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The potential roles of RNA N6-methyladenosine in atherosclerosis

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Abstract. – OBJECTIVE: Atherosclerosis, characterized by endothelial injury, multicellular involvement, chronic inflammation, and lipid deposition, can lead to acute cardiovascular events. N^6-methyladenosine (m^{6}A) is the most abundant, prevalent RNA modification in mammalian cells. m^{6}A, a reversible modification, can be catalyzed by m^{6}A methyltransferase complexes (writers), reverted by demethylases (erasers), and recognized by m^{6}A-binding proteins (readers). Emerging evidence suggests that m^{6}A modification plays a significant role in regulating many biological and cellular processes in atherosclerosis. In this review, we highlight the biological function of m^{6}A modification and give a brief perspective on its future applications in atherosclerosis.

MATERIALS AND METHODS: This is a narrative review. The literature search strategy for indexed Scopus articles was performed randomly using PubMed and MEDLINE as the primary sources. No specific term was used.

RESULTS: As the mechanism of the relationship between inflammatory response and atherosclerosis, m^{6}A has become a new focus in the study of clinical treatment strategies for atherosclerosis. METTL14-dependent m^{6}A modification may be a target for atherosclerosis therapy. A variety of m^{6}A regulatory factors promote the progression of atherosclerosis by regulating polarization and inflammation of macrophages. WTAP and METTL14 can affect the phenotypic modulation of VSMCs through m^{6}A modification.

CONCLUSIONS: The existence of m^{6}A in cardiovascular transcripts is necessary to maintain cardiac function, and the level of m^{6}A modification is increased in a variety of atherosclerotic vascular cells, indicating that m^{6}A modification is involved in the pathophysiological process of atherosclerosis. m^{6}A modification plays an important character in atherosclerosis.

Key Words:
Atherosclerosis, N^6-Methyladenosine, Inflammation, Lipid metabolism, Vascular calcification.

Abbreviations

N6-methyladenosine: m6A; methyltransferase-like 3: METTL3; methyltransferase-like 14: METTL14; methyltransferase-like 16: METTL16; Wilms tumor 1-associating protein: WTAP; RNA-binding motif protein 15: RBM15; methyltransferase complex: MTC: myeloid ecotropic viral integration site 1; MEIS1: human artery smooth muscle cells: HASMCs; abdominal aortic aneurysm: AAA: fat mass and obesity-associated protein: FTO; bone marrow differentiation factor 88: My88; signal transducer and activator of transcription 1: STAT1; lipopolysaccharide: LPS; vascular smooth muscle cells: VSMCs; insulin-like growth factor 2: IGF2; Interferon regulatory factor-1: IRF-1; Macrophage scavenger receptor 1: MSR1; dead box protein 5: DDX5; mono-(2-ethylhexyl) phthalate: MEHP; scavenger receptor B type 1: SR-B1; nonalcoholic fatty liver disease: NAFLD; peroxisome proliferator-activated receptor γ: PPARγ; fatty acid synthase: FAS; stearoyl-CoA desaturase 1; SCD1: single nucleotide polymorphism: SNP; total panax notoginseng saponins: TPNS.

Introduction

Atherosclerosis is a chronic inflammatory and lipid metabolic disorder disease which is a primary cause of vascular death worldwide[1]. Understanding the potential mechanisms that drive the pathological development of atherosclerosis are indispensable for solving clinical problems and developing new therapeutic strategies. Atherosclerosis is considered to occur in large and medium arteries triggered by risk factors which lead to endothelial dysfunction and atherosclerotic plaques[1]. N^6-methyladenosine (m^{6}A) is the most prevalent and abundant post-transcriptional

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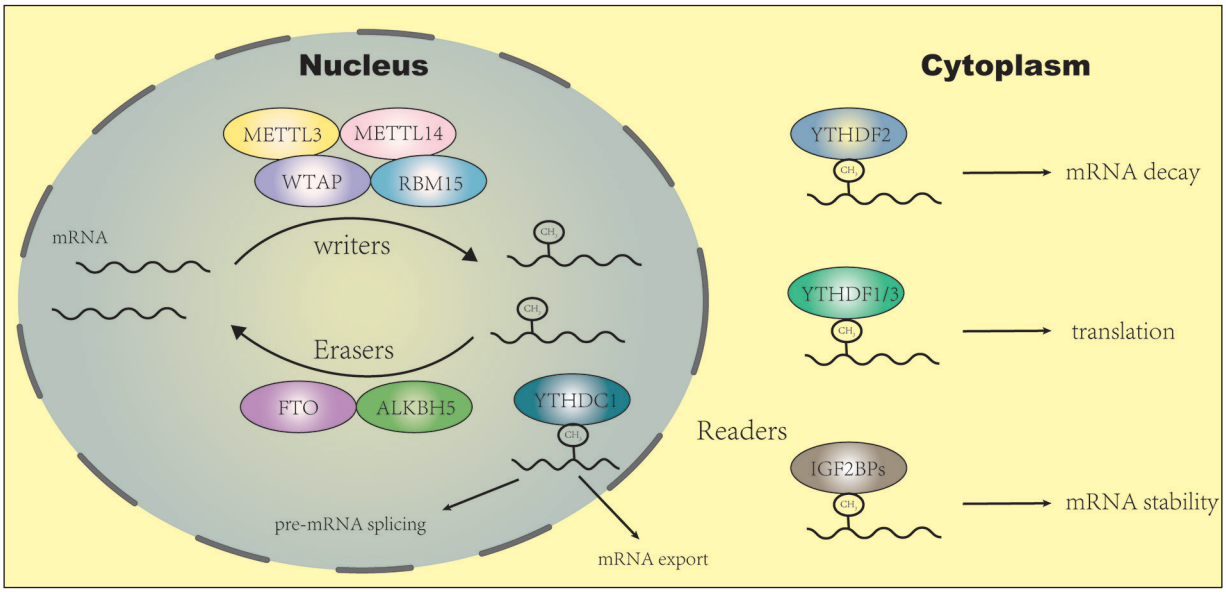

