Epitranscriptomics

Major finding: Reversible methylation of spliceosome snRNAs is controlled by the RNA demethylase FTO.

Clinical relevance: Increased 2-HG levels caused by *IDH1/2* mutation inhibit FTO and prevent snRNA demethylation.

Impact: Altered epitranscriptomic regulation of snRNAs in cancer cells may affect alternative splicing.

snRNAs EXIST AS DIFFERENT METHYL ISOFORMS AND ARE TARGETS OF FTO

Small nuclear RNAs (snRNA) are uridine-rich noncoding RNAs that are incorporated into small nuclear ribonucleoproteins as part of the spliceosome to regulate pre-mRNA splicing. snRNAs undergo a series of nucleotide modifications over the course of their biogenesis that are essential for their function, with mature snRNAs thought to harbor a single set of final modifications. Mauer and colleagues made the unexpected discovery that a subset of spliceosomal snRNAs exist in two distinct isoforms differing in the methylation state of the adenosine residue immediately adjacent to the snRNA cap: a single-methylated m_1 isoform with 2′-O-methyladenosine (Am) and a dimethylated m_2 isoform with N^6,2′-*O*-dimethyladenosine (m^6Am). The methylation is reversible, with demethylation of m_2 snRNAs mediated by fat mass and obesity-associated protein (FTO), an RNA demethylase thought to primarily target N^6-methyladenosine on mRNA. However, mRNAs are predominantly cytoplasmic whereas FTO is nuclear, raising the possibility that snRNAs are the major cellular targets of FTO. Of note, FTO is an α-KG–dependent dioxygenase that is inhibited by the metabolite D-2-hydroxyglutarate, which is generated at abnormally high levels in the presence of cancer-associated isocitrate dehydrogenase 1 and 2 (IDH1/2) mutations. m_2 snRNA levels were significantly increased upon FTO knockout or in the presence of mutant IDH1 or IDH2 and could be restored by mutant isoform-selective IDH inhibitors. FTO deficiency also led to increased exon inclusion, and preliminary data suggested that higher m^6Am in the absence of FTO might affect total levels of some snRNAs and spliceosome composition. Although further work is needed to determine the exact roles of snRNA methyl isoforms, these findings expand our understanding of epitranscriptomic regulation by FTO, suggest that reversible methylation of snRNA may affect alternative splicing, and link cancer-associated mutations to altered snRNA methylation. ■

Mauer J, Sindelar M, Despic V, Guez T, Hawley BR, Vasseur JJ, et al. FTO controls reversible m^6Am RNA methylation during snRNA biogenesis. Nat Chem Biol 2019 Feb 18 [Epub ahead of print].

Leukemia

Major finding: A network of RNA-binding proteins maintains splicing and survival in AML.

Concept: The RNA-binding protein RBM39 is critical for splicing of essential HOXA9 target gene mRNAs.

Impact: Pharmacologic degradation of RBM39 is a potential treatment strategy in spliceosomal-mutant AML.

AML IS DEPENDENT ON AN RNA-BINDING PROTEIN NETWORK

Recurrent mutations in RNA-binding proteins (RBP) that regulate splicing have been identified in acute myeloid leukemia (AML), and dependencies on several nonmutated RBPs have been discovered. Wang, Lu, Pastore, and colleagues systematically analyzed RBP dependencies in AML by performing a CRISPR/Cas9 RNA binding domain-focused screen against 490 classic RBPs and ultimately identified 8 that were essential for AML survival, were highly expressed in patient samples, and were selectively upregulated in AML. Among the top-scoring candidates was RBM39, whose suppression resulted in growth inhibition and induction of apoptosis in AML cell lines. Mice injected with *Rbm39*-deleted AML cells exhibited delayed progression, reduced circulating leukemia cells, and prolonged survival, indicating that RBM39 is required for AML survival *in vivo*. Mass spectrometry analysis revealed an RBM39 interaction network consisting primarily of proteins with roles in the spliceosome complex and ribosome biogenesis, including many other RBPs essential for AML survival. Depletion of RBM39 resulted in several altered splicing events, predominantly repression of cassette exon inclusion and promotion of intron retention. Among the pre-mRNAs most sensitive to RBM39 loss were several HOXA9 targets including *BMI1* and *GATA2*, whose expression is required for leukemogenesis. Pharmacologic inhibition of RBM39 with indisulam (a sulfonamide compound that selectively degrades RBM39) enhanced aberrant splicing and decreased protein levels of HOXA9 targets, leading to cell-cycle arrest and apoptosis in AML cell lines. AML cell lines harboring mutations in splicing factors were significantly more sensitive to indisulam treatment than their wild-type counterparts. In several AML xenograft models, treatment with indisulam elicited strong antileukemic effects, delaying or reducing leukemia burden and prolonging survival. Collectively, these results demonstrate that an RBM39-centered RBP network is critical for survival in AML, particularly those harboring mutations in the spliceosomal machinery, and that targeting RBM39 in this context is a potentially effective therapeutic strategy. ■

Wang E, Lu SX, Pastore A, Chen X, Imig J, Lee SC, et al. Targeting an RNA-binding protein network in acute myeloid leukemia. Cancer Cell 2019 Feb 21 [Epub ahead of print].