



Mammalian SWI/SNF Enzymes and the Epigenetics of Tumor Cell Metabolic Reprogramming

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Tumor cells reprogram their metabolism to survive and grow in a challenging micro-environment. Some of this reprogramming is performed by epigenetic mechanisms. Epigenetics is in turn affected by metabolism; chromatin modifying enzymes are dependent on substrates that are also key metabolic intermediates. We have shown that the chromatin remodeling enzyme Brahma-related gene 1 (BRG1), an epigenetic regulator, is necessary for rapid breast cancer cell proliferation. The mechanism for this requirement is the BRG1-dependent transcription of key lipogenic enzymes and regulators. Reduction in lipid synthesis lowers proliferation rates, which can be restored by palmitate supplementation. This work has established BRG1 as an attractive target for breast cancer therapy. Unlike genetic alterations, epigenetic mechanisms are reversible, promising gentler therapies without permanent off-target effects at distant sites.

Keywords: SMARCA4, breast cancer, SWI/SNF, fatty acid synthesis pathway, chromatin remodeling, epigenetic regulation, cancer metabolism

Tumor cells reprogram their metabolism to support growth in their unique and challenging micro-environment, a hypoxic environment with inadequate blood supply for normal nutrient replenishment. As first observed by Otto Warburg (1, 2), tumor cells develop a glycolytic metabolism where energy is derived primarily from nutrient catabolism to lactate and not from the mitochondrial Krebs cycle where cells in normal tissue derive most of their energy. Although adaptive to a hypoxic tumor microenvironment, this preference for glycolysis persists even when oxygen is abundant. The nutrient fuel for glycolysis is glucose, but tumor cells also become “addicted” to the normally non-essential amino acid glutamine (3), as first observed in cultured cells by Harry Eagle (4). Glutamine can serve as a carbon and nitrogen source for amino acid synthesis and can fuel the residual Krebs cycle after conversion to glutamate and then α -ketoglutarate. After a period of neglect, cancer metabolism is now recognized as central to the cancer phenotype and as an important target for the development of therapies (5).

REGULATION OF METABOLISM

Cells carefully regulate their metabolism with nested levels of controls (6). First, levels of circulating molecules that serve as feedstock for metabolic pathways change with diet. These include plasma-free fatty acids and amino acids that increase after a meal (7) or plasma ketone bodies and free fatty acids that increase after a prolonged fast (8, 9). Second, allosteric regulation of metabolic enzymes changes flux rates through metabolic pathways in response to concentrations of substrates or products (10–12). Third, there is regulation by hormones (13), often through posttranslational modification

of metabolic enzymes. For example, glycogen deposition or depletion is regulated by a protein kinase cascade-modifying glycogen synthase and glycogen phosphorylase downstream of insulin or glucagon (14, 15). AMP-activated Kinase (AMPK) is a master regulator of metabolism that can sense cellular energy status and respond by switching on and off pathways to achieve energy homeostasis (16, 17). AMPK is activated in response to cellular ATP depletion, which can result from low glucose levels, hypoxia, and heat shock. Upon activation, AMPK upregulates pathways replenishing ATP, including fatty acid β -oxidation and autophagy, and downregulates ATP-consuming processes, including lipid synthesis and protein synthesis.

The protein kinase mTOR (mechanistic target of rapamycin) (18, 19) is the core Ser/Thr protein kinase in two signal transduction complexes, mTORC1 and mTORC2. mTORC1 is a master growth regulator that senses and integrates diverse signals, including levels of growth factors, amino acids, other metabolites, and cellular stress. mTORC2 activates the cell signaling Ser/Thr protein kinase AKT, promotes cellular survival, regulates cytoskeletal dynamics, and regulates growth *via* SGK1 phosphorylation. mTOR complexes promote cell growth through regulation of anabolic and catabolic metabolic processes by multiple mechanisms, as well as through control of cell proliferation. An altered interplay of all of these mechanisms participates in the progressive reprogramming of metabolism with tumor progression.

