

TET2: A Novel Epigenetic Regulator and Potential Intervention Target for Atherosclerosis

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Atherosclerosis is the underlying cause of cardio-cerebrovascular disease. However, the mechanisms of atherosclerosis are still unclear. The modification of DNA methylation has an important role in atherosclerosis development. As a member of the Ten-eleven translocation (TET) family, TET methylcytosine dioxygenase 2 (*TET2*) can modify DNA methylation by catalyzing 5-methylcytosine to 5-hydroxymethylcytosine and mediate DNA demethylation. Recent findings suggest that *TET2* is related to the phenotype transformation of vascular smooth muscle cells, endothelial dysfunction, and inflammation of macrophage, the key factors of atherosclerosis. Therefore, *TET2* may be a potential target for atherosclerosis treatment. This review will elaborate the recent findings that suggest the role of *TET2* in atherosclerosis.

Keywords: *TET2*, DNA demethylation, atherosclerosis, inflammation

Introduction

ATHEROSCLEROSIS, A MULTIFACTORIAL disease that preferentially occurs in large and medium-size arteries, is the main pathological basis of cardio-cerebrovascular diseases. It is also a chronic inflammatory arterial vessel disease, which is initiated by the accumulation of lipoproteins in the intimal layer of the arterial vascular wall. It is closely related to endothelial cell (EC) dysfunction, vascular smooth muscle cell (VSMC) proliferation and phenotype transformation, and macrophage invasion (van Diepen *et al.*, 2013; Yu *et al.*, 2013a; Wang *et al.*, 2015; Studentova *et al.*, 2016), but its underlying regulatory mechanism is less clear.

In recent years, the role of epigenetics in atherosclerosis has become a topic of intense research interest, especially DNA methylation modification. Newman first proposed that DNA methylation modification is related to atherosclerosis (Newman, 1999). As a reverse process of DNA methylation, DNA demethylation is a passive or active process. In 2009, the human Ten-Eleven translocation methylcytosine dioxygenase 1 (*TET1*) was discovered to catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) (Tahiliani *et al.*, 2009). Then, other mouse TET family members (*Tet1*, *Tet2*, and *Tet3*) were found (Ito *et al.*, 2010).

The roles of *TET* in diseases, especially in cancer, have been widely studied in recent years (Rasmussen and Helin, 2016). Furthermore, studies also found that *TET2* was downregulated in atherosclerotic lesions and involved in pathophysiological progression of atherosclerosis (Li *et al.*, 2015). In this review, we summarized the studies on the protective role of *TET2* in atherosclerosis, transcription regulation of *TET2* gene expression, and activity regulation of *TET2* enzyme. Moreover, we aimed to provide a novel effective regulator and potential intervention target for atherosclerosis.

Protective Role of *TET2* in Atherosclerosis

Atherosclerosis is a chronic inflammatory disease that exhibits monocyte and foam cell infiltration, vascular EC dysfunction, and proliferation and phenotype transformation of VSMCs. Studies have found that almost all the above processes can be regulated by *TET2* (Table 1, Fig. 1).

TET2 regulates the transfer of smooth muscle cell phenotype

As a major component of atherosclerotic plaques, VSMCs play an important role in the initiation of atherosclerosis

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TABLE 1. ROLE OF TEN-ELEVEN TRANSLOCATION METHYLCYTOSINE DIOXYGENASE 2 IN ATHEROSCLEROSIS

