Keeping A Breast of Recent Developments in Cancer Metabolism

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Abstract: For decades, it has been recognized that cancer cells display a unique metabolism; specifically, cancer cells have been shown to preferentially utilize glycolysis instead of mitochondrial respiration. This phenomenon is commonly known as the “Warburg effect” after Otto Warburg who first made this observation in 1927. The discovery of the Warburg effect has lead to new methods of detection and differentiation of cancerous tissue and normal tissue. More recently, alterations in cancer metabolism have been researched as a possible target for chemotherapeutic intervention in a number of cancers. The push to understand the metabolism of cancer cells has been particularly acute in breast cancer cells, where multiple novel metabolic mechanisms have recently been described and characterized. However, despite this recent progress, the completion of additional studies on the cellular metabolism of breast cancer cells is necessary before drugs that target cancer cell metabolism could be available to disease-afflicted women. Here, we review recent discoveries in breast cancer cell metabolism as well as current logical drug targets that could be used to alter cell metabolism to promote the selective elimination of breast cancer cells.

Keywords: Breast cancer, cell metabolism, cell death, glucose metabolism, hypoxia, Warburg effect, autophagy, cancer metabolism.

INTRODUCTION

In the last quarter century, normal cells have been demonstrated to have the capacity to develop numerous epigenetic and genetic changes that can eventually result in the formation of tumors and the development of cancer. For the last decade, six functional characteristics, or hallmarks, have been widely accepted to characterize tumorigenic cells: 1) self-sufficiency in growth signals, 2) insensitivity to anti-growth signals, 3) evading apoptosis, 4) limitless replicative potential, 5) sustained angiogenesis, and 6) ability to invade tissue and metastasis [1].

However, the recent decade has also seen our understanding of the basic biological mechanisms that control cancer cells improve dramatically. A specific alteration seen in the vast majority of cancers has recently reappeared in the spotlight after over 40 years in purgatory—the fact that cancer cells have very unique metabolism. Otto Warburg first made the observation that cancer cells rely heavily on glycolysis in 1927 [2]. At the time, he believed that cancer cells preferentially utilized glycolysis due to a defect in mitochondrial respiration [3, 4]. While current evidence suggests that defective mitochondrial metabolism does not cause what is now known as the “Warburg effect”, interest in this phenomenon has taken off in recent years. Studies aimed at understanding the molecular mechanisms responsible for these metabolic changes and the potential advantages for cancer cells to utilize such a strategy are now appearing with increasing frequency. In the clinic, this striking metabolic phenomenon is now frequently used to detect and differentiate tumor cells from normal cells. This is done using F18-fluorodeoxyglucose positron emission tomography (PET) which is an indicator of glucose consumption and can easily differentiate normal tissue from cancerous tissue [5].

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Work done by Craig Thompson’s laboratory has really driven this resurrection of interest in cancer metabolism. The recent discoveries by the Thompson laboratory include the discovery that the inhibition of ATP citrate lyase (ACL), a protein involved in the conversion of citrate to acetyl-CoA and in the regeneration of oxaloacetate, can suppress the growth of tumors [6]. In addition, another paper from Thompson’s group has revealed that ACL is involved in the stimulation of histone acetylation, thus linking glucose metabolism to transcription and gene expression [7]. Other important findings from the Thompson group include the recent discoveries of the functions of isocitrate dehydrogenase 1 (IDH1)/isocitrate dehydrogenase 2 (IDH2) genes in brain tumors and acute myelogenous leukemia (AML) [8,9].

As a result of Thompson and other like-minded investigators, breast cancer researchers have also turned their attention to understanding cancer metabolism. The last decade has also seen a substantial increase in our understanding of metabolic alterations in breast cancer. As is the case with a number of other tumors, breast cancer cells have a high rate of glycolysis as well as an increased rate of glucose uptake and glucose phosphorylation even in the presence of abundant oxygen [10]. A plethora of recent papers examining this phenomenon in breast cancer have revealed much about the molecular mechanisms involved in this metabolic change and it is these discoveries that will be the focus of this review.

