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Opinion

Adipocyte–Tumor Cell Metabolic Crosstalk in Breast Cancer

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The tumor stroma is a heterogeneous ecosystem comprising matrix, fibroblasts, and immune cells and has an important role in cancer progression. Adipocytes constitute a major component of breast stroma, and significant emerging evidence demonstrates a reciprocal metabolic adaptation between stromal adipocytes and breast cancer (BC) cells. Recent observations promote a model where adipocytes respond to cancer cell-derived endocrine and paracrine signaling to provide metabolic substrates, which in turn drive enhanced cancer cell proliferation, invasion, and treatment resistance. Further defining the mechanisms that underpin this dynamic interaction between stromal adipocytes and BC cells, especially in the context of obesity, may identify novel therapeutic strategies. These will become increasingly important in addressing the clinical challenges presented by obesity and metabolic syndromes.

Reciprocal Interactions between the Local Microenvironment and Tumor Cells Support Disease Progression

Cell-to-cell and organ-to-organ crosstalk is a characteristic of complex organisms, and disruptions in these interactions are central to the pathogenesis of many diseases. In a cancer context, the cells of the surrounding or nearby tissue, including fibroblasts, endothelial cells, and immune cells [1–3], have altered biological properties compared with the normal state. In turn, these cells can influence the tumor microenvironment and tumor cell biology (including metabolism) to support disease progression (Figure 1). Hence, a spectrum of reciprocal interactions between stroma and tumor drives several aspects of cancer progression. The predominant cell population in the breast is the **adipocyte** (see Glossary), and its role in BC progression has only recently received significant attention. This is somewhat surprising given the close juxtaposition of adipocytes and BC cells during early local invasion [4–6]. In the context of tumor metabolic reprogramming, relatively little attention has been paid to the potential role of mammary stromal adipocyte lipolysis in supporting BC progression.

In recent years, the concept of tumor–stroma interactions, in particular between tumor cells and fibroblasts, has been broadened to include tumor metabolism [7]. The metabolic interaction between these two compartments (i.e., tumor and stroma) results in a metabolic synergy that promotes tumor progression. Specifically, this two-compartment tumor metabolism model explains the scenario whereby tumors act as metabolic parasites, sequestering metabolic substrates, including lactate, glutamine, and fatty acids, from local and/or stromal sources via the stimulation of catabolic pathways, such as **autophagy**, **glycolysis**, and **lipolysis**. These

Trends

Reciprocal metabolic interactions between adipocytes and tumor cells can drive several aspects of cancer progression.

Adipocytes stimulate BC cell growth, proliferation, and migration in a range of model systems, as well as inducing therapeutic resistance.

BC cells signal to adipocytes to mobilize metabolic substrates, especially fatty acids, resulting in smaller adipocytes near tumors.

Adipocytes supply metabolic substrates, lipid-signaling agonists, and growth factors, and alter intermediary metabolism, especially fatty acid oxidation, in BC cells to promote BC cell growth, migration and migration.

An altered metabolic microenvironment in obese patients can amplify the reciprocal interactions between tumor cells and adipocytes, to potentially enhance BC progression.

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metabolites are utilized as substrates for anabolic metabolism in cancer cells. In this context, stromal adipocytes can act as supporting cells, contributing fatty acids via lipolysis to BC cells [8].

In this opinion, we argue that stromal adipocytes drive metabolic reprogramming in tumor cells to promote progression, and that tumor cells exert reciprocal effects on adipocyte function to activate catabolic metabolism, providing substrates for tumor anabolic metabolism. We also discuss emerging evidence for the underlying mechanisms, including the role of signaling mediators, direct transfer of metabolic substrates, and genetic regulation. These have important implications for understanding the links between obesity and BC, and highlight the potential for novel therapeutic strategies.

Adipocytes Modify Breast Cancer Cell Behavior

Numerous studies using various combinations of human and animal cell co-cultures, conditioned media, and animal models (discussed in detail below) have demonstrated that adipocytes promote the proliferation, migration, and invasion of BC cells, along with the development of resistance to chemotherapy and radiotherapy. Following seminal discoveries of adipocyte-derived hormones, including **leptin** [9] and **adiponectin** [10,11], adipocytes are no longer viewed merely as inert lipid storage cells. In fact, over 600 **adipokines** have now been identified [12], including a complex mixture of growth factors, such as insulin-like growth factor 1 (IGF-1). The levels of these adipokines secreted by adipocytes are altered in mammalian diseases, such as obesity and type 2 diabetes mellitus [13], likely due to the altered levels of extracellular stimuli, including glucose, amino acids, and lipids, as well as hormones, such as insulin [14,15].

