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·专家笔谈·

TET2:a Potential Epigenetic Biomarker and Therapeutic Target for Atherosclerosis

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Abstract: Atherosclerosis, a slow and progressive pathological change, is the usual cause of cardiovascular diseases. Finding new biomarkers focusing on the nature of atherosclerosis is necessary to effectively diagnose and prevent clinical events. Recent studies find the functions of TET2 are deficient or aberrant in many neoplastic disorders. Ten-eleven-translocation 2 (TET2) is a member of the TET family that functions as a regulator of DNA methylation by oxidizing 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), which contributes to DNA demethylation, repair, and genomic stability. DNA methylation pattern alterations occur in the development of atherosclerosis. Recent studies demonstrated that TET2 has a significant function in the self-renewal and differentiation of hematopoietic stem cells. Its mutation and dysfunction contribute to numerous specific diseases, such as hematopoiesis and hematopoietic diseases. Based on its biochemical, genetic, and functional implications, TET2 is a potential "ideal" epigenetic biomarker and therapeutic target for atherosclerosis.

Key words: TET2; atherosclerosis; epigenetic; biomarker

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TET2:潜在的动脉粥样硬化表观遗传生物标记物和治疗靶点

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摘 要: 动脉粥样硬化是一种慢性炎症性的病理改变,是心血管疾病最主要的病因,寻找新的生物标记物对有效诊断和治疗动脉粥样硬化有非常重要的作用。最新研究发现在很多肿瘤疾病中出现了 TET2 的功能缺失或异常。甲基双加氧酶 TET2 为 TET 蛋白家族成员,主要功能为催化 5 甲基胞嘧啶(5mC) 生成 5 羟甲基胞嘧啶(5hmC),参与 DNA 的去甲基化和修复及基因组的稳定。DNA 甲基化模式的改变发生于动脉粥样硬化的发生发展过程中。最近的研究表明,TET2 在造血干细胞的自我更新及分化过程中起着非常重要的作用,TET2 突变及功能失调与多种疾病,特别是血液性疾病的发生、发展密切相关。基于 TET2 的生物学作用,我们推测 TET2 可能是动脉粥样硬化的理想的表观遗传学生物标记物以及防治的靶点。

关键词: 甲基双加氧酶 TET2; 动脉粥样硬化; 表观遗传; 生物标记物

1 *Introduction*

Atherosclerosis, a pathological change that occurs in large- and medium-sized arteries, is the main pathological basis of cardiovascular diseases. Atherosclerosis can lead to ischemic stroke, myocardial infarction, kid-

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ney disease, intermittent claudication, and other serious complications. In the last two decades, a substantial number of molecules have been identified to be involved in the atherosclerotic process. As of these writings, some molecules have been detected and used as biomarkers to assess future cardiovascular events. However, their clinical effects have not yet been established. Although atherosclerotic plaque imaging is a precise predictor of atherosclerotic lesions, it is costly and impractical for the diagnosis of the early stages of atherosclerosis. Therefore, atherosclerotic biomarkers with good sensitivity, specificity, predictive value, and cost-effectiveness for the prediction, diagnosis, and treatment of the disease need to be identified.

DNA methylation is a major epigenetic modification of a genome that can be inherited, and contributes to gene silencing without changing the DNA sequences[1]. Both global and gene-specific alterations in DNA methylation are associated with abnormal phenotypes. Epigenetic changes, the heritable nature of these changes, lock in the early stages of pathology, and induce the development of the disease. In human carcinogenesis, DNA methylation pattern alterations are characterized by global genomic DNA hypomethylation and specific gene hypermethylation. Genomic hypomethylation contributes to transformation, tumor progression, and oncogene expression, whereas regional DNA hypermethylation is related to the inactivation of tumor suppressor genes and enhances cell proliferation[2]. Similar DNA methylation pattern alterations occur in early atherosclerosis[3-5]. Aberrant genomic DNA methylation patterns occur earlier than the pathological and morphological changes of atherosclerosis[6-10]. Therefore, the identification of specific changes in DNA methylation and its regulatory mechanism were valued to justify the expectations for novel diagnostic and therapeutic techniques for atherosclerosis.

The DNA methylation landscape of a genome includes two patterns of methylation and demethylation, and is established by methylation and demethylation enzymes. DNA methylation occurs at carbon 5 of cytosine in CpG dinucleotides, and dramatically suppresses transcription in the gene promoter regions[11]. Thus, DNA methylation is frequently described as a "silencing" epigenetic mark, whereas DNA demethylation is described as an "activating" mark. Ten-eleven translocation (TET) proteins (TET1-3), a family of DNA demethylases, can catalyze the conversion of 5mC to 5hmC, 5-formylcytosine (5fC), and 5-carboxycytosine (5caC), which is a well-characterized epigenetic modification that has crucial functions in regulating gene expression and maintaining cellular identity[12,13].

In the last decade, numerous studies have suggested a significant function for these enzymes in the epigenetic transcriptional regulation of eukaryotes primarily by hydroxylation reactions[14]. TET2 is one of the most frequently mutated genes in myelodysplastic syndromes[15,16], and the loss of TET2 and 5hmC is an early key epigenetic event in aggressive melanoma[17]. Studies have shown that the deletion of TET2 alone is sufficient to initiate myeloid transformation. TET2-null mouse models mainly exhibit chronic myelomonocytic leukemia-like disease. Patients with TET2 mutations show low levels of genomic 5hmC and global hypomethylation in the marrow compared with those of wild-type TET2, which is similar to the DNA methylation pattern in atherosclerotic lesions[18,19]. TET-mediated oxidative demethylation was recently established to have a key function in reprogramming fibroblasts to pluripotency[20]. The depletion of TET2 in mouse hematopoietic progenitors results in monocyte/macrophage differentiation dysfunction, leading to an impaired upregulation of macrophage markers as well as phagocytic capacity[16]. Therefore, measurements of TET2 may carry important prognostic information and subsequent clinical complications, which are independent of traditional risk factors.