Going back to the hallmarks of cancer, given that this switch in metabolism is being used as an indicator of cancerous cells in the lab as well as in the clinic, alterations in metabolism could represent the seventh hallmark of cancer [11]. While much still needs to be understood regarding the mechanisms that differentiate malignant and normal cell metabolism, the advances in our understanding of this topic over the last few years have been remarkable. In this review, we will discuss these recent discoveries involving glucose metabolism, p53, cell death, autophagy, and hypoxia in breast cancer cells as well as logical drug targets that

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could possibly be used to treat cancer by altering cellular metabolism. By no means will this review be completely comprehensive, as the number of papers revealing key information about breast cancer cell metabolism are too numerous for the space we have available. However, we will examine what we believe constitute some of the most substantial advances in our understanding of breast cancer metabolism and how these mechanisms have revealed potential drug targets.

As described above, the interest in understanding the cellular metabolism of breast cancer cells has increased substantially over the past few years. In fact, a recent systematic examination of genomic aberrations in human breast cancer samples revealed 66 genes that were highly amplified and correlated with a poor prognosis [12]. Of these 66 genes, only 9 are considered to be druggable based on presence of a predicted protein structure that would interact with drug like compounds. One of these genes was found to be ACACA which encodes the protein Acetyl-CoA carboxylase (ACC), a protein that functions downstream of Akt to control the rate-limiting step in fatty acid synthesis [12]. These results suggest that breast cancer cells rely on fatty acid synthesis for some aspect of their growth and that inhibiting this function may be beneficial and more importantly, be practical. This recent paper is just one of the many alterations in cellular metabolism recently discovered to impact breast cancer, and we will detail many of these other significant findings in the sections below.

**Estradiol Stimulation**

Currently, one of the most common breast cancer therapies is the treatment with selective estrogen receptor modulators (SERMs), which act to suppress endogenous estrogen stimulation of breast cancer cell proliferation [13]. It is known that estrogen stimulates breast cancer cell proliferation; however, the exact mechanisms by which estrogen stimulates proliferation and increases intracellular metabolic activity in breast cancer cells are unknown [14,15]. SERMs have been found to be successful in treating and preventing breast cancer [13,16]. However, patients displaying estrogen receptor negative (ER-negative) or SERM-resistant tumors cannot be successfully treated with SERMs [17-19]. Therefore, gaining a better understanding of metabolism in breast cancer cells could result in discovering other drug targets available for treatment of ER-negative and SERM-resistant tumors [13, 20].

Recently, the metabolic mechanisms of estradiol-stimulated breast cancer cell growth have been examined. Interestingly, estradiol treatments significantly increase the consumption of glucose and glutamine. Glutamine consumption is approximately 30% of glucose consumption, which suggests that glutamine is an important nutrient for ER-positive breast cancer cells. In addition, treatment of MCF-7 cells with estradiol increases the production of lactate as a result of direct stimulation of glycolysis [19].

In addition to stimulating glycolysis, estradiol treatments cause a significant increase in flux through the pentose phosphate pathway (PPP) as well as pyruvate carboxylase in MCF-7 cells. The flux through these pathways contributes to the increase in uptake of glucose and glutamine as well as the increase in production of lactate [21]. This increase in flux through the pentose phosphate pathway leads to enhanced levels of nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH) in breast cancer cells, thus only small amounts of malate and NADH are transferred into the mitochondrion. The larger amounts of NADH in the cytosol result in an increase of the cytosolic NADH/NAD+ ratio, which causes lactate dehydrogenase to produce more lactate even in the presence of high levels of oxygen, a phenomenon referred to as aerobic glycolysis [21]. Instead of being oxidized within the mitochondrion, the excess glutamine is predominantly used for biosynthesis, a crucial mechanism in survival of breast cancer cells [22-24].

Thus, it is clear that estradiol has multiple effects on the metabolic mechanisms of MCF-7 breast cancer cells. Potential candidates for targeting the selective elimination of ER positive breast cancer cells have been suggested. For example, Glucose-6-phosphate dehydrogenase (G6PDH) represents a potential target for chemotherapeutic intervention. G6PDH is involved in directing glucose into the pentose phosphate pathway to produce NADPH and ribose, which are both necessary for biosynthesis. Disrupting flux through the PPP in some capacity could result in the disruption of biosynthesis that is necessary for proliferation in ER positive cells. In addition, the aspartate/glutamate antiporter could be a potential candidate for the design of novel therapeutics. The aspartate/glutamate antiporter allows the mitochondria to convert glutamate into aspartate, an essential biosynthetic precursor [21], and its inhibition could possibly disrupt important biosynthesis mechanisms that affect the viability of breast cancer cells.