Indeed, a recent *in vitro* study demonstrated that both primary human adipocytes and mouse 3T3-L1-derived adipocytes directly provide metabolic substrates to help support the altered metabolic demands in BC cells [8]. Specifically, by exposing BC cells of various molecular subtypes [including **ER$^+$ BC** and **triple-negative BC (TNBC)**] to adipocyte-conditioned media, or by co-culturing with adipocytes, key tumor cell behaviors (i.e., proliferation, migration, and invasion) were enhanced [5,8,16–22]. Similar adipocyte-stimulated effects on tumor cell proliferation, migration, and invasion have been observed in 3D cultures of human BC cells [23,24] and xenograft models using human cells in mice [20,25]. This adipocyte-stimulated BC cell invasion is due to BC cell fatty acid oxidation, as reduced levels of CPT1 (carnitine palmitoyltransferase 1) – the rate limiting enzyme in fatty acid oxidation – in ZR-75-1 BC cells blunted cell invasion into Matrigel during co-culture with mouse 3T3-F442A adipocytes [82]. Further, pharmacological inhibition of fatty acid oxidation in murine TS/A BC cells co-cultured with mouse 3T3-F442A adipocytes reduced liver metastasis in mice following tail-vein injection. Importantly, these effects were shown to be predominantly mediated by mature adipocytes rather than by other cell types found in adipose tissue, such as resident immune cells, preadipocytes, and fibroblasts (Box 1). For example, the stromal vascular fraction (a heterogeneous mix of cells that are isolated by the collagenase treatment of adipose tissue that does not contain mature adipocytes) does not alter **MCF-7** cell proliferation [17] or viability [19], or **MCF-10CA** cell migration in culture [16]. These *in vitro* observations indicate that BC progression could be promoted specifically by stromal adipocytes. Subsequent *in vivo* experiments using subcutaneous co-injection of **F442A** preadipocytes with MFC-7 cells in SCID mice supported these *in vitro* findings, with larger tumors forming when MCF-7 cells were co-injected with F442A preadipocytes compared with injection of MCF-7 cells alone [26]. Collectively, these studies demonstrate that mature adipocytes can induce various processes of BC cell biology (i.e., proliferation, migration, and invasion) consistent with an ability to promote disease progression.

A range of mechanisms has been proposed to explain how mature adipocytes alter BC cell behavior. The best described of these involves secreted factors (adipokines). For example,

Glossary

3T3F442A: clonal fibroblast cell lines derived from 3T3 mouse embryonic fibroblasts; can be differentiated to adipocytes.
Acetyl-CoA carboxylase (ACC): key regulator of fatty acid synthesis and degradation via catalyzing the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA.
Adipocyte: differentiated cell type specialized in the synthesis and storage of lipids.
Adipokine: cell signaling proteins (cytokines) secreted by adipose tissue.
Adiponectin: protein hormone produced and secreted by adipocytes to regulate lipid and glucose metabolism.
Adipose triglyceride lipase (ATGL): encoded by *PNPLA2* and highly expressed in adipose tissue; catalyzes the removal of fatty acid from triacylglycerol to generate fatty acids and diacylglycerol.
Autophagy: proteolytic degradation of cytosolic components at the lysosome; important for energy homeostasis in development and in response to nutrient stress, and for removing misfolded and aggregated proteins and damaged organelles.
BT474: breast ductal carcinoma cell line isolated from a 60-year-old Caucasian female.
Co-culture: cell culture system containing two distinct cell types.
Docetaxel: a member of the taxane family of microtubule-disrupting drugs; inhibits cell division.
E0771: murine medullary mammary adenocarcinoma cell line, isolated from a spontaneous tumor from a C57BL/6 mouse.
ER$^+$ breast cancer: BCs expressing receptors for the hormone estrogen.
Fatty acid synthase (FASN): a multi-enzyme protein complex that catalyzes fatty acid synthesis. Encoded by the *FASN* gene in human.
Glycolysis: metabolic pathway catalyzing the breakdown of glucose into pyruvate to generate ATP.
Hormone-sensitive lipase (HSL): an intracellular neutral lipase that catalyzes the hydrolysis of complex lipid species; encoded by *LIPE*.
Human epidermal growth factor receptor 2 (HER2): a receptor tyrosine-protein kinase encoded by *ERBB2* and frequently amplified or overexpressed in some BC subtypes.

Box 1. Adipocytes versus Adipose Tissue: Not the Same Thing

Adipose tissue is a heterogeneous mix of cell types comprising mature adipocytes, resident immune cells such as macrophages, fibroblasts, and the stem cell population termed 'preadipocytes'. Several studies have generated conditioned media from explants of adipose tissue and then exposed these to cancer cells [24,75,76]. Given that these conditioned media contain a mixture of factors secreted from the various cell types present in adipose tissue, assigning cell-specific mechanisms of action can be difficult. Below, we discuss examples of known effects of other nonadipocyte cells types in adipose tissue on BC cells.

Adipocytokines from Resident and/or Infiltrating Immune Cells

The interaction between immune and cancer cells has received significant attention, especially with the deployment of immunotherapies into clinical trials [77]. Several studies have reported roles for adipose tissue-derived cytokines. For example, adipocytes within human and mouse breast tissues recruited and activated human and mouse macrophages via the CCL2/IL-1β/CXCL12 signaling pathway, which in turn promoted stromal vascularization and angiogenesis [75]. In another study, secretion of oncostatin M by $CD45^+$ leucocytes in BC-associated adipose tissue stimulated MCF-7 and SKBR3 cell invasion [24]. Cytokine-induced effects on BC cells by adipocytes resulted from signaling pathways, such as Src, Sox2, and miR-302b [52]. While adipocytes do express mRNA for many cytokines, including TNFα, IL-6, IL-8, and CCL2, the relative amount secreted by mature adipocytes is miniscule compared with cultured stromal vascular fractions, which contain various immune cells [52]. As such, caution is required when interpreting gene expression data in adipocytes and extrapolating this to the *in vivo* setting.