Figure 1. m^{6}A modification by m6A "writers", "erasers" and "readers". m^{6}A modification mediated by regulators: "writers" (METTL3, METTL14, WTAP, RBM15), "erasers" (FTO, ALKBH5) and "readers" (YTHDC1, YTHDF1, YTHDF2, YTHDF3, IGF2BPs).

modifications in eukaryotic mRNA[2]. It was firstly reported in the 1970s[3], the function of m^{6}A stayed unclear until recent years. Subsequent studies[4-6] showed that m^{6}A peak sites mainly existed in the RRACH consensus sequence (R = A, G; H = A, C, U), which is located around stop codons and in 3' UTR (untranslated region) and within long internal exons. The m^{6}A levels of modification in mRNA were found to be dynamic and reversible, by varying in development of cellular process and in regulation of gene expression[7].

Recently, the role of m^{6}A modification in atherosclerosis has been increasingly recognized[8]. More importantly, epigenetic processes, m^{6}A modification in particular, have specific "writers" (methyltransferases), "erasers" (demethylases) and "readers" (m^{6}A binding proteins)[9]. Increasingly epigenetic evidence[8,9] support that RNA methylation plays a critical role in atherosclerosis, giving a new perspective to treat atherosclerosis by intervening these epigenetic processes.

In this review, we highlight the biological function of m6A modification as well as the relationships between the m^{6}A modification and pathological process of atherosclerosis, to provide new points for the study of molecules targeting atherosclerosis and its future applications.

m6A "Writers", "Erasers" and "Readers"

Similar to dynamic modifications of histone protein and DNA methylation, m^{6}A modification is mediated by three types of regulators: "writers" (methyltransferases), "erasers" (demethylases) and "readers" (m^{6}A binding proteins), respectively (Figure 1).

Cross-talk among writers, readers and erasers of m^{6}A modification is involved in the regulation of RNA life cycle including pre-mRNA splicing, pri-miRNA processing, RNA translation and RNA degradation.

Writers

N^6-methyladenosine modification usually refers to the methylation of the sixth nitrogen of adenosine in mRNA. The m^{6}A methyltransferases consist of methyltransferase-like 3 (METTL3)[10], methyltransferase-like 14 (METTL14)[11], methyltransferase-like 16 (METTL16)[12], Wilms tumor 1-associating protein (WTAP)[13], KIAA1429[14], RNA-binding motif protein 15 (RBM15), and its paralogue RBM15B[15]. m^{6}A modification is catalyzed by a methyltransferase complex (MTC) which is mainly composed of METTL3, METTL14 and WTAP. METTL3 is the core catalytic component in the complex, whereas METTL14 plays an active role that forms a stable structure with METTL3 and contributes to substrate recognition[16]. WTAP, which is known to interact with METTL3 and METTL14, co-localizes with METTL3-METTL14 into nuclear speckles and functions as an adaptor to recruit MTC to mRNA targets[17]. Besides, METTL16, KIAA1429, RBM15 and RBM15B are the

newly found methyltransferases of the m^{6}A complex, but their functions are not clear.

METTL3

METTL3 has been identified to be involved in various biological processes associated with the development and progression of diseases. METTL3 can promote the proliferation and transition of cardiac fibroblasts and collagen accumulation, providing a molecular target for the regulation of fibrosis and the related cardiac diseases[18]. Hypoxic stress upregulating the level of METTL3-mediated m^{6}A modification adjust the proliferation, migration, viability of endothelial cells and tube formation *in vitro*[19]. METTL3 can enhance the M1 macrophage polarization and potentially playing as an anti-inflammatory role *via* targeting the coding sequence and 3'-untranslated regions of STAT1, a transcription factor promoting M1 macrophage polarization, to install the m^{6}A modification[20]. METTL3 methylating TFEB, a transcription factor controlling lysosomal biogenesis and autophagy genes, in the 3'-UTR inhibits autophagic flux and enhances apoptosis in H/R-treated cardiomyocytes[21]. METTL3-mediated m^{6}A methylation promotes the result of compensated cardiac hypertrophy and weakened m^{6}A leads to cardiomyocyte remodeling and dysfunction[22].

METTL14

Studies[23] have demonstrated that METTL14 was also related with the development of cardiovascular diseases. METTL14 promotes atherosclerotic vascular endothelial cell proliferation and invasion through regulating N^6-methyladenosine modified primary miR-19a. In pulmonary hypertension, myeloid ecotropic viral integration site 1 (MEIS1) mediates hypoxia-induced the proliferation and migration of pulmonary artery smooth muscle cells through METTL14/MEIS1/p21 signaling pathway[24]. Mechanistical study demonstrated that downregulated METTL14 in calcified arteries substantially abolishes the increase of indoxyl sulfate-induced m^{6}A modifications and the decrease of human artery smooth muscle cells (HASMCs) calcification[25]. In addition, comparing to healthy aortic tissues, m^{6}A modification significantly increased in abdominal aortic aneurysm (AAA). METTL14 has been proved to relate to inflammatory infiltrates and neovascularization in AAA[26].