**GLUCOSE METABOLISM**

In normal epithelial cells exposed to physiological levels of oxygen, production of ATP from glucose occurs through glycolysis and oxidative phosphorylation [25]. However, tumor cells have been found to specifically utilize glycolysis in the presence of abundant oxygen and in the absence of discernable oxidative phosphorylation resulting in the production of large amounts of lactic acid. This phenomenon in tumor cells is known as the Warburg effect. It has also been found that this high rate of conversion of glucose to lactate is coupled with an increased rate of glucose uptake when oxygen is present. Oncogenes such as Ras, Src, and ErbB2 have been found to facilitate these changes in glucose metabolism by stabilizing HIF-1α, which subsequently results in the upregulation of enzymes involved in the glycolytic pathway. One of these enzymes is lactate dehydrogenase (LDH-A), which is responsible for the conversion of pyruvate to lactate at the end of glycolysis [26,27]. LDH-A can also be regulated by oncogenes as the transcription of LDH-A has been shown to be regulated by c-Myc [28].

**LDH-A**

Recently, much progress has been made in understanding the molecular mechanisms underlying the switch to aerobic glycolysis in breast tumors. A landmark paper from Philip Leder’s group revealed that LDH-A levels can regulate the tumorigenicity of mammary epithelial cells by enhancing
glycolytic metabolism [10]. In hypoxic conditions, tumor cells that have increased levels of LDH-A protein show higher proliferation and ATP production than those with decreased LDH-A protein.

Therefore, an LDH-A deficiency can dramatically alter the mitochondrial activity in breast cancer cells. Using a lipophilic cation (F16) that can localize to the mitochondria [29], LDH-A positive tumor cells accumulated F16 in their mitochondrial compartment. In contrast, cells derived from LDH-A deficient mice did not accumulate F16 in their mitochondrial compartment. The expression of LDH-A in these LDH-A deficient cells allowed these cells to restore F16 accumulation. However, these alterations in F16 accumulation were not the result of differences in mitochondrial morphology or volume density between LDH-A deficient and wild type tumor cells [10].

In further examining the effects of LDH-A on mitochondrial respiration, the effects of LDH-A withdrawal on coupled and uncoupled respiration were examined. Coupled respiration is the fraction of mitochondrial respiration used for ATP production, whereas uncoupled respiration is the fraction of mitochondrial respiration used to drive protons across the biological membrane, often referred to as “proton leak”. In the presence of LDH-A, a decrease in total ATP turnover and proton leak result in a decrease in total mitochondrial respiration. Thus, this suggests that high levels of LDH-A could promote aerobic glycolysis at the expense of mitochondrial respiration. To examine the functional consequences of LDH-A-mediated metabolic alterations on the formation of breast tumors, LDH-A positive and negative cells were injected into the mammary fat pad. Tumors derived from LDH-A negative cells showed a diminished tumor volume and retarded tumor cell proliferation; whereas tumors derived from LDH-A positive cells had larger tumor volumes and enhanced proliferation. In addition, LDH-A negative tumor cells produced tumors that had a limited ability to endure the tumor microenvironment and could not metastasize or grow beyond the initial lesions [10].

Therefore, LDH-A can dramatically alter the method by which breast tumor cells metabolize glucose and mitochondrial respiration (while reduced) is still functional in mammary tumor cells. Furthermore LDH-A expression can promote aerobic glycolysis and facilitate the formation of tumors that are larger and more aggressive. These data dramatically enhance our knowledge of how breast tumor cells can alter their consumption of glucose to facilitate tumor progression [10].

SGLT1

In addition to the discovery that LDH-A expression can promote the Warburg effect in breast cancer cells, additional breakthroughs in the understanding of breast cancer cell metabolism have been made through the investigation of the molecular mechanisms underlying enhanced glucose uptake in tumor cells. Recently, using breast cancer and other cell lines, it was discovered that the epidermal growth factor receptor (EGFR) can prevent cancer cells from undergoing autophagic cell death by maintaining basal intracellular glucose levels. Cancer cells were susceptible to death induced by siRNA-mediated knockdown of EGFR, but were unaffected when the kinase activity of EGFR was inhibited. These findings suggested the presence of a novel kinase-independent mechanism of EGFR that could promote tumor cell viability. Contrary to what one would predict, EGFR downregulation caused an increase in phospho-Akt and phospho-ERK levels and resulted in no activation of caspase-3 or caspase-9, suggesting that this cell death was not due to the classical apoptosis program. However, these EGFR knockdown cells contained autophagosomes in the cytoplasm, further suggesting that cell death in EGFR deficient cells was not due to apoptosis, but rather autophagy. The reintroduction of wild-type EGFR could successfully save tumor cells from autophagic death [30].

