Elucidating the role of adipose tissue secreted factors in malignant transformation

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ABSTRACT
Although there is a growing number of incidences of obesity and obesity-linked cancers, how excess adiposity actually causes cancer has not been fully explained. Our previous study showed that removal of visceral adipose tissue significantly reduced the number of ultraviolet radiation (UVR)-initiated, high-fat diet-promoted skin cancers. This commentary focuses on our recently published study (Chakraborty, et al., 2017) which demonstrated that fibroblast growth factor-2 (FGF2) released from visceral adipose tissue is a key factor in the malignant transformation of epithelial cells. Within this commentary we have provided additional interpretations and new data in support of the role of FGF2 in adiposity-associated tumorigenesis.

Cancer is the second leading cause of death in the United States (US), exceeded only by heart disease.1 It has been estimated that obesity contributes to 20% of US cancer deaths by promoting cancer occurrence.2 There is ample evidence that obesity is an oncogenic factor:3 Epidemiological studies demonstrate excess body weight increases the risk of developing many types of cancers, including breast, prostate, colon, skin (melanoma) and esophageal cancer.4-9 For a review of cancers associated with a high body mass index (BMI) see ref. 10 The mechanism by which excess adipose tissue drives carcinogenesis is hypothesized to involve stimulating low-grade chronic inflammation, inducing growth factors and hormones, reprogramming metabolism, and altering the gut microbiome. Unraveling the complicated interplay between these various biological mechanisms is challenging given existing studies are mainly associative and many of these physiological aspects affect different people in different ways; for example, not everyone who is obese has chronic inflammation and/or metabolic syndrome.

In our recent publication, “Fibroblast growth factor receptor is a mechanistic link between visceral adiposity and cancer”, published in the journal Oncogene, we identified a factor, fibroblast growth factor-2 (FGF2), released from visceral adipose tissue that appears to play a direct role in epithelial cell malignant transformation.11 In this study, we suggest that blocking FGF2-FGFR-1, either with drugs or dietary modulation, may interfere with adiposity-associated tumor formation.

Our previous work demonstrated that visceral adipose tissue promotes ultraviolet radiation (UVR)-induced skin carcinogenesis. We compared UVR-induced tumor development rates in visceral adipose tissue-lipectomized mice with those seen in sham-operated controls. The visceral adipose tissue depot we excised was parametrial adipose tissue, the largest visceral fat pad in female mice, and closest analog of human omental adipose tissue, which contributes to cardiovascular disease, hypertension and diabetes.12-15 We discovered that surgical lipectomy of visceral adipose tissue in HFD (45% kcal from fat)-fed mice inhibited UVR-induced formation of non-melanoma skin tumors by 75-80% and inhibited the conversion of benign papillomas to malignant SCCs vs. the rates seen in controls.16 By the end of the study (31 weeks post-lipectomy), HFD-fed lipectomized mice had little to no residual visceral adipose tissue, but exhibited markedly increased subcutaneous adipose tissue depot mass, presumably as a compensatory effect, and/or result of removing an inhibitory influence of the visceral adipose tissue.16 However, the increased mass of subcutaneous adipose tissue failed to exert the tumor-promoting effect of visceral adipose tissue revealed by lipectomy. These findings directly support a role of visceral adipose tissue in HFD-promoted tumorigenesis.

We sought to investigate which factors released from this fat pad stimulated malignant transformation in epithelial cells. In our recent study, we demonstrated that FGF2 was selectively induced in the visceral adipose tissue compared with the subcutaneous adipose tissue when mice were fed a HFD.11 Moreover, treatment with recombinant FGF2, at levels present in the serum of HFD-fed mice, stimulated growth in soft agar
(transformation, surrogate marker of tumorigenicity) of skin epithelial JB6 P<sup>+</sup> cells. Cells that lacked the receptor for FGF2, FGFR-1, failed to transform in the presence of visceral adipose tissue. The subcutaneously obese mice had significantly less serum FGF2 than the viscerally obese mice, indicating that this factor may be a systemic promoter of tumorigenicity in HFD-fed, viscerally obese mice.

FGF2 is a known mitogen that has been studied for its role in tumor progression. Elevated levels of urinary FGF2 correlate with metastatic disease of several types of cancers. FGF2 promotes tumor progression by stimulating both angiogenesis and the proliferation of tumor cells however, how FGF2 may influence cancer susceptibility is less clear. FGF2 can stimulate the growth of non-tumorigenic cells and, the greater the number of cell divisions, the more likely a tissue is to get cancer. However, the levels of FGF2 that stimulated transformation (growth in soft agar, 3D culture) did not stimulate cellular proliferation as measured by growth in 2D culture, suggesting that the transforming activity of FGF2 is separate from its proliferative activity. To demonstrate that stimulating proliferation 2D culture is not sufficient to stimulate growth in soft agar, JB6 P<sup>+</sup> cells were treated with leptin. Leptin is a mitogenic factor in several cancers and is significantly elevated in obese individuals and in the visceral adipose tissue of HFD-fed mice. Leptin significantly increased proliferation of JB6 P<sup>+</sup> cells (not shown), but had no effect on cellular transformation (Fig. 1). Our studies indicate that the mechanisms involved with growth in soft agar are different than the mechanisms for 2D proliferation. How FGF2 stimulates cell transformation will be the subject of future studies.