Treatment	Object	<i>TET2</i> expression level	Pathway	Effect on AS	Reference
Ox-LDL	THP-1-derived macrophages	mRNA ↓ protein ↓	Autophagy (Beclin1 ↓, Lc3-II ↓)	AS ↑	Li <i>et al.</i> (2015)
Rapamycin	Smooth muscle cell	Protein ↓ 5hmC ↓	Knockdown of <i>TET2</i> can inhibit key contractile genes (<i>MYOCD</i> , <i>SRF</i>), upregulation of synthetic gene transcription (<i>KLF4</i>)	/	Liu <i>et al.</i> (2013)
Ox-LDL	HUVECs	5hmC ↓	Autophagy (Beclin1 ↓, Lc3 ↓), Autophagic flux ↓	AS ↑	Peng <i>et al.</i> (2016)
Low shear stress	Endothelial cell	protein ↓	Autophagy (Beclin1 ↓ and Lc3-II/I ↓)	AS ↑	Yang <i>et al.</i> (2016)
LPS	<i>Tet2</i> -deficient BMDC and macrophages and <i>Tet2</i> -silenced human dendritic cells	/	<i>Tet2</i> recruited Hdac2 and repressed transcription of <i>IL-6</i> through histone deacetylation. (independent of DNA methylation and hydroxymethylation)	AS ↓	Zhang <i>et al.</i> (2015)
Bone marrow transplantation	LDLR ^{-/-} chimeric mice with 10% of <i>TET2</i> -deficient HSPCs	/	Inflammation (NLRP3 inflammasome-mediated IL-1β production)	AS ↑	Fuster <i>et al.</i> (2017)

AS, atherosclerosis; BMDC, bone marrow-derived dendritic cells; IL-6, interleukin-6; HSPCs, hematopoietic stem progenitor cells; HUVECs, human umbilical vascular endothelial cell lines; SRF, serum response factor; TET2, ten-eleven translocation methylcytosine dioxygenase 2.

(Doran *et al.*, 2008), especially the phenotype change (from contractile to synthetic phenotype) in the development and progression of atherosclerotic lesions. The synthetic phenotype has a stronger proliferation and migration ability than the contractile phenotype. Moreover, it can transfer VSMCs from the tunica media to the intima, leading to atherosclerosis (Bennett *et al.*, 2016). Of course, the inhibition of VSMC phenotypic transformation is beneficial for atherosclerosis. *TET2* and 5hmC are enriched in contractile VSMCs, but reduced in synthetic phenotype VSMCs. In addition, *TET2* knockdown inhibits the expression of critical precontractual genes, such as myocardia (*MYOCD*) and serum response factor (*SRF*), and upregulates *KLF4* simultaneously. *TET2* and 5hmC are enriched in the CARG-rich regions of active SMC contractile promoters (*MYOCD*, *SRF*, and *MYH11*). In addition, the loss of *TET2* prevents rapamycin-induced VSMC differentiation, whereas *TET2* overexpression induces a contractile phenotype (Liu *et al.*, 2013). These findings suggested that *TET2* is a novel epigenetic regulator of VSMC phenotype transformation.

TET2 ameliorates vascular EC dysfunction

Endothelial damage and dysfunction is the starting point of vascular disease. The reduction of nitric oxide (NO) synthesis by endothelial nitric oxide synthase (eNOS) in vascular ECs is a marker of endothelial dysfunction (Hahn and Schwartz, 2008). As a crucial regulator of intracellular homeostasis, autophagy can serve as an antiatherosclerotic biological mechanism (Martinet and De Meyer, 2009). Autophagy inhibition can lead to EC dysfunction and atherosclerotic lesion formation. Studies have indicated that autophagy can be regulated by

TET2 through demethylation modification in atherosclerosis. Autophagy was assessed primarily through detecting the expression level of Beclin1 and microtubule-associated protein1 light chain 3 (LC3). Beclin1 is a component of the class III PI3K complex that is required for autophagy initiation. LC3 is involved in the formation of autophagosome membranes, including two interconvertible forms, LC3-I and LC3-II. The proportion of LC3-II or LC3-II/LC3-I was positively correlated with the number of autophagosomes (Lamb *et al.*, 2013). For example, low shear stress repressed EC autophagy through the downregulated expression of *TET2* and decrease of autophagy-related gene (*Beclin1*, *Lc3 II*) expression (Yang *et al.*, 2016). By contrast, autophagy level and autophagy-related gene (*Beclin1*, *Lc3 II*) expression were upregulated by *TET2* overexpression, and also with an improvement of endothelial function, with increased eNOS expression and release of NO. In the ApoE^{-/-} mice animal model, autophagy was upregulated by *TET2* overexpression, whereas autophagy level decreased by *TET2* silencing. Furthermore, in ox-LDL-treated vascular ECs, autophagy and autophagic flux were improved by *TET2* overexpression and decreased by *TET2* silencing (Peng *et al.*, 2016).

