Perspective
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Inflammatory Talk: Linking Obesity, NF-κB, and Aromatase

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Abstract

Obesity is associated with increased risk and worsened prognosis for postmenopausal breast cancer, but the underlying mechanisms remain unclear. Elegant work reported by Subbaramaiah and colleagues in this issue of the journal (beginning on page **329**) adds important insights, particularly into the connections between obesity, inflammation, and aromatase via cross-talk among adipocytes, macrophages, and epithelial cells. This work provides several new molecular targets and strategies to test in model systems for preventing or controlling obesity-related breast cancer and provides a framework for studying the linkages among the complex mechanistic pathways underlying the obesity-cancer relationship. *Cancer Prev Res; 4(3); 285–7. ©2011 AACR.*

Findings from epidemiological and animal studies have established obesity as an important risk and prognostic factor for postmenopausal breast cancer. Unfortunately, we still lack a clear understanding of the mechanisms underlying the association of obesity with postmenopausal breast cancer. Given that the prevalence of obesity in U.S. women remains very high and that childhood obesity rates are rising, we urgently need new targets and strategies for breaking the obesity–breast cancer link.

Historically, discussions about the potential mechanisms through which obesity enhances postmenopausal breast cancer risk or progression often involve 4 putatively independent pathways (1, 2). These pathways are as follows: (i) growth factor signaling circuitry, particularly involving increased systemic insulin/insulin-like growth factor 1 (IGF-1) levels and increased signaling through the mammalian target of rapamycin (mTOR) pathway; (ii) altered estrogen metabolism (particularly involving increased aromatase activity and conversion of androgens to estradiol) and/or estrogen receptor (ER) signaling; (iii) adipokine signaling, particularly involving increased leptin and decreased adiponectin; and (iv) a variety of possible (and poorly characterized) inflammatory signals. Evidence for cross-talk between these pathways is mounting, but the critical interactions are not well understood (2). The elegant work reported in this issue of the journal by Subbaramaiah and colleagues (3) adds several important insights, particularly the previously underappreciated connection between obesity, inflammation, and aromatase via

cross-talk between adipocytes and macrophages. As evidenced by the paper's 11 figures in the main text (and 3 Supplementary figures), the demonstration and characterization of this cross-talk required a rather long story, but one well worth reading. Although I summarize and highlight here some of the key findings and important new information, I encourage you to carve out the time to read the well-written and groundbreaking original article (2).

Subbaramaiah and colleagues (3) began with the observation that a 10-week diet-induced obesity (DIO) regimen of a calorically dense high-fat (60 kcal%) diet in female C57BL/6 mice resulted in weight gain [vs. a low-fat diet (10 kcal% fat) in control mice], particularly in ovariectomized mice. Using similar diets and mice, we previously reported that ovariectomized mice are more susceptible than intact mice to developing obesity, particularly when administered a calorically dense DIO regimen (4). As discussed by Subbaramaiah and colleagues (3), this finding in mice is consistent with observations of increased susceptibility to weight gain following menopause in women. We also showed that obesity increased the growth of orthotopically transplanted Wnt-1 mammary tumors in ovariectomized but not intact mice (4), consistent with the long-established relationship between obesity and breast cancer in postmenopausal women. Thus, DIO mice provide a relevant model for studying the biology of obesity and breast cancer occurring in women. A burning question remains, however: How do we block the adverse effects of obesity on postmenopausal breast cancer?

Several novel findings of Subbaramaiah and colleagues (3) bring the answer to this question closer. They report that the DIO regimen causes an increase in inflammatory lesions, referred to as crown-like structures (CLS), in the mammary gland and peripheral adipose tissue. Furthermore, their immunohistochemical data establish that these CLS are necrotic adipocytes rimmed by macrophages. Their finding is consistent with recent reports by others (in tissues other than the mammary gland) suggesting that the obese state results in enhanced adipocyte necrosis,

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with concomitant macrophage infiltration to sequester and scavenge the residual lipid droplets (5,6). This process of obesity-related adipocyte death leads to activated macrophages (often called giant cells because they are engorged with lipid) that contribute importantly to chronic inflammation. We have also observed increased CLS in response to DIO in our MMTV-Wnt-1 (4) mammary tumor model. We failed to understand what these structures were, however, or whether they were of any importance to the obesity-cancer connection; we plan to revisit the role of CLS in our own studies.

