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IDH mutation, competitive inhibition of FTO and RNA methylation

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A recent report published in Cancer Cell used elaborate in vitro and in vivo systems to link FTO expression to the pathogenesis of acute myeloid leukemia (AML) (Li et al., 2017). The N6-methyladenosine (m^6A) RNA demethylase encoded by *FTO* is an alpha-ketoglutarate (α-KG)-dependent dioxygenase (Fedeles et al., 2015). Alpha-KG-dependent dioxygenases are competitively inhibited by the structurally related metabolite D-2-hydorxyglutarate (D2-HG), which aberrantly accumulates in isocitrate dehydrogenase 1 or 2 (IDH1/2)-mutant tumors, including ~20% of AMLs (Cairns and Mak, 2013). Competitive inhibition of the α-KG-dependent DNA hydroxylases (TET family) and Jumonji family histone demethylases, and an attendant hypermethylator phenotype, is characteristic of IDH-mutant AMLs (Garrett-Bakelman and Melnick, 2016). However, whether D2-HG-producing mutant IDH also inhibits FTO and consequently deregulates RNA methylation is unknown. If positive, these observations would suggest that Li et al. (Li et al., 2017) need to take into account the IDH mutational status when attributing an oncogenic role for FTO in AML.

To test if the neomorphic IDH enzyme could influence m^6A levels, we stably expressed IDH2 wild-type (WT), IDH2 R140Q and IDH2 R172K in HEK-293T cells and quantified RNA methylation using an m^6A dot blot assay and a capture-detection ELISA-based test (Supplemental experimental procedures). Cells expressing either IDH2 mutant displayed significantly higher levels of m^6A RNA than the isogenic IDH2 WT expressing cells (Figure S1A). Suggestive of a D2-HG role, exposing HEK-293T cells to the α-KG analog and competitive inhibitor dimethyloxalylglycine (DMOG) also significantly increased m^6A levels (Figure S1A). To link these data to the aberrantly produced D2-HG, we exposed the

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cells to the IDH2-mutant selective inhibitor AG-221, and quantified D2-HG using liquid chromatography-mass spectrometry, as we described (Lin et al., 2015). As expected, R140Q- and R172K-expressing cells displayed extraordinarily high levels of D2-HG, which were significantly suppressed following exposure to AG-221 (Figure S1B). In agreement with a role for IDH2 mutation/D2-HG accumulation in regulating global m^6A levels, exposure to AG-211 restored RNA methylation in the R140Q and R172K cells to levels comparable to those of IDH2-WT isogenic controls (Figure S1B). Importantly, AG-221 did not influence m^6A levels of IDH2-WT cells, nor did it modify FTO expression in any of the cell models (Figure S1B). To more precisely establish the role of FTO in mediating the effects of IDH mutant on m^6A levels, we used CRISPR-Cas9 to knockout (KO) this RNA demethylase in our cell panel. We reasoned that if competitive inhibition of FTO activity by D2-HG was central to the heightened RNA methylation found in IDH2-mutant cells, then deletion of FTO would have limited or no effect on R140Q- and R172K-expressing models but it would significantly increase m^6A levels in IDH2 WT cells. Further, we propose that in this setting, FTO KO is a more informative model than its ectopic expression for in the latter RNA methylation could be suppressed irrespective of how IDH mutation influences it. In agreement with our hypothesis, we found that in comparison to their FTO-expressing isogenic counterparts, m^6A abundance nearly doubled in IDH2-WT/FTO-KO cells, whereas a negligible increase was noted in IDH2-mutant cells after FTO KO (Figure S1C). Notably, the RNA methylation levels of IDH2-WT/FTO-KO cells became as high as those of IDH2-mutant/FTO-WT cells, supporting the idea that most of the effects of R140Q and R172K on RNA methylation are FTO-mediated. In spite of these data, at the moment we cannot exclude the possibility that in IDH1/2-mutant tumors both FTO and ALKBH5, another α-KG-dependent m^6A RNA demethylase, are dysfunctional. Lastly, we examined a small series of well-characterized primary AMLs (IDH2-mutant n=5, IDH1-mutant n=2, IDH1/2-WT n=5), to test the hypothesis that global m^6A levels are higher in IDH-mutant than WT AMLs, and that this dichotomy is not driven by *FTO* expression levels. We found that m^6A levels were significantly higher in IDH1/2-mutant than in IDH1/2 WT AMLs, even though FTO expression was comparable between these two groups (Figure S1D). Of note, expression of *ALKBH5* in these two groups also did not explain the observed differences in m^6A levels (Figure S1D). Although these findings fully align with the cell line models (Figures S1A-S1C) it will be important to validate them in future studies of larger primary AML series.

In agreement with Li et al., four of the five AMLs with highest *FTO* expression in our series were *NPM1*-mutant and/or had *FLT3* internal tandem duplication, including two cases also harboring IDH1/2 mutations; the described association of *NPM1/IDH* mutations in AML adds another layer of complexity to the correlation between FTO expression and activity. We suggest that in the setting of IDH1/2-mutant AMLs it is very difficult to validate the proposed correlation between *FTO* expression, m^6A levels and target gene regulation. This is particularly problematic because, in addition to deregulating RNA methylation that we describe here, IDH1/2 mutations also influence target gene expression in a TET- and histone demethylase-dependent manner. Deconvoluting the individual contribution of each of these dioxygenase families to the epigenetic reprograming that typifies IDH1/2-mutant tumors is an important goal for future studies.

In summary, we propose that: 1) IDH1/2 mutant cells, likely via a D2-HG-mediated competitive inhibition of the α-KG-dependent RNA demethylase FTO, display significantly elevated RNA methylation; 2) Deregulated RNA methylation should be considered part of the pathogenesis of IDH-mutant tumors, which alongside with the well-characterized DNA and histone disturbances defines a “hypermethylation triad”; 3) the effects of FTO expression on AML pathogenesis need to be interpreted in the context of IDH1/2 mutation since in this setting FTO activity is probably low, irrespective of its expression level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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