Research progress on the molecular mechanism of coronary microvascular endothelial cell dysfunction

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A R T I C L E   I N F O

A B S T R A C T

Coronary microvascular disease is a high-risk factor for many cardiovascular events. However, due to its high concealment and many etiologies, the current understanding of its pathophysiological mechanism is very limited, which greatly limits its clinical diagnosis and treatment. In the process of the occurrence and development of coronary microvascular disease, the damage of coronary microvascular endothelial cell (CMEC) is the core link. CMEC's stress, metabolism, inflammation and other dysfunctions have a causal relationship with coronary microvascular disease, and are also the main features of coronary microvascular disease in the early stage. This article mainly reviews the molecular mechanisms of CMEC damage.

Contents

1. Introduction

In recent years, coronary microvascular disease (CMVD) has received increasing attention. CMVD is a clinical syndrome with objective evidence such as exertional angina or myocardial ischemia caused by abnormalities in the structure and/or function of the coronary pre-arterioles and arterioles under the action of multiple pathogenic factors. Recent studies [1,2] have found that the incidence of CMVD in people with chest pain symptoms but normal coronary angiography is as high as 45% to 60%, and such patients have significant cardiovascular events such as myocardial ischemia, angina pectoris, and myocardial infarction, as well as significant mortality. It is speculated that CMVD may be an important reason for the poor prognosis of these patients. In addition, the complicated etiology of CMVD, the wide range of people involved, and the lack of standardized and effective detection methods have brought great difficulties to the systematic prevention and management of coronary heart disease, and seriously affected the prognosis of patients. It is necessary to systematically and in-depth understanding of CMVD [3,4].

The risk factors of CMVD are similar to coronary atherosclerosis, mainly including hypertension, diabetes, high free fatty acids and aging, all of which are damage to the function or structure of

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vascular endothelial cells as the main pathological characteristics. Studies [5,6] have found that coronary microvascular endothelial cells (CMEC) account for about 1/3 of the total number of cells in the heart, and play an important role in maintaining the normal function of coronary microvessels, and CMEC dysfunction often precedes myocardial injury. CMEC dysfunction means that under the stimulation of pathological factors, the normal functions of CMEC such as proliferation, adhesion, migration, apoptosis and secretion are damaged, leading to coronary microvascular dysfunction. Clinically, it can cause coronary microvascular constriction, decreased coronary blood flow reserve, and insufficient blood supply to the myocardium, which are the early manifestations of CMVD [1,7]. At present, there have been some reports about the mechanism of CMEC functional damage. This article mainly reviews the research progress of the molecular mechanism of CMEC dysfunction.

2. Mitochondrial reactive oxygen species (ROS) accumulation

Cells produce ROS during normal growth and metabolism, but they maintain the balance of redox state through a variety of mechanisms. In patients with type 2 diabetes or hypertension, the oxidative stress level of endothelial cells is significantly increased due to the stimulation of high blood sugar, high insulin or high blood pressure on the coronary microvascular endothelium, which are considered important factors to cause the microvascular endothelial dysfunction in such patients [8,9]. Angiotensin (Ang) Ⅱ level increases during hypertension, activates protein kinase C (PKC) dependent nicotinamide dehydrogenase/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase system, and induces superoxide Anion (O_2^-) levels increase [10,11]. Under other stimulating factors (such as high sugar, high fat, inflammatory factors), the activity of pro-oxidant enzymes (such as xanthine oxidase, NAD(P)H oxidase and PKC) in vascular endothelial cells increases, and the production of antioxidant glutathione decreases, which leads to an increase in ROS levels [12–14]. Excessive accumulation of ROS will interfere with the nitric oxide (NO) signaling pathway, thereby reducing the bioavailability of NO, and ultimately leading to microvascular endothelial dysfunction [14,15].