Erasers

m^{6}A methylation is a dynamic and reversible RNA modification as discovered of two demethylases, fat mass and obesity-associated protein (FTO) and ALKBH5[27,28]. The two identified demethylases of FTO and ALKBH5 both belong to the alpha-ketoglutarate-dependent dioxygenase family. FTO was the first m^{6}A-associated demethylase to be discovered, and has an efficient oxidative demethylation activity[27]. FTO-dependent m^{6}A modification can contribute to human obesity and regulating energy homeostasis, its biological function as a demethylation in cardiovascular system is essential[30,31]. The second identified m^{6}A demethylase is ALKBH5, which has been shown to regulate mRNA export, RNA metabolism and the assembly of mRNA in nuclear speckles[28]. Moreover, demethylase ALKBH5 also plays the key roles in biological processes, such as cell cycle, stress response, apoptosis, and RNA metabolism[32].

Readers

The reader of m^{6}A methylation can recognize and bind with the m^{6}A-modified mRNA to regulate gene expression *via* modulating various processes, such as mRNA transcription, stability, splicing, nuclear export, and stability[33]. Different readers have different biological functions, members, including YTHDC1, YTHDF1, YTHDF2, YTHDF3, HNRNPA2B1, EIF3 and HNRNPC[34].

m^{6}A and Atherosclerosis (Figure 2)

m^{6}A and Inflammation

Various chronic inflammatory diseases can stimulate the occurrence and development of cardiovascular diseases. These abnormal biochemical characteristics are caused by the interaction of oxidative stress pathway, cytokines and renin-angiotensin system[35]. Inflammatory response is one of the main and basic risk factors in all stages of atherosclerosis[36]. As the specific mechanism of the relationship between inflammatory response and atherosclerosis has not been fully clarified, m^{6}A has become a new focus in the study of clinical treatment strategies for atherosclerosis.

Dysfunction of vascular endothelial cells is a key factor in the pathogenesis of atherosclerosis[37]. The TNF-α-induced high expression of METTL14 in endothelial cells promoted the translation of FOXO1 mRNA through YTHDF1 recognition, thus increasing the expression of adhesion molecules and mediating endothelial-monocyte adhesion. METTL14 gene knockout significantly inhibits the development of atherosclerosis, which proves the potential of METTL14 in the treatment

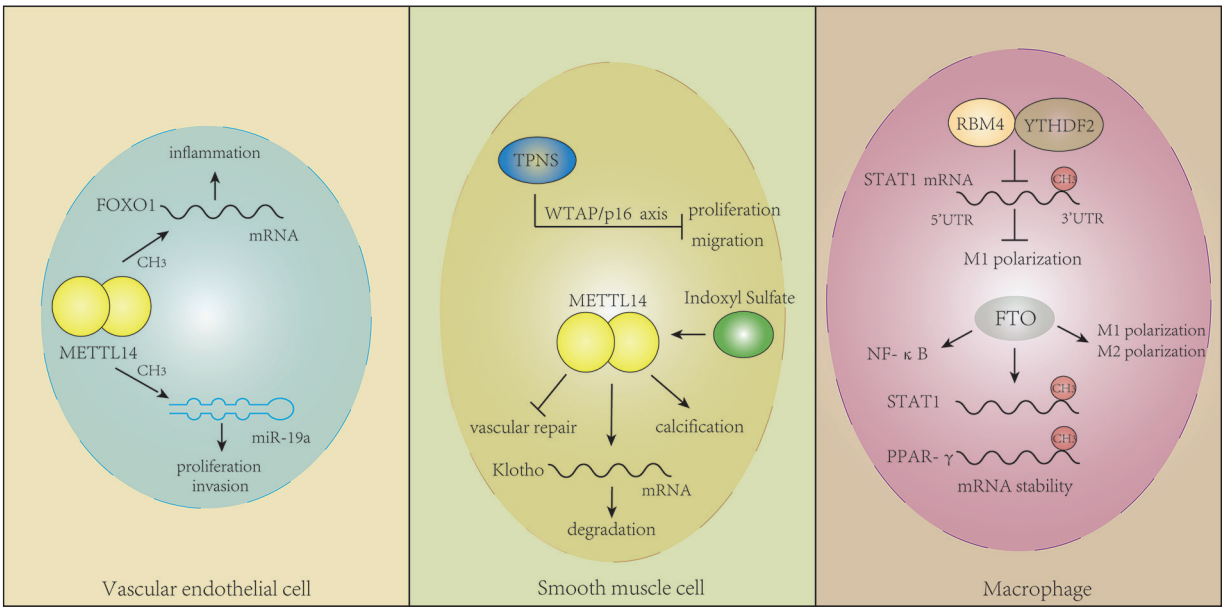

Figure 2. Biological process of m6A modification in different cells.

of atherosclerosis[38]. It has been shown[23] that METTL14 increases the m^{6}A modification of pri-miR-19a and promotes the processing of mature miR-19a, thus promoting the proliferation and invasion of atherosclerotic vascular endothelial cells. This means that METTL14-dependent m^{6}A modification may be a target for atherosclerosis therapy.

