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Get the fat out!

Natalia A. Ignatenko^{1,2} and Eugene W. Gerner^{1,3}

¹The University of Arizona, Department of Cellular and Molecular Medicine, Tucson, Arizona

²Arizona Cancer Center, Tucson, Arizona

³BIO5 Institute, Tucson, Arizona

Abstract

Obesity is associated with increased risk of a number of cancers in humans, but the mechanism(s) responsible for these associations have not been established. It is estimated that 68% of adults are overweight or obese and that obesity may be causative in 4–7% of cancers in the United States. Several hypotheses have been put forward to explain the association between obesity and cancer including adipose-directed signaling (e.g. mTOR, AMPK), production of factors (e.g. insulin growth factor 1, fibroblast growth factor 1 (FGF1)) and/or chronic inflammation associated with obesity. Huffman and colleagues (beginning on page XXXX) used surgical methods to determine if visceral fat (VF) was causally related to intestinal tumorigenesis in the *Apc^{1638/N+}* mouse in a manner independent of confounding factors such as caloric restriction. They found that caloric restriction could extend survival in both male and female *Apc^{1638/N+}* mice but found that surgical removal of VF was only effective in reducing macroadenomas in females. The results of this study do not identify the specific mechanism of association between VF and intestinal carcinogenesis in female mice but do support the rationale for future cancer prevention trials that evaluate pharmacological and behavioral strategies to reduce abdominal obesity in humans.

Colorectal cancer is one of the most common cancers in the world with the incidence rising particularly in developed countries (1). Colorectal carcinogenesis results from progressive accumulation of genetic and epigenetic alterations and causes deregulation of different signaling pathways governing cellular metabolism, proliferation, differentiation and survival (2). Up to 90% of colorectal cancer cases are related to lifestyle and eating behavior particularly excessive consumption of calorie-rich food and low physical activity, which results in a significant overweight and even obese phenotype (with body mass index (BMI) of 30 kg/m² or higher; ref. 3). Obesity is a worldwide epidemic and presents a major health problem. Significant epidemiological evidence indicates that obesity increases the risk of development of different cancers such as colon and rectum, endometrium, esophagus, kidney, pancreas, breast (postmenopausal), gallbladder, and hematologic malignancies (4). Moreover, obesity is a poor prognostic factor for some neoplastic tumors (4, 5). Cohort studies demonstrate that risk of developing of colon cancer increases by 15% in overweight people, and obesity increases this risk by up to 33% compared to the risk of people with a normal BMI (4).

The obese phenotype is characterized by increase of volume of white adipose tissue (WAT), which is a major source of energy storage in the form of triglycerides. WAT not only serves

Corresponding Author: Eugene W. Gerner, Ph.D., BIO5 Oro Valley, 1580 E. Hanley Blvd, Tucson, Arizona 85737; Phone: 520-626-2197; egerner@email.arizona.edu.

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as an energy depot but is also the largest endocrine organ in the body, which secretes hormones, adipokines, proinflammatory cytokines and growth factors (6).

A number of obesity-related carcinogenic pathways uncover a positive relation between increased body size and colorectal malignancy. Obesity influences carcinogenesis through the production of multiple circulating signaling molecules such as hormones and growth factors (adipokines, insulin, and insulin growth factor 1) and inflammatory cytokines (TNF- α , IL-6, IL-1 β , monocyte chemoattractive protein and C-reactive protein). These signaling molecules promote tumor cell growth and support the inflammatory state by acting locally on the neighboring tissues and systemically via serum circulation. Obesity-produced factors activate multiple intracellular signaling pathways including PI3K-AKT-mTOR (7), JAK2-STAT3-MAPK, EGFR-Notch1-Survivin, 5'-adenosine monophosphate-activated protein kinase (AMPK) (8), PPAR γ -FGF1 (9), and TGF- β /SMAD3 signaling (6). Cytokine production in obese adipose tissue creates a chronic inflammatory microenvironment that favors tumor cell motility, invasion, and epithelial-mesenchymal transition to enhance the metastatic potential of tumor cells (10). Despite the growing evidence for crosstalk between these pathways, it remains difficult to investigate the insights into mechanistic links between adipose tissue and cancer.

