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Obesity-Activated Adipose-Derived Stromal Cells Promote Breast Cancer Growth and Invasion[1,2]

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Abstract

Obese women diagnosed with breast cancer have an increased risk for metastasis, and the underlying mechanisms are not well established. Within the mammary gland, adipose-derived stromal cells (ASCs) are heterogeneous cells with the capacity to differentiate into multiple mesenchymal lineages. To study the effects of obesity on ASCs, mice were fed a control diet (CD) or high-fat diet (HFD) to induce obesity, and ASCs were isolated from the mammary glands of lean and obese mice. We observed that obesity increased ASCs proliferation, decreased differentiation potential, and upregulated expression of α-smooth muscle actin, a marker of activated fibroblasts, compared to ASCs from lean mice. To determine how ASCs from obese mice impacted tumor growth, we mixed ASCs isolated from CD- or HFD-fed mice with mammary tumor cells and injected them into the mammary glands of lean mice. Tumor cells mixed with ASCs from obese mice grew significantly larger tumors and had increased invasion into surrounding adipose tissue than tumor cells mixed with control ASCs. ASCs from obese mice demonstrated enhanced tumor cell invasion in culture, a phenotype associated with increased expression of insulin-like growth factor-1 (IGF-1) and abrogated by IGF-1 neutralizing antibodies. Weight loss induced in obese mice significantly decreased expression of IGF-1 from ASCs and reduced the ability of the ASCs to induce an invasive phenotype. Together, these results suggest that obesity enhances local invasion of breast cancer cells through increased expression of IGF-1 by mammary ASCs, and weight loss may reverse this tumor-promoting phenotype.

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Introduction

Obesity rates in the United States and around the world have more than doubled in the last 40 years [1]. Obesity is associated with increased risk for development of several types of cancer, including breast cancer in postmenopausal women [2–4]. Regardless of menopausal status, breast cancer patients with an obese body mass index (BMI) are more frequently diagnosed with poorly differentiated, larger primary tumors and lymph node metastases than lean patients [5,6]. Increased BMI is significantly correlated with elevated rates of breast cancer–related mortality [7]. Understanding how obesity promotes the growth of aggressive breast tumors is of great clinical importance in order to develop targeted therapies for obese patients.

In obesity, adipose tissue expansion results in a chronic inflammatory state that contributes to obesity-related insulin resistance [8–10]. This

Abbreviations: ASC, adipose-derived stromal cells; BMI, body mass index; CD, control diet; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFP, green fluorescent protein; HFD, high fat diet; IGF-1, insulin-like growth factor-1; SMA, alpha-smooth muscle actin; SVF, stromal vascular fraction; WL, weight loss.

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inflammatory state leads to elevated circulating levels of proinflammatory cytokines, leptin, and insulin; many of these factors have been implicated in breast cancer progression [11–15]. Given the complexity of dysregulated factors in obesity, understanding how local changes within the adipose tissue of the breast may contribute to breast tumor growth and progression has been challenging to define.

Within the mammary gland, white adipose tissue is capable of undergoing expansion and retraction in response to changes in energy balance. Adipose-derived stromal cells (ASCs) are a heterogeneous group of cells within the extracellular matrix of the adipose tissue surrounding mature adipocytes [16]. ASCs, which are present in normal breast adipose tissue, have been shown to induce tissue remodeling through angiogenesis [17], proliferation [18], and deposition of extracellular matrix proteins [19]. One cell type within the ASC population is adipose stem cells which have the ability to differentiate into mature adipocytes *in vivo* as well as into multiple mesenchymal lineages in response to lineage-specific stimuli *in vitro* [20]. The differentiation potential of adipose stem cells in culture is influenced by multiple factors including fat depot–specific origin [21–23], alterations in the extracellular matrix [24], sex-specific hormones [25], and increasing BMI [26–29]. Multiple studies have shown that secreted factors from ASCs promote growth of breast cancer cells within the tumor microenvironment [30–33]. However, the effects of obesity on breast ASCs and their ability to promote cancer have not been well explored.

Here, we sought to address how obesity impacts the function of ASCs and how these cells within the microenvironment of the breast may impact tumor progression. We show that obesity increases ASC proliferation and reduces adipose stem cell differentiation potential *in vitro*. The ASCs from obese mice promote rapid mammary tumor growth and invasion into surrounding mammary adipose tissue. In addition, we demonstrate that some, but not all, obesity-induced changes to ASCs are reversible. Following weight loss, ASCs have less capacity to promote mammary tumor cell invasion. Overall, our findings suggest that obesity-induced changes in ASCs may contribute to the increased local invasion observed clinically in the breast tumors of obese women.