Adipose Tissue and Local Estrogen Production

A major hypothesis linking obesity to BC is the increased local production of estrogen due to the expansion of adipose beds. A recently proposed model states that adipocyte-derived factors stimulate aromatase expression in adipose stromal cell populations and that these cells then convert androgen in estrogen to increase local estrogen levels, which then stimulate the growth estrogen-sensitive BC [78].

Preadipocytes, Stem Cells, and Fibroblasts

The adipose stem cell pool, also known as preadipocytes, supports tumor growth. Recent studies demonstrated that co-culture of adipose stem cells enhanced MCF-7 and MDA-MB-231 cell proliferation [79]. While co-injection of human adipose tissue-derived stromal and/or stem cells with MDA-MB-231 cells in a xenograft mouse model did not alter tumor size compared with MDA-MB-231-only xenografts, they did facilitate enhanced metastasis to the liver, lung, lymph node, and spleen [25]. Furthermore, adipocyte-derived fibroblasts stimulated the invasive capacity of 2D and spheroid cultures by secreting fibronectin and collagen I [55].

leptin enhances the proliferation of MCF-7 and T47D cells *in vitro* [27,28]. Most recently, leptin has been shown to promote epithelial–mesenchymal transition (EMT) in MCF-7, SK-BR-3, and MDA-MB-468 BC cells via the upregulation of pyruvate kinase M2 expression, as well as via activation of the **phosphatidylinositol-4,5-bisphosphate 3-kinase (**PI3K)/Akt signaling pathway [28]. Moreover, leptin knockdown by short hairpin (sh)RNA in human adipocytes attenuated the proproliferative effect of adipocytes on ER^+ BC cell lines MCF-7, **ZR75,** and T47D *in vitro* [29]. The other major adipokine, adiponectin, selectively stimulated apoptosis in MCF-7, T47D, and **SK-BR-3** (ER^-) cells but not in **MDA-MB-361** (ER^+) or MDA-MB-231 (ER^-) cells [30], suggesting that the response of cancer cells to adiponectin reflects a heterogeneous response to other signaling mediators, such as steroid hormones and growth factors [31]. Conversely, a cleaved adiponectin isoform (globular adiponectin) did not alter proliferation but did stimulate migration and invasion in **MDA-MB-231** cells [32], possibly influenced by the ligand-determined specificity of adiponectin, based on its molecular structure. These adipokines do not act in isolation *in vivo,* and experiments comparing different combinations of leptin and adiponectin added to MDA-MB-231 cell culture media have demonstrated that their effects on proliferation are dependent on the relative abundance of leptin relative to adiponectin. Specifically, added in isolation, leptin was reported to exert a proproliferative effect on cells, while adiponectin was found to have a proapoptotic effect; by contrast, when added in combination, the net effect was influenced by the predominant adipokine in culture [33]. Importantly, this adipokine balance has been found to be altered in obese humans [34].

Leptin: peptide hormone produced by adipose tissue; acts through the leptin receptor to regulate energy expenditure and endocrine function.
Lipogenesis: involved in the *de novo* synthesis of new fatty acids from acetyl-CoA, catalyzed by FASN; mediates the generation of new complex lipids, such triacylglycerols, sphingolipids, and so on.
Lipolysis: hydrolysis of lipids to produce glycerol and fatty acids.
MBA-MD-361: breast adenocarcinoma cell line isolated from brain metastasis from a 40-year-old Caucasian female.
MCF-10A: a spontaneously immortalized human breast epithelial cell line isolated from a 36-year-old Caucasian female
MCF-7: ER/PR^+ BC cell line-isolated pleural effusion of invasive ductal carcinoma from a 69-year-old Caucasian female.
MDA-MB-231: basal (ER^-, PR^-, $Her2^-$) BC cell line isolated from pleural effusion of invasive ductal carcinoma from a Caucasian female.
Omental adipocytes: cells derived from the omentum (a fatty tissue that overlays the abdominal organs within the peritoneal cavity).
Organotypic: tissue culture systems modeling the complex 3D organization of cells and extracellular matrix (ECM) within tissues and organs.
Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K): key signal transduction enzyme catalyzing phosphorylation of the 3-position hydroxyl group of the inositol ring of phosphatidylinositol.
Preadipocyte: stem-like cell within the adipose differentiation lineage.
SK-BR-3: breast adenocarcinoma cell line isolated from pleural effusion from a 43-year-old Caucasian female.
SUM159PT: basal (ER^-, PR^-, $Her2^-$) BC cell line derived from a primary human anaplastic breast carcinoma.
T-47D: ER/PR^+ BC cell line isolated from a pleural effusion of infiltrating ductal carcinoma from a 54-year-old female patient.
Triple-negative breast cancer (TNBC): BC that does not express estrogen receptor (ER), progesterone receptor (PR), or HER2 (e.g., ER^-, PR^-, and $Her2^-$).
Trastuzumab: (Herceptin) monoclonal antibody-based therapy used to treat $HER2^+$ BC.

