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Review Article

Mechanisms of VSMC Proliferation, Migration, and Phenotypic Transformation in Homocysteine-Induced Atherosclerosis: A Review

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Abstract

Evidence-based medical evidence has shown that homocysteine (Hcy) is an independent risk factor for atherosclerosis (As). If the pathogenesis of Hcy induction As can be elucidated, it will provide an important theoretical basis for the prevention and clinical treatment of As. Vascular smooth muscle cells (VSMC) are the main components of the vascular media, and the proliferation, migration, and phenotypic transformation of VSMC play an important role in the pathogenesis of Hcy induced As. This paper reviews the mechanisms related to the proliferation, migration, and phenotypic transformation of VSMC during Hcy induction of As.

Keywords: Homocysteine; Atherosclerosis; Vascular smooth muscle cells; Phenotypic transformation

Introduction

Atherosclerosis (As) is an important basis lesion in many cardiovascular and cerebrovascular diseases such as ischemic cardiomyopathy and cerebral stroke. Homocysteine (Hcy) is an independent risk factor for atherosclerosis (As). Hyperhomocysteinemia (HHcy) can induce As, but the pathogenesis is unclear. Vascular smooth muscle cells (VSMC) are the main components of the vascular media and the main source of foam cells in As plaques. Hcy can induce the proliferation, migration, and phenotypic transformation of VSMC, which plays an important role in the pathogenesis of As induced by Hcy. This paper reviews the mechanisms related to the proliferation, migration and phenotypic transformation of VSMC during Hcy induction of As.

The Main Pathogenesis of Atherosclerosis

Atherosclerosis (As) is a chronic compensatory arterial inflammatory response associated with abnormal lipid metabolism and changes in the composition of blood vessel walls, which is the main cause of cardiovascular disease (CVD). It is considered to be the common pathological basis of most cardiovascular diseases such as myocardial infarction, stroke, and peripheral artery disease [1]. The formation of As lesions is a chronic inflammatory process involving complex signal networks and a variety of effector molecules [2-4]. Currently, the main theories on the pathogenesis of As include [5]: inflammatory response theory [6], oxidative stress theory [7], lipid infiltration theory [8,9], homocysteine theory, thrombus formation theory [10], and immune injury theory. It is believed that the formation of As is mainly caused by circulating factors, cholesterol, low density lipoprotein, inflammatory factors, chemokines, and a variety of cells in the vascular wall. Including the results of interactions among vascular endothelial cells, lymphocytes, monocytes/macrophages, and vascular smooth muscle cells (VSMC) [11], which are specifically manifested as damage of vascular intima, activation of chemokines and inflammatory factors, lipid infiltration, and dysfunction

of endothelial cells [12]. VSMC proliferation and phenotypic transformation [13], macrophage-like cell formation, migration, foam cell formation [14], and atheromatous plaque formation are main pathologic processes in As formation. VSMC is one of the active cells in the As plaque proliferation system [15,16], and the proliferation, migration and phenotypic transformation of VSMC and the injury of vascular endothelial cells are considered to be the main initial links in the formation of As [17,18]. Although many studies have been conducted on As, the mechanisms that cause proliferation, migration and phenotypic transformation of VSMC remain unclear.

Proliferation, Migration, and Phenotypic Transformation of VSMC Play an Important Role in the Pathogenesis of As