In recent years, in addition to the classic redox-related proteins directly involved, it has also been found that other proteins are involved in the regulation of the CMEC damage process caused by ROS-related signaling pathways. (1) Under high glucose conditions, the accumulation of ROS is stimulated to activate the forkhead box protein O3A (FOXO3A) protein. On the one hand, the activation of FOXO3A can reduce the level of ROS, and at the same time can inhibit the anti-apoptotic protein recombinant human B cell lymphoma factor 2xL (Bcl2- xL) level, which ultimately triggers CMEC apoptosis [13,14]. (2) Bax inhibitor 1 (BL1) regulates mitochondrial fusion by inhibiting the xanthine oxidase (XO)/ROS/F-actin (F-actin) signaling pathway, thereby reducing CMEC damage caused by ischemia–reperfusion [16]. (3) The activation of phosphatidylinositol 3-kinase/serine kinase/threonine kinase (PI3K/AKt) signal can reduce the CMEC damage due to hypoxia by inhibiting the sarcoplasmic reticulum (SR)-Ca^{2+}-XO-ROS signal axis. Also, in the hypoxia/reoxygenation model, the ROS/mitogen-activated protein kinase (MAPK) signaling pathway activates the translocation of early growth response factor-1 (Egr-1) to the nucleus and initiates the expression of downstream genes, resulting in CMEC damage [16,17]. Micro (mi)RNA-200a affects ROS accumulation and CMEC damage due to hypoxia/reoxygenation by regulating thymosin β4 (Tβ4) [18,19].

In summary, the mechanism of ROS in CMEC injury is multi-layered, which may be related to the stress background of cells, the relative level of ROS and the space-time relationship. In the case of low ROS levels, antioxidant-related proteins such as glutathione that directly participate in redox balance in mitochondria may be in a preferential regulatory position. When ROS levels accumulate to a certain extent and break the redox balance, ROS may act more as a signaling molecule to activate downstream proteins and signaling pathways to cause biological reactions. At present, the downstream pathways involved in the mechanism of ROS in CMEC damage need to be further studied. But what can be determined is that the stress background (such as hypoxia, high glucose, high free fatty acid, inflammation) and its degree of cells have an important influence on ROS-mediated signal regulation and cell outcome.

3. Dysregulation of nitric oxide synthase (NOS) signaling pathway

Nitric oxide (NO) is a key vascular function homeostasis regulator, which is synthesized by L-arginine under the catalysis of NOS. At present, it is known that there are two kinds of NOS in endothelial cells, namely endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS). eNOS is a Ca^{2+} dependent enzyme, and eNOS catalyzes low levels of NO (nmol level), and it often plays a role in the steady-state regulation of vascular function. iNOS is an enzyme that is not Ca^{2+} dependent but regulated by cytokines and endotoxins. iNOS catalyzes high levels of NO (μmol level) and participates in a variety of pathophysiological processes [20,21]. Under the stress of endothelial cells, intracellular Ca^{2+} is released rapidly, and Ca^{2+} combines with calmodulin to form a complex to activate eNOS. Under the regulation of cofactors [such as flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), tetrahydrobiopterin (BH4), heme], eNOS oxidizes the terminal guanidine nitrogen atom of L-arginine to generate NO. NO has a short half-life and is quickly released into the blood and binds to the heme of vascular smooth muscle cells, thereby activating guanylate cyclase (cGMPase) and increasing the level of cyclic guanosinc monophosphate (cGMP). After cGMP is activated by protein kinase G, it regulates vasodilation and maintains vascular homeostasis [22,23].