Adipocytes also secrete growth factors and hormones that are proproliferative, including IGF-1, and their binding proteins. For example, mature adipocyte-stimulated MFC-7 cell growth has been associated with levels of adipocyte-derived IGF-1, and pharmacological inhibition of cancer cell IGF-1 signaling attenuated this response [19]. This may be due in part to the actions of IGFBP-2, because knockdown of IGFBP-2 in human adipocytes blunted adipocyte-induced MCF-7 invasion into a Matrigel extracellular matrix environment *in vitro* [20].

'Warburg'-like metabolism: the tendency of tumor cells to adopt a metabolic state (aerobic glycolysis) favoring 'fermentation' of glucose into lactate in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation.
ZR-75-1: ER^+ BC cell line isolated from ascites of ductal carcinoma from a 63-year-old female.

Adipocytes Drive Breast Cancer Cell Metabolic Reprogramming

In the context of BC, the role of adipocytes as professional long-term energy storage reservoirs is often not considered. In this role, adipocytes are capable of secreting significant quantities of metabolic substrates, such as glycerol and fatty acids, alongside growth factors and adipokines (discussed above). Cancer cells adapt intracellular metabolic processes to address redox stress, generate the macromolecule building blocks to support proliferation, and provide the energy required for biomass synthesis, migration, and invasion [35]. These building blocks include fatty acids and complex lipids for membrane synthesis, nucleotides for DNA and/or

Key Figure

Reciprocal Interactions between Human Stromal Adipocytes and Breast Cancer Cells

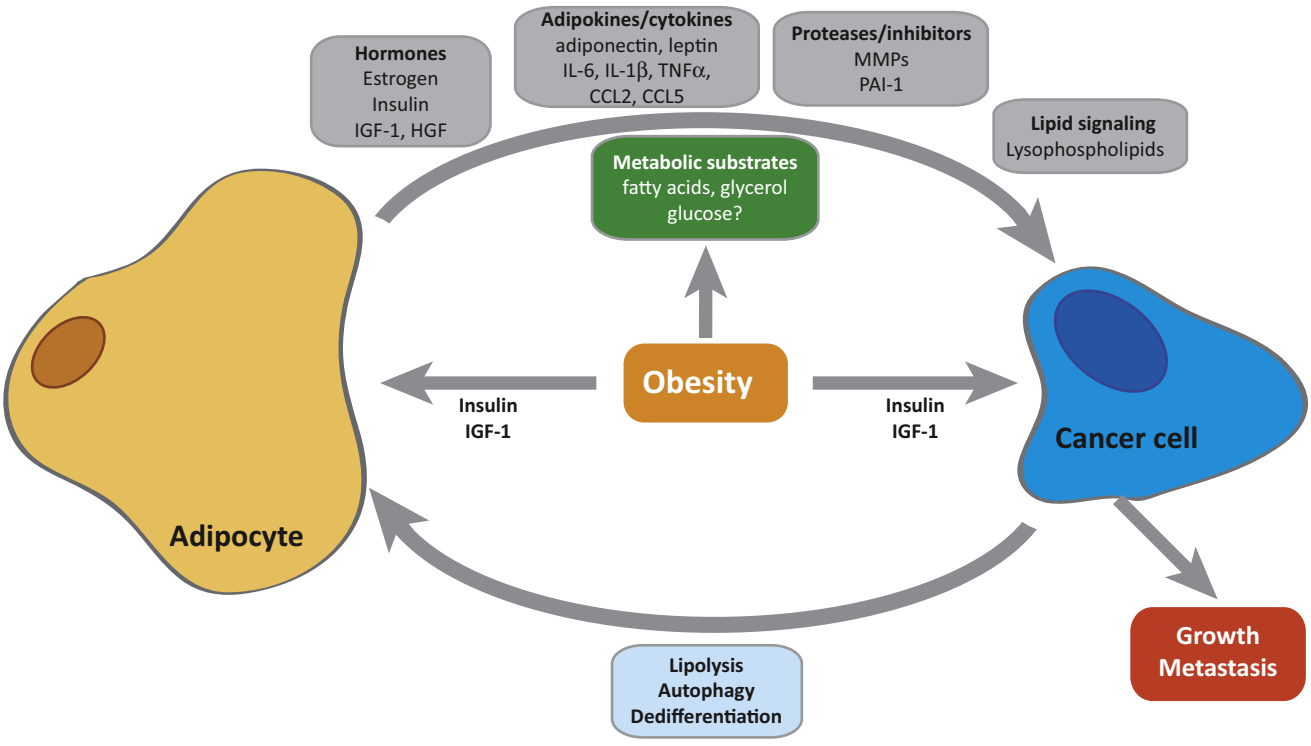

Figure 1. Within the breast cancer microenvironment, tumor cells and adipocytes are in close proximity and can exert a variety of reciprocal effects on each other. Breast cancer cells induce the production of endocrine and paracrine signaling mediators, proteolytic enzymes, and bioactive lipids, along with metabolic substrates by adipocytes. These in turn drive increased growth and invasion of tumor cells along with therapeutic resistance. Obesity leads to the increased production of some signaling factors, such as hormones and adipokines and/or cytokines, and increased availability of metabolic substrates, accentuating in turn, cancer cell growth and metastasis. Abbreviations: CCL2, C-C motif chemokine ligand 2; CCL5, C-C motif chemokine ligand 5; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; IL-1β, interleukin 1β; IL-6, interleukin 6; MMP, matrix metalloproteinases; PAI-1, plasminogen activator inhibitor-1; TNFα, tumor necrosis factor α.