VSMC is a highly specific cell located in the middle layer of the arterial wall and is the main source of macrophage-like and foam cells in As plaques [8,19]. The main function of VSMC is to regulate vascular elasticity and maintain vascular tone. During embryonic vascular development, VSMC gradually differentiated from immature undifferentiated/poorly differentiated phenotype to mature highly differentiated phenotype. When the vascular wall is stimulated by internal and external environmental factors or the VSMC is stimulated by inflammatory factors, cytokines, vasoactive peptides, oxidative stress, drug damage, mechanical effects, and other pathological factors, on the one hand, the imbalance of VSMC proliferation and apoptosis can be caused, resulting in VSMC proliferation and migration. At the same time, VSMC can transform from a highly mature differentiated phenotype to a dedifferentiated/poorly differentiated phenotype, and obtain proliferation and phagocytosis capabilities, and eventually form macrophage-like cells. This pathological process is called the phenotypic transformation of VSMC [20]. The proliferation, migration, and cell phenotypic transformation of VSMC may be key events in the pathological process of various vascular diseases such As, and play an important role in the occurrence and development of As [1,5,13]. According to the maturity of VSMC differentiation, VSMC can be divided into two phenotypes: highly differentiated systolic phenotype (differentiated type) and poorly differentiated synthetic phenotype (dedifferentiated or undifferentiated type) [21,22]. The systolic phenotype of VSMC is highly differentiated, spindle-shaped, rich in intracellular muscle fibers, and mainly manifested as systolic function, poor proliferation and migration ability. The main molecular markers were α-smooth muscle actin (α-SMA) [23], smooth muscle 22α(Smooth Muscle 22α, SM22α) [24,25], calponin, Smooth muscle myosin heavy chain (SM-MHC), smooth muscle cell actin [13,26-28]. However, the synthetic phenotype of VSMC has a low differentiation degree, strong proliferation and migration ability, reduced intracellular muscle fibers, reduced or disappeared systolic function, and increased contents of intracellular rough endoplasmic reticulum, Golgi apparatus and ribosome, and stronger ability to secrete and synthesize cytokines and extracellular matrix than the systolic phenotype. In addition, VSMCS undergoing phenotypic transformation can also acquire macrophage-like markers and properties, including LGALS3/Mac2, CD11b, F4/80, and CD68 [13], and have phagocytic function. The ability of synthetic phenotypic VSMCS to absorb lipids was also increased, The unique molecular markers of osteopontin (OPN) and thrombospondin-1 were Osteopontin (OPN) and Thrombospondin-1. TSP1), epidermal growth factor, epiregulin, etc. [29-31]. The phenotypic transformation of VSMC from systolic phenotype to synthetic phenotype has long been considered important for As. The phenotypic transformation of VSMC during the occurrence of As is not only the main feature of VSMC in As, but also the premise for VSMC to form macrophage-like cells and myogenic foam cells and play an important role in plaque formation and development [13,32]. Under normal conditions, VSMC exists in the media of arterial wall with a contractile phenotype, which is conducive to maintaining the contraction of smooth muscle cells and vascular tone to maintain vascular wall homeostasis. However, when VSMC is subjected to different pathologic and physiological stimuli, changes occur in the surrounding environment of the cells, including growth factors, extracellular matrix, mechanical forces, oxidative stress, intercellular interactions, and neuroregulation, resulting in phenotypic changes of VSMC, from a highly differentiated contractiles phenotype to an undifferentiated or poorly differentiated synthetic phenotype. They began to proliferate, migrate and synthesize excessive extracellular matrix to form macrophage-like cells, resulting in increased lipid phagocytosis of VSMC, which could take in large amounts of oxidized low-density lipoprotein, and migrate from the arterial media to the inner artery to transform into foam cells [13,15,19]. Foam cells are deposited under the intima of the damaged blood vessels, causing local vascular lumen stenosis [33,34] and changes in hemodynamics, eventually leading to the formation of atherosclerotic plaques. Literature has shown that, in plaque formation of As, 70% of plaque components are composed of VSMC and its derivatives [35,36], and 40% of foam cells, which constitute an important part of lesions, are from VSMC, which is called smooth muscle-derived foam cells [37,38]. It is suggested that VSMC is an important component in the formation of As. At the same time, the transformation from systolic phenotype to synthetic phenotype of VSMC can promote endometrial hyperplasia and the formation of As lesions and abnormal phenotype transformation of VSMC is therefore considered to be one of the main markers of the progression of As lesions [39], As well as an important step in the development of AS restenosis, vascular remodelling and other pathophysiological processes [40,41]. It is certain that the transformation of VSMC into foam cells not only damages the vasoconstrictive function, but also promotes its own proliferation, migration and secretion of pro-inflammatory mediators [42], and the expression of different phenotypic markers is regulated by various factors. Such as platelet-derived growth factor (PDGF), transforming growth factor β, TGF-β), interleukin, endothelin, Angiotensin and microRNAs, etc. [13,15]. In carotid artery ligation animal models, blocking the phenotypic transformation of VSMC can inhibit the formation of

intima damage [43,44], and promoting this process can accelerate the development of As in mice [45]. Meanwhile, the phenotypic transformation of VSMC also plays an important role in the stability of As and plaque, and inhibiting the phenotypic transformation of VSMC may be beneficial for advanced As [15]. Although there have been some reports on the proliferation, migration and phenotypic transformation of VSMC, the specific molecular mechanism has not been clarified and needs further study.