Scholars[39] have shown that METTL3 knockout can inhibit the production of inflammatory cytokines and the expression of various genes related to inflammatory response, mainly by changing the phosphorylation level of related signaling pathways, such as the splicing variant of bone marrow differentiation factor 88 (My88) in lipopolysaccharide-induced human pulpitis. It is suggested that m^{6}A may be involved in the pathophysiological process of atherosclerotic inflammation. METTL3 has been postulated to play a pro-inflammatory role by driving macrophages polarization toward pro-inflammatory M1 phenotype. METTL3 upregulates the expression of key transcription factor, such as STAT1, initiating pro-inflammatory macrophages polarization. In view of the key role of M1 macrophages in the pathogenesis of various inflammatory diseases, METTL3-STAT1-mediated macrophage polarization may lead to the occurrence and development of atherosclerosis[20].

Transforming macrophages into an inflammatory phenotype are closely related to the progression of atherosclerosis. METTL3 directly methylates the signal transducer and activator of transcription 1 (STAT1) mRNA in the CDS and 3'UTR regions leading to the increases of STAT1 mRNA stability and the promotion of the polarization of pro-inflammatory M1 macrophages[20]. It was demonstrated that RNA binding motif protein 4 (RBM4) interacted with reader YTHDF2 to reduce the level of m^{6}A modified STAT1 mRNA and inhibit the polarization of M1 macrophages induced by interferon-γ[40]. In addition, FTO gene knockout of m^{6}A demethylase inhibits the phosphorylation of key proteins in NF-κB signal pathway, such as IKK α/β, IκBα and p6, and reduces the mRNA stability of STAT1 and PPAR-γ through YTHDF2 involvement, thus hindering the polarization of macrophages[41]. Macrophage-mediated inflammation is an important mechanism in the development of atherosclerosis. Downregulation of YTHDF2 significantly increased the LPS-induced the expression of proinflammatory cytokines, such as IL-6, TNF-α, IL-1β, and IL-12 and activated MAPK and NF-κB signaling pathways in RAW 264.7 cells[42,43]. However, upregulation of METTL3 significantly attenuated the inflammatory response dependence on NF-κB signaling pathway in lipopolysaccharide (LPS)-induced macrophages[42,43]. All the above studies suggested that METTL3 and YTHDF2 may be the target of anti-inflammatory therapy. Circular RNAs (circRNAs) have been regarded as critical regulators in the progression of

atherosclerosis. Circ_0029589 silence inhibits the proliferation, migration and invasion of vascular smooth muscle cells (VSMCs) meanwhile induces the apoptosis of VSMCs by regulating miR-424-5p/ insulin-like growth factor 2 (IGF2) axis[44]. The levels of m^{6}A and METTL3 of has_circ_0029589 in macrophages of patients with acute coronary syndrome are increased. Interferon regulatory factor-1 (IRF-1) inhibits circ_0029589, by promoting the m^{6}A modification of circ_0029589, thus promoting macrophage pyrogenesis and inflammation of atherosclerosis[45]. Based on the above results, a variety of m^{6}A regulatory factors promote the progression of atherosclerosis by regulating polarization and inflammation of macrophages.

m^{6}A and Lipid Metabolism

Atherosclerosis is a chronic pathological process characterized by the gradual accumulation of lipids, cells and fibers on the arterial wall[46]. Macrophages play a key role in all stages of atherosclerosis. When arterial walls are damaged, the monocytes differentiate into macrophages in the intima. Macrophages absorb and metabolize excessive ox-LDL, resulting in the deposition of esterified cholesterol in the cytoplasm and the generation of foam cells. Macrophage scavenger receptor 1 (MSR1) and CD36 are highly expressed on the surface of macrophages and the main receptors for binding, uptake and clearance of ox-LDL[47]. Studies have shown that ox-LDL can induce the expression of dead box protein 5 (DDX5), and then, promote the expression of MSR1 in macrophages. DDX5 is upregulated in macrophage treated with ox-LDL, thereby inhibiting the methyltransferase activity of METTL3 on MSR1 mRNA, maintaining the stability of MSR1 mRNA and promoting lipid uptake. This study[48] suggests that DDX5 inhibits the activity of METTL3 and the binding of METTL3 to MSR1 mRNA. However, the specific mechanism of DDX5 inhibiting the activity of METTL3 is unclear, and how DDX5 affects secretion of pro-inflammatory factors from macrophages remains to be further studied.

Macrophages are important immune cells in the necrotic core area of atherosclerotic plaque, coordinating a series of inflammatory processes. Cholesterol efflux from macrophages plays an important role in reversing the transport of cholesterol in the arterial wall. Study[49] have shown that a major bioactive metabolite, mono-(2-ethylhexyl) phthalate (MEHP), increased the m^{6}A modification of scavenger receptor B type 1 (SR-B1) through reducing the METTL14 expression in macrophage Raw 264.7 cells, thus suppressing SR-B1 expression and leading to the formation of foam cells.

The liver is the main site of endogenous lipid production and plays an important role in lipid metabolism. Curcumin can improve the LPS-induced liver lipid metabolism disorder by increasing the mRNA expression of METTL3 and METTL14[50]. Animal experiments found that FTO is increased in the liver, while nonalcoholic fatty liver disease (NAFLD) and the overexpression of FTO could promote inflammation and lead to excessive accumulation of lipids in hepatocytes[51]. LPS can increase the expression of fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1) and other lipid metabolism-related enzymes through FTO-mediated m^{6}A hypomethylation[52]. In addition, overexpression of METTL3 can inhibit the expression of peroxisome proliferator-activated receptor γ (PPARγ), reducing lipid deposition and TG content in adipocytes[53]. m^{6}A methylation can also regulate the stability and half-life of peroxisome proliferator-activated receptor α (PPARα) mRNA, an important gene of liver lipid metabolism, thus regulating the transcription and translation of PPARα gene through YTHDF2 binding to PPAR-α and affecting the circadian rhythm of lipid metabolism[54]. It has been found that many m^{6}A- single nucleotide polymorphism (SNP) are associated with TG, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) levels, some of which are significantly associated with HDL-C and TG, suggesting that, in addition to liver lipid metabolism disorders, m^{6}A methylation may also be involved in lipid metabolism in other tissues and organs, such as hyperlipidemia and atherosclerotic diseases[55]. The precise mechanism of how m^{6}A methylation influences the development of lipid disorders need to be further clarified.