The knockdown of EGFR in MDA-MB-231 breast cancer cells also resulted in a marked decrease in intracellular glucose levels. When additional glucose was supplemented to rescue the lower glucose in EGFR knockdown cells, both cell death and autophagy were inhibited. This supplementation with high levels of glucose also caused a decrease in phosphorylated Akt and phosphorylated ERK suggesting that phosphorylation of these two kinases could be a stress response to the lowering intracellular levels of glucose [30].

When examining the molecular mechanisms underlying the loss of intracellular glucose in EGFR knockdown cells, Hung’s group found a striking role for the protein sodium/glucose cotransporter 1 (SGLT1). SGLT1 is known to translocate from the intracellular compartments to the cell membrane, and it is responsible for transporting glucose along a glucose gradient in response to stress or other stimuli [31]. Interestingly, Weihua et al. demonstrate that SGLT1 levels are downregulated when EGFR is knocked down. In addition, knockdown of SGLT1 on its own resulted in the presence of abundant autophagosomes which closely mirrors that of the EGFR knockdown cells. Subsequently, it was discovered that EGFR directly interacts with and stabilizes SGLT1 independent of its kinase activity to promote glucose uptake and tumor cell survival. The significance of this stabilization was demonstrated by the data that reveals that EGFR/SGLT1 expressing cells were able to survive in lower levels of glucose, suggesting that EGFR and SGLT1 are crucial for maintaining necessary intracellular glucose levels in tumor cells for survival. These results dovetail nicely with the presence of the Warburg effect in tumor cells and reveal a novel mechanism (EGFR stabilization of SGLT1) that could be targeted to induce cell death in tumors by impeding their access to glucose [30].

p53 AND TIGAR

The multiple functions of the p53 tumor-suppressor protein strongly imply a role for p53 in breast cancer metabolism. p53 has a widely demonstrated importance for the cell stress response and inhibiting malignant cellular behavior [32] and has been demonstrated to be involved in apoptosis, cell-cycle arrest, senescence, differentiation, and repair of damaged DNA. [33,34]. p53 can also be induced under hypoxic conditions and is stabilized by hypoxia inducible factor 1α (HIF-1α), which is known to stimulate transcription of several other genes involved in hypoxia [35]. p53 activates multiple different groups of target genes to induce cell cycle
arrest and allow DNA damage to be resolved without activating apoptotic target genes [36]. Furthermore, p53 activates numerous genes that increase reactive oxygen species (ROS), thereby contributing to apoptosis and feedback signaling for additional activation of p53 [37,41]. However, p53 can also induce proteins that lower ROS levels, which is crucial for the prevention of DNA damage and tumor development [42].

Using breast cancer cells and a variety of other cell types, Karen Vousden's group has recently discovered the protein TIGAR (TP53-induced glycolysis and apoptosis regulator), which is a novel p53-inducible protein that regulates glycolysis and oxidative stress (See Fig. 1) [43]. The TIGAR gene is located on chromosome 12p13-3 and contains 6 potential coding exons as well as two binding sites to properly bind p53. The TIGAR protein is approximately 30 kDa and was found to have a conserved amino acid sequence throughout vertebrate species. Vousden's group determined that induction of p53 results in a corresponding induction of TIGAR; however, TIGAR expression can also be regulated by other p53-independent mechanisms. In line with the dual role for p53 in cell cycle and apoptosis control, Vousden's group noticed that lower levels of stress that activate p53 could also adequately induce TIGAR; whereas higher stress levels elevated the expression of PIG3, an apoptotic protein, while decreasing the expression of TIGAR. This evidence suggests that TIGAR may be important in the switch in p53 response from cell cycle arrest and apoptosis [43].