TRANSCRIPTIONAL REGULATION OF METABOLISM

Metabolic pathways can also be regulated by transcriptional mechanisms increasing or decreasing levels of enzymes. Take the example of lipid metabolism. The Sterol Regulatory Element Binding Protein (SREBP) transcription factors are the master regulators that control the expression of nearly all lipogenic enzymes. The mTORC1 complex regulates lipid synthesis (20) through SREBP by multiple mechanisms. In response to cellular signaling, mTORC1 regulates SREBP processing through S6K and increases SREBP nuclear accumulation through Lipin 1, a phosphatidic acid phosphatase that is also a transcriptional coactivator (21–25). mTORC1 phosphorylates Lipin1, preventing its translocation into the nucleus where it can inhibit SREBP1/2-dependent transcription (24). mTORC1 also increases the activity and expression of peroxisome proliferator-activated receptor γ (PPAR γ), another transcriptional regulator of lipogenic genes (26, 27). By these mechanisms, mTORC1 increases the transcription of lipogenic genes, including key enzymes in fatty acid synthesis, such as acetyl CoA carboxylase (ACC), ATP citrate lyase (ACLY), and fatty acid synthase (FASN). As we shall discuss, we have shown Lipin1 and each of these enzymes involved in fatty acid synthesis to be transcriptionally regulated by Brahma-related gene 1 (BRG1), a chromatin remodeling enzyme (28).

CANCER EPIGENETICS

Epigenetic mechanisms control heritable phenotypes without changes in DNA sequence, often changing chromatin structure by

modulating DNA methylation, the posttranslational modification of histones and non-histone chromatin associated proteins, and the regulation of ATP-dependent chromatin remodeling enzymes that control genome accessibility (29). Epigenetic mechanisms regulate normal development and maintain tissue-specific gene expression patterns while their disruption can cause altered gene function and contribute to malignant cellular transformation. The initiation and progression of cancer has been seen as a genetic disease, but we now realize that epigenetic abnormalities contribute to the development of cancer. Cancer cells often have altered levels or activities of epigenetic regulatory proteins with consequences including altered chromatin structure and altered regulation of gene expression (30, 31). These are so common and numerous that global changes in the epigenetic landscape are now considered a hallmark of cancer (5).

THE ROLE OF BRG1 IN CANCER EPIGENETICS IS CONTEXT DEPENDENT

Chromatin structure presents a barrier to transcription factors and polymerases accessing DNA. Several multiprotein complexes alter chromatin structure using the energy derived from ATP-hydrolysis (32–34), including the mammalian SWI/SNF family of chromatin modifiers, which are large, multisubunit enzymes that contain one of two closely related ATPases called BRM or BRG1 (35–37). SWI/SNF complexes containing either catalytic subunit alter nucleosome structure and facilitate binding of transcription factors to nucleosomal DNA in an ATP-dependent manner (38, 39). Subunits of the mammalian SWI/SNF complexes are important for gene activation and repression, development and differentiation, recombination and repair, cell cycle control, and tumorigenesis (40–43). For example, the SNF5 (INI1) subunit is required for embryonic development and functions as a tumor suppressor (44–46).

Brahma-related gene 1 (BRG1) function in cancer is context dependent. BRG1 is mutated in lung and other cancers, where it may function as a tumor suppressor (30, 47). Cancers that have lost the SWI/SNF INI1 subunit require BRG1 (48), suggesting that targeting BRG1 may be therapeutic for these tumors. Similarly, targeting BRM might be an effective strategy for targeting BRG1-deficient tumors (49, 50). As we and others have shown, BRG1 is upregulated but rarely mutated in primary breast and prostate tumors, in melanoma and neuroblastoma, and in pancreatic, gastric, and colorectal carcinomas (51–60). Mice heterozygous for *Brg1* develop mammary tumors (61, 62). However, conditional knockout of *Brg1* in mammary gland does not cause mammary tumors (63). Genome sequencing of more than 500 primary breast cancers showed none with mutations in BRG1 (64). The evidence suggests that BRG1 can be a driver of cancer as well as a tumor suppressor.