m^{6}A and Vascular Calcification

Vascular calcification, particularly coronary artery calcification, is a major independent risk factor associated with cardiovascular events and death. Phenotypic changes of vascular smooth muscle cells (VSMCs) also promote the progression of cardiovascular disease. The total panax notoginseng saponins (TPNS) can regulate the WTAP/p16 signal axis through m^{6}A modification, thus inhibiting the balloon catheter injury-induced proliferation, migration and intimal hyperplasia of VSMCs in rat carotid arteries, suggest-

Table I. Multiple functions exerted by m6A regulators in various cells.

Cell type	Molecule	Expression	Target gene	m6A Levels	Main Functions	References
EC	METTL14	upregulated	miR-19a	increased	promote the proliferation and invasion	Zhang et al[20], 2020
EC	METTL14	upregulated	FOXO1	increased	induce inflammatory response and plaque formation	Jian et al[40], 2020
Macrophage	METTL14	downregulated	SR-B1	decreased	regulate cholesterol efflux	Park et al[41], 2020
Macrophage	METTL3	upregulated	STAT1	increased	facilitate M1 macrophage	Liu et al[56], 2019
Macrophage	RBM4	upregulated	STAT1	/	polarization regulate glycolysis and M1 macrophage polarization	Huang et al[25], 2020
Macrophage	FTO	downregulated	STAT1, PPAR-γ	/	inhibit both M1 and M2 macrophage polarization	Gu et al[41], 2020
Macrophage	YTHDF2	upregulated	/	/	regulate the expression of pro-inflammatory cytokines	Yu et al[42], 2019
Macrophage	METTL3	upregulated	/	/	attenuate the inflammatory response	Wang et al[43], 2019
Macrophage	METTL3	upregulated	circ_0029589	increased	promote macrophage pyroptosis	Guo et al[45], 2020
SMC	WTAP	upregulated	/	/	inhibit the viability, proliferation, and migration	Zhu et al[56], 2020
SMC	METTL14	upregulated	/	increased	increase the calcification and decrease the vascular repair function	Chen et al[25], 2019

ing that m^{6}A modification-mediated regulation of gene expression may be a potential target for arterial restenosis[56]. Moreover, vascular calcification in VSMCs increases the risk of atherosclerotic plaque rupture[57]. METTL14-catalyzed klotho degradation induced by indole sulfate, while the downregulation of the expression of METTL14 can reduce calcification and enhance vascular repair function[25]. The above studies suggest that WTAP and METTL14 can affect the phenotypic modulation of VSMCs through m^{6}A modification and can be used as potential targets for the treatment of atherosclerosis.

Conclusions

The existence of m^{6}A in cardiovascular transcripts is necessary to maintain cardiac function, and the level of m^{6}A modification is increased in a variety of atherosclerotic vascular cells, indicating that m^{6}A modification is involved in the pathophysiological process of atherosclerosis. At present, m^{6}A modification in cardiovascular diseases is mainly focused on methylase, which regulates the targets and signal pathways. In summary, m^{6}A modification plays an important character in atherosclerosis.