The work by Huffman and colleagues reported in this issue provides a critical evaluation of the contribution of adipose tissue apart from caloric restriction in the deregulation of energy homeostasis in the colon and colon tumor growth (11). Huffman and colleagues have a long-standing interest in dissecting the obesity-linked pathways. In 2007, using a transgenic mouse model of prostate cancer (TRAMP), they reported that changes in energy balance, body mass, and/or body composition rather than food intake *per se* conferred cancer risk (12). Earlier studies by the same group established a direct relationship between insulin resistance, type 2 diabetes and lifespan using a surgical model of visceral fat removal in rats (13–15). The current study offers an important addition to our understanding of the obesity-associated colorectal cancer risks as it establishes the important role of visceral fat in obesity-associated intestinal tumorigenesis (11). Epidemiological evidence points to visceral adipose tissue as a major contributor to the development of metabolic diseases such as type 2 diabetes, cardiovascular disease, dyslipidemia, inflammation and hypertension (16). A strong association exists between abdominal or visceral fat tissue and risk of colorectal and pancreatic cancers independent of BMI (17). Huffman et al. used a surgical approach to eliminate visceral fat (VF). The experimental animals were assigned to three groups: sham operated, visceral fat removal (VF-), and sham operated and caloric restricted (CR). This approach allowed evaluating the independent contribution of visceral fat and caloric restriction to the intestinal tumor formation and survival in animals genetically predisposed to colon carcinogenesis (*Apc*^{1638N/+} mouse model).

Several novel findings came out of this work. First, VF removal was an effective mode of suppressing experimental intestinal carcinogenesis, and it improved the animal survival rates similarly to CR. The beneficial effect of CR was in line with the previously reported experimental data that a calorie-rich diet augments colorectal carcinogenesis, whereas CR reduces colorectal tumor incidence (18, 19). The suppression of carcinogenesis induced by caloric restriction was likely associated with the morphological and biochemical changes in adipocytes. Specifically, CR reduced the adipocyte size, decreased triglyceride and proinflammatory cytokines production but stimulated adiponectin secretion and AMPK activation (8, 20).

Second, the gender-specific differences in anti-tumorigenic efficacy of VF- and CR were observed, specifically in relation to the size of the formed adenomas (microadenomas $\sim$ < 0.5 mm diameter versus macroadenomas, $\sim$ > 0.5 mm diameter). Although in the current study,

CR restriction was a very effective mode of suppressing colon carcinogenesis and improving survival rates of mice of both sexes, gender-specific analysis showed that CR was effective in suppressing both micro- and macroadenoma formation in males but had no effect on tumorigenesis in females.

In contrast, VF removal suppressed the macroadenoma formation in females but not in males. The authors explained this difference by the shift in regrown fat distribution among VR- males, particularly, due to an increase in mesenteric fat after removal of the epididymal and perinephric fat deposits, which was not observed in VR- females.

At the same time, VF removal in females unexpectedly increased the number of microadenomas. This interesting observation was explained by the blockage of progression of tumor development due to “a systemic change in factor(s)”. Indeed, the visceral fat removal may disrupt normal adipocyte maturation. Although the state of adipocyte differentiation was not evaluated by Huffman et al., they found that serum leptin levels were significantly higher and adiponectin levels were lower in VF- females. Adiponectin is a known antiangiogenic and antiproliferative adipokine (21). It promotes the differentiation of adipocytes and is normally elevated in mature adipose tissue. Mature adipocytes produce both leptin and adiponectin, but preadipocytes secrete high levels of leptin (22). It has been shown that *in vitro*, leptin produced by both mature adipocytes and preadipocytes was able to enhance the proliferation of colon cancer cells (23). Females produce more leptin than males, which is related to gender differences in fat depots and to leptin-suppressive effects of testosterone. Furthermore, VF- females retained the elevated level of insulin and the proinflammatory chemokine CXCL-1. This chemokine and possibly others secreted by preadipocytes attract monocytes, and high leptin levels promote monocyte conversion to macrophages, which contribute to local production of proinflammatory cytokines and proangiogenic factors, as has been reported in breast cancer (24). Such a microenvironment in adipose tissue may favor microadenoma initiation.

Still, important questions remain. What are the factors that can block the progression of tumor development at the microadenoma-macroadenoma transition? What factors contribute to the different outcome after visceral fat removal in male and female mice? What are the genetic interactions for the sex-related increase of microadenoma formation?

