OUTLOOK

Acetyl-CoA-directed gene transcription in cancer cells

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Fluctuations in acetyl-coenzyme A (acetyl-CoA) levels have been previously associated with changes in global histone acetylation and gene expression. The study by Lee and colleagues (pp. 497–511) in this issue of *Genes & Development* demonstrates that acetyl-CoA can promote the up-regulation of cell migration- and adhesion-related genes in glioblastoma by controlling Ca^{2+} -NFAT (nuclear factor of activated T cells) signaling.

Tumor cells need a reprogrammed metabolism to cope with the increased metabolic demands imposed by an uncontrolled proliferative state. Metabolites from the TCA cycle in mitochondria have long been appreciated for being the precursors for macromolecule biosynthesis. Recently, there has been a lot of interest in the participation of these metabolites in signaling in various aspects of tumor cell biology. One of the most prominent findings is the link between metabolism and the regulation of the epigenome, where acetyl-coenzyme A (acetyl-CoA) has a main role as an essential cofactor in the post-translational acetylation reactions of histones (Shi and Tu 2015; Sivanand et al. 2018).

Acetyl-CoA is produced by the oxidative decarboxylation of pyruvate from glycolysis, the oxidation of long chain fatty acids, or the oxidative degradation of certain amino acids. Acetyl-CoA then enters the TCA cycle, where it combines with oxaloacetate to generate citrate (Fig. 1). In a dynamic metabolic cross-talk, citrate from the TCA cycle travels from the mitochondria and gets converted to acetyl-CoA by the enzyme ATP-citrate lyase (ACLY) in both the cytosol and the nucleus (Fig. 1). Interestingly, inhibition of ACLY has been shown to suppress tumorigenesis (Hatzivassiliou et al. 2005). The abundance of acetyl-CoA is highly dependent on glucose availability, fatty acid oxidation, and mitochondrial respiratory function and has been shown to correlate with the levels of global histone acetylation (Lee et al. 2014; Martínez-Reyes et al. 2016; McDonnell et al. 2016). Remarkably, exogenous acetate, which generates acetyl-CoA by acetyl-CoA

synthetase enzymes, can maintain global histone acetylation when ACLY activity is impaired (Fig. 1; Zhao et al. 2016). Previous studies by Wellen and colleagues (Lee et al. 2014) showed that limiting glucose availability diminished acetyl-CoA levels and global histone acetylation, which was rescued by exogenous acetate in glioblastoma multiforme (GBM) cell lines. Furthermore, expression of a subset of glucose-regulated genes was rescued by acetate supplementation. These acetyl-CoA-dependent genes include genes that are involved in cell adhesion, cytoskeletal dynamics and extracellular matrix (ECM) interactions, and integrin signaling. Although a strong correlation between acetyl-CoA levels, histone acetylation, and gene expression was established, it remained unclear whether alterations in acetyl-CoA levels result in histone acetylation changes at specific loci and whether these changes have a direct impact on gene expression.

Building on their previous work, Wellen and colleagues (Lee et al. 2014, 2018) demonstrate that low-glucose conditions impair cell migration and adhesion in GBM cells. This effect was rescued by the addition of acetate, which also restored histone acetylation. Furthermore, the inhibition of ACLY also diminished cell migration and adhesion as well as the expression of the related genes. Next, they defined the specific loci where histone acetylation is differentially regulated upon limited glucose availability and rescued by acetate supplementation. In particular, they focused on H3K27 acetylation (H3K27ac). H3K27ac is regulated by the lysine acetyltransferase (KAT) p300 whose inhibition prevented the glucose- and acetate-dependent increase in global H3K27ac and cell adhesion to the ECM. They observed that H3K27ac was significantly more suppressed at acetyl-CoA-dependent genes when compared with all genes in glucose-limited conditions, raising the question of how such specificity is regulated in these acetyl-CoA-dependent genes. The investigators observed that cells needed at least 4 h to rescue the cell adhesion phenotype when supplemented with glucose or acetate after being cultured in low-glucose conditions. This

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Figure 1. Acetyl-CoA promotes cell migration and adhesion by controlling Ca^{2+} -NFAT (nuclear factor of activated T cells) signaling. Acetyl-CoA is produced by the oxidative decarboxylation of pyruvate from glycolysis and enters the TCA cycle. Citrate is released from mitochondria and converted to acetyl-CoA by ACLY. Acetate generates acetyl-CoA by ACSS2. High levels of acetyl-CoA promote an increased influx of calcium into the cells, activating and translocating NFAT to the nucleus. In combination with p300, NFAT drives site-specific regulation of H3K27 acetylation (H3K27ac) and increases the expression of genes related to cell migration and adhesion to the ECM.

result indicated that changes in gene expression are likely to involve specific acetyl-CoA-responsive transcription factors. The nuclear factor of activated T cells (NFAT) was identified as the most enriched transcription factor, and the data confirmed a novel interaction between the activation and nuclear localization of NFAT and acetyl-CoA production. The silencing of NFAT1 reduced acetyl-CoA-dependent gene expression, cell migration, and cell adhesion, while the overexpression of a constitutively active NFAT1 rescued defects in cell adhesion in acetyl-CoA-limited conditions. Thus, NFAT is necessary and sufficient in regulating acetyl-CoA-dependent gene expression and cell adhesion to the ECM in glioblastoma cells. NFAT nuclear localization is controlled by Ca^{2+} -dependent dephosphorylation. Further analysis demonstrated that acetyl-CoA production promoted NFAT dephosphorylation and nuclear localization by regulating Ca^{2+} influx (Fig. 1). Indeed, treatment with ionomycin, which floods the cells with calcium, was able to rescue cell adhesion in low-glucose conditions or upon ACLY inhibition.

At first glance, the observation that constitutively active NFAT is able to rescue cell adhesion in low-acetyl-CoA conditions suggests that gene expression can be uncoupled from histone acetylation and that acetyl-CoA controls gene expression only at the level of dictating translocation of transcription factors into the nucleus. However, p300 inhibition also diminishes cell adhesion, indicating that NFAT likely brings p300 to site-specific loci for histone acetylation. The limited acetyl-CoA levels in the presence of constitutively active NFAT under low-glucose conditions are likely able to sustain site-specific p300-dependent histone acetylation for gene

transcription. Thus, acetyl-CoA levels might not be rate-limiting for site-specific histone acetylation. This is analogous to ATP not being rate-limiting for protein phosphorylation.

A fascinating finding of this study is the novel connection between acetyl-CoA and the regulation of calcium homeostasis. This observation demonstrates that the effects observed upon changes in acetyl-CoA abundance involve not only changes in histone acetylation reactions but tightly coordinated signaling pathways. Calcium levels are known to regulate a wide variety of processes. Further investigation is needed to determine how calcium specifically regulates NFAT or other transcription factors. Such regulation is likely to be context-dependent. For example, the production of acetyl-CoA by fatty acid β -oxidation regulates lymphangiogenesis through epigenetic changes regulated by the transcription factor PROX1 (Wong et al. 2017). The microenvironment might be critical in defining the preferred pathways in which acetyl-CoA is used.

An intriguing but unanswered question is to what extent the proposed mechanism is common in different cancer types or other highly proliferative cells, such as T cells, where NFAT, ACLY, and mitochondrial respiratory chain function have been shown previously to control T-cell activation and proliferation (Mehta et al. 2017). Given the success of immunotherapy, it will be important to know whether targeting ACLY would negatively impact immune checkpoint inhibitors. These studies will be important to determine the potential therapeutic opportunities to treat GBM (the most common brain tumor in adults with no cure to date) by targeting ACLY.

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