TET2 inhibits inflammation

Interestingly, DNMT1 in macrophages has been reported to aggravate chronic inflammation release and atherosclerosis development by weakening the PPAR-γ signaling in mice (Yu *et al.*, 2016). It indicated that inflammation may be regulated by methylation or demethylation. As an important demethylase, *TET2* can regulate autophagy not only in ECs, but also in macrophages (Li *et al.*, 2015). *TET2* selectively mediates the repression of interleukin-6 (IL-6) transcription

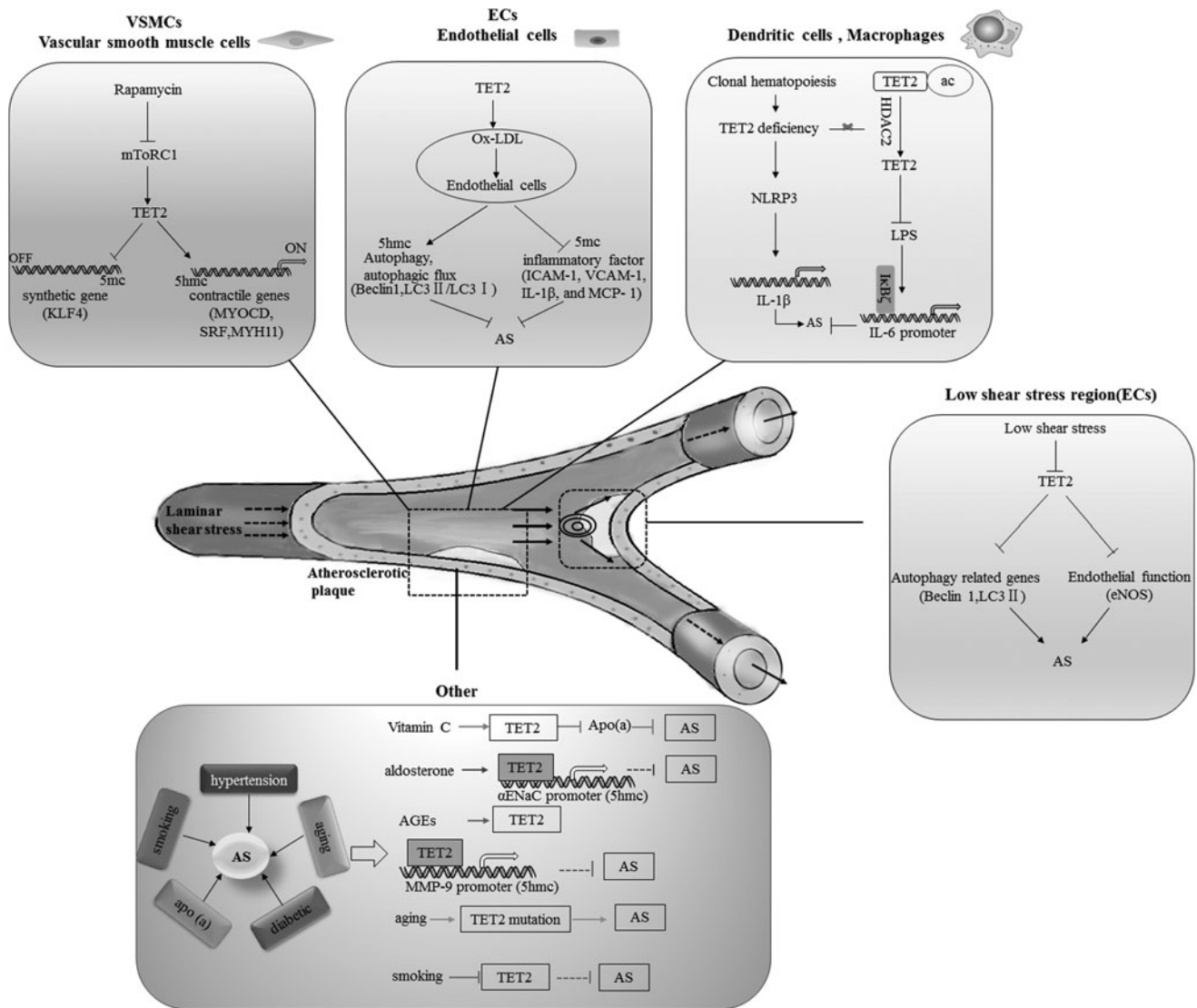


FIG. 1. Schematic of *TET2* signaling pathways involved in AS. *TET2* as a potential target for regulation of AS processes, including the transformation of smooth muscle cells, autophagy of endothelial cells, endothelial dysfunction, and chronic inflammation. In addition, it is presumed that *TET2* may indirectly regulate the development of AS by regulating the genes related to AS-related diseases. The *dotted line* represents the results to be verified, and the *solid line* represents the confirmed conclusion. AS, atherosclerosis; *TET2*, Ten-Eleven translocation methylcytosine dioxygenase 2.

in dendritic cells and macrophages and several inflammatory mediators by histone deacetylation (Zhang *et al.*, 2015). Clonal hematopoiesis of indeterminate potential, which is defined as the presence of expanded somatic blood cell clone in persons without other hematological abnormalities, is common in the elderly and is associated with an increased risk of blood cancer (Jaiswal *et al.