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RNA Methylation: A New Regulator of Vascular Remodeling in Pulmonary Hypertension

Pulmonary hypertension (PH) is a chronic and progressive vascular disease characterized by a major constrictive remodeling of the distal pulmonary vasculature leading to increase in pulmonary artery pressure, resistance, and ultimately heart failure (1). Although there is a good understanding of the cellular processes occurring during the development of the disease, including endothelial cell dysfunction and apoptosis and smooth muscle cell (SMC) proliferation, the therapeutic options to limit or revert its progression are limited (2). This may be explained by a lack of understanding and knowledge of the intracellular mechanisms driving cellular dysfunction. Recent investigations have pointed out the contribution of nuclear, transcriptional, and epigenetic mechanisms in mediating PH-associated environmental changes into cell phenotypic and functional perturbations (1, 3). In this issue of the *Journal*, Hu and colleagues (pp. 1158–1172) identified a novel epigenetic mechanism, namely RNA methylation, as a driver of vascular remodeling and SMC proliferation in PH (4).

Epigenetics is an ensemble of mechanisms regulating genome organization, stability, and gene expression without modification of the DNA sequence. These mechanisms include DNA adenine and cytosine modifications, posttranslational modifications of histone residues, and expression of noncoding RNA. All these regulatory systems play a role in regulating gene expression by acting on chromatin conformation and gene accessibility to transcription machinery, transcription factor binding, and mRNA stability and degradation. More recently, mRNA base modifications have also been described, among them N^6 adenosine methylation or m^6A . Like other epigenetic systems, mRNA methylation dynamics and functions require the participation of three types of proteins: “writers” catalyzing N^6 adenosine methylation, “erasers” reverting

these modifications, and “readers” recognizing and utilizing methylated mRNA residues for the recruitment of translational complexes (5). Importantly, the role of methylation on mRNA highly depends on which “reader” is involved and may be diametrically different. Although m^6A “reader” YTHDF1 (YTH domain-containing family protein 1) promotes mRNA translation, YTHDF2 causes mRNA instability and degradation (6). This reflects the complexity and versatility of mRNA methylation on gene regulation and protein expression.

Hu and colleagues found a robust increase in mRNA methylation levels and m^6A “reader” YTHDF1 expression in the pulmonary vasculature of patients with PH as well as in animal and *in vitro* models of PH (Figure 1). By performing global genetic deletion of YTHDF1, they provide strong evidence that m^6A -mediated YTHDF1 recruitment on a subset of transcripts contributes to detrimental vascular remodeling in Sugen/hypoxia-treated mice. Mechanistically, m^6A and YTHDF1 exacerbate SMC proliferation, at least in part by increasing the translation of *MAGED1*. *MAGED1* transcript is methylated by the methyltransferase METTL3 and targeted by YTHDF1 in mice with PH. *MAGED1* knockout phenocopies YTHDF1 deletion and prevents PH development. These studies are compelling in demonstrating a causal role of this central epigenetic mechanism in the development of PH and draw an interesting parallel with recent discoveries in other proliferative disorders such as cancer, in which studies have already identified alteration of mRNA methylation homeostasis as a driver of tumoral cell proliferation. An elevated YTHDF1 expression has been reported in multiple cancers, and functional studies have demonstrated that YTHDF1 plays a detrimental role with respect to tumor growth, metastasis, and antitumor immunity (7, 8). These reports suggest a common YTHDF1-dependent pathway driving hyperproliferative processes in cancer and PH, thus reinforcing the cancer theory of PH (9). In contrast, *MAGED1* appears to display opposite roles in these diseases. In human and mouse models of PH, *MAGED1* is overexpressed due to an increase in translation mediated by YTHDF1. Knockout and knockdown experiments

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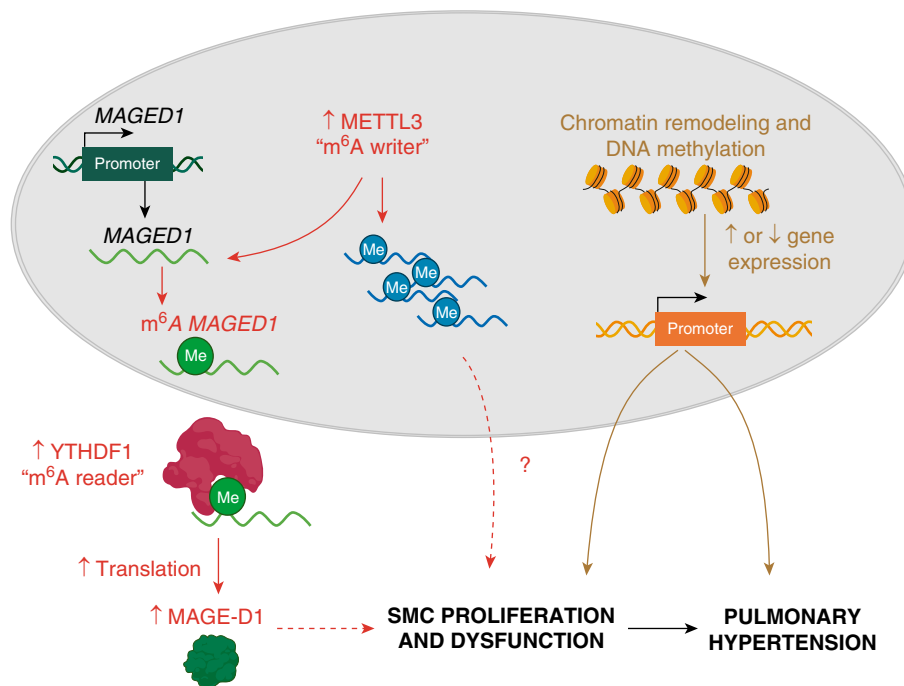


