How Does Obesity Drive Human Carcinogenesis? 
Challenges in Dissecting the Mechanisms of Adipose–Epithelial Signaling

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ABSTRACT

Obesity is the second leading environmental association with cancer risk; yet, the mechanisms by which obesity drives carcinogenesis are poorly understood. The paper published in this issue of Cancer Prevention Research by Holowatyj and colleagues explores the mechanisms of human visceral adipose–epithelial signaling using samples collected at surgery in patients with invasive colorectal cancer. They identify pathway intermediates potentially involved in the regulation of fibrosis, inflammation, glycosis, and epithelial–mesenchymal transition in neoplastic tissue. Omics-based profiling of perioperative human biosamples has potential for inherent biases (preoperative and intraoperative drug therapies, hydration, dynamics, inflammatory response to surgical intervention) and appropriate control samples are difficult to identify and collect. Solutions to this dilemma may include strategies to identify patients undergoing similar surgical procedures but who are without neoplasms, for example, patients with gynecologic problems, abdominal exploration for suspected appendicitis, symptoms or resection of gall stone disease or undergoing bariatric surgery. As the field continues to grow, studies incorporating robust statistical analyses, validation of findings in diverse cohorts, and public data sharing will be essential to identify biological pathways linking obesity and carcinogenesis to be further interrogated using focused, hypothesis-driven approaches.

Findings from the American Cancer Society, the American Institute for Cancer Research, and an umbrella review of the literature are consistent with regard to clear evidence of obesity-associated increased risks of gastrointestinal cancers (colorectal, esophageal, pancreatic), postmenopausal breast, and intraoperative drug therapies, hydration, dynamics, inflammatory response to surgical intervention) and how this chronic inflammatory state drives transformation of vulnerable epithelia.

Excess energy intake results in a shift of metabolic and hormonal pathways to increase adipose stores. Over time, the limits of healthy adipose cell expansion are exceeded, eliciting a wide variety of responses thought to be a source of the clinical metabolic syndrome. A consequence of stressed adipocyte expansion and subsequent necrosis drives chronic local inflammation from macrophage recruitment to the necrotic adipose. The resulting “crown-like structures” identified in human adipose by the Dannenberg laboratory are associated with enhanced epithelial carcinogenesis. Adipose tissue is not the only storage depot for excess fatty acids. They are stored in a wide variety of cell types within cytoplasmic lipid droplets. In intestinal epithelium, for example, obesity induces lipid droplet formation. Lipid droplets are sites of active membrane based synthesis of proinflammatory molecules, for example, prostaglandins and leukotrienes. In neoplastic cells, lipid synthesis is induced and can contribute to the expansion of lipid droplets. Fatty acid synthase expression is identified as an early marker of colorectal neoplasia.

Some, including our group, suggest that the metabolic milieu resulting from excess adiposity increases cancer risk through either local tissue signaling, paracrine signaling or through systemic signals, namely, endocrine signaling that converge through their control of epithelial cellular stemness and shifts within stemness pools to more self-renewal and...
proliferation. Microenvironmental factors such as tissue inflammation facilitate this process (7). Multiple obesity-related pathways, for example, insulin-related pathways and the activation of NFKB by prostaglandin E2 critically, induce expansion of the cancer stem cell pools (8).

Physiologic modeling of this complex milieu that addresses the precise mechanisms by which lipid excess either in the form of adipose stores or intracellular lipids drive epithelial carcinogenesis has eluded investigators to date. The precise mechanism by which signals from nearby or distant adipose stores reach and interact with target epithelia, remain unclear. In part, this gap is driven by technical barriers such as identifying and characterizing the contributions of stromal components (fibrous tissue, circulating inflammatory cells, adipocytes) to the epithelial environment in situ. Methods used to collect, disaggregate, and generate in vitro preparations of tissue components necessarily create bias from the physical and chemical reagents necessary to process primary tissues. While recent progress in three-dimensional tissue culture systems have resulted in more informative models than the standard two-dimensional culture systems of the past, the creation of true organoids requires a stromal component that may not entirely reproduce the stromal–epithelial signaling environment in vivo. These challenges are further amplified when attempting to model adipose. Adipose tissue (visceral, primarily abdominal or subcutaneous from rodents or humans) adapts well to three-dimensional culture conditions and remains viable for at least 10 days. Key physiologic differences among visceral and subcutaneous adipose and potentially other adipose depots make it imperative to characterize the local and systemic secretomes produced by individual depots.