Future studies should focus on how crosstalk between methylases and demethylases affects protein expression, and how m^{6}A binding proteins specifically recognize the role of m^{6}A modification in the initiation and development of atherosclerosis. In addition, whether m^{6}A modification is possible to reverse the process by which some special cells, such as macrophages and VSMCs, undergo phenotypic changes remains to be further studied.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1) Herrington W, Lacey B, Sherliker P, Armitage J, and Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circ Res 2016; 118: 535-546.
2) Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, Weng X, Chen K, Shi H, and He C. N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell 2015; 161: 1388-1399.
3) Desrosiers R, Friderici K, and Rottman F. Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc Natl Acad Sci U S A 1974; 71: 3971-3975.
4) Csepany T, Lin A, Baldick CJ, Jr., and Beemon K. Sequence specificity of mRNA N6-adenosine methyltransferase. J Biol Chem 1990; 265: 20117-20122.
5) Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, and Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 2012; 149: 1635-1646.
6) Harper JE, Miceli SM, Roberts RJ, and Manley JL. Sequence specificity of the human mRNA N6-adenosine methylase in vitro. Nucleic Acids Res 1990; 18: 5735-5741.
7) Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, and Rechavi G. Topology of the human and mouse m^{6}A RNA methylomes revealed by m^{6}A-seq. Nature 2012; 485: 201-206.
8) Quiles-Jimenez A, Gregersen I, Mittelstedt Leal de Sousa M, Abbas A, Kong XY, Alseth I, Holm S, Dahl TB, Skagen K, Skjelland M, Aukrust P, Bjoras M, and Halvorsen B. N6-methyladenosine in RNA of atherosclerotic plaques: An epitranscriptomic signature of human carotid atherosclerosis. Biochem Biophys Res Commun 2020: 533; 631-637.
9) Zaccara S, Ries RJ, and Jaffrey SR. Reading, writing and erasing mRNA methylation. Nat Rev Mol Cell Biol 2019; 20: 608-624.
10) Bokar JA, Shambaugh ME, Polayes D, Matera AG, and Rottman FM. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA 1997; 3: 1233-1247.
11) Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, Dai Q, Chen W, and He C. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol 2014; 10: 93-95.
12) Fitzsimmons CM, and Batista PJ. It's complicated... m(6)A-dependent regulation of gene expression in cancer. Biochim Biophys Acta Gene Regul Mech 2019; 1862: 382-393.
13) Su J, Li SJ, Chen ZH, Zeng CH, Zhou H, Li LS, and Liu ZH. Evaluation of podocyte lesion in patients with diabetic nephropathy: Wilms' tumor-1 protein used as a podocyte marker. Diabetes Res Clin Pract 2010; 87: 167-175.
14) Schwartz S, Mumbach MR, Jovanovic M, Wang T, Maciag K, Bushkin GG, Mertins P, Ter-Ovanesyan D, Habib N, Cacchiarelli D, Sanjana NE, Freinkman E, Pacold ME, Satija R, Mikkelsen TS, Hacohen N, Zhang F, Carr SA, Lander ES, and Regev A. Perturbation of m^{6}A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. Cell Rep 2014; 8: 284-296.
15) Patil DP, Chen CK, Pickering BF, Chow A, Jackson C, Guttman M, and Jaffrey SR. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature 2016; 537: 369-373.
16) Wang P, Doxtader KA, and Nam Y. Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases. Mol Cell 2016; 63: 306-317.
17) Ping XL, Sun BF, Wang L, Xiao W, Yang X, Wang WJ, Adhikari S, Shi Y, Lv Y, Chen YS, Zhao X, Li A, Yang Y, Dahal U, Lou XM, Liu X, Huang J, Yuan WP, Zhu XF, Cheng T, Zhao YL, Wang X, Rendtlew Danielsen JM, Liu F, and Yang YG. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res 2014; 24: 177-189.
18) Li T, Zhuang Y, Yang W, Xie Y, Shang W, Su S, Dong X, Wu J, Jiang W, Zhou Y, Li Y, Zhou X, Zhang M, Lu Y, and Pan Z. Silencing of METTL3 attenuates cardiac fibrosis induced by myocardial infarction via inhibiting the activation of cardiac fibroblasts. FASEB J 2021; 35: e21162.
19) Yao MD, Jiang Q, Ma Y, Liu C, Zhu CY, Sun YN, Shan K, Ge HM, Zhang QY, Zhang HY, Yao J, Li XM, and Yan B. Role of METTL3-Dependent N(6)-Methyladenosine mRNA Modification in the Promotion of Angiogenesis. Mol Ther 2020; 28: 2191-2202.
20) Liu Y, Liu Z, Tang H, Shen Y, Gong Z, Xie N, Zhang X, Wang W, Kong W, Zhou Y, Fu Y. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 facilitates M1 macrophage polarization through the methylation of STAT1 mRNA. Am J Physiol Cell Physiol 2019; 317: C762-C775.
21) Song H, Feng X, Zhang H, Luo Y, Huang J, Lin M, Jin J, Ding X, Wu S, Huang H, Yu T, Zhang M, Hong H, Yao S, Zhao Y, Zhang Z. METTL3 and ALKBH5 oppositely regulate m(6)A modification of TFEB mRNA, which dictates the fate of hypoxia/reoxygenation-treated cardiomyocytes. Autophagy 2019; 15: 1419-1437.
22) Dorn LE, Lasman L, Chen J, Xu X, Hund TJ, Medvedovic M, Hanna JH, van Berlo JH, Accornero F. The N(6)-Methyladenosine mRNA Methylase METTL3 Controls Cardiac Homeostasis and Hypertrophy. Circulation 2019; 139: 533-545.
23) Zhang BY, Han L, Tang YF, Zhang GX, Fan XL, Zhang JJ, Xue Q, Xu ZY. METTL14 regulates M^{6}A

methylation-modified primary miR-19a to promote cardiovascular endothelial cell proliferation and invasion. European Rev Med Pharmacol Sci 2020; 24: 7015-7023.

24) Yao MZ, Ge XY, Liu T, Huang N, Liu H, Chen Y, Zhang Z, Hu CP. MEIS1 regulated proliferation and migration of pulmonary artery smooth muscle cells in hypoxia-induced pulmonary hypertension. Life Sci 2020; 255: 117822.

25) Chen J, Ning Y, Zhang H, Song N, Gu Y, Shi Y, Cai J, Ding X, Zhang X. METTL14-dependent m^{6}A regulates vascular calcification induced by indoxyl sulfate. Life Sci 2019; 239: 117034.

26) He Y, Xing J, Wang S, Xin S, Han Y, Zhang J. Increased m^{6}A methylation level is associated with the progression of human abdominal aortic aneurysm. Ann Transl Med 2019; 7: 797.

27) Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011; 7: 885-887.

28) Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vagbo CB, Shi Y, Wang WL, Song SH, Lu Z, Bosmans RP, Dai Q, Hao YJ, Yang X, Zhao WM, Tong WM, Wang XJ, Bogdan F, Furu K, Fu Y, Jia G, Zhao X, Liu J, Krokan HE, Klungland A, Yang YG, He C. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 2013; 49: 18-29.