Hcy Can Induce the Occurrence of As

Homocysteine (Hcy), also known as homocysteine, is a kind of sulfur-containing amino acid, which is the intermediate product of methionine metabolism. Normally, plasma Hcy levels are very low; When the plasma Hcy concentration is higher than 15 μmol/L, it is called hyperhomocysteinemia (HHcy). Evidence of evidence-based medicine shows that HHcy is an independent risk factor for As [46,47], and Hcy induces As through a variety of pathway interactions and correlations. Every 5 μmol/L increase in plasma Hcy is equivalent to a 0.5 mmol/L increase in cholesterol, and the vascular risk increases by about 1/3 [48]. Hcy is closely associated with the risk of coronary heart disease, stroke, and peripheral vascular diseases, and is no less harmful than hyperlipidemia. Known as the "cholesterol of the 21st century", HCY has been identified as a potentially important risk factor for cardiovascular diseases [49-51]. The Chinese Guidelines for the Prevention and Treatment of Hypertension (2018 Revision) clearly proposed serum Hcy concentration as a selective item for laboratory tests in the diagnostic assessment of hypertension. HHcy(≧15 μmol/L), together with old age, smoking, impaired glucose tolerance, dyslipidemia, abdominal obesity and other factors, are considered to be important cardiovascular risk factors affecting the cardiovascular prognosis of patients with hypertension [52], indicating that reducing blood Hcy is an important strategy for collaborative prevention and treatment of cardiovascular diseases. As an inflammatory stimulant, Hcy promotes the occurrence and development of AS through various mechanisms such as affecting the function of endothelial cells and VSMC, participating in oxidative stress and inflammatory response, and altering gene expression activity [53,54].

Hcy Promotes the Proliferation, Migration, and Phenotypic Transformation of VSMC

At present, the mechanism of Hcy causing As through vascular endothelial cell injury has been relatively clear [55,56]. It includes the following aspects: (1) direct endothelial injury through oxidation and inflammation [57,58]; (2) Induced oxidative stress mitochondrial dysfunction and endoplasmic reticulum stress [59-61]: (3) the protective effect of NO was weakened [62]; (4) Interference with DNA and protein methylation [63,64]; (5) Promote adhesion and penetration [65]. The promotion effect of Hcy on the proliferation of VSMC is considered to be one of the important pathological basis of As [66], however, the mechanism of Hcy causing the proliferation, migration and expression transformation of VSMC is not very clear.

Studies have shown that, on the one hand, Hcy can act on related growth factors and genes, activate VSMC, and promote the proliferation and migration of VSMC. PDGF is an important mitogenic factor, which can stimulate specific cell mitosis and promote cell proliferation. Hcy can up-regulate PDGF levels through DNA demethylation of human and mice vascular endothelial cells, affect the cross-linking between vascular endothelial cells and VSMC, and lead to activation of VSMC [46]. Hcy affects the methylation of As-related genes and mediates the overall methylation state of VSMC proliferation [67,68]. Hcy can be hypermethylated through promoter regions of p53, PTEN, MFN2, etc. Demethylation of PDGF promoter region affects epigenetic regulation of p53, PTEN, MFN2, PDGF, etc., thus promoting proliferation of VSMC [69-71]. Hcy can cause dysregulation of the expression and activity of metalloproteinase 2/9(MMP-2/9) and tissue inhibitor of metalloproteinase 2(TIMP-2) in VSMC, affect the dynamic balance of extracellular matrix, degrade extracellular matrix such as basement membrane, destroy the physiological barrier of VSMC migration, and induce the proliferation and migration of VSMC in rats [72]. Hcy (50-1000 μmol/L) increased the production and activation of MMP-2 and the expression of TIMP-2 in rat VSMC in a dose-dependent manner, while the expression of MMP-2 was up-regulated and the activity was down-regulated when incubated with 5000 μmol/L Hcy [73]. It is possible that Hcy promotes the expression of the protein Concave protein-1, which inhibits the activity of endothelial nitric oxide synthase (eNOS) and the production of NO and activates the expression of PI3K and p-Akt. The proliferation and migration of thoracic aorta smooth muscle cells cultured in vitro in SD rats were induced, leading to As [74,75]. Meanwhile, compared with the control group, the proliferation and migration ability of VSMC in aorta of rats in the Hcy stimulation group were significantly enhanced, the mRNA and protein expression levels of MMP-2, MMP-9, and p-P70S6K were significantly increased, and the expression levels of p21 and p27 were significantly decreased [76]. These results suggest that Hcy can induce the proliferation and migration of VSMC, and play an important role in the pathogenesis of As induced by Hcy.

