A Fresh Take on the "TCA" Cycle: TETs, Citrate, and Asthma

Asthma, a condition that affects more than 300 million individuals of all races, both sexes, and all ages worldwide, is a classic example that is often used to illustrate how genes and environment interact in complex ways to result in disease. Although genetics clearly plays an important role in the development of asthma, the overall susceptibility, age of onset, and disease severity are also strongly influenced by the environment, which may include exposure to allergens, air pollutants, and factors that influence immunologic tolerance and the lung microbiome. Epigenetics, which is often influenced by the environment and leads to modulation of gene expression in a persistent, often heritable manner, lies at the crossroads of many gene–environment interactions. Thus, not surprisingly, a wealth of evidence shows how epigenetic marks, such as DNA methylation, play a crucial role in the developmental origins of asthma (1–3).

"DNA methylation" refers to the addition of methyl groups to predominantly cytosine base pairs of DNA and is critical for both normal development and the pathogenesis of cancer (4), autoimmune disease (5), and other lung diseases such as pulmonary fibrosis (6). Increased DNA methylation, or hypermethylation, is described in tumor suppressor genes in cancer (7) and antifibrotic genes in pulmonary fibrosis (8). Although many genes are hypermethylated in asthma, genome-wide studies have found DNA hypomethylation to be just as common in the population with asthma (1–3). DNA methyltransferases(DNMTs) are the enzymes long recognized as responsible for the addition of methyl groups to DNA, and for many years, loss of methylation in mammals was believed to occur only passively because of loss of DNMT activity. However, the discovery of TET (ten-eleven translocation) proteins and their actions (9), now nearly 10 years ago, has led to the identification of robust mechanisms for the active loss of methyl groups from DNA.

TET, which consists of a family of three enzymes, TET1, TET2, and TET3, converts methylcytosine to hydroxymethylcytosine (9). This unstable base pair can be further modified, also by TETs, to formylcytosine and carboxycytosine. Hydroxymethylcytosine, formylcytosine, and carboxycytosine are all recognized by the DNA base pair excision machinery of the cell, which replaces the base pair with unmethylated cytosine (10). The importance of TET enzymes is now recognized in many diseases (11); indeed, they were initially discovered and named because the gene was commonly found translocated in acute myelogenous leukemia. In asthma, TET enzymes have been shown to be upregulated in the presence of air pollution (12). An important question in many diseases, including asthma, is, How is TET activity modulated?

TET enzymes are dependent on α-ketoglutarate (α-KG) (9, 10), a metabolite of the tricarboxylic acid, or Krebs/citric acid cycle. Citrate is converted to isocitrate, which is then converted to α-KG by isocitrate dehydrogenase (IDH). This results in production of NAD^+ reduced, which is used in the electron transport chain to generate ATP and energy. The activity of TET is dependent on the activity of IDH and concentrations of α-KG. Studies have shown that mutations in IDH, which results in production of 2-hydroxyglutarate instead of α-KG, lead to diminished TET activity, as found in some cancers (13). The dependence of TET on citric acid cycle metabolites highlights one of many potential mechanisms by which cellular metabolism influences epigenetic machinery (Figure 1A).

In this issue of the *Journal*, the paper by Yeung and colleagues (pp. 36–45) nicely brings together these concepts in asthmatic smooth muscle (SM) cells (14). Airway SM cells are responsible for bronchoconstriction and are often the cells that define the hyperresponsiveness and airway remodeling that are the hallmarks of asthma. Multiple studies have shown that SM cells from individuals with asthma are phenotypically distinct from those in the airways of individuals without asthma (15). Yeung and colleagues demonstrate that asthmatic SM cells exhibit hypomethylation of the *TGFB2* (transforming growth factor-β2) and *COL3A* (collagen, type IIIα), which leads to the increased expression of these genes. Hypomethylation of these genes is due to increased concentrations of IDH, particularly IDH2, which leads to increased production of the metabolite α-KG and activation of TET enzymes, specifically TET1 and TET2 (Figure 1B).

These findings provide a novel and potentially important mechanism that further links metabolism, epigenetics, and asthma. As mentioned earlier, the link between epigenetics and asthma is already well appreciated; changes in DNA methylation are critical to explaining how environmental exposures in the pre- and perinatal periods contribute to the development of asthma in the offspring. Asthma is also influenced by air pollution, which itself has been shown to upregulate TET activity (12). Alterations in cellular metabolism, including obesity, are also a well-known risk factor for asthma. The findings reported by Yeung and colleagues (14) provide mechanistic links for how cellular metabolism influences epigenetics and ultimately asthma.