29) Su R, Dong L, Li C, Nachtergaele S, Wunderlich M, Qing Y, Deng X, Wang Y, Weng X, Hu C, Yu M, Skibbe J, Dai Q, Zou D, Wu T, Yu K, Weng H, Huang H, Ferchen K, Qin X, Zhang B, Qi J, Sasaki AT, Plas DR, Bradner JE, Wei M, Marcucci G, Jiang X, Mulloy JC, Jin J, He C, Chen J. R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. Cell 2018; 172: 90-105 e123.

30) Boissel S, Reish O, Proulx K, Kawagoe-Takaki H, Sedgwick B, Yeo GS, Meyre D, Golzio C, Molinari F, Kadhom N, Etchevers HC, Saudek V, Farooqi IS, Froguel P, Lindahl T, O'Rahilly S, Munnich A, Colleaux L. Loss-of-function mutation in the dioxygenase-encoding FTO gene causes severe growth retardation and multiple malformations. Am J Hum Genet 2009; 85: 106-111.

31) Yang J, Loos RJ, Powell JE, Medland SE, Speliotes EK, Chasman DI, Rose LM, Thorleifsson G, Steinthorsdottir V, Magi R, Waite L, Smith AV, Yerges-Armstrong LM, Monda KL, Hadley D, Mahajan A, Li G, Kapur K, Vitart V, Huffman JE, Wang SR, Palmer C, Esko T, Fischer K, Zhao JH, Demirkan A, Isaacs A, Feitosa MF, Luan J, Heard-Costa NL, White C, Jackson AU, Preuss M, Ziegler A, Eriksson J, Kutalik Z, Frau F, Nolte IM, Van Vliet-Ostaptchouk JV, Hottenga JJ, Jacobs KB, Verweij N, Goel A, Medina-Gomez C, Estrada K, Bragg-Gresham JL, Sanna S, Sidore C, Tyrer J, Teumer A, Prokopenko I, Mangino M, Lindgren CM, Assimes TL, Shuldiner AR, Hui J, Beilby JP, McArdle WL, Hall P, Haritunians T, Zgaga L, Kolcic I, Polasek O, Zemunik T, Oostra BA, Junttila MJ, Gronberg H, Schreiber S, Peters A, Hicks AA, Stephens J, Foad NS, Laitinen J, Pouta A, Kaakinen M, Willemsen G, Vink JM, Wild SH, Navis G, Asselbergs FW, Homuth G, John U, Iribarren C, Harris T, Launer L, Gudnason V, O'Connell JR, Boerwinkle E, Cadby G, Palmer LJ, James AL, Musk AW, Ingelsson E, Psaty BM, Beckmann JS, Waeber G, Vollenweider P, Hayward C, Wright AF, Rudan I, Groop LC, Metspalu A, Khaw KT, van Duijn CM, Borecki IB, Province MA, Wareham NJ, Tardif JC, Huikuri HV, Cupples LA, Atwood LD, Fox CS, Boehnke M, Collins FS, Mohlke KL, Erdmann J, Schunkert H, Hengstenberg C, Stark K, Lorentzon M, Ohlsson C, Cusi D, Staessen JA, Van der Klauw MM, Pramstaller PP, Kathiresan S, Jolley JD, Ripatti S, Jarvelin MR, de Geus EJ, Boomsma DI, Penninx B, Wilson JF, Campbell H, Chanock SJ, van der Harst P, Hamsten A, Watkins H, Hofman A, Witteman JC, Zillikens MC, Uitterlinden AG, Rivadeneira F, Zillikens MC, Kiemeney LA, Vermeulen SH, Abecasis GR, Schlessinger D, Schipf S, Stumvoll M, Tonjes A, Spector TD, North KE, Lettre G, McCarthy MI, Berndt SI, Heath AC, Madden PA, Nyholt DR, Montgomery GW, Martin NG, McKnight B, Strachan DP, Hill WG, Snieder H, Ridker PM, Thorsteinsdottir U, Stefansson K, Frayling TM, Hirschhorn JN, Goddard ME, Visscher PM. FTO genotype is associated with phenotypic variability of body mass index. Nature 2012; 490: 267-272.

32) Shah A, Rashid F, Awan HM, Hu S, Wang X, Chen L, and Shan G. The DEAD-Box RNA Helicase DDX3 Interacts with m(6)A RNA Demethylase ALKBH5. Stem Cells Int 2017; 2017: 8596135.

33) Niu Y, Zhao X, Wu YS, Li MM, Wang XJ, Yang YG. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinformatics 2013; 11: 8-17.

34) Dai D, Wang H, Zhu L, Jin H, Wang X. N6-methyladenosine links RNA metabolism to cancer progression. Cell Death Dis 2018; 9: 124.

35) Fiechter M, Haider A, Bengs S, Maredziak M, Burger IA, Roggo A, Portmann A, Warnock GI, Schade K, Treyer V, Becker AS, Messerli M, Felten EV, Benz DC, Fuchs TA, Grani C, Pazhenkottil AP, Buechel RR, Kaufmann PA, aGebhard C. Sex Differences in the Association between Inflammation and Ischemic Heart Disease. Thromb Haemost 2019; 119: 1471-1480.

36) Ketelhuth DFJ, Lutgens E, Back M, Binder CJ, Van den Bossche J, Daniel C, Dumitriu IE, Hoefer I, Libby P, O'Neill L, Weber C, Evans PC. Immunometabolism and atherosclerosis: perspectives and clinical significance: a position paper from the Working Group on Atherosclerosis and Vascular Biology of the European Society of Cardiology. Cardiovasc Res 2019; 115: 1385-1392.