*, 2014). Clonal hematopoiesis contributes to the development of human atherosclerosis. Moreover, the *LDLR*^{-/-} mice model, which was transplanted with bone marrow obtained from *TET2*^{+/-} or *TET2*^{-/-} mice, had larger atherosclerotic lesions in the aortic root and aorta than did mice that had received control bone marrow (Jaiswal *et al.*, 2014, 2017). Interestingly, Fuster *et al.* (2017) also suggested a causal link between accelerated atherosclerosis and clonal hematopoiesis induced by somatic *TET2* mutations. The results revealed that increased

NLRP3 inflammasome-mediated IL-1 β production is necessary for aggravated atherosclerosis in clonal hematopoiesis because of *TET2* deficiency.

TET2 and other atherosclerotic pathogenic factors

Clonal hematopoiesis, associated with human aging, leads to the development of human atherosclerosis (Jaiswal *et al.*, 2014, 2017), and somatic *TET2* mutations in blood cells accelerate atherosclerosis development (Fuster *et al.*, 2017). As an independent risk factor of atherosclerosis, the unique glycoprotein apolipoprotein(a) [apo(a)] composition of Lipoprotein(a) [Lp(a)] can be regulated by vitamin C, and enhances 5mC generation *in vivo* and in cultured cells, most probably by acting as a cofactor for TET to hydroxylate 5 mC (Minor *et al.*, 2013). Vitamin C can downregulate apo(a) expression