FATTY ACID METABOLISM AND CANCER

In tissues with high rates of lipogenesis such as liver, lactating mammary gland, and adipose tissue, the fatty acid synthesis pathway has three principal functions: storage of excess energy as fat,

synthesis of lipids from carbohydrate or protein precursors when dietary lipids are scarce, and synthesis of milk fats during lactation. Most normal cells in other tissues do not synthesize fatty acids *de novo* but preferentially use circulating lipids. However, upregulation of both lipogenic genes and overall lipogenesis are observed widely in tumors in those non-lipogenic tissues (65). Depending on the tumor type, tumor cells synthesize up to 95% of saturated and mono-unsaturated fatty acids *de novo* from acetyl CoA despite a sufficient exogenous supply of fatty acids (66). Lipogenic enzymes, such as FASN, ACC, and ACLY that are required for fatty acid biosynthesis, and SREBP1, the master regulator of lipogenic gene expression, are overexpressed in many cancers, including breast (67–70). FASN is a key enzyme involved in energy storage from excess carbohydrates to fat in liver and adipose tissue, during lactation in breast, and in support of reproduction in endometrium and decidua. But FASN expression during these processes is strictly regulated by nutrition and hormonal levels. In contrast, FASN is highly expressed in many cancer and precancerous lesions. The expression of FASN is independent of nutrition, in many cancers, as well as independent of hormonal regulation. Whereas various tumor types have elevated endogenous fatty acid biosynthesis irrespective of extracellular lipid availability, most normal cells, even those proliferating rapidly, preferentially use exogenous lipids for synthesis of new structural lipids (65, 71).

The activation of the *de novo* fatty acid synthesis pathway is not only observed in tumors but also may be required for malignant progression (65, 72, 73). For example, elevated levels of FASN, the enzyme catalyzing the synthesis of palmitate and thereby required for long chain and unsaturated fatty acid synthesis, are correlated with poor prognosis in breast cancer patients (65, 72). Increases in FASN activity and expression are observed early in cancer development and correlate with cancer progression, while high FASN levels correlate with more aggressive malignant phenotypes (65). Inhibiting key enzymes involved in fatty acid synthesis, including FASN, ACC, and ACLY, with small molecules or knockdowns reduces cell proliferation, induces the apoptosis of cancer cells, and decreases the growth of human tumors grown as mouse xenografts (65, 71, 74–77).