However, their study, of course, just touches the tip of the iceberg. They focused on SM cells, but whether changes in TET activity and α-KG are found in other important cells in asthma, such as T cells, mast cells, macrophages, or bronchial epithelial cells, is unknown. Interestingly, these investigators also found increases in the expression of TET1 protein itself in asthmatic airway SM, indicating that increased concentrations of α-KG may not be the only mechanism that promotes hypomethylation. Genome-wide studies of DNA methylation and hydroxymethylation could further identify the extent to which the genome is affected by increased TET activity. Finally, animal

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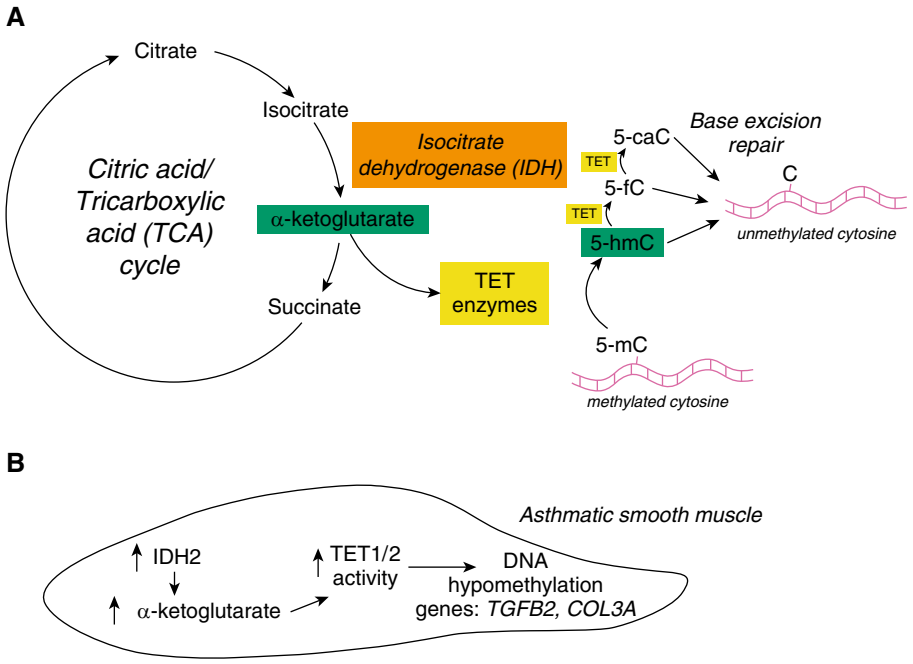

Figure 1. Relationship between tricarboxylic acid cycle metabolites, DNA hydroxymethylation, and asthma. (*A*) α-Ketoglutarate, synthesized from isocitrate by isocitrate dehydrogenase (IDH), are necessary for the activity of ten-eleven translocation (TET) enzymes in the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) as well as 5-formylcytosine (5-fC) and 5-carboxycytosine (5-caC). These are recognized by the base excision repair machinery of the cell and replaced by unmethylated cytosine (C). (*B*) Yeung and colleagues (14) identified increased expression of IDH2, increased levels of α-ketoglutarate, and increased TET activity in smooth muscle cells of patients with asthma. *COL3A* = collagen, type IIIα; TCA = tricarboxylic acid; *TGFB2* = transforming growth factor-β2.

models would be critical to demonstrating the importance of IDH2 and α-KG concentrations *in vivo*. How much of a shift in glucose metabolism is needed to alter the epigenetics of SM cells? How much do α-KG concentrations need to change *in vivo* to affect TET expression? The data reported by Yeung and colleagues suggest that even small changes in α-KG concentrations may be sufficient. Finally, asthma is a heterogeneous disease, and the experiments that they performed were in SM cells from a select few individuals with asthma in whom clinical phenotyping was not performed. It remains to be determined whether these observations are universal to all individuals with asthma or only in the SM of certain individuals who se airways may be more hyperresponsive or demonstrate airway remodeling. The significance of these findings in patients of different endotypes, such as eosinophilic versus neutrophilic or T-helper cell type 2 (Th2) versus non-Th2 disease, is also unknown.

How glucose is metabolized and the mechanisms by which a cell shifts from oxidative phosphorylation to the glycolytic pathway (often referred to as the "Warburg effect") are increasingly recognized as important in health, aging, and the development of many diseases. The importance of these metabolic pathways is likewise critical to asthma and may explain how diet, oxidative stress, and obesity influence the asthma phenotype. Just as diets rich in methyl donors can have important effects on DNMT activity, the importance of α-KG to TET activity provides another dimension by which alterations in cellular metabolism and bioenergetics affect key machinery to produce changes in the epigenetic profile. ■

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