2 *Proposed hypothesis*

We propose that pro-atherosclerotic risk factors down-regulate TET2 expression, which leads to anti-atherosclerosis genes promoter DNA hypermethylation and atherosclerosis (Figure 1). Therefore, TET2 has the potential to be utilized as an ideal epigenetic biomarker for atherosclerosis, and contributes to the prediction and diagnosis of the disease. Notably, TET2 may be an ideal therapeutic target for the prevention or therapy of atherosclerosis because the TET2-mediated dynamic changes in DNA methylation and gene expression are reversible.

3 *The value of the hypothesis*

Atherosclerosis, as well as the resulting coronary heart disease and cerebral stroke, is still the leading cause of death and disability worldwide[21]. Currently, atherosclerotic lesions cannot be effectively predicted and prevented. Thus, better biomarkers and therapeutic targets need to be identified.

DNA methylation regulates fundamental biological

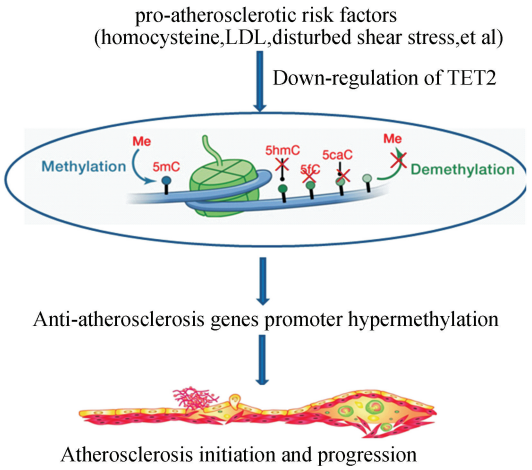

Figure 1 Schematic view of the hypothesis of TET2 as a potential idea biomarker of atherosclerosis

processes, such as gene expression, genomic stability, mutation rate, genomic imprinting, and X-chromosome inactivation. Both global and gene-specific alterations in DNA methylation are associated with abnormal phenotypes. DNA methylation has been described as an early diagnostic marker, and is a valuable molecular treatment strategy for cancer because of its dynamic nature[22]. In atherosclerosis-prone apolipoprotein E knockout mouse, aortas, and peripheral blood leukocytes, specific changes in DNA methylation precede the formation of aortic lesions and are detectable in as early as four weeks[10]. Low DNA methylation content has also been observed in peripheral blood leukocytes of patients with atherosclerotic cardiovascular disease[3]. Alterations in DNA methylation affect the transcription of critical regulatory genes that induce a pro-atherogenic cellular phenotype[23]. DNA methylation in leukocytes is associated with the expression of soluble mediators and surface molecules, which contribute to margination, adhesion, and trans-intimal migration. In particular, DNA methylation modification has been demonstrated as the bridge that links pro-atherosclerotic risk factors to atherosclerosis. Studies showed that homocysteine, a prevalent risk factor for cardiovascular events, increases the DNA methylation level of the ATP-binding cassette transporter A 1 (ABCA1) gene and decreases acetyl-CoA acetyltransferase 1 (ACAT1) DNA methylation to promote the accumulation of cholesterol in monocyte-derived foam cells[24,25]. Low-density lipoprotein, a pro-atherosclerotic risk factor, represses endothelial KLF2 expression via DNA methylation, and downregulates several target genes, namely, endothelial NO synthase, plasminogen activator inhibitor-1, and thrombomodulin, to promote the development and progression of atherosclerosis[26,27]. Notably, a study showed that pro-atherosclerotic risk factor disturbed flow resulted in mechanosensitive genes promoter hypermethylation and that the DNA methyltransferases inhibitor 5Aza treatment restored normal methylation patterns and antiatherogenic gene expression. Those confirmed evidences showed that DNA methylation dysfunction is a critic event in process of atherosclerotic. Further studies for the mechanism might improve the clinic outcome of atherosclerotic patients.

Recent studies discovered that TET2 has a critical function in regulating the expansion and function of hematopoietic stem cells[28] by controlling the 5hmC levels, and erythrocyte development by regulating lineage-specific genes via DNA oxidative demethylation[29]. In hematopoietic systems, the deletion of TET2 is sufficient to cause a significant loss of 5hmC in genomic DNA. TET2 mutation is a plausible cause for aberrant epigenetic regulation of gene expression.

Smooth muscle cell (SMC) dedifferentiation significantly contributes to atherosclerosis[30]. Studies showed that SMCs in advanced atherosclerotic plaques proliferate and are characterized by hypomethylation. Hypomethylation of collagen, type XV, and alpha 1 occurs during SMC proliferation, and the increase in gene expression contributes to the SMC phenotype and atherosclerosis formation[31]. TET2 was recently demonstrated to be a novel and necessary master epigenetic regulator of SMC dedifferentiation[32]. TET2 mutation promotes phenotypic alterations in vascular SMCs (VSMCs) from the "contractile" phenotype to the active "synthetic" phenotype. Consequently, these active VSMCs migrate from the media to the intima, proliferating and producing excessive amounts of extracellular matrix[32].

Therefore, the loss of TET2 may be an essential manifestation in atherosclerosis development. Alterations in TET2-mediated DNA demethylation precede and parallel the development of atherosclerosis, and are reversible. TET2 may be an "epigenetic biomarker" for risk stratification and "epigenetic therapeutic target" for the prevention of atherosclerosis.

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