BRG1 IS NECESSARY FOR FATTY ACID BIOSYNTHESIS IN SUPPORT OF PROLIFERATION IN BREAST CANCER

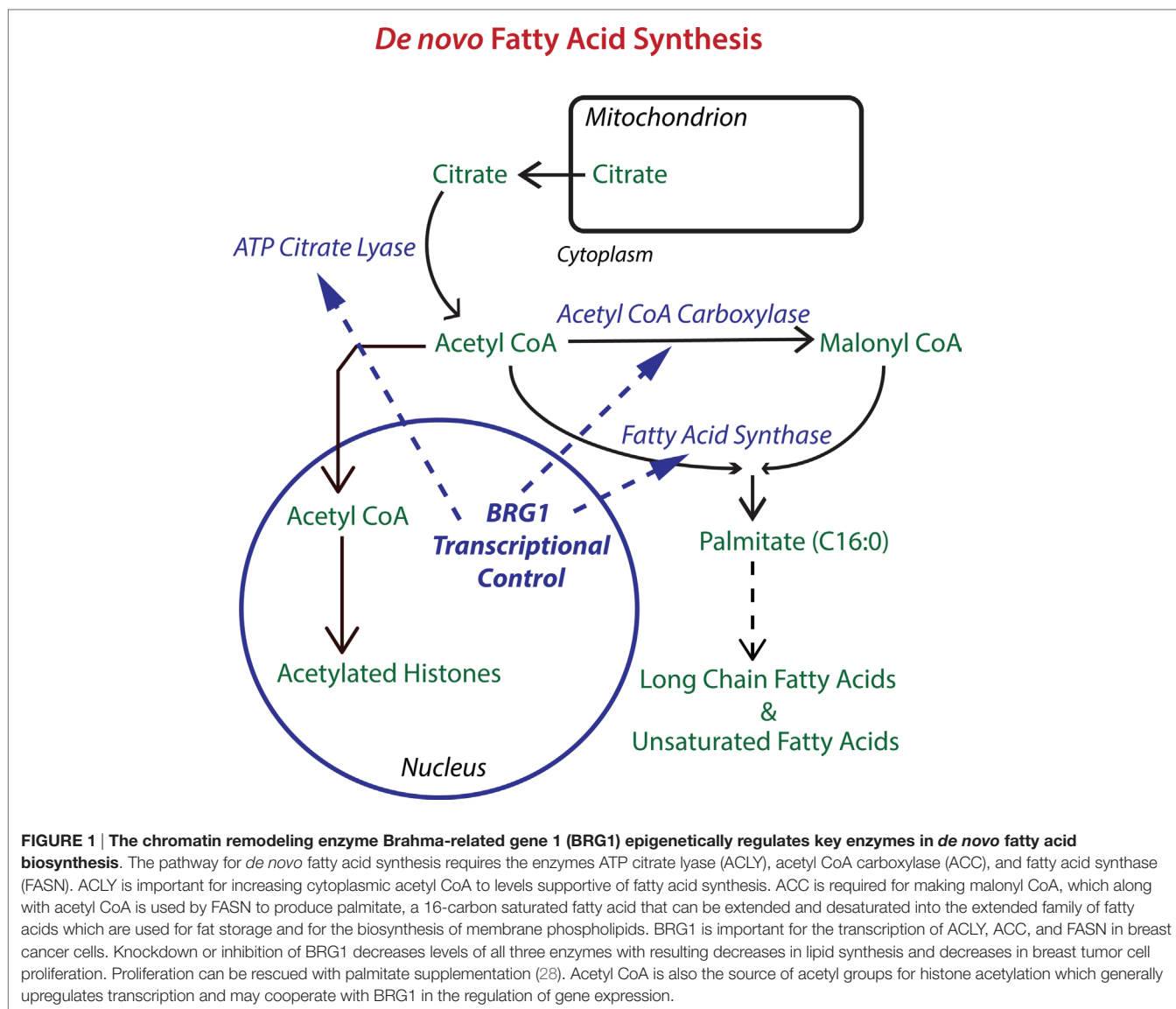
We first reported that the alternative SWI/SNF chromatin remodeling enzyme ATPases BRG1 and BRM are required for proliferation of breast cancer cells (59). Western blots of biopsies showed that BRG1 protein levels were higher in tumor than in normal tissue. Analysis of TCGA Breast Cancer patient data revealed an approximate twofold increase in BRG1 mRNA levels (64) and in BRG1 protein levels (78) in tumors compared to normal tissue across all subtypes. These are not well-controlled comparisons because of the great heterogeneity in normal tissue cell types. More convincingly, immunohistochemistry confirmed that the BRG1 and BRM proteins are greatly overexpressed in most primary breast cancers independent of receptor status (55, 59). BRG1 staining was rarely observed in the normal ductal

epithelial cells from which most breast tumors derive but was seen in normal myoepithelial cells. However, in tumors BRG1 staining was observed in almost every cell. Because of the heterogeneity of breast cancer subtypes our further experimental work focused on triple-negative breast cancer, the most aggressive and deadly type.

Knockdown of either ATPase in triple-negative breast cancer cell lines reduced cell proliferation *in vitro* and tumor formation in xenografts. An extended cell cycle progression time was observed without apoptosis, without senescence, or without alterations in migration or attachment. Combined knockdown of BRM and BRG1 produced additive effects, suggesting that these enzymes function, at least in part, through independent mechanisms. Knockout of BRG1 or BRM using CRISPR/Cas9 technology caused cell death. Our work supports the novel idea that overexpression of BRG1 and BRM is common in breast cancer and that BRG1 and BRM are required for breast cancer cell proliferation and survival. These results are in direct contrast to other tumors where BRG1 acts as a tumor suppressor (79). For example, it is mutated in lung and other cancers. We and others have now shown that BRG1 is upregulated but rarely mutated in primary breast and prostate tumors, in melanoma and neuroblastoma, and in pancreatic, gastric, and colorectal carcinomas (51–60, 80).