For studies of human adipose, accessibility of subcutaneous adipose with needle aspiration makes longitudinal collection and primary three-dimensional culture feasible. This strategy permits interrogation of the stromal–epithelial environment as environmental and other physiologic events unfold over time. For example, our laboratory has studied the adipokine milieu associated with obesity by culturing subcutaneous adipose tissue explants and assessing stemness in organoid cultures obtained from normal reduction mammoplasty tissue (manuscript in preparation). Such an approach misses the role of visceral adipose that, arguably, the adipose proximate to the colon and, therefore, more relevant to understanding local tissue stromal–epithelial signaling upon colonic epithelium. In the case of other vulnerable epithelia, for example, breast, modeling adipose–epithelial interactions using subcutaneous adipose sampling may have more merit. The precise signaling molecules dominant in adipose paracrine signaling among local adipose–stromal–epithelial structures remains to be dissected. It is conceivable that phenotypic and genomic differences in transformation are caused, in part, by differences in proximate adipose physiology resulting in microenvironmental signals that are unique to that local anatomic site.

The manuscript of Holowatyj and colleagues published in this issue of Cancer Prevention Research, addresses the issue of adipose–epithelial signaling in humans in a unique experimental model. Matched human visceral adipose, invasive neoplastic, and normal colonic tissue from patients undergoing surgical resection of a colorectal neoplasm was extracted and genomically interrogated an Illumina gene expression microarray. Matched serum was interrogated for inflammatory mediators using a Mesoscale Discovery platform. In analyzing the profiles generated by the broad transcriptomic, metabolomic, and proteomic approaches, they report evidence of a relationship between adipose signaling to neoplasms driving fibrosis, inflammation, glycosis, and EMT in neoplastic tissue. A careful review of the molecular classifiers identified from the expression microarray leads one to suggest that acute phase reactants account for many of the adipose-based signaling molecules, for example, α-2 macroglobulin, ALOX5, cadherens, cathepsins, chemokines, and common cytokines. Of course, these same molecules are implicated in the early carcinogenesis phases and subsequent emergence of invasive clones. The interventions and procedures required for surgery—bowel preparation, antibiotics, incisions, and tissue trauma—all generate an acute inflammatory response. Interrogating blood samples and tissues resected in such a situation and generating a signature of upregulation of acute phase reactants leaves one with associations that might be surgery-associated bias. Most acute phase reactants impact on prostaglandin H synthase-2 expression that was used to dichotomize the colon tumor tissues because prostaglandin H synthase-2 is rapidly induced to produce prostaglandin E2, a critical component of the wound repair process in the colon (9). The stratification criteria used in analysis of these data, PPARγ and prostaglandin H synthase-2 expression, could in part reflect this acute phase response and ultimately influence the interpretation of stromal–epithelial signaling.

One can sympathize that the availability of true controls, resected bowel from noncancer patients, would be very difficult to obtain and likely biased with other pathologies then neoplasm. The authors used each individual’s adjacent normal colon tissue gene expression as a “normalization” factor for tumor gene expression. This “internal control” is a reasonable first approach to understand tumor-specific biology in each patient. Future studies can additionally implement methods to better assess the cancer field effect. For example, using matched normal tissue as an internal control may mask the true effect of adipose if an individual has a higher “inflammatory tone,” that is, a baseline of increased inflammatory mediators, throughout their colon driven by dysregulated adipose biology. Consideration and quantification of tissue heterogeneity from bulk expression analyses can help to quantify this field effect and predict impacts on particularly susceptible cell types, such as stem cells.

Is sampling of human visceral adipose feasible without encountering the barriers of surgical procedural bias? Patients undergoing laparoscopic surgery may not require general anesthesia (although most do), but the drug and bowel preparation procedures remain. Short of reverting to the
acknowledged shortcomings of rodent models, are there strategies that might overcome the limitation of inadequate controls to enable correction of surgical bias in large omics datasets? One approach might be to include controls patients who are undergoing similar surgical procedures but do not have neoplasms. For example, patients undergoing abdominal procedures for gynecologic problems, patients undergoing abdominal exploration for abdominal symptoms such as appendicitis, or resection of gall stone disease could be useful controls to enable classification of benign inflammation versus neoplasm-induced changes in gene expression or circulating inflammatory proteins. Select patients undergoing bariatric surgery might serve as strong controls, although the majority of these patients will have the obesity-associated metabolic syndrome.

Dissection of the paracrine signaling pathways that drive carcinogenesis progression will require human biosamples. Collections of visceral adipose and matched inflammatory, normal and neoplastic tissues from a variety of patients undergoing surgery for nonneoplasm indications and for those with known neoplasms may provide a more accurate comparison of molecular profiles versus those reported here. As the field continues to grow and evolve, researchers should continue to incorporate best practices to enhance rigor and reproducibility:

- Performing robust and transparent statistical analyses that adjust for multiple comparisons, validating findings in external cohorts of diverse study participants, and making data publicly available for use in meta-analysis and consortium-level efforts.
- Ultimately, the signals identified in these “big data” experiments will need to be investigated using more focused, pathway-driven approaches. These will need to utilize the full armamentarium of conditional transgenic rodents, three-dimensional coculturing technologies of biosamples collected from both controlled rodent systems and less controlled human biosamples, and complex statistical modeling tools to discern valid neoplastic-driven signals from biases induced by clinically required interventions.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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