The translational relevance of visceral adipose tissue-derived FGF2 for the promotion of human cancers is currently under investigation. In our manuscript, we demonstrated that human visceral adipose samples with higher levels of FGF2 had a greater capacity to stimulate growth in soft agar. Since visceral adipose tissue samples require surgery to obtain and evidence suggests that circulating FGF2 can originate in adipose tissue, the capacity of human serum samples to stimulate growth in soft agar was investigated. Human serum was obtained from 23 subjects from The Spectrum Health Universal Biorepository (SHUB, Grand Rapids, MI) undergoing abdominal surgeries for inflammatory gastro-intestinal conditions. Samples were chosen based upon body mass index to provide a distribution of body weights. JB6 P<sup>+</sup> cells were cultured with 5% human serum for 14 days in soft agar. Human serum samples with higher levels of FGF2 demonstrated a greater ability to stimulate JB6 P<sup>+</sup> cell growth in soft agar (Fig. 2A), building evidence for the potential of FGF2 to be a biomarker of risk for obesity-associated tumor formation. Interestingly, the levels of FGF2 in the serum had no association with BMI (Fig. 2B) and BMI had no association with the ability of the samples to stimulate growth in soft agar. Conversely, an epidemiological study showed serum FGF2 levels were elevated in an obese population when compared to those with a normal BMI. Two major limitations of our study are the small sample size and that the samples are not from healthy subjects. However, these serum data are in support of our prior work on FGF2 in visceral adipose tissue stimulating malignant transformation. Further work is needed to clarify the relationship between FGF2, visceral adiposity and cancer risk.

Further understanding what specific cell types synthesize and secrete FGF2 would provide additional targets for chemoprevention. While previously reported to be synthesized and secreted from adipocytes, our work demonstrated that FGF2 protein is expressed in both the

**Figure 1.** Transforming potential of human serum is associated FGF2 protein expression. Human serum samples were collected from 23 donors with a spectrum of BMIs. (A) Percentage of clones growing in soft agar (% colony formation) significantly increases in JB6 P<sup>+</sup> with serum that has higher concentrations of FGF2 compared to no treatment (control; Cont); R<sup>2</sup> = 0.753. (B) Percentage of clones growing in soft agar (% Colony Formation) is not associated with BMI; R<sup>2</sup> = 0.132. Data were analyzed with the Pearson’s correlation coefficient.
adipocyte and stromal vascular fraction of the visceral adipose tissue, and therefore, could potentially be secreted from many cell types. However, questions still remain to be answered in vivo: Is the adipocyte-immune cell interaction important for FGF2 synthesis and secretion? Which cell types secrete FGF2? Is it the quantity of the visceral adipose tissue or immune cells and their function (adipose tissue quality) that is most critical for circulating FGF2 and tumor promotion? How is FGF2 secreted? How is it stabilized? How does it get into the target tissues? One way to study adipose tissue quality in vivo would be to feed animals a HFD (40% kcal from fat) rich in either omega-3 fatty acids or omega-6 fatty acids. It has been shown that mice fed either of these two diets have the same amount of visceral adiposity, but the omega-3 fatty acid diet is protective against UVR-induced skin carcinogenesis whereas the omega-6 fatty acid diet promotes UVR-induced skin carcinogenesis. Additionally, animals fed the omega-3 HFD have fewer circulating pro-inflammatory cytokines. It would be interesting to compare FGF2 levels in both the visceral adipose tissue and the serum of animals fed these different diets. Understanding how different dietary fats impact circulatory FGF2 may lead to dietary interventions for chemoprevention.

Overall our study suggests that FGF2 is released from visceral adipose tissue to stimulate malignant transformation in FGFR-1 expressing epithelial tissues (Fig. 3). The completion of this study suggested a new function of the FGF2-FGFR-1 axis in visceral adiposity-associated tumorigenesis. We are interested in studying this mechanism in vivo and in other cancer models. Determining the impact of excess visceral adipose tissue on tumor formation will lead to mechanism-based strategies to help prevent adiposity-associated cancers and determine individuals at risk for disease.

**Disclosure of potential conflicts of interest**

The authors declare no conflict of interest.

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**References**

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90. doi:10.3322/caac.20107. PubMed PMID:21296855.
2. Colditz GA, Wolin KY, Gehlert S. Applying what we know to accelerate cancer prevention. Sci Transl Med. 2012;4(127):127rv4. doi:10.1126/scitranslmed.3003218. PubMed PMID:22461645; PMCID: PMC3343638.
3. 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(17):1625–38. doi:10.1056/NEJMoa021423. PubMed PMID:12711737.
4. Carmichael AR. Obesity as a risk factor for development and poor prognosis of breast cancer. BJOG. 2006;113(10):1160–6. doi:10.1111/j.1471-0528.2006.01021.x. PubMed PMID:16945118.
5. Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: a systematic review. Obesity Reviews: an Official Journal of the International Association for the Study of Obesity. 2003;4(3):157–73. PubMed PMID:12916817.
6. Huffman DM, Augenlicht LH, Zhang X, Lofrese JJ, Atzmon G, Chamberland JP, Mantzoros CS. Abdominal obesity, independent from caloric intake, accounts for the development of intestinal tumors in Apc(1638N/C) female mice. Cancer Prev Res (Phila). 2013;6(3):177–87. doi:10.1158/1940-6207.CAPR-12-0414. PubMed PMID:23466815; PMCID: PMC3595118.
7. Karimi K, Lindgren TH, Koch CA, Brodell RT. Obesity as a risk factor for malignant melanoma and non-melanoma skin

