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Cancer mistunes methylation

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Abstract

Metabolic aberrations affecting protein and DNA methylation are a potential source of cancer. A new study shows that the metabolic enzyme nicotinamide N-methyl-transferase, which is overexpressed in several types of tumors, can enhance cancer aggressiveness by draining methyl groups from *S*-adenosyl-methionine.

Signal transduction pathways, induced by hormones and growth factors, stimulate metabolic activity to promote cellular proliferation and differentiation. On the flip side, metabolism regulates signaling pathways, for example, by nucleotide regulation of AMP-activated protein kinase (AMPK), amino acid activation of mammalian target of rapamycin complex 1 (mTORC1) and succinate and fumarate inhibition of degradation of hypoxia induction factor (HIF1). In addition to these specific regulatory interactions, metabolite concentrations can more globally influence rates of protein modifications, such as methylation, acetylation and glycosylation. In this issue, Ulanovskaya *et al.*¹ identify that the metabolic enzyme nicotinamide N-methyltransferase (NNMT), which is overexpressed in a variety of human cancers, broadly alters protein methylation profiles and thereby promotes oncogenesis.

Transmethylation is the transfer of methyl groups between organic molecules. A central methylation pathway in mammalian cells is the cycle between the reactive methyl donor *S*-adenosyl-methionine (SAM), its demethylated form *S*-adenosyl-homocysteine (SAH), homocysteine and methionine (**Fig. 1**). The ratio of SAM to SAH is commonly considered an indicator of cellular methylation potential. For methyltransferase enzymes with high K_m for SAM, however, the absolute concentration of SAM in the cell may be more important.

Methylation has a dual role in cancer: hypermethylation in some cases inhibits tumor suppressors, whereas hypomethylation may activate oncogenes. Several metabolic enzyme aberrations affecting methylation promote tumorigenesis. Active site mutations of the citric acid cycle enzyme isocitrate dehydrogenase frequently occur in glioma and acute myeloid leukemia. The mutant enzymes produce the metabolic error product 2-hydroxyglutarate², which inhibits multiple α -ketoglutarate-dependent dioxygenases, including histone demethylases and 5-methylcytosine hydroxylases³. This inhibition results in genome-wide histone and DNA hypermethylation that blocks cell differentiation; thus, 2-hydroxyglutarate is now referred to as an oncometabolite. Another example is the deletion of methylthioadenosine phosphorylase (MTAP) in various cancers. MTAP is an enzyme

involved in the regeneration of SAM that serves not as a methyl donor but as a propylamine donor (for example, for polyamine synthesis). The high frequency of homozygous loss of MTAP in tumors may be primarily explained by its genomic location adjacent to the known tumor suppressor p16, though MTAP might also be a tumor suppressor⁴. MTAP deletion leads to an increased concentration of its substrate, *S*-methylthioadenosine, an inhibitor of protein methylation⁵. The potential for either excessive or insufficient methylation to promote cancer is also evident in the case of glycine N-methyltransferase, which consumes SAM to methylate glycine, yielding sarcosine (*N*-methylglycine). Loss of glycine N-methyltransferase activity is observed in hepatocellular carcinoma⁶, whereas high activity is found in prostate cancers⁷ with associated increased amounts of sarcosine⁸. Whether sarcosine has important bioactivity separate from being a methyl acceptor remains to be determined.

The findings of Ulanovskaya *et al.*¹ introduce another metabolic enzyme that can promote cancer by disrupting methylation balance. NNMT has been previously found to be overexpressed in a variety of tumors and to promote oncogenesis. The underlying biochemical mechanism remained, however, ill defined. To identify it, Ulanovskaya *et al.*¹ overexpressed NNMT (or its catalytically inactive form) in nonaggressive cancer cell lines and monitored the metabolic consequences. By untargeted metabolomics, they found only two metabolic changes that were consistently induced across cell lines by NNMT overexpression: elevation of the amounts of two direct enzymatic products of NNMT, 1-methylnicotinamide (1MNA) and SAH. Treatment of cells with 1MNA did not enhance aggressiveness, nor was 1MNA converted to other metabolites. These findings suggest that in these cancer cells, 1MNA is a sink for methyl units. The increased SAH resulted in at least a 50% decrease in the SAM/SAH ratio in all studied cell lines. However, in standard tissue culture medium, NNMT overexpression did not decrease the amount of SAM. Standard tissue culture medium contains high concentrations of methionine (~100 μ M), about five times the typical amount in plasma. In medium with physiological concentrations of methionine (10–20 μ M), however, NNMT overexpression reliably decreased SAM, whereas siRNA knockdown of NNMT in aggressive cancers with high endogenous NNMT activity increased SAM. The altered SAM concentration and SAM:SAH ratio were accompanied by parallel broad-based changes in histone (but not DNA) methylation and with associated changes in gene expression and cellular invasiveness. Notably, by ruling out pro-oncogenic mechanisms involving 1MNA or a noncatalytic activity of NNMT, the study by Ulanovskaya *et al.*¹ provides particularly persuasive evidence that decreased methylation potential per se is sufficient to promote cancer.

1MNA has been long considered to be an inactive form of nicotinamide, though recent evidence suggests that it may have anti-inflammatory, antithrombotic, vasoprotective and gastroprotective properties⁹. 1MNA is produced in both liver and kidney and is a major urinary product of nicotinamide metabolism. Tumor-bearing rats have increased renal excretion of 1MNA¹⁰, and serum amounts of 1MNA have been proposed as a biomarkers for colorectal cancer¹¹. In addition to its role as a sink for methyl groups, 1MNA can also serve as a sink for nicotinamide units, and 1MNA inhibits NAD⁺ synthesis in cell culture¹². Although Ulanovskaya *et al.*¹ do not report a change in NAD⁺ when modulating the expression of NNMT, in standard cell culture medium nicotinamide, like methionine, is present at supraphysiological concentrations. Thus, *in vivo* NNMT may exert epigenetic effects by draining both methyl units and NAD⁺, which is a substrate of sirtuins and whose concentration may affect protein acetylation and ribosylation.

Drugs that inhibit methylation, most notably the DNA methyltransferase inhibitor 5-azacytidine, are used in the clinic¹³. However, there are currently no approved drugs that selectively enhance protein methylation. In this respect, NNMT is an interesting target, as

inhibition of NNMT would presumably enhance methylation potential and thereby could have therapeutic benefits in cancers associated with insufficient histone methylation, including those driven by NNMT itself.

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Figure 1. NNMT drains methyl groups from the methionine cycle and thereby decreases protein methylation. Red arrows highlight methyltransferase reactions that consume SAM, including the NNMT reaction, in which nicotinamide's nitrogen engages in nucleophilic attack on SAM's electrophilic methyl carbon. NNMT depletes SAM and produces SAH; SAH is a product inhibitor of SAM-driven methyltransferase enzymes. THF, tetrahydrofolate.