RNA synthesis, and amino acids for protein synthesis. Given that most BC cells adopt a **'Warburg'-like metabolism** (i.e., aerobic glycolysis [35]), and exhibit overexpression of key regulators of *de novo* **lipogenesis**, such as **fatty acid synthase** (FASN) and **acetyl-CoA carboxylase** (ACC) [36,37], much attention has focused on the contribution of newly synthesized fatty acids generated from glucose and glutamine in metabolic reprogramming [38].

Until recently, the specific metabolic characteristics of adipocyte biology that can influence BC cell behavior were largely overlooked, with only a few studies addressing the topic. Most recently, mouse 3T3-L1 and human primary adipocytes were shown to alter MCF-7 and MDA-MB-231 BC cell substrate metabolism, as evidenced by increased fatty acid storage and oxidation, increased conversion of glucose into lipid, as well as oxidation of glucose and glutamine [8]. Similarly, another recent study further elucidated the metabolic link between adipocytes and breast cancer cells. Specifically, co-culturing ZR-75-1 BC cells with mouse 3T3-F442A adipocytes increased TAG content and fatty acid oxidation due to increased mitochondrial DNA levels and protein levels of the rate limiting enzyme in fatty acid oxidation carnitine palmitoyltransferase 1 in ZR-75-1 BC cells [82]. This adipocyte-induced metabolic reprogramming resulted in increased TAG content of BC cells, accumulating as lipid droplets [8]. Similarly, lipid accumulation was observed in MDA-MB-231 and T47D BC cells following co-culture with primary human **omental adipocytes** [39]. This lipid accumulation in BC cells is a direct downstream consequence of BC cell-stimulated **HSL/hormone-sensitive lipase**/**adipose triglyceride lipase** (HSL/ATGL)-mediated lipolysis of **TAG,** release of fatty acids by adipocytes, and subsequent transfer to adjacent BC cells [8].

Alongside these metabolic interactions, lysophospholipids secreted by mouse mammary adipose tissue were shown to promote the proliferation of mouse mammary tumor cells: Pharmacological and genetic inhibition of the G-protein-coupled lysophosphatidic acid receptor attenuated this lysophospholipid-stimulated proliferation [40], demonstrating an additional lipid-specific mechanism that underpins the influence of adipocytes on BC cell biology. Consequently, it appears that the mechanisms underpinning adipocyte-induced changes in BC cell behavior may include both paracrine signaling effects and the direct provision of metabolic substrates. However, further validation in *in vivo* models is required to firmly establish a definitive lipid-dependent signaling mechanism in BC. Notably, a recent study showed that inhibition of the fatty acid transporter CD36 impaired the metastasis of human oral squamous carcinoma cells in a xenograft mouse model [41].

In vivo studies on a variety of other cancer types, including pancreatic, prostate, ovarian, oral, and melanoma, support the importance of tumor–adipocyte interactions for tumor growth and metastasis more broadly, through provision of metabolic fuel and/or signaling mediators to drive the proliferation and invasion of tumor cells [39,41–48]. While many of these studies focus on the role of adipocytes in modulating cancer cell behavior (including proliferation, migration, and invasion), few have demonstrated reciprocal effects between adipocytes and tumor cells [39]. Beyond mechanistic insight into tumor biology, these studies also highlight the potential for the characterization of tumor–adipocyte interactions to provide not only a better understanding of drug resistance, but also avenues for novel therapeutic development [41,49,50].

Breast Cancer Cells Alter Adipocyte Secretory Profiles and Lipid Stores

While adipocytes can clearly modify BC cell behavior (discussed in detail above), reciprocal effects of BC cells on adipocyte behavior have also been observed. These include modified expression and secretion of paracrine signaling factors, proteases, and inhibitors, as well as delipidation and release of fatty acids (discussed in detail below).

Indeed, as with other cell types that constitute the tumor microenvironment, cancer-associated adipocytes exhibit a significantly altered biological profile that can enhance and support tumor progression. For example, one study reported that human preadipocytes in the stromal vascular fraction (SVF) collected from mammary fat immediately adjacent to malignant breast tumors had reduced differentiation capacity and lower lipid droplet formation compared with preadipocytes adjacent to benign lesions [51]. This demonstrated that tumor cells were able to exert significant effects on adjacent adipocytes in patients with BC. Conversely, in *ex vivo* **organotypic culture models**, BC cell lines (MCF-7, MDA-MB-231) led to a stronger effect on stimulating preadipocyte differentiation than did immortalized mammary epithelial cells (MCF-10A) [23].

