

# Crown-like Structures in Breast Adipose Tissue from Normal Weight Women: Important Impact

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## Abstract

Crown-like structures (CLS), composed of macrophages surrounding dead or dying adipocytes, are a histologic hallmark of the proinflammatory process by which adipose tissue contributes to the increased risk and worse prognosis of breast cancer in obese, postmenopausal patients. In this issue of *Cancer Prevention Research*, Iyengar and colleagues report the intriguing finding that CLS can be identified in a significant proportion of normal-BMI women undergoing mastectomy for breast cancer risk reduction or therapy. This surprising observation suggests that some normal weight women may

have similar mechanisms driving initiation and/or progression of breast cancer as those contributing to the increased incidence and worse prognosis of breast cancer in obese postmenopausal women. The possibility of a common mechanism in both lean and obese women provides added impetus to more fully define this process and evaluate its important implications for prevention and screening strategies as well as therapeutic interventions. *Cancer Prev Res*; 10(4); 1–3. ©2017 AACR.

See related article by Iyengar et al., p. 235–43

Articles by Iyengar et al. published in this issue *Cancer Prev Res* (1) as well as in 2015 (2) provide important insight into the potential mechanism(s) by which adipose tissue promotes cancer, especially in the breast, in both obese and some normal weight women. Many malignancies, including postmenopausal breast cancer, are epidemiologically and etiologically associated with obesity, which has been linked to all phases of carcinogenesis, including initiation, promotion, and progression (3, 4). The linkage between obesity and cancer has been attributed to multiple interacting factors, including changes in physical structure, altered adipokine secretion, increased secretion of growth factors, shifts in microbiome composition, dysregulation of hormone balance, and increased local and circulating inflammatory factors (4). Adipose tissue has long been recognized as an energy storage depot that accumulates or releases triglycerides, respectively, in response to energy excess or needs (5). It also is an active metabolic organ, communicating and regulating many physiologic and metabolic processes (appetite, hunger, satiety) through synthesis and secretion of a host of adipokines, signaling protein molecules synthesized primarily in adipocytes (5, 6). Among the multitude of these adipokines is leptin, whose synthesis is upregulated in obesity (6). Under physiologic conditions, leptin serves an anorexigenic function. It also functions to stimulate proinflammatory pathways in monocytes and macrophages and has been shown to promote tumor growth in mammary tumor systems (7). In contrast, synthesis and secretion of adiponectin, an anti-inflammatory and proapoptotic adipokine, is often downregulated in obesity (6). Adipose tissue serves also as the source for

localized and circulating factors characterizing a state of chronic low-grade inflammation associated with obesity (5, 6, 8). This metabolic inflammation (metaflammation) has been attributed to areas of adipose tissue where infiltrating bone marrow–derived macrophages (5, 9), showing a proinflammatory phenotype, cluster around the rim of enlarged, hypertrophied, dead and dying adipocytes, to form crown-like structures (CLS; refs. 8, 10–12). CLSs show greater frequency in adipose tissue from obese compared with lean individuals. They have been identified in most adipose deposits and show greater frequency in visceral compared with subcutaneous adipose tissue. These adipose tissue macrophages (ATM) in CLS phagocytose cellular debris, lipid droplets, and release triglycerides and free fatty acids (13, 14), products that can lead to insulin resistance and hyperinsulinemia (11–13, 15). Proinflammatory ATMs generate reactive oxygen species and reactive nitrogen species (5), both of which are potential mutagens. CLSs are associated with NF- $\kappa$ B activation and secretion of multiple inflammatory factors, including TNF $\alpha$ , IL1 $\beta$ , IL6, and COX-2–derived prostaglandin E2 (5, 6, 16). A variety of additional important immune abnormalities occur in inflamed adipose tissue (5). Adipose tissue is responsible for synthesis of aromatase, the rate-limiting enzyme that converts androgens to estrogens, and aromatase expression and enzymatic activity are increased in inflamed breast white adipose tissue (WAT; ref. 17). Importantly, an increase in the estrone:androstenedione ratio was found in breast tissue from postmenopausal breast cancer patients that contained CLS (18). Interestingly, inflamed adipose tissue has recently been shown to release cell-free DNA capable of stimulating insulin resistance (19). In addition, adipose tissue has been shown to be a source of circulating exosomal miRNA with a capacity for regulating glucose tolerance (20). These recent studies indicate that it will be important to investigate the role of circulating nucleic acids in contributing to the carcinogenic impact of inflamed WAT.