Figure 1. Epigenetic mechanisms regulating pulmonary hypertension. Several epigenetic mechanisms have been implicated in the development and progression of pulmonary hypertension, including chromatin remodeling and DNA methylation, impacting the expression of various genes. Hu and colleagues describe a new epigenetic pathway consisting of the increase in mRNA methylation (m⁶A) and the YTHDF1-mediated increase in the translation of *MAGED1*. This pathway enhances smooth muscle cell proliferation, vascular remodeling, and pulmonary hypertension. The potential role of other transcripts targeted by the m⁶A/YTHDF1 pathway remains unknown. Created with Biorender.com. Me = methylation; SMC = smooth muscle cell.

provide supportive evidence that *MAGED1* promotes detrimental SMC proliferation and vascular remodeling. In cancer, *MAGED1* expression is significantly reduced, whereas *MAGED1* has a marked antitumorigenesis effect by inhibiting cell proliferation (10). These differences suggest that although RNA methylation and YTHDF1 promote development of both PH and cancer, the transcripts targeted by this mechanism are likely cell-type and disease specific.

Although giving compelling loss-of-function, the work by Hu and colleagues presents the inherent limitation of relying exclusively on global knockouts of *YTHDF1* and *MAGED1*, limiting our understanding of the specific effect of these proteins in the main cell types involved in PH development (e.g., endothelial cell vs. SMC). The development of conditional and inducible knockout mouse models would allow for a clear characterization of the impact of alteration of the m⁶A/YTHDF1/*MAGED1* axis in a cell-specific manner, without the possible developmental and disease-independent impact of noninducible global knockouts. Future experiments should further investigate the full spectrum of transcripts and their downstream pathways impacted by global and cell-type-specific inhibition of m⁶A writer (METTL3) and reader (YTHDF1) *in vivo*. Finally, the identification of several epigenetic pathways involved in PH, namely, DNA methylation, chromatin remodeling, and mRNA modifications, interrogates the interrelationship and interdependence of these mechanisms (11, 12).

From a therapeutic perspective, the findings from Hu and colleagues open a new therapeutic avenue for PH and beyond. Although the findings are promising, several other steps will be necessary prior to clinical translation, including the following (13): 1) confirm the feasibility to exploit m⁶A reader modulators alone or in

combination with other therapies for treating PH, such as that recently published for other epigenetic readers (BET [Bromodomain and extraterminal motif]) (14); 2) identify and characterize appropriate biomarkers to evaluate efficacy of RNA methylation-targeted approaches in patients with PH; and 3) establish the impact beyond the lungs, as PH is now recognized as a systemic disease (15). ■

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⊕ Prospective Cohort Studies of Major Disorders Can Facilitate Phenotyping for Sleep Apnea

A few decades ago, several prospective cohort studies were initiated with the support of epidemiologists and often focused on a specific disorder or risk factors (1). One example of such a cohort study is MESA (Multi-Ethnic Study of Artherosclerosis), which was designed to investigate risk factors for cardiovascular diseases (2). Assessments of cardiovascular disease, including severity, risk factors, and comorbidities, were carefully chosen according to the methodology and physiological knowledge available 20 years ago. The hypotheses in the studies begun decades ago are borne out by the results that have been published more recently (3). However, sleep recording was not part of the original methodology. Today, however, this information can be added, as was done in the study by Borker and colleagues (pp. 1173–1182) in this issue of the *Journal* (4); this addition offers new insights on the meaning of sleep apnea in medicine.

Today, it is increasingly recognized that sleep apnea is more than a diagnostic entity; it has been found to be a contributor to many cardiovascular, respiratory, and metabolic disorders (5). And vice versa: sleep medicine also views cardiovascular, respiratory, and metabolic disorders as contributors to sleep apnea. Respiratory

events during sleep such as obstructive, mixed, or central apneas and obstructive and central hypopneas, or even less well-defined events such as respiratory-related arousal or airflow flattening, are carefully scored in sleep centers and then counted and used as metrics for sleep apnea severity. It is now recognized that apnea–hypopnea index is not an adequate measure of severity. Counting oxygen desaturations and calculating the oxygen desaturation index is not much better, but instead distracts from the core problem of pathophysiological mechanisms.

Sleep apnea, as defined by apnea–hypopnea index (or oxygen desaturation index), is heterogenous. Sleep apnea may be the cause of cardiovascular, respiratory, or metabolic disorders, or it may be the consequence of these. For an appropriate treatment, this does not matter much. However, for an understanding of pathophysiological pathways, and thus for prevention, this is important. The assessment of sleep apnea can be regarded as being similar to that used for high blood pressure. It is a sign, and a finding, that a basic physiological regulation (of blood pressure or, respectively, of respiratory stability during sleep) is losing its physiological boundaries. Different parameters are used to characterize the regulation. All these parameters are recorded by polysomnography and can be analyzed by exploiting the recordings more (6). Not only the number, but also the duration, of respiratory events is important for phenotyping patients (7). Analyzing subgroups related to event duration may provide surprising results (8). Event duration may even allow a prediction of mortality, as recently reported based on a sleep cohort study (9).

To change the perspective on sleep-disordered breathing and change the view on the pathophysiology of sleep apnea, it is valuable

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