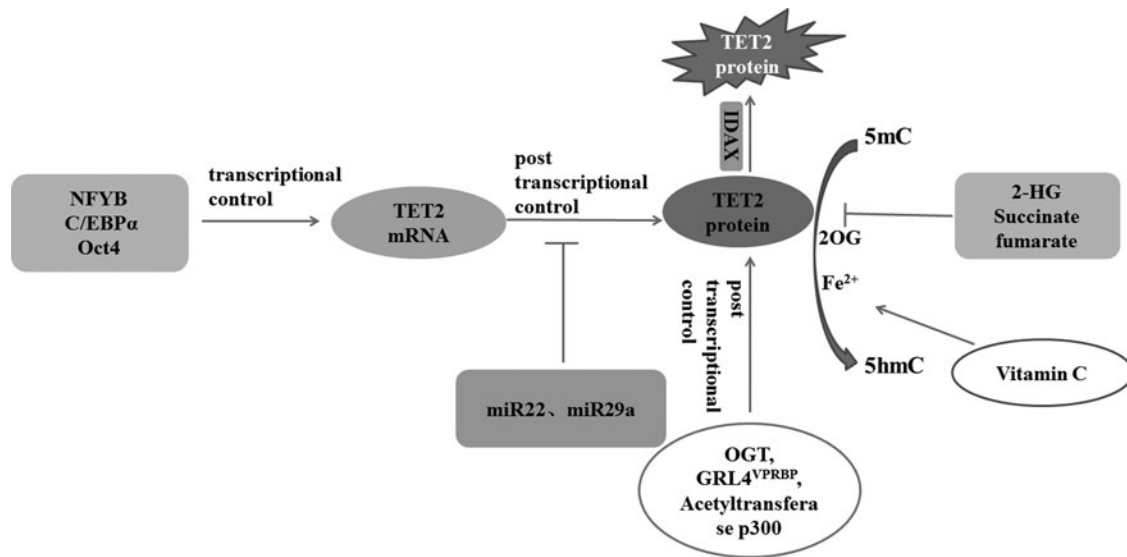


FIG. 2. Factors involved in regulation of *TET2* gene expression and enzyme activity. Transcription factors (*NFYB*, *C/EBPα*, *Oct4*) regulate *TET2* mRNA levels in various developmental stages or cell types. microRNA inhibited TET protein expression in post-transcription level. TET protein can also be regulated by O-linked N-acetylglucosamine transferase or cullin-RING ubiquitin ligase 4-CRL4VprBP, acetyltransferase p300 in post-transcription level. IDAX results in *TET2* protein degradation through caspase activation. Vitamin C acts as a cofactor for Fe^{2+} and 2-OG-dependent TET dioxygenase and can directly enhance the catalytic activity of TET protein. Pathological accumulation of three metabolites, namely, 2-hydroxyglutarate (2-HG), succinate, and fumarate, which are structurally similar to α -OG, leading to a competitive inhibition of α -OG-dependent TET activity. *NFYB*, nuclear transcriptional factor Y subunit beta.

through *TET2*-dependent DNA demethylation in HepG2 cells (Qu *et al.*, 2017). A number of studies certified that *TET2* is significantly increased in diabetic wound (Tan *et al.*, 2016; Zhang *et al.*, 2016), and advanced glycation end products can upregulate *TET2* gene expression and activity in human keratinocytes. Additionally, *TET2* proteins directly bind to the promoter region of *MMP-9* and reduce the percentage of methylation in the *MMP-9* promoter (Zhang *et al.*, 2016), which results in the development of diabetic retinopathy (Kowluru *et al.*, 2016). The epithelial sodium channel (ENaC) is a key player in the regulation of renal sodium homeostasis and blood pressure (Schild, 2010; Rossier, 2014). Aldosterone increases the reabsorption of sodium ions by increasing the transcription of α ENaC in the collecting duct cells (Yu *et al.*, 2013b). Previous studies have demonstrated that aldosterone stimulates the recruitment of *TET2* to demethylate the α ENaC promoter to induce α ENaC transcription (Yu *et al.*, 2013b). Martina *et al.* hypothesized that *TET2* can also induce ENaC transcription, which affects cardiovascular outcomes and the regulation of blood pressure by hydrochlorothiazide (Chittani *et al.*, 2015). In 2005, ~673,000 people died of tobacco-related diseases in China, and the three major causes of deaths among 40–79-year-olds were cancer, cardiovascular diseases, and respiratory diseases (Chen, 2009). Wain *et al.* (2015) reported that the *TET2* expression was higher in individuals who have not smoked than in heavy smokers (mean 25 pack-years). Unfortunately, the relationship between *TET2* and smoking has not been well addressed in cardiovascular studies.

Factors Involved in the Regulation of *TET2* Gene Expression and Enzyme Activity

With more evidences showing the importance of *TET2* in atherosclerosis, it became very important to elucidate the fac-

tors involved in the expression and activity regulation of *TET2* gene and enzyme. Some transcription factors can regulate *TET2* gene expression and the activity of *TET2* enzyme (Fig. 2).

Factors involved in *TET2* gene expression regulation

DNA demethylation can be controlled by regulating the transcription of *TET* genes (Wu and Zhang, 2014). *TET2* gene transcription is regulated by different transcription factors in various stages or cell types. *TET2* is broadly expressed in adult tissues. For instance, hydrogen sulfide may facilitate the sulfhydrylation of the nuclear transcriptional factor Y subunit beta (*NFYB*) binding to *TET2* promoters, resulting in increased transcription of the *TET2* gene (Oh and Li, 2015). During the transformation of B cells into macrophages, *CEBPα* is directly associated with *TET2* promoter and upregulated mRNA levels by binding to the *TET2* enhancer (Kallin *et al.*, 2012). In pluripotent stem cells, the key pluripotency factor *Oct4* regulates *TET2* transcription (Koh *et al.*, 2011).

