30.
- Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 2001;280:E745-51.
- Bachelot T, Ray-Coquard I, Menetrier-Caux C, Rastkha M, Duc A, Blay JY. Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. *Br J Cancer* 2003;88:1721-6.
- Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* 1998;83:2907-10.
- Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* 2001;9:414-7.
- Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, et al. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. *Cancer Prev Res* 2011;4:329-46.
- Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol* 2005;5:749-59.
- Subbaramaiah K, Hudis C, Chang SH, Hla T, Dannenberg AJ. EP2 and EP4 receptors regulate aromatase expression in human adipocytes and breast cancer cells. Evidence of a BRCA1 and p300 exchange. *J Biol Chem* 2008;283:3433-44.
- King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* 2003;302:643-6.
- Bulun SE, Simpson ER. Competitive reverse transcription-polymerase chain reaction analysis indicates that levels of aromatase cytochrome P450 transcripts in adipose tissue of buttocks, thighs, and abdomen of women increase with advancing age. *J Clin Endocrinol Metab* 1994;78:428-32.
- Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. *Pharmacol Rev* 2005;57:359-83.
- Subbaramaiah K, Howe LR, Port ER, Brogi E, Fishman J, Liu CH, et al. HER-2/neu status is a determinant of mammary aromatase activity *in vivo*: evidence for a cyclooxygenase-2-dependent mechanism. *Cancer Res* 2006;66:5504-11.
- Bellacosa A, Godwin AK, Peri S, Devarajan K, Caretti E, Vanderveer L, et al. Altered gene expression in morphologically normal epithelial cells from heterozygous carriers of BRCA1 or BRCA2 mutations. *Cancer Prev Res* 2010;3:48-61.
- Sestak I, Distler W, Forbes JF, Dowsett M, Howell A, Cuzick J. Effect of body mass index on recurrences in tamoxifen and anastrozole

- treated women: an exploratory analysis from the ATAC trial. *J Clin Oncol* 2010;28:3411-5.
37. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006;66:605-12.
38. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996;56:4625-9.
39. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin* 2006;56:69-83.
40. Phipps AI, Chlebowski RT, Prentice R, McTiernan A, Stefanick ML, Wactawski-Wende J, et al. Body size, physical activity, and risk of triple-negative and estrogen receptor-positive breast cancer. *Cancer Epidemiol Biomarkers Prev* 2011;20:454-63.