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**Figure 2.** Leptin stimulates growth of JB6 P+ cells in soft agar (anchorage-independent 3D-culture). JB6 P+ cells were treated with leptin at indicated concentrations. Leptin failed to stimulate JB6 P+ cell growth in soft agar after 14 days of incubation. FGF2 was used as a positive control. Data are presented as mean ± S.D. of values from triplicates and statistical significance was determined using a one-way ANOVA (**p**<0.001).

**Figure 3.** Malignant transformation of non-tumorigenic epithelial cells by FGF2 secreted from visceral adipose tissue (VAT). FGF2 secreted from VAT binds with FGFR1 and activates the FGF2-FGFR1 axis. This further initiates downstream signals to induce c-Myc protein expression. c-Myc levels are maintained in FGF2 and VAT-transformed cells. FGF2: Fibroblast growth factor 2; VAT: Visceral adipose tissue; FGFR1: Fibroblast growth factor receptor 1.
18. Zhang X, Xu J, Jiang T, Liu G, Wang D, Lu Y. MicroRNA-195 suppresses colorectal cancer cells proliferation via targeting FGF2 and regulating Wnt/beta-catenin pathway. Am J Cancer Res. 2016;6(11):2631–40. PubMed PMID:27904776; PMCID: PMC5126278.

19. Nguyen M, Watanabe H, Hudson AE, Richie JP, Hayes DF, Folkman J. Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. Journal of the National Cancer Institute. 1994;86(5):356–61. PubMed PMID:7508518.

20. Scarpese PJ, Zhang Y. Elevated leptin: consequence or cause of obesity? Front Biosci. 2007;12:3531–44. PubMed PMID:17485319.

21. Amjadi F, Mehdipoor R, Zarkesh-Esfahani H, Javanmard SH. Leptin serves as angiogenic/mitogenic factor in melanoma tumor growth. Adv Biomed Res. 2016;5:127. doi:10.4103/2277-9175.187005. PubMed PMID:27563637; PMCID: PMC4976532.

22. Hoda MR, Popken G. Mitogenic and anti-apoptotic actions of adipocyte-derived hormone leptin in prostate cancer cells. BJU Int. 2008;102(3):383–8. doi:10.1111/j.1464-410X.2008.07534.x. PubMed PMID:18341625.

23. Hoda MR, Keely SJ, Bertelsen LS, Junger WG, Dharmasena D, Barrett KE. Leptin acts as a mitogenic and antiapoptotic factor for colonic cancer cells. Br J Surg. 2007;94(3):346–54. doi:10.1002/bjs.5530. PubMed PMID:17212381.

24. Bifulco G, Trenca A, Caruso M, Tommaselli GA, Miele C, di Carlo C, Beggunit F, Nappi C. Leptin induces mitogenic and antiapoptotic effect on human choriocarcinoma cell line (JAr) via MAP kinase activation in a glucose-dependent fashion. Placenta. 2003;24(4):385–91. PubMed PMID:12657513.

25. Kuhn MC, Willenberg HS, Schott M, Papewalis C, Stump U, Flohe S, Scherbaum WA, Schinner S. Adipocyte-secreted factors increase osteoblast proliferation and the OPG/RANKL ratio to influence osteoclast formation. Mol Cell Endocrinol. 2012;349(2):180–8. doi:10.1016/j.mce.2011.10.018. PubMed PMID:22040599.

26. Hao RH, Guo Y, Dong SS, Weng GZ, Yan H, Zhu DL, Chen XF, Chen JB, Yang TL. Associations of Plasma FGF2 Levels and Polymorphisms in the FGF2 Gene with Obesity Phenotypes in Han Chinese Population. Sci Rep. 2016;6:19868. doi:10.1038/srep19868. PubMed PMID:26879180; PMCID: PMC4754629.

27. Mydlo JH, Kral JG, Macchia RJ. Preliminary results comparing the recovery of basic fibroblast growth factor (FGF-2) in adipose tissue and benign and malignant renal tissue. J Urol. 1998;159(6):2159–63. PubMed PMID:9598562.

28. Lou YR, Peng QY, Li T, Medvecky CM, Lin Y, Shih WJ, Conney AH, Shapses S, Wagner GC, Lu YP. Effects of high-fat diets rich in either omega-3 or omega-6 fatty acids on UVB-induced skin carcinogenesis in SKH-1 mice. Carcinogenesis. 2011;32(7):1078–84. doi:10.1093/carcin/bgr074. PubMed PMID:21525235; PMCID: PMC3128560.