37) Gimbrone MA, Garcia-Cardena G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circ Res 2016; 118: 620-636.

38) Jian D, Wang Y, Jian L, Tang H, Rao L, Chen K, Jia Z, Zhang W, Liu Y, Chen X, Shen X, Gao C, Wang S, Li M. METTL14 aggravates endothelial inflammation and atherosclerosis by increasing

FOXO1 N6-methyladeosine modifications. Theranostics 2020; 10: 8939-8956.

39) Feng Z, Li Q, Meng R, Yi B, Xu Q. METTL3 regulates alternative splicing of MyD88 upon the lipopolysaccharide-induced inflammatory response in human dental pulp cells. J Cell Mol Med 2018; 22: 2558-2568.

40) Huangfu N, Zheng W, Xu Z, Wang S, Wang Y, Cheng J, Li Z, Cheng K, Zhang S, Chen X, Zhu J. RBM4 regulates M1 macrophages polarization through targeting STAT1-mediated glycolysis. International immunopharmacology 2020; 83: 106432.

41) Gu X, Zhang Y, Li D, Cai H, Cai L, Xu Q. N6-methyladenosine demethylase FTO promotes M1 and M2 macrophage activation. Cellular signalling 2020; 69: 109553.

42) Yu R, Li Q, Feng Z, Cai L, Xu Q. m6A Reader YTHDF2 Regulates LPS-Induced Inflammatory Response. Int J Mol Sci 2019; 20.

43) Wang J, Yan S, Lu H, Wang S, Xu D. METTL3 Attenuates LPS-Induced Inflammatory Response in Macrophages via NF-kappaB Signaling Pathway. Mediators Inflamm 2019; 2019: 3120391.

44) Yu H, Zhao L, Zhao Y, Fei J, Zhang W. Circular RNA circ_0029589 regulates proliferation, migration, invasion, and apoptosis in ox-LDL-stimulated VSMCs by regulating miR-424-5p/IGF2 axis. Vascul Pharmacol 2020; 135: 106782.

45) Guo M, Yan R, Ji Q, Yao H, Sun M, Duan L, Xue Z, Jia Y. IFN regulatory Factor-1 induced macrophage pyroptosis by modulating m6A modification of circ_0029589 in patients with acute coronary syndrome. Int Immunopharmacol 2020; 86: 106800.

46) Yvan-Charvet L, Bonacina F, Guinamard RR, Norata GD. Immunometabolic function of cholesterol in cardiovascular disease and beyond. Cardiovasc Res 2019; 115: 1393-1407.

47) Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. J Biol Chem 2002; 277: 49982-49988.

48) Zhao W, Wang Z, Sun Z, He Y, Jian D, Hu X, Zhang W, Zheng L. RNA helicase DDX5 participates in oxLDL-induced macrophage scavenger receptor 1 expression by suppressing mRNA degradation. Exp Cell Res 2018; 366: 114-120.

49) Park MH, Jeong E, Choudhury M. Mono-(2-Ethylhexyl)phthalate Regulates Cholesterol Efflux via MicroRNAs Regulated m(6)A RNA Methylation. Chem Res Toxicol 2020; 33: 461-469.

50) Lu N, Li X, Yu J, Li Y, Wang C, Zhang L, Wang T, Zhong X. Curcumin Attenuates Lipopolysaccharide-Induced Hepatic Lipid Metabolism Disorder by Modification of m(6) A RNA Methylation in Piglets. Lipids 2018; 53: 53-63.

51) Guo J, Ren W, Li A, Ding Y, Guo W, Su D, Hu C, Xu K, Chen H, Xu X, Yang T, Jia W. Fat mass and obesity-associated gene enhances oxidative stress and lipogenesis in nonalcoholic fatty liver disease. Dig Dis Sci 2013; 58: 1004-1009.

52) Jia X, Nie Q, Lamont SJ, Zhang X. Variation in sequence and expression of the avian FTO, and association with glucose metabolism, body weight, fatness and body composition in chickens. Int J Obes (Lond) 2012; 36: 1054-1061.

53) Wang X, Zhu L, Chen J, Wang Y. mRNA m(6)A methylation downregulates adipogenesis in porcine adipocytes. Biochem Biophys Res Commun 2015; 459: 201-207.

54) Zhong X, Yu J, Frazier K, Weng X, Li Y, Cham CM, Dolan K, Zhu X, Hubert N, Tao Y, Lin F, Martinez-Guryn K, Huang Y, Wang T, Liu J, He C, Chang EB, Leone V. Circadian Clock Regulation of Hepatic Lipid Metabolism by Modulation of m(6)A mRNA Methylation. Cell Rep 2018; 25: 1816-1828 e1814.

55) Mo X, Lei S, Zhang Y, Zhang H. Genome-wide enrichment of m(6)A-associated single-nucleotide polymorphisms in the lipid loci. Pharmacogenomics J 2019; 19: 347-357.

56) Zhu B, Gong Y, Shen L, Li J, Han J, Song B, Hu L, Wang Q, Wang Z. Total Panax notoginseng saponin inhibits vascular smooth muscle cell proliferation and migration and intimal hyperplasia by regulating WTAP/p16 signals via m(6)A modulation. Biomed Pharmacother 2020; 124: 109935.

57) Durham AL, Speer MY, Scatena M, Giachelli CM, Shanahan CM. Role of smooth muscle cells in vascular calcification: implications in atherosclerosis and arterial stiffness. Cardiovasc Res 2018; 114: 590-600.