Huffman et al. did not address the potential Wnt signaling effects on adipogenesis and specifically, visceral fat growth factor signaling. Figure 1 depicts these signaling relationships and those influenced by caloric restriction and identifies key responses in male and female mice. The *Apc*^{L638N/+} mouse used in the Huffman et al. study produces the adenomatous polyposis coli (APC) protein truncated in position 1638, leading to the increase in β -catenin stability and overexpression of Wnt target genes. It has been shown *in vitro* that Wnt-10b signaling protein stabilizes free β -catenin and blocks adipogenesis in 3T3-L1 mouse preadipocytes (25). It is tempting to assume that in *Apc*^{L638N/+} mice, the adipose tissue is not fully differentiated due to the aberrant Wnt signaling. Thus, there is a great possibility that peroxisome proliferator-activated receptor γ (PPAR γ) plays a significant role in the observed findings. PPAR γ has been shown to be necessary and sufficient for adipocytes differentiation (26). PPAR γ function is also required for the maintenance of the mature adipocytes, as expression of a dominant-negative PPAR γ in differentiated mouse 3T3-L1 adipocytes results in dedifferentiation of adipocytes with loss of lipid accumulation and decreased expression of adipocyte markers (27). *In vivo*, knockdown of PPAR γ in mature adipocytes via adipocyte-specific aP2-driven expression of Cre-recombinase results in lipodystrophy accompanied by susceptibility to high-fat-induced steatosis, hyperinsulinemia, and insulin resistance (28). In fully formed adipocytes, PPAR γ activation is important for both lipid and glucose metabolism. Specifically, the control of

glucose homeostasis and insulin sensitivity by PPAR γ occurs upon its activation by the insulin-sensitizing synthetic ligands for PPAR γ , thiazolidinediones, that have been used to treat insulin resistance associated with type 2 diabetes (29). Ligand-dependent PPAR γ activation regulates a number of genes involved in lipid uptake and storage in adipose tissue and fatty acid utilization such as lipoprotein lipase, which catalyzes hydrolysis of triglycerides (30). This latter reference provides support for the relationships depicted in Figure 1 for the role of adipogenesis, VF and carcinogenesis. In that paper, hyperlipidemia was observed in *Apc*^{Min/+} mice, which express a truncated APC protein, and PPAR γ agonists suppressed both the hyperlipidemia and intestinal carcinogenesis in treated animals. Others have described the PPAR γ -mediated regulation of fibroblast growth factor 1 (FGF1), which is required for adaptive adipose remodeling and metabolic homeostasis (9). All the mentioned PPAR γ -mediated effects in adipocytes may be critical for colon carcinogenesis.

In conclusion, the exciting work by Huffman et al. provides important data regarding the contribution of visceral adipose tissue to intestinal carcinogenesis in the *Apc*^{I638/N+} mouse model. Given the significant role of the *APC* tumor suppressor gene in human colon carcinogenesis, it is likely that these results have relevance to colon carcinogenesis in humans. These new data address complex relationships between multiple signals associated with the obese state (summarized in Figure 1) and gender. Results from this study do not support a current hypothesis relating to excess production of estrogen by fat tissue, as serum estradiol levels were not different in control animals and mice in which VF had been surgically removed. The study findings do distinguish the role of visceral fat from confounding factors such as caloric restriction. It will be important to validate these findings in human studies as they may provide new opportunities for the prevention of colorectal cancer in patients with sporadic or genetic risk factors for colon cancer. The authors correctly point out that future clinical trials can exploit both pharmaceutical and behavioral strategies to address abdominal adiposity as a cancer risk factor in humans.

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Figure 1.

Relationships between mutant APC signaling pathways and experimental interventions, including caloric restriction and visceral fat (VF) removal in male and female mice.

Abbreviations include: AMPK – 5' adenosine monophosphate-activated protein kinase; mTOR – mammalian target of rapamycin; NF-κB – nuclear factor kappaB; IKKβ – inhibitor of NF-κB; CXCL-1 – chemokine (C-X-C motif) ligand 1; c-MYC – oncogene; ERK – extracellular signal-regulated kinase; ROS – reactive oxygen species; PKC – protein kinase C; LPL – lipoprotein lipase.