Further investigation revealed a striking role for TIGAR in metabolism. TIGAR was found to share a similar bisphosphatase domain with 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) suggesting that TIGAR may act as a fructose bisphosphatase. This mode of regulation involves both the synthesis and the degradation of intracellular fructose-2, 6-bisphosphate which is a positive allosteric effector of 6-phosphofructo-1-kinase (PFK-1) and fructose-1,6-bisphosphatase (FBPase-1). PFK-1 stimulates glycolysis whereas FBPase-1 is a regulator of gluconeogenesis [44]. Given that TIGAR is induced by p53, this type of regulation would intimately link TIGAR and p53 function with glucose metabolism.

Indeed, in the absence of TIGAR expression, there was an increase in fructose-2,6-bisphosphate levels; whereas there was a decrease in fructose-2,6-bisphosphate in presence of TIGAR. The knock down of TIGAR expression resulted in a substantial increase in glycolytic rates. Conversely, decreased overexpression of TIGAR decreased glycolytic rates and resulted in subsequent apoptosis. Overexpression of solely the FBPase-2 domain was shown to result in the inhibition of PFK-1 causing an increased amount of fructose-6-phosphate resulting in the isomerization of fructose-6-phosphate to glucose-6-phosphate.

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**Fig. (1).** Functions of TIGAR within the cell. TIGAR is a p53-inducible protein that is involved in glycolysis regulation, protection from oxidative stress, and decreasing sensitivity to p53 and other apoptotic signals.
Accumulated glucose-6-phosphate is subsequently diverted into the pentose phosphate pathway which will produce NADPH and cause a reduction in oxidative stress. This decrease in ROS led to a dramatic inhibition in apoptosis. Using specific inhibitors that target the pentose phosphate pathway, Vousden’s group demonstrated that flux through the pentose phosphate pathway is necessary for the anti-apoptotic activity of TIGAR. Vousden’s group has further characterized the ability of TIGAR to modulate ROS levels through the pentose phosphate pathway, which can have a substantial impact on autophagy. When TIGAR expression is knocked down, there is an increase in ROS which results in enhanced autophagy and reduced apoptosis. TIGAR expression can also modulate autophagy independently of p53 [45].

**CELL DEATH AND SURVIVAL**

Recent work from Joan Brugge’s laboratory has revealed an additional role for pentose phosphate pathway (PPP) flux and oxidative stress in breast cancer cells that is independent of apoptosis (See Fig. 2) [46]. During the progression of breast cancer, cells become detached from the basement membrane/extracellular matrix (ECM), and eventually will proliferate into the luminal space of mammary acini. There are a number of cellular programs responsible for eliminating these matrix-detached cells to prevent their survival in the luminal space. However, cancer cells utilize multiple mechanisms to evade these death programs and thus thrive in the absence of ECM attachment [47-50]. Previously, ErbB2 has been found to rescue matrix-deprived cells by blocking the induction of anoikis and preventing the clearing of cells.

**Fig. (2).** Detached mammary epithelial cells display an increase in ROS and lower ATP levels which facilitates luminal clearing. ErbB2 expression rescues detached cells by neutralizing ROS and increasing cellular ATP. The antioxidant compounds n-acetyl-l-cysteine (NAC) and Trolox also cause an increase in ATP production which can lead to luminal filling.
from the luminal space [51]. However, our recent work suggests that in addition to inhibiting anoikis, cancer cells must also compensate for detachment-induced metabolic alterations to survive in the absence of ECM attachment [46].

We directly examined metabolism in detached and attached human mammary epithelial cells (MCF-10A) and discovered that detached cells suffer from a marked reduction in ATP levels. However, the expression of ErbB2 in the MCF-10A cells completely rescued the loss of ATP caused by ECM detachment. The molecular mechanism underlying the ability of ErbB2 to rescue ATP levels involved in the stabilization of EGFR and a subsequent enhancement in PI3K/Akt signaling to restore glucose transport. Interestingly, it was not traditional glucose metabolism through glycolysis and the TCA cycle that led to this rescue by ErbB2, as flux through the PPP was found to be critical for the enhanced ATP levels [46].

Given the importance of the PPP in reducing oxidative stress and the evidence of high levels of ROS in detached cells, we were interested in examining the contribution of ROS to the loss of cellular ATP in detached cells. Neutralization of ROS with the antioxidants N-acetyl-L-cysteine (NAC) or Trolox (water-soluble vitamin E derivative) rescued the cellular ATP in detached cells through a mechanism that is independent of glucose uptake [46]. This led us to examine fatty acid oxidation (FAO), which previous research had demonstrated was used by glucose-starved cancer cells to produce ATP [52]. Surprisingly, increased ROS production in detached cells was found to inhibit FAO; however, antioxidant treatment was found to substantially elevate FAO in detached cells [46].