TET expression may also be post-transcriptionally regulated through miRNAs. miR-22 exerts its invasiveness and metastasis potential depending on the silencing of miR-200 through direct targeting of *TET* in breast cancer (Song *et al.*, 2013c). In addition, miR-22 is upregulated in myelodysplastic syndrome (MDS), and its aberrant expression is correlated with poor survival. Conversely, *TET2* is a key target of miR-22, and its ectopic expression suppresses miR-22-induced phenotypes. Hematopoietic stem cells exhibit increased self-renewal and are involved in MDS development because of the reduced levels of 5hmC by deregulating of *TET2* proteins through miR-22 (Song *et al.*, 2013b). Takayama *et al.* reported that the miR-29 family targets *TET2*, reduces *TET2* or 5hmC expression levels in the

animal model of hormone refractory PCa, and induces tumor growth (Takayama *et al.*, 2015).

Factors involved in the regulation of TET2 enzymatic activity

TET proteins are the members of Fe²⁺/α-ketoglutarate (α-KG)-dependent dioxygenases, which require Fe²⁺ as a metal cofactor and α-KG as a cosubstrate. Withdrawing Fe²⁺ dramatically inhibited the conversion of 5mC to 5hmC (Ito *et al.*, 2010). The activity of TET2 can also be regulated by metabolites. In various types of tumors, the pathological accumulation of three metabolites, namely, 2-hydroxyglutarate, succinate, and fumarate, which are structurally similar to α-KG, leads to a competitive inhibition of α-KG-dependent TET2 activity, resulting in a hypermethylation phenotype. Intraperitoneal injection of glucose in mice rapidly increased the levels of blood glucose and hepatic α-KG. Interestingly, genome-wide 5hmC levels were rapidly increased in various mouse tissues. However, the mRNA and protein level of TET2 was not changed in mouse livers after glucose injection. The results show that α-KG is important for activity regulation of TET2 enzyme (Yang *et al.*, 2014).

O-linked N-acetylglucosamine transferase (OGT) modifies O-linked N-acetylglucosamine (O-GlcNAc) on various proteins, such as transcription factors, epigenetic regulators, and histones. Various studies have demonstrated that OGT as a protein interacts with TET2 proteins and catalyzes O-GlcNAcylation of TET2 (Chen *et al.*, 2013; Deplus *et al.*, 2013; Vella *et al.*, 2013). O-GlcNAcylation occurs at serine or threonine residues of TET protein, and phosphorylation of TET protein can also happen in these amino acids. Interestingly, the phosphorylation extends of TET2 are higher than TET3. Phosphorylation of TET2 protein can be suppressed through O-GlcNAcylation by the glycosyltransferase OGT (Bauer *et al.*, 2015). However, the relationship between TET2 phosphorylation and TET enzymatic activity has not been addressed in the literature.

The cullin-RING finger ligase-4 (CRL4) complex is crucial in regulating mammalian oocyte survival and reprogramming. Oocyte-specific deletion of the CRL4 linker protein DDB1 or its substrate adaptor VPRBP (also known as DCAF1) causes rapid oocyte loss, premature ovarian insufficiency, and silencing of fertility-maintaining genes (Yu *et al.*, 2013). TET2 was highly expressed in oocytes and early embryos. The 5hmC generation was reduced in DDB1- or VPRBP-deleted oocytes and CRL4^{VPRBP} might regulate TET2 activities. CRL4^{VPRBP} enters the nucleus and activates TET2. Additionally, VPRBP-mediated monoubiquitylation plays a critical role in TET2 activation by promoting the ability of TET2 to bind DNA, but not the catalytic activity of TET2 enzyme. Then, deletion of the VPRBP gene causes substantial reduction of 5hmC, suggesting that the function of VPRBP is important for TET2 activity (Nakagawa *et al.*, 2015).