Most studies in the field of tumor cell–adipocyte crosstalk have focused on changes in adipocyte gene expression and paracrine signaling factor secretion induced by tumor cells. These have documented increased gene expression of a range of adipokines and adipocytokines, such as *TNF*, *IL6*, *IL1B*, and other members of the interleukin family. Genes encoding CCL2 and CCL5 as well as LEP, along with those encoding proteases and inhibitors, including *MMP11* and *SERPINE1*, have also been shown to be upregulated [5,51–53]. By contrast, decreased adiponectin gene expression has been observed in human mammary fat [51]. Importantly, some of these changes at the gene expression level are reflected in the altered protein secretion of IL-6, IL-1β, and other members of the IL family, along with CCL2 and CCL5 [5,52].

Alongside changes in gene expression and secretory profiles, a limited number of studies have shown that BC cells exert significant effects on lipid storage and mobilization by adipocytes. Adipocytes in close proximity to a tumor in primary human samples are smaller compared with adipocytes that are distal to the tumor [5,51,53,54], suggesting that there are reduced triacylglycerol (**TAG**) stores in proximal adipocytes. Furthermore, co-culture and conditioned media models have been used to show that human BC cells reduce adipocyte TAG stores [5,8,51,55]. For instance, as discussed above, both MCF-7 and MDA-MB-231 BC cells stimulate the release of fatty acids by mouse 3T3-L1 adipocytes in co-culture, and these fatty acids are transferred to BC cells for storage and mitochondrial oxidation, as shown using radiometric approaches [8]. This effect was shown to be partially reduced by small interfering (si)RNA-mediated knockdown of the TAG lipases ATGL and HSL in adipocytes [8].

Intriguingly, cancer cell-induced adipocyte delipidation is associated with reduced gene expression of markers of adipocyte differentiation in human tissues, including *LIPE* and *FABP4* [5,51]. This is somewhat counterintuitive, because a reduction in the proteins encoded by these genes (HSL and FABP4, respectively) would be expected to reduce adipocyte lipolysis (Box 2). Hence, cancer cells may regulate delipidation via mechanisms that are independent of altered expression of lipolytic genes. Future studies may help elucidate the relationship between gene expression and metabolic flux and how this is affected by BC cells.

Further underlining the reciprocal nature of the interaction between cancer cells and adipocytes, the altered metabolic and paracrine microenvironment in BC not only drives proproliferative signaling (see above), but in some instances, also stimulates increased substrate uptake by cancer cells. However, the underlying regulatory and transport mechanisms are not well described in cancer cells. In other tissues, insulin-stimulated glucose and fatty acid uptake into mouse and human skeletal muscle and adipocytes is PI3K dependent [56,57]. Similarly, other adipokines, including IL-6 [58], TNFα [59], and leptin [60], increase glucose uptake in murine and human skeletal muscle, whereas the effects on fatty acid uptake are not clear. Even though the mechanisms are yet to be deciphered, it is clear that BC cells exert distinct effects on adipocyte behavior that are likely relevant to promoting disease progression. Consequently,

Box 2. Adipocyte Lipolysis

The release of stored fatty acids from adipocytes is termed 'lipolysis', a highly regulated process involving protein phosphorylation, protein–protein interactions, and protein translocation (Figure 2, main text) [80,81]. Alongside the proteins discussed below, lipid droplets are coated with a large number of proteins whose roles in lipid droplet biology are yet to be fully defined. Known lipid droplet proteins include members of the perilipin family, such as PLIN 2 (ADRP, ADFP, and adipophilin), PLIN3 (TIP47) and PLIN4 (S3-12), as well as cell death-inducing DFFA-like effector A (Cidea) and Cidec (FSP27 and FPLD5).

Basal State

Adipose triacylglycerol lipase (ATGL) is located both at the lipid droplet and cytosol. It is complexed with its negative regulator G0S2, while its positive regulator CGI-58 is complexed with PLIN1 to result in low ATGL activity. HSL is predominantly located in the cytosol and collectively this results in the minimal release of fatty acids and glycerol [81].

Stimulated State

Increased lipolytic flux is mediated by PKA-dependent, as well as PKG, signaling. ATGL, HSL, PLIN1, and CGI-58 are all phosphorylated by PKA to alter the enzymatic activity of ATGL and HSL as well as protein–protein interactions and protein translocation. Upon phosphorylation of PLIN1, CGI-58 is released and interacts with ATGL, while G0S2 disassociates, leading to increased ATGL activity [81]. At the same time, PKA phosphorylates HSL on multiple sites, leading to translocation to the lipid droplet and association with phosphorylated PLIN1, resulting in increased HSL activity. The fatty acid chaperone FABP4 also interacts with phosphorylated HSL, and through mechanisms not well defined, regulates lipolysis [81].

understanding these crosstalk mechanisms may indeed provide a basis for new therapeutic strategies.

Are Adipocytes Mediators of the Obesity–Breast Cancer Link?

Much of the rationale for investigating the role of adipocytes in promoting BC progression is linked to epidemiological evidence demonstrating that survival is lower in obese women, independent of menopausal status [61]. A key question is whether the influence of adipocytes on BC behavior is altered in obesity compared with a lean body composition. Could the BC cell–adipocyte link help explain some of the epidemiological evidence?

As discussed above, there are clear reciprocal interactions between adipocytes and BC cells, yet most of these studies have not considered the obesity context. This is a key point, because there are significant differences in adipocyte biology between lean and obese individuals, including altered secretome profiles, adipocyte size, and insulin sensitivity [62]. The experimental design of most *in vitro* studies using co-culture or conditioned media have not considered the influence of obesity on either adipocyte or cancer cell behavior. As such, we have little understanding of the potential cellular mechanisms driving altered cancer progression in obesity.