Subbaramaiah and colleagues (3) went on to show that mRNA levels of several proinflammatory mediators, including cyclooxygenase-2 (COX-2), tumor necrosis factor alpha (TNF-α), and interleukin 1 beta (IL-1β), were elevated in mammary and visceral white adipose tissue from DIO mice and from ob/ob mice with genetically induced obesity. Furthermore, this inflammatory profile was associated with increased aromatase expression (the paper's *first critical new finding*, in my opinion), suggesting that inflammatory signals, possibly resulting from the presence of activated macrophages surrounding the necrotic adipocytes in CLS in mammary and adipose tissue in obese mice (regardless of mode of obesity induction), trigger aromatase upregulation. The use of both a DIO regimen and a genetic model of obesity (ob/ob mice, which are hyperphagic and consume greater amounts of control diet because of their alterations in leptin signaling) was a clever way to rule out the possibility that the effects of the high-fat DIO regimen were a consequence of increased lipid intake *per se*, independent of increased adiposity. Unfortunately, ob/ob mice have severe alterations in mammary gland morphology, limiting their usefulness in mammary tumorigenesis studies. Nonetheless, the obesity-related inflammatory and aromatase changes appeared to be virtually identical in the DIO and ob/ob mouse models, making this a highly informative experiment.

These investigators' findings also show that leptin may not be an important contributor to the adiposity–inflammation–aromatase axis, as the obesity-related increases of CLS, inflammatory mediators, and aromatase in ob/ob mice, which have mutant leptin, were similar to increases in DIO mice, which have high leptin levels. Our previous findings with A-Zip/F1 mice, which lack white adipose tissue (and thus lack adipokines, including leptin) but develop lipoatrophic diabetes including elevated systemic insulin, IGF-1, and proinflammatory cytokines, also suggest that inflammatory factors and growth-factor signaling may be more important than are adipokines in the mammary tumor–enhancing effects of obesity (7).

A *second critical new finding* from the new work reported in this issue (3) is that the production of inflammatory signals in the mammary gland occurs in the stromal-vascular fraction. The stromal-vascular fraction, relative to the mature adipocyte fraction, is enriched in preadipocytes/adipose-derived stems cells and macrophages (8). Using *ex vivo* and *in vitro* studies, the investigators found that the stromal-vascular fraction isolated from the mammary

glands of DIO mice produced high levels of TNF-α, IL-1β, and COX-2–derived prostaglandin E2 (vs. lean mice), and that these inflammatory factors were responsible, at least in large measure, for the induction of aromatase in adipocytes.

Saturated fatty acids released from adipocytes activate macrophages and trigger a proinflammatory response. Subbaramaiah and colleagues (3) established that saturated fatty acids, varying in chain length from C12 to C18, increased expression of COX-2 and other inflammatory mediators in THP-1 cells, a human monocyte–derived macrophage cell line. Inhibiting either TNF-α or IL-1β attenuated the aromatase induction, as did inhibition of COX-2 with celecoxib or COX-2 small-interfering RNA (siRNA), suggesting that saturated fatty acid release from adipocytes induces TNF-α, IL-1β, and COX-2 in macrophages, which in turn trigger the induction of aromatase in preadipocytes and adipocytes. These results represent the paper's *third critical new finding*.

To specifically test the hypothesis that the master inflammatory regulator nuclear factor kappa B (NF-κB) plays a role in saturated fatty acid–mediated induction of inflammatory mediators, Subbaramaiah and colleagues (3) conducted a series of *in vitro* experiments in THP-1 cells. They found that saturated fatty acids (i) increased NF-κB–luciferase activity (in a reporter assay); (ii) increased nuclear translocation and functional binding of p65 (a key NF-κB subunit) to the nuclear binding complex (in gel shift and supershift assays); (iii) increased phosphorylation of p65 and its translocation to the nucleus (in Western blots); and (iv) increased phospho-p65 binding to the promoters of proinflammatory genes (in ChIP assays). In determining the role of p65 in regulating the proinflammatory mediators and aromatase, they found that silencing NF-κB with siRNA to p65 or inhibiting NF-κB with BAY11-7082 in THP-1 cells decreased the production of the proinflammatory mediators TNF-α, IL-1β, and prostaglandin E2 after saturated fatty acid treatment. THP-1 conditioned media from the silencing or inhibitor experiment also showed attenuated aromatase induction in preadipocytes.