The Dannenberg laboratory has demonstrated the presence of CLS in WAT of the breast (CLS-B) in women with and without breast cancer, as well as in the noninvolved breast in women with

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breast cancer (16). CLS number and density increased with body mass index (BMI) and increased adipocyte size. CLS-B were more prevalent in post- compared with premenopausal women, and within each group, this difference was proportional to BMI. Thus, the highest percentage of patients positive for CLS-B occurred in postmenopausal women with BMI greater than 25 kg/m<sup>2</sup> (2, 21). They showed that the presence of CLS-B was associated with features of the metabolic syndrome. The presence of CLS-B was also associated with insulin resistance and changes in levels of circulating triglycerides, and increased proinflammatory factors, including leptin, hs-CRP, and IL6. These studies provide strong support for the premise that both obesity and postmenopausal status contribute to the development of CLS-B. As CLS-B occur both in the absence and presence of breast cancer and in the uninvolved breast in patients with cancer, these studies collectively suggest that WAT inflammation manifested as CLS-B may be causally linked to the increased incidence of hormone receptor-positive postmenopausal breast cancer in obese women. Interestingly, it has recently been reported that breast WAT from Black women, who have worse prognosis with breast cancer, have higher density of CLS-B than their non-Black counterparts (22). The probability that WAT inflammation helps to explain the obesity-inflammation-carcinogenesis axis is further supported by observations showing the occurrence of CLS in adipose tissue associated with other malignancies, including oral squamous cell carcinoma of the tongue (23), endometrial cancer (24), and in the report of liver macrophages, identified as Kupffer cells, forming CLS with lipid-laden hepatocytes in NAFLD, a disorder now considered to be a precursor for hepatocellular cancer (14, 25).

In this report by Iyengar and colleagues, in which CLS-B were found in women with normal BMI, there were, nonetheless, hypertrophied adipocytes, elevated levels of aromatase, increased circulating levels of proinflammatory factors, including hsCRP and leptin as well as elevated insulin and dyslipidemia, the latter contributing to a diagnosis of metabolic syndrome (1). These patients constitute a group of individuals with characteristics of metabolic syndrome with normal BMI now recognized as the metabolic equivalent of obesity. In addition to the likelihood that these normal-BMI women are at increased risk for breast cancer, occult WAT inflammation, a systemic process, is likely to place them at elevated risk for diabetes mellitus and cardiovascular disease. The practical implication of this observation is that these women deserve special attention regarding screening and risk reduction strategies. Successful

strategies to noninvasively identify these women, if coupled with interventions that attenuate WAT inflammation, could lead to a multitude of clinical benefits, including reducing the risk of breast cancer. Moreover, the findings of Iyengar and colleagues raise the question of what stimulates the carcinogenic process. Is it a consequence of obesity or do the causes of obesity independently promote the carcinogenic process? In response to this intriguing question, it is interesting to note that multiple models have now been reported wherein transplanted and/or spontaneous tumors, including breast cancer, are promoted by high-fat diet in the absence of obesity (26–30).

In a different tumor system, APC<sup>Min/+</sup> mice, we have recently shown that a high-fat coconut oil diet promotes obesity, inflammation, and aggressive tumor growth in some mouse strains, whereas in other strains, the same high-fat diet promotes inflammation and tumor growth independent of obesity (30). We showed, moreover, that an isocaloric high-fat, corn oil diet promoted inflammation and tumor growth in the absence of obesity, whereas in contrast, a high-fat olive oil diet promoted obesity but not inflammation or tumor growth. Thus, depending on the quality and quantity of dietary fat and its interaction with genetic background, it is clear that all combinations of obesity, inflammation, and carcinogenesis can be stimulated. Given these findings, it would be of considerable interest to determine whether the type and/or amount of dietary fat impacts the incidence of breast WAT inflammation reported by Iyengar and colleagues in this issue of *Cancer Prevention Research* (1). In addition, the human counterpart of our study may explain differential beneficial versus carcinogenic effects of the Mediterranean versus the Western diet relative to obesity, inflammation, and cancer (Di Daniele and colleagues 2017; ref. 31). The important issue that these studies raise regarding primary and secondary cancer prevention is whether it is more important to control the quality and/or quantity of dietary fat. The public health implications are significant.

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No potential conflicts of interest were disclosed.

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