To translate these results back to the issue of cell survival in the absence of ECM attachment, we analyzed the ability of antioxidant treatment to promote the viability of cells in the luminal space of 3D MCF-10A acinar cultures. Treatment with antioxidants (in the presence or absence of apoptosis), caused substantial survival of cells in the luminal space thus suggesting that the relief of the ROS-mediated effects on FAO could allow these cells to produce ATP and thus survive in the absence of ECM attachment. These results have interesting implications for some of the more conventional thinking about antioxidants which are typically touted for their anti-tumor capacity. These data suggest that oxidative stress can be tumor suppressive in its ability to kill off detached cells and that antioxidants may promote the survival of tumors cells that have departed from their normal niche.

Often as a result of some of the metabolic changes described above, cells will initiate autophagy, which is a catabolic process in which organelles and cytoplasmic protein are degraded through lysosomes [53-55]. The induction of autophagy can be temporarily used for cell survival by digesting its own components as a source of energy [56,57]. In addition to functioning as an alternative energy source, autophagy can be involved in clearing organelles, [58-60], and defective autophagy can contribute to tumorigenesis. For example, Beclin-1 is involved in the formation of autophagosomes during autophagy, and Beclin-1 is often monoallelically deleted in forms of human breast cancer [61]. Human breast carcinomas often show low Beclin-1 levels, and an increase in Beclin-1 levels has been shown to suppress tumorigenesis in MCF-7 cells [62].

Eileen White’s group has discovered a mechanism that may help explain why breast cancer cells typically are defective in autophagy. They discovered that autophagy is involved in the survival of mammary epithelial cells under metabolic stress, DNA damage, and nutrient and oxygen deprivation. Under normal conditions, immortalized mouse mammary epithelial cells (iMMECs) that are derived from either Beclin-1 +/- or Beclin-1 +/- mice display similar amounts of autophagy. However, when these cells are placed under metabolic stress, Beclin-1 +/- iMMECs showed a significant increase in autophagy activation, whereas Beclin-1 +/- iMMECs showed a slower and weaker autophagic response under metabolic stress. In addition, the anti-apoptotic protein Bcl-2 was found to collaborate with Beclin-1 +/- iMMECs to be more resistant to cell death [63]. Furthermore, when Bcl-2 expressing Beclin-1 +/- iMMECs were grown in 3D cell culture, lumen formation was inhibited and instead filled with abnormally large cells; whereas Bcl-2 expressing Beclin-1 +/- iMMECs formed polarized acini with an accelerated lumen formation [63]. These intriguing results suggest a collaboration between apoptosis inhibition and autophagy to promote the survival of mammary epithelial cells.

White’s group further demonstrated that Bcl-2 expressing cells that were heterozygous for Beclin-1 could more efficiently form tumors when compared with Bcl-2-expressing cells with two wild type copies of Beclin-1. The tumors that formed from Beclin-1 +/- iMMECs displayed tumors with altered glandular morphology and exhibited accelerated growth. In addition, this allelic loss of Beclin-1 resulted in the induction of the DNA damage response suggesting that cells deficient in autophagy may cause genomic damage when under metabolic stress [63]. As tumor cells typically inhibit most forms of cell death, the fact that a defective survival pathway could contribute to mammary tumorigenesis was somewhat surprising. However, this apparent paradox is resolved by the demonstration that genomic damage resulting from metabolic stress may collaborate with apoptotic inhibition to promote tumorigenesis.

Other studies from White’s group using immortalized primary mouse kidney epithelial cells (iBMK cells) have revealed that this survival role for autophagy under conditions of metabolic stress is not limited to mammary tumorigenesis. In this model they examined the contribution of the key autophagy regulator, Atg5. Using cells derived from Atg5 +/- , Atg5 +/+ , and Atg5 -/- mice, they demonstrated a dramatic induction of autophagy in Atg5 +/- cells under metabolic stress. However, cells derived from Atg5 +/- and Atg5 -/- mice had accelerated apoptosis and interestingly, enhanced aneuyploidy and genomic instability. These results seem to validate the results seen in the mammary model and further suggest that the induction of autophagy may be an effective chemotherapeutic strategy in certain conditions [64].