A limited number of studies have directly focused on the differential effects of lean and obese adipocytes on cancer cell behavior. One laboratory reported that conditioned media generated from adipocytes isolated from obese women (body mass index 30–35 kg/m^2) increased MCF-7 proliferation compared with lean donors [19]. A more recent study showed that xenografts of estrogen-dependent **E0771 cells** in the mammary fat pad of mice fed a high-fat diet [diet-induced obesity (DIO) and hyperinsulinemia model] produced an increased tumor volume compared with animals fed normal chow [21]. Others have demonstrated that the stromal vascular fraction taken from the abdominal subcutaneous adipose bed of obese women increased MFC-7 and MDA-MB-231 BC cell proliferation *in vitro,* as well as MCF-7 tumorigenicity in SCID mice [29]. Conversely, others have shown that conditioned media from adipose tissue isolated from male rats could delay cell cycle progression of MCF-7 cells, but no effect

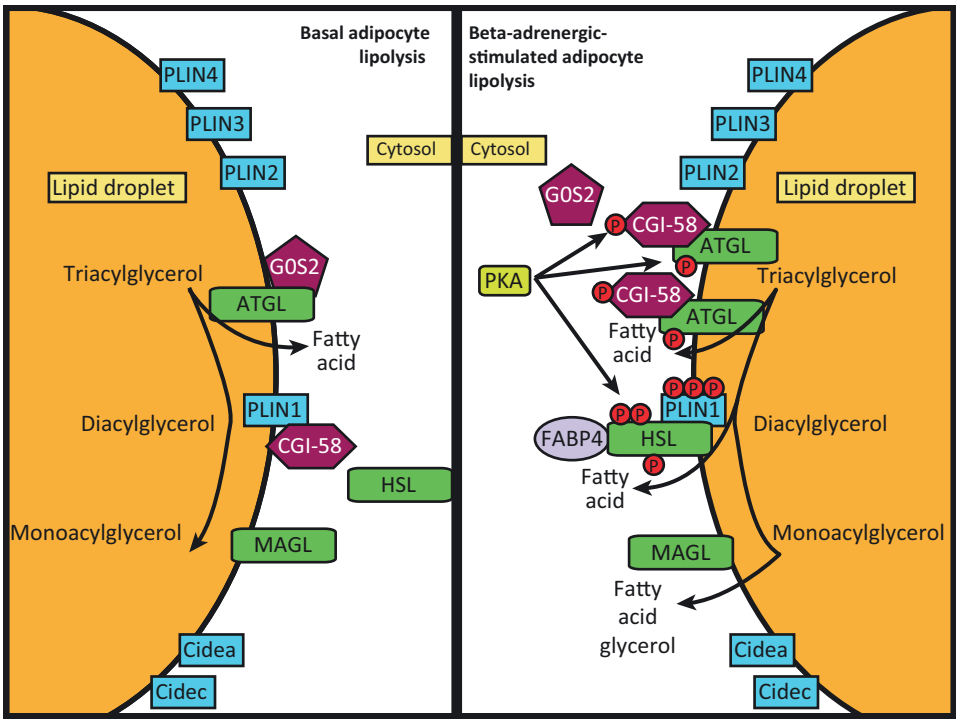

Figure 2. Adipocyte Lipolysis. The release of fatty acids and glycerol from triacylglycerols stored in intracellular lipid droplets of adipocytes is regulated by a network of protein interactions and post-translational modifications. Proteins in green are lipases, those in purple are co-activators and co-repressors, and those in blue are lipid droplet proteins that have no enzymatic activity, Abbreviations: ATGL, adipose triglyceride lipase; Cidea, cell death-inducing DFFA-like effector A; Cidec, cell death-inducing DFFA-like effector C; FABP4, fatty acid-binding protein 4; G0-S2, G0/G1 switch 2; HSL, hormone-sensitive lipase; MAGL, monacylglyerol lipase; P, phosphorylation; PKA, protein kinase A; PLIN1, perilipin 1; PLIN2, perilipin 2; PLIN3, perilipin 3; PLIN4, perilipin 4.

was observed in DIO mice [18]. These two studies indicate obesity-specific effects on BC cell proliferation. However, there are few mechanistic studies that directly assess the differential effects of lean and obese environments and, consequently, the adipocyte-specific mechanisms that might explain the epidemiological and clinical observations of altered BC outcomes in obese women remain to be elucidated.