On the other hand, Hcy affects collagen synthesis and metabolism in VSMC. In Cbs-/- mice aortic VSMC, the effect of Hcy on collagen secretion was observed. It was found that Cbs-/- mice with severe HHcy had significantly thickened vascular intima, a higher percentage of lumen stenosis, and significantly increased the deposition of elastin and collagen in the newborn intima and the secretion of collagen in VSMC. These results suggest that Hcy stimulates increased neointima formation, elastin and collagen deposition, contributing to the development of vascular remodelling [77].

Thirdly, Hcy can promote lipid deposition in blood vessel walls. Hcy can induce lipid deposition in the arterial wall, an

increase of foam cells, plaque calcification, and low-density lipoprotein oxidation. Hcy can promote oxidative stress reaction in VSMC and generate reactive oxygen species (ROS). Through oxidative modification of low-density lipoprotein, oxidized low-density lipoprotein (ox-LDL) increases in oxidized low-density lipoprotein (Oxidized low-density lipoprotein), thereby promoting foam cell generation [49]. At the same time, ox-LDL itself is highly cytotoxic to vascular endothelial cells, macrophages and VSMC. ox-LDL can directly damage vascular endothelial cells, activate monocytes in blood to swim to the wall of the bleeding tube and transform into macrophages, and activate VSMC to phagocytic ox-LDL, and eventually form foam cells. Aggravate the occurrence and development of As [37-39,78]. Our research group also found that Hcy induced proliferation, migration and phenotypic transformation of VSMC by activating PI3K/Akt and mTOR signalling pathways. miRNA-145 can inhibit the activity of PI3K/Akt and mTOR pathways, and reduce the proliferation, migration and phenotypic transformation of VSMC induced by Hcy [79]. Lycium barbarum polysaccharide inhibits the PI3K/Akt signalling pathway by up-regulating the expression of miRNA-145 and alleviates the proliferation and phenotypic transformation of Hcy-induced VSMC [80]. By inhibiting the expression of CTRP9, Hcy negatively regulates the occurrence of endoplasmic reticulum stress, thereby inducing VSMC migration. Meanwhile, the up-regulation of DNMT1 plays an important role in this process, suggesting that CTRP9 may be regulated by methylation [81].

Conclusion

These studies suggest that Hcy can affect the extracellular matrix, destroy the vascular basement membrane, and activate growth factors and related genes in VSMC, ultimately promoting the proliferation and migration of VSMC. Hcy induced proliferation and migration of VSMC is one of the important mechanisms of Hcy induced As [82]. VSMC over proliferate and migrate through basement membrane to endovascular subcutaneous, transforming into foam cells by phagocytosis of lipids, and eventually developing into fibrous plaques [83]. These results indicate that Hcy is closely related to the damage of As VSMC. Hcy can induce the proliferation, migration and phenotypic transformation of VSMC, and ultimately promote the formation of foam cells in As plaques. However, the above studies also have some shortcomings, such as the relatively independent and lack of internal correlation between the mechanisms, and it is not clear whether there is interaction between them. Furthermore, whether there are other unknown molecular mechanisms in the process of Hcy promoting the proliferation, migration and phenotypic transformation of VSMC, so As to promote the occurrence and development of As also needs further study.

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Author Contributions

ZMH designed the idea of the article. WXY, MT, MX, ZY and XLB collected relevant literature and analyzed it. ZMH and WXY wrote the manuscript. All authors have read and approved the manuscript.

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Conflict of Interest

All authors declare that they have no conflict of interest.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

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