In an interesting follow up study examining the molecular mechanisms underlying the ability of autophagy to inhibit tumorigenesis, White’s group focused on the p62 protein. p62 has been found to direct damaged or unfolded proteins to autophagy by binding to polyubiquitinated pro-
teins and aggregates and targeting them to the autophagosome [65-66]. Using their aforementioned iBMK cell model, they demonstrated that autophagy-defective tumor cells accumulate p62 along with ER chaperones and protein disulphide isomerases (PDIs), proving that tumor cells lacking autophagy exhibit poor protein quality control [67]. In addition, defects in autophagy resulting in accumulation of p62 in tumor cells caused damaged mitochondria, elevated oxidative stress, and the activation of the DNA damage response. This accumulation of p62 in tumor cells resulted in alterations in NF-κB regulation of gene expression that could contribute to tumorigenesis. This study now provides a direct link between deficient autophagy, metabolic stress, and an oncogenic signaling pathway and reveals an additional mechanism could be targeted in cancer cells.

HYPOXIA

Following up on the fact that cancer cells can encounter metabolic stress, it has become abundantly clear that hypoxia (an inadequate amount of oxygen) is commonly found in breast cancer [68-71]. An important context where breast cancer cells encounter hypoxia is during metastasis, more specifically to the bone. Breast cancer cells commonly metastasize to the bone, which has serious complications for the patient including severe bone pain, pathologic bone fracture, hypercalcemia, nerve compression syndromes, and eventually death [72]. A critical transcription factor involved in allowing cancer cells to adapt to environments of inadequate oxygen, is Hypoxia-inducible factor-1 (HIF-1), which is composed of a heterodimer of HIF-1α and HIF1-β. The targets of the HIF-1 transcription factor include glycolytic enzymes, glucose transporters, angiogenic factors, growth factors, enzymes, and other proteins involved in tumor invasiveness and metastasis. Therefore, a recent study examined the importance of hypoxic signaling to the successful metastasis of breast cancer cells to bone [73].

Using MDA-MB-231 cells expressing constitutively active HIF-1, a recent study revealed that the expression of this hyperactive HIF-1 resulted in an enhanced tumor burden in metastatic bone lesions following injection into nude mice. In contrast, the introduction of a dominant-negative version of HIF-1 resulted in the inhibition of bone metastases and decreased osteolytic activity. In subsequent experiments examining the effects of HIF-1 signaling on breast cancer cells that metastasize to the bone, it was discovered that cells expressing constitutively active HIF-1 could inhibit the differentiation of osteoprogenitors into osteoblasts. In stark contrast, constitutively active HIF-1 would promote the differentiation and subsequent accumulation of osteoclasts, resulting in lesions with high osteolytic capacity. These results suggest that antagonists of HIF-1 could be beneficial to patients suffering from breast to bone metastases by blocking the osteolytic activities of the metastatic lesion [73].

Additional studies examining the importance of HIF-1 signaling in breast cancer revealed that mice with a conditional deficiency of HIF-1α in the mammary epithelium had retarded tumor progression as a result of changes in tumor cell proliferation, reduced vascularization, and decreased invasiveness [74]. These HIF-1α deficient mice were also found to have a substantial reduction in pulmonary metastasis resulting from genetically-induced tumors and this reduction in metastasis correlated with improved survival rates for mice bearing these tumors.

METABOLIC ALTERATIONS AS THERAPEUTIC TARGETS?

In summary, there are a number of metabolic mechanisms in breast cancer cells that are now better understood and could potentially be targeted by chemotherapeutics. However, despite these substantial advances, our overall knowledge of cellular metabolism in breast cancer cells is still poorly characterized. For example, as discussed above, oxidative stress has been shown to inhibit fatty acid oxidation in detached mammary epithelial cells, to decrease the production of ATP, and ultimately to result in cell death [46]. However, we do not yet understand the molecular mechanism of fatty acid oxidation inhibition by oxidative stress. A better understanding of this mechanism could potentially lead to a more specific and effective chemotherapeutic strategy. In addition to better characterizing the multiple metabolic mechanisms involved in breast cancer progression, clinical studies aimed at actually targeting these mechanisms would need to be conducted in model systems that could predict efficacy in humans. However, if the knowledge of cellular metabolism in breast cancer cells continues to grow as it has in the past several years, the possibility of treating afflicted women with drugs that disrupt cancer cell metabolism may not be too far off the horizon.

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REFERENCES