It has been found that eNOS signal obstruction and decreased activity are the core features of many cardiovascular diseases [24]. At present, nicorandil, the first-choice drug used to interfere with angina pectoris caused by coronary microvascular disorders, works by activating cGMPase in vascular smooth muscles to increase the production of cGMP, thereby causing coronary vasodilation and improving microcirculation. Affecting the activity of NOS signal related substrates and cofactors will directly interfere with the NOS signal pathway. Animal experiments have found that increasing the level of CMEC BH4 can alleviate the impairment of NO-dependent vasodilation caused by diabetes [25]. Moreover, the accumulation of ROS, an important pathological factor of CMEC damage, will accelerate the degradation of NO. Intervention with CMEC active oxygen scavengers can significantly increase the bioavailability of NO and improve microvascular function, suggesting that ROS has a negative regulatory effect on the NOS signaling pathway [24,25]. In addition, some signaling pathways are also involved in the regulation of NOS activity. Studies have found [26,27] that activating the PI3K/AKT signaling pathway can also activate eNOS, thereby promoting the release of NO from CMEC, and ultimately alleviating the damage of microvascular function caused by ischemia–reperfusion. The PI3K/protein kinase B (PKB) signaling pathway is activated under hypoxia. The activated PKB (serine phosphorylation at position 473) phosphorylates the serine 1177 (Ser1177) position of eNOS, thereby activating eNOS, and then promoting the release of NO from CMEC, and finally reducing

the vascular dysfunction caused by hypoxia. Moreover, the PI3K inhibitor wortmannin will reduce the eNOS activity of CMEC [28,29].

In summary, the NOS signaling pathway in CMEC is regulated by a variety of enzymes, substrates and cofactors, and PI3K signaling may play an important role in regulating eNOS enzyme activity.

4. Disorders of cellular metabolic pathways

Cell metabolism is essential for maintaining the normal biochemical process and biological function of cells. Metabolic disorders such as chronic hyperglycemia and high free fatty acids increase the risk of cardiovascular events in patients [30,31]. Studies [32,33] have shown that changes in the metabolic status of myocardium and vascular endothelial cells in diabetes may be an important cause of cardiovascular events in patients with type 2 diabetes. At the same time, changes in coronary artery function can lead to an imbalance between myocardial supply and demand, which can lead to diabetic ischemic heart disease [33].

Among the metabolic abnormalities induced by high glucose, ROS accumulation and activation of cell death signaling pathways are the key molecular events that cause CMEC damage [34,35]. In a high glucose environment, CMEC has a higher level of glucose metabolism. At this time, the level of ROS production is significantly increased. It has been found that the competitive glycolysis inhibitor 2-DG can reduce the superoxide production of vascular endothelial cells induced by high glucose. It is suggested that the elevated glycolysis level caused by high sugar is an important factor in the production of superoxide in vascular endothelial cells [36,37]. Studies have found that high glucose does not change the levels of eNOS and iNOS, but high glucose reduces the level of the antioxidant protein glutathione (GSH) in the cell and increases the membrane-bound component of NAD(P)H oxidase p22-phox level. Studies [38,39] have confirmed that NAD(P)H is the main source of oxygen free radicals in CMEC. Therefore, the oxidative stress imbalance of CMEC induced by high glucose may be caused by the increase of pro-oxidase activity and the decrease of antioxidant protein level. The mechanism by which high glucose regulates GSH has not yet been elucidated. The results of studies on vascular smooth muscle indicate that the changes in antioxidant protein levels caused by high glucose may be related to the effect of high glucose on the expression and activity of the rate-limiting enzyme glutamylcysteine synthase (γ-GCS) [40]. But whether it can be analogized to CMEC is still uncertain. In addition, the metabolic disorder caused by high glucose can also activate the Akt/mammalian target of rapamycin (mTOR) signaling pathway, and then inhibit the autophagy process as a cellular stress protection program, and promote the apoptosis of CMEC [12]. Recent studies [12,40,41] have shown that the drug nicorandil for the treatment of CMVD can protect CMEC damage due to advanced glycosylation products by promoting autophagy. Therefore, autophagy may be an intervention target for CMEC damage caused by high glucose. In summary, the mechanism of CMEC damage caused by high glucose may be multifaceted.