To validate *in vivo* their *in vitro* findings of a mediating role of NF-κB in inflammatory mediator production and aromatase expression, they performed similar NF-κB characterization assays (described previously) on mammary tissues from their DIO mice. They found (i) that NF-κB nuclear binding activity was increased in the DIO group relative to controls, and p65 was part of the binding complex, and (ii) that higher levels of NF-κB binding activity occurred in the stromal-vascular fractions (which had previously been shown to contain higher levels of inflammatory mediators) relative to the adipocyte fractions prepared from mammary glands of DIO mice. Thus, NF-κB indeed appears to regulate the production of proinflammatory mediators in macrophages *in vivo* in response to obesity. These inflammatory signals trigger the induction of aromatase in the adipocytes present in the mammary gland, which in turn increases estrogen biosynthesis and

presumably ER-dependent and -independent signaling. This proposed cascade of obesity-induced signals in the mammary gland, mediated by NF-κB and resulting in aromatase induction, represents Subbaramaiah and colleague's (3) *fourth critical new finding* (and the most important, in my opinion). NF-κB is thus a promising molecular target for blocking the enhancing effects of obesity on risk and/or progression of postmenopausal breast cancer.

These investigators did not address the potential interactions of obesity-related growth factor signaling with this newly identified obesity–inflammation–aromatase axis in the mammary gland and visceral white adipose tissues(3). Important cross-talk between IGF-1 and estrogen signaling has previously been established (9). Not only do ER and IGF-1 receptor expressions typically correlate with each other in cancer tissue, but also estradiol and IGF-1 can synergistically enhance breast cancer cell proliferation *in vitro* (10). The synergistic relationship between these 2 pathways occurs as a biological consequence of an interrelated network governed at multiple levels. For example, exposure of breast cancer cells to IGF-1 results in an increased expression of ERα as well as enhancement of its transcriptional activity (11). In addition, estradiol causes a complementary augmentation of IGF-1 signaling by increasing expression of IGF-1, IGF-1 receptor, IGF binding proteins, and insulin receptor substrate-1 (11).

Metabolic hormones, such as IGF-1 and insulin, have also been shown to interact with inflammation-related pathways, such as NF-κB signaling. In addition to our previously mentioned studies with A-Zip/F1 mice, which lack white adipose tissue but are diabetic, display high levels of insulin, IGF-1, and inflammatory cytokines, and are highly susceptible to mammary cancer development (7), there is additional support for the hypothesis that components of the insulin/IGF-1 and inflammatory pathways may be interrelated targets for breaking the obesity–

cancer link. For example, liver IGF-1-deficient (LID) mice demonstrate reductions in tumor development and in serum IGF-1 and a broad panel of cytokine levels (12, 13). Restoration of IGF-1 levels in LID mice also restored pancreatic tumor development, cytokines, and inflammation-related signaling (including NF-κB) to control levels (13). Once bound to their cognate receptor, IGF-1, insulin, and other energy balance–related growth factors can activate Akt, which is an established upstream kinase of the IκB kinase (IKK) complex (14). Subsequently, the activated IKK complex targets IκB-α for degradation and allows the p50/p65 subunits of NF-κB to translocate to the nucleus and initiate transcription. IGF-1 increases NF-κB DNA binding activity, comparable with TNF-α, and induces expression of FLICE-inhibitor protein (FLIP), X-linked inhibitor of apoptosis protein (XIAP), cellular inhibitor of apoptosis 2 (cIAP-2), Al/Bfl-1, and survivin, which are downstream targets mediated by NF-κB (14).

In conclusion, multiple signals associated with the obese state may contribute to the inflammatory cross-talk occurring between macrophages, adipocytes, and epithelial cells in breast and other cancers. Thanks to the exciting work by Subbaramaiah and colleagues (3), we have several new molecular targets and strategies to test in our model systems for preventing or controlling obesity-related cancer. We also now have a clearer framework for understanding the linkages among the complex mechanistic pathways underlying the obesity-cancer relationship, specifically growth factor signaling, estrogen metabolism, and/or ER signaling, adipokine signaling, and (perhaps the key trigger) inflammation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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