When we began our studies, it was expected that BRG1 was a weak tumor suppressor in mammary gland because about 10% of Brg1+/- mice eventually developed mammary tumors (61, 62) and because there were functional interactions between BRG1 and cell cycle regulatory proteins, including RB and p53 (30, 42, 81). This tentative identification of BRG1 as a mammary tumor suppressor was challenged by our work (59) and by others (55). The conditional knockout of Brg1 in the mouse mammary gland did not cause mammary tumors (63). We observed that fewer than 2% of BRG1 sequences in the TCGA database contained mutations. Breast cancer is not alone in this requirement for BRG1. BRG1 is also required for the proliferation of HeLa cells and mouse fibroblasts (82, 83).

What is the mechanism for the BRG1 requirement for breast cancer cell proliferation? We discovered that BRG1 promotes breast cancer by reprogramming lipid synthesis (28) as shown in **Figure 1**. BRG1 knockdown reduced the rate of chloroform/methanol extractable lipid synthesis by 35% while glucose uptake remained unchanged. mRNA and protein levels for ACC, ACLY, and FASN, the key enzymes in *de novo* fatty acid synthesis, were all significantly decreased in BRG1 knockdown cells as were other important proteins performing or regulating lipid synthesis such as Lipin1. BRG1 bound to the promoters of all of these genes, and the promoter binding was diminished in BRG1 knockdown cells, evidence of direct BRG1 transcriptional control. Treatment with either an ACC inhibitor or a FASN inhibitor decreased cell number, and BRG1 knockdown cells showed increased sensitivity to these inhibitors. Remarkably, addition of exogenous palmitate, the key intermediate in fatty acid synthesis, completely rescued proliferation. Our work supports a mechanism in which BRG1 transcriptionally promotes *de novo* lipid synthesis, which is necessary for maintaining high rates of proliferation. In these cells, exogenous palmitate can substitute for endogenous FASN-generated palmitate. Furthermore, BRG1 regulation of proliferation through fatty acid metabolism is breast cancer specific. We



showed that key fatty acid synthesis enzymes are not upregulated by BRG1 in non-tumorigenic MCF-10A mammary epithelial cells (59). Though MCF-10A cells also require BRG1 for proliferation (84), this requirement has a different mechanism. Restoration of BRG1 expression in cells depleted for both BRG1 and BRM rescued lipid synthesis, the expression of lipogenic enzymes and cell proliferation so BRM is not required for these effects in this system.

TARGETING BRG1 FOR BREAST CANCER THERAPY

Chromatin remodeling complexes have not been viewed as a drugable target until recently, but our work shows that the BRG1 chromatin remodeling enzyme is an especially promising target for epigenetic breast cancer chemotherapy (28, 59, 85). Inhibition

of BRG1 function decreases tumor cell proliferation, decreases tumor mass in mouse models, and potentiates tumor cell killing by clinically used chemotherapy drugs.

Only two BRG1 inhibitors have been reported. PFI-3, a Pfizer/Structural Genomics Consortium candidate, is a small molecule inhibitor that specifically targets the bromo domains of BRG1, BRM, and PB1 (86, 87). We treated three triple-negative breast cancer cell lines, MDA-MB-231, MDA-MB-468, and HDQ-P1, with PFI-3 at different doses (85). No inhibition of cell proliferation was observed. This is consistent with recent results demonstrating that PFI-3 does not affect the proliferation rate of other cancer cell lines (87). While PFI-3 does have an effect on some BRG1 functions, it does not dislodge full length BRG1 from chromatin (87) and this may be necessary for inhibiting proliferation through control of lipid synthesis.