In addition to high glucose, it has also been clinically found that hyperlipidemia can damage the coronary microvascular. Experimental studies have also confirmed that the disorder of free fatty acid metabolism can cause CMEC damage. Perilipid protein 5 (PLIN5) is an important protein in fatty acid metabolism. Loss of PLIN5 in CMEC will cause a decrease in intracellular lipid droplets and an increase in free fatty acid levels, followed by an increase in mitochondrial fatty acid β oxidation reaction, resulting in a large amount of ROS, which leads to a decrease in eNOS level and activity, and ultimately reduces NO production in CMEC and triggers cells apoptosis [42,43]. The main metabolic mode of normal vascular endothelial cells is glycolysis. The stimulation of high levels of fatty acids causes endothelial cells to turn to rely on oxidized fatty acids to provide energy. During this process, a large amount of ROS will be generated, which will cause endothelial cell dysfunction [44–46]. Therefore, fatty acid metabolism disorders cannot be ignored.

5. Cell senescence signaling pathway activation

Aging is a recognized risk factor for cardiovascular disease [47]. Animal experiments have shown that the coronary blood flow reserve and the expansion of endothelium-dependent resistance vessels decrease with age [48,49]. The mechanism may be related to the decreased secretion and inactivation of endothelium-dependent relaxing factors in coronary vascular endothelial cells and the release of contractile factors. The function of microvascular endothelial cells is impaired, and the inhibitory effect of endothelial-dependent relaxing factors is reduced, which accelerates the aggregation of immune cells and platelets, directly affecting the function of microvascular endothelial cells.

In recent years, studies have found that a class of nicotinamide adenine dinucleotide (NAD) dependent deacetylase sirtuins family proteins that are closely related to aging are involved in aging-related CMEC dysfunction. During aging and atherosclerosis, the expression of miRNA-217 in endothelial cells is up-regulated. Inhibition of sirtuins family protein silent information regulator 1 (SIRT1) can lead to changes in endothelial signals and decreased eNOS activity, and ultimately cause vascular endothelial cell dysfunction [50–52]. Moreover, SIRT3, another protein member of the Sirtuins family, can decrease with aging. It is mainly located in mitochondria and regulates mitochondrial function and cell metabolism, thereby protecting cardiomyocytes against aging, hypertrophy and oxidative stress damage [53,54]. Animal experiments found that the peak blood flow velocity and coronary blood flow reserve of SIRT3 knockout mice were significantly reduced, leading to aggravation of cardiac dysfunction and impaired repair after myocardial ischemia [55,56]. The influence of Sirtuins family proteins on the function of microvascular endothelial cells in aging may be related to their role as deacylating enzymes in regulating mitochondrial metabolism and maintaining the balance of oxidative stress. In addition, ROS also participates in the aging of CMEC. Studies [57,58] have found that elevated ROS can cause CMEC cell cycle arrest, proliferation inhibition and other cell senescence phenotypes, and FOXO3A can improve CMEC senescence by inhibiting the ROS/cycle-dependent kinase specific inhibitor P27 (p27^{kip1}) signaling pathway. Furthermore, animal experiments [59,60] have also found that aging increases the levels of fat-derived cytokines [such as tumor necrosis factor-α (TNF-α)], which is also a key factor leading to vascular endothelial dysfunction.

6. Inflammatory signal activation

Inflammatory signals play an important role in the occurrence and development of coronary microvascular endothelial dysfunction. Inflammatory factors not only cause vascular endothelial damage and intimal thickening, but also reduce the synthesis of NO and prostacyclin in endothelial cells. At the same time, activation of immune cells releases human endothelin (ET) and ET immune-like activators, resulting in abnormal endothelial function [61,62]. Under the condition of inflammatory signal activation, the expression of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) increases. At the same time, as the affinity of monocyte integrin receptors increases, the rolling of monocytes on the inner wall of blood vessels is inhibited. Under the action of chemokines, monocytes migrate and infiltrate

Table 1
Pharmacotherapy of coronary microvascular disease.

Medical treatment	Effect of medication
Conventional anti-angina drugs [1]	
Nitrates	+/−
Calcium channel antagonists	+/−
β-receptor blockers	+/−
Alpha beta-blockers	+/−
Ranolazine	+/−
Conventional anti-atherosclerosis drugs [1