Materials and Methods

Animal Studies

All procedures involving animals were approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee. Female C57BL/6 (000664) and FVB/N (001800) mice were purchased from Jackson Laboratories and maintained according to the Guide for Care and Use of Laboratory Animals in AAALAC-accredited facilities. Eight-week-old female C57BL/6 and three-week-old FVB/N mice were fed control diet (CD, 10% kcal from fat, Test Diet 58Y1) or high-fat diet (HFD, 60% kcal from fat, Test Diet 58Y2) for 16 weeks to induce obesity. Purified diets contained equal amounts of vitamins and micronutrients. Body weights were measured weekly. For weight loss experiments, mice were fed HFD for 15 weeks and then switched to the CD for 5 weeks. Following euthanasia, thoracic and inguinal mammary glands were collected. Mammary tissue was minced and digested with collagenase I (Sigma; 1148089) for 1 hour. The mammary organoids, which are enriched for epithelial cells, were separated from the stromal vascular fraction (SVF) as described [34], and the SVF was cryopreserved for use in studies. SVF cells were plated in DMEM (Corning, 10-017-CV) containing 10% FBS (Gibco, 10437-28) and 1% antibiotic/antimycotic solution (Mediatech, 30-004-CI), and adherent cells were expanded in culture for no more than three passages prior to use in assays.

Human Tissue Isolation

All human breast tissues were obtained in compliance with the law and institutional guidelines as approved by the Institutional Review Board at the University of Wisconsin-Madison. Disease-free, deidentified breast tissues were obtained from patients undergoing elective reduction mammoplasty with informed consent through the Translational Science BioCore BioBank at the Carbone Cancer Center at the University of Wisconsin-Madison. This research study was approved by Institutional Review Board as Not Human Subject Research with a limited patient data set including patient age, date of service, and BMI. Tissue samples from patients aged 18-45 were included in these studies. Breast tissue from reduction mammoplasty surgeries was enzymatically dissociated for 8 hours using collagenase I as described [35,36]. The digested tissue was allowed to settle at room temperature for 10 minutes. The lipid-rich fraction was removed, and the SVF was isolated, incubated with red blood cell lysis buffer (ACK Lysing Buffer, Lonza, 10-548E), and plated in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic solution to generate adherent stromal cells.

Cell Lines

EO771 cells were derived from a spontaneous mammary adenocarcinoma from a C57Bl/6 mouse [37] and were provided by Dr. Mikhail Kolonin. Met-1 cells were derived from metastasis from a MMTV-PyMT tumor from an FVB/N female mouse [38] and were provided by Dr. Alexander Borowsky. Met-1 cells were transduced with lentivirus encoding green fluorescent protein (GFP), and GFP^+ cells were selected using fluorescence-activated cell sorting. MCF-7 cells were derived from pleural effusion from a metastatic estrogen receptor alpha–positive breast carcinoma [39] and were purchased from ATCC (30-2101). All tumor cell lines were cultured in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic solution at 37°C at 5% CO_2.

Tumor and Stromal Cell Transplantations

To generate tumors, 1×10^6 EO771 or 5×10^5 Met-1 cells were mixed with 2.5×10^5 ASCs isolated from obese or lean C57Bl/6 or FVB/N mice, respectively. These ratios of tumor cells to stromal cells were based on previous studies examining tumor and stromal cell interactions [40–42]. Tumor cells and ASCs were pelleted and resuspended in 2:1 Matrigel (Corning, 354234):DMEM and injected bilaterally into the inguinal mammary glands of 8-week-old C57Bl/6 or FVB/N female mice fed CD. Tumor diameters were measured using calipers three times each week. Tumor volume was calculated using the formula $4/3\pi r^3$. When tumors reached the humane endpoint of 1.5 cm in diameter, mice were euthanized. Tumors were weighed and then sectioned for fixation in formalin or collagenase digestion. To isolate single tumor cells, tumors were minced, incubated in DMEM:F12 (Corning, 10-090-CV) supplemented with collagenase I, and further treated 0.25% trypsin-EDTA (Corning, 25-053-CI) as described [43].

Conditioned Media Collection and Treatment