The IDAX gene known as CXXC4 originates from the ancestral TET2 gene fission during vertebrate evolution and is transcribed in the opposite direction. The IDAX CXXC domain binds DNA sequences enriched in unmethylated CpG dinucleotides and interacts directly with the catalytic domain of TET2. IDAX binds DNA sequences containing unmethylated CpG dinucleotides in gene promoter and recruits TET2 to DNA, which results in caspase activation and

TET2 protein degradation, without directly affecting the enzymatic activity of TET2 (Ko *et al.*, 2013).

As an antioxidant, free radical scavenger, and a cofactor for Fe²⁺- and 2-α-KG-dependent dioxygenases, vitamin C enhances 5hmC generation, most probably by acting as a cofactor for TET2 (Minor *et al.*, 2013). It can directly enhance the catalytic activity of TET2 dioxygenases to oxidize 5mC. Furthermore, the results showed that vitamin C can uniquely interact with the C-terminal catalytic domain of TET2 enzymes, which probably promotes their folding and/or recycling of the cofactor Fe²⁺. However, in the TET1/TET2 double-knockout cells, vitamin C did not alter 5mC oxidation or the overall level of 5mC (Yin *et al.*, 2013). Blaschke demonstrated that the addition of vitamin C to mouse embryonic stem (ES) cells promotes TET activity, leading to the rapid and global increase in 5hmC. Interestingly, dot blot analysis revealed that TET1/TET2 double-knockout ESCs also show significantly reduced 5hmC signal, which was not increased by vitamin C treatment (Blaschke *et al.*, 2013). These events indicated that the effects of vitamin C are TET dependent.

5hmC and 5fC are significantly enriched at the binding site of acetyltransferase p300 throughout the genome (Yu *et al.*, 2012; Song *et al.*, 2013a). TET2 is regulated by acetyltransferase p300, which enhances its enzymatic activity, protein stability, and partnering with DNMT1. The TET2/DNMT complex targeting chromatin is a key regulator during oxidative stress, which protects against abnormal DNA methylation (Zhang *et al.*, 2017).

Conclusions and Perspectives

In this study, we showed that *TET2* plays an important role in preventing atherosclerosis by repressing VSMC phenotype transformation, protecting ECs from damage and dysfunction, and inhibiting inflammation. The underlying mechanisms were mostly associated with the methylation/demethylation regulation of related gene expression, such as *MYOCD*, *SRF*, *KLF4*, *eNOS*, and autophagy-related genes. Also, several factors were associated with the expression and activity regulation of the *TET2* gene and protein. *TET2* gene expression could be regulated at the transcription and post-transcription level by *NFYB*, *CEBPα*, *OCT4*, and miRNAs. *TET2* activity could be regulated by Fe²⁺, α-KG, OGT, VPRBP, vitamin C, IDAX, and acetyltransferase p300. The protective roles of *TET2* in atherosclerosis are mentioned above. *TET2* selectively mediates the repression of IL-6 transcription in dendritic cells and macrophages by histone deacetylation, and vitamin C can downregulate apo(a) expression through *TET2* in HepG2 cells. *TET2* enzyme activity-related factors, vitamin C, and acetyltransferase p300 may play an important role in atherosclerosis through regulating *TET2* activity. In addition, miRNAs affect the pathogenesis of atherosclerosis. miRNAs may target *TET2* and accelerate the process of atherosclerosis. Further studies should determine other regulatory factors related to *TET2* and atherosclerosis.

Studies have confirmed that the early stage of atherosclerosis occurs during DNA methylation. Thus, DNA methylation is necessary for atherosclerosis development, or an epiphenomenon, and this condition has not been confirmed until now. Based on the effects of DNA methylation on the development of atherosclerosis, *TET2*, as an important demethylase, inevitably participates in most biological and

pathological processes. However, the mechanisms related to atherosclerosis development have only been studied recently. We believe that in-depth studies of *TET2* and its mechanisms related to atherosclerosis development will provide a new target for the effective prevention and treatment of atherosclerosis.

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No competing financial interests exist.

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