More recently, MDA-MB-231 cells in co-culture with obese adipocytes displayed increased growth and migration properties relative to the less pronounced effects observed with lean adipocyte co-cultures [8]. Specifically, a greater transfer of adipocyte-derived radiolabeled fatty acids to MDA-MB-231 cells occurred under obese conditions relative to lean adipocytes; this, in turn, resulted in elevated TAG synthesis and mitochondrial fatty acid oxidation in MDA-MB-231 cells [8]. However, no increase in mitochondrial fatty acid oxidation and/or growth and migration were observed in MCF-7 cells under obese conditions, despite increased transfer of adipocyte-derived fatty acids. Hence, individual cancer cell lines displayed a heterogeneous response to altered availability of adipocyte-derived fatty acids in the context of obesity. Unlike MDA-MB-231 cells, MCF-7 cells are estrogen responsive, supporting the concept that the endocrine context is an important consideration in understanding the effect of obesity on BC [63]. To our knowledge, this is the first demonstration of adipocyte-specific mechanisms driving BC cell metabolic changes through the provision of key substrates needed for energy, growth, and perhaps even migration. Furthermore, a huge body of literature has defined a clear role for insulin and IGF-1 signaling in adipocyte and cancer cell biology [64], which has important

Box 3. Clinicians Corner

Tumor behavior is influenced by the microenvironment, in particular the metabolic interaction with local and/or stromal adipocytes.

Reciprocal interactions between BC and adipocytes could influence current prognostic and diagnostic understanding.

Better understanding of the mechanisms of metabolic interactions between adipocytes and breast tumors may identify new biomarkers and potential druggable targets.

The influence of the metabolic environment at both a micro- and whole body-level dictates consideration of the metabolic health of patients in determining therapeutic strategies.

Outstanding Questions

Do opportunities exist to reposition drugs targeting lipid metabolic pathways either as adjuvant or stand-alone treatments in a precision medicine path?

How does the culture environment affect the lipidomic and metabolomic profiling of cancer model systems and tumors?

Do the observations made in cell lines and xenografts hold true in other preclinical models, such as patient-derived explants, patient-derived xenografts, cell lines, or organoids?

What are the factor(s) secreted by cancer cells that mediate cell-to-cell crosstalk, in particular with stromal adipocytes?

Does cancer cell–adipocyte crosstalk explain the observed epidemiological and clinical data in obese patients?

What are the precise mechanistic pathways that govern these interactions in lean and obese environments, and in what way do these pathways promote tumorigenesis?

relevance in the establishment of a hyperinsulinemic state commonly associated with obesity (see below). Obesity-associated hyperinsulinemia is one mechanism proposed to explain the epidemiological observations of increased BC incidence in obese women (reviewed in [65]). However, there are limited mechanistic molecular studies that have directly investigated the effects of obesity on tumorigenesis. Therefore, future studies should aim to elucidate the complex interplay between macro- and micro-metabolic and endocrine environments (a defining characteristic of the obese phenotype) with tumorigenesis and BC progression.

Obesity as a Driver of Therapeutic Resistance

The increasing incidence of obesity is creating significant challenges for the clinical management of BC, particularly because obese women display a reduced response and increased resistance to chemotherapy [66,67]. This effect has been replicated in the laboratory, with the development of **trastuzumab** resistance in **human epidermal growth factor receptor 2** (HER2)-overexpressing TNBC **BT474 cells** when cultured with human adipocytes [68]. Similarly, adipocytes derived from human multipotent adipose-derived stem cells were shown to inhibit trastuzumab-mediated cellular cytotoxicity in a range of BC cell lines (BT-474, MDA-MB-453) in culture [69]. Increased IGF-1R expression has been proposed as one mechanism underlying acquired trastuzumab resistance (reviewed in [70]). Therefore, it is possible that an increased release of IGF-1 from obese adipocytes [19] is also relevant in the context of trastuzumab resistance.

Obesity has also been implicated in resistance to chemotherapy and radiotherapy in BC. For example, in the presence of sera from obese patients with BC, the sensitivity of MDA-MB-231 cells to **docetaxel** was reduced *in vitro* [71]. Notably, this study did not directly demonstrate adipocyte-specific effects, leaving the possibility that other circulating mediators influenced this effect. In another study, co-culture of **SUM159PT** cells with **3T3F442A adipocytes** conferred radioresistance compared with cells cultured in isolation [72].

While obesity might have a role in therapeutic resistance, the underlying mechanisms are not well defined. Both systemic and paracrine signaling pathways have been implicated, including inflammatory and growth factor signaling, both of which are altered in various ways in obesity [73,74]. Better understanding these mechanisms may lead to the development of adjuvant therapies targeting metabolic pathways for a more effective and durable patient response to existing therapies. This is especially pressing, given the rapidly changing metabolic profile of the community.

Concluding Remarks

Here, we have collated the current and emerging evidence supporting our hypothesis that the metabolic microenvironment of BC is an important driver of disease progression and may

represent a potential target for the development of new therapies. Advancing our understanding of disease mechanisms from both tumor cell and adipocyte perspectives (see Outstanding Questions and Box 3), in particular in the obese setting, has clear clinical relevance. Obesity has the potential to diminish important advances that have been made in efforts to treat cancer because it influences cancer incidence, progression, and treatment efficacy.

Generating new knowledge about the molecular links between adipocytes and cancer cells may enable the development and implementation of novel therapies in the long term using either novel compounds or repurposing existing drugs aimed at specific pathways in these cells that have been developed in other disease contexts. These agents may directly target adipocyte and/or cancer cells as stand-alone therapies or be used in an adjuvant setting for achieving maximum efficacy of existing treatments. Concurrent development of novel metabolic biomarkers of prognosis and treatment response will be equally important. Furthermore, more immediate lifestyle interventions targeting obesity (diet and exercise) may represent simple and effective strategies for improved outcomes.

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