The natural product ADAADi (Active DNA-dependent ATPase A Domain inhibitor) inhibits the ATPase activity of the SWI2/

SNF2 family of ATPases (88, 89). Enzymes from other families of DNA-dependent ATPases have no or greatly reduced sensitivity to ADAADi, and DNA-independent or RNA-dependent ATPases are not affected (88). ADAADi inhibits BRG1 nucleosome remodeling activity *in vitro* (88). We tested the ADAADi inhibitor on TNBC cell lines: MDA-MB-231, MDA-MB-468, and HDQ-P1. ADAADiN significantly decreased cell proliferation in these cell lines (85). However, ADAADi failed to decrease cell proliferation significantly in cells with experimentally reduced BRG1 expression. This observation strongly suggests that ADAADiN specifically targeted BRG1 in these cells by interfering with its ATPase function.

ADAADi decreases lipid biosynthesis in breast cancer cells (28) and also sensitizes cells to chemotherapy drugs, just as BRG1 knockdown does (85). After pretreatment with ADAADi, cells were exposed to different doses of six clinically used chemotherapy drugs and cell viability was assayed by MTT. ADAADi significantly increased the drug killing efficacy in MDA-MB-231 and MDA-MB-468 cells from 3-fold to over 10-fold. Therefore, chemical inhibition of the BRG1 ATPase domain targets BRG1-mediated pro-survival pathways in breast cancer cells, decreasing levels of the ABC transporters that pump chemotherapy drugs out of cells and contribute to treatment failure (85).

METABOLOEPIGENETICS

At the level of organisms, food intake affects patterns of gene expression. At the level of cells, levels of nutrients and metabolites regulate patterns of gene expression. Multiple mechanisms have been described and many remain to be discovered (90–92). Epigenetic controls are often exerted through covalent modifications of chromatin proteins or through modification of DNA itself. The essential donor groups for these modifications are important metabolic intermediates including Acetyl CoA, S-adenosylmethionine, ATP, and NAD⁺.

Here, we will concentrate on histone acetylation and metabolism. In one form of epigenetic regulation, histones can be acetylated at multiple positions on their N-terminal tail domains, affecting gene expression at the proximate genes. The extent of histone acetylation at specific sites depends on relative rates of deposition by histone acetyl transferases and removal by Histone Deacetylases (HDACs). The acetyl donor for histone acetylases is acetyl CoA, a metabolite that is produced downstream of glycolysis by the mitochondrial trichloroacetic acid cycle, by the β -oxidation of fatty acids, or by amino acid catabolism. Acetyl CoA is required for both fatty acid and cholesterol synthesis. ACLY generates acetyl CoA from citrate, ATP, and CoA (Figure 1). It partitions to both nucleus and cytoplasm, suggesting that nuclear acetyl CoA can be made locally (93) and that nucleocytoplasmic levels change with the metabolic status of cells, for example with glucose levels (94, 95). Knocking down ACLY reduces the acetylation of core histones H2B, H3, and H4 with consequent reductions in the expression proximate genes (93). As we have found ACLY to be transcriptionally regulated by the chromatin remodeling enzyme BRG1 in triple-negative breast

cancer cells (28), BRG1-mediated chromatin remodeling may tune the relationship between metabolism and histone acetylation, linking two distinct mechanisms for epigenetic regulation.

Histones can also be acylated with at least eight other species of short chain carboxyl groups: propionyl, butyryl, 2-hydroxyisobutyryl, succinyl, malonyl, glutaryl, crotonyl and β -hydroxybutyryl (94, 96). The levels of these modifications may be controlled by the metabolic pathways producing these carboxyl groups. This may be a mechanism for integrating readouts from these pathways to control patterns of transcription. There is now evidence that histones are acylated with longer chain fatty acids (97). Such a mechanism would directly link fatty acid levels with histone epigenetics.

Many HDACs exist in mammalian cells. Class III HDACs, also known as sirtuins, are nicotinamide adenine dinucleotide (NAD)-dependent deacetylases (98). NAD is a coenzyme carrying electrons between redox reactions in its reduced form NADH. More than 200 metabolic enzymes use NAD⁺/NADH as a cofactor, most functioning in catabolism. For example, starting with one glucose molecule, two NAD⁺ molecules are reduced to NADH in glycolysis, at the step catalyzed by glyceraldehyde 3-phosphate dehydrogenase. The highly related NADP⁺/NADPH performs the same role for enzyme catalyzed anabolic reactions, for example in the *de novo* synthesis of palmitate by FASN.

As first shown for SIRT2 (99–101), a cytoplasmically localized protein, sirtuins have a deacetylation activity requiring NAD⁺, but not as an electron carrier. Instead, their reactions use NAD⁺ in equal stoichiometry to the acetyl group and cleave NAD, generating nicotinamide and 2'-O-acetyl-ADP-ribose. Of the seven mammalian sirtuins, SIRT1, SIRT 6, and SIRT 7 are nuclear proteins, enriched in the nucleoplasm, in heterochromatin, and in nucleoli, respectively, and positioned to deacetylate histones and other nuclear proteins (102). SIRT1 efficiently deacetylates p53 (102).

It has been proposed that this unusual use of NAD⁺ makes these sirtuins sensors of cellular NAD⁺ levels. Cellular and nuclear NAD⁺ levels are close enough to the K_m of SIRT1 for NAD⁺ to make this plausible (103). In this view, cellular NAD⁺ levels would change in response to metabolic fluxes or stresses and cause changes in histone and other nuclear protein acetylation with consequences on gene expression. Conflicts have been noted between this model and early studies on NAD⁺ levels that showed little response to starvation (92). NAD⁺ levels do cycle with circadian rhythms (104) and increase with exercise (105). NAD⁺/NADH ratios decrease in response to elevated glucose levels in C2C12 skeletal muscle cells while in the muscles of fasted mice SIRT1 decreases expression of AMPK targets in control animals and is necessary for their induction after fasting (106). In mouse liver, NAD⁺ levels are increased by 33% after fasting for 24 h and return to control levels after 24 h after refeeding (107). SIRT1 protein levels were induced after refeeding, showing a second mechanism for SIRT1 activity regulation. The energy sensor AMPK increases cellular NAD⁺ levels, increasing SIRT1 deacetylation of downstream SIRT1 targets (108). SIRT1 is proposed to activate AMPK creating a feedback loop between SIRT1 and AMPK that controls energy metabolism.

THERAPEUTIC INTERVENTION IN BREAST CANCER EPIGENETICS AND METABOLISM

The reciprocal relationships between metabolism and epigenetic regulation are attractive opportunities for targeted cancer therapy. Multiple drug candidates targeting epigenetic mechanisms are currently in trials for breast cancer. Among those with published promising results are the HDAC inhibitors SAHA (Vorinostat) (109–111), entinostat (112), valproate (113), and romidepsin (114). Romidepsin and vorinostat have been FDA approved for treatment of T-cell lymphomas (115, 116). An inhibitor of a DNA methyltransferase, 5-aza-2'-deoxycytidine (5azaC), causes DNA hypomethylation, is FDA approved for treatment of myelodysplastic syndrome (117, 118), and has early promise for breast cancer (119, 120). The great promise of these drugs should drive the search for other epigenetic targets in cancer therapy.

In the work, we have reviewed here, the chromatin remodeling enzyme BRG1 and its breast cancer-specific effects on lipid

metabolism are an attractive target for breast cancer therapy. Our work establishes that one part of the anti-cancer mechanism of BRG1-targeted drugs is an effect on fatty acid synthesis decreasing proliferation. Unlike genetic alterations, epigenetic mechanisms are reversible, promising gentler therapies without permanent off-target effects at distant sites.

AUTHOR CONTRIBUTIONS

JN wrote sections and edited the contributions of the other authors. AI and QW wrote sections and edited the manuscript. All three authors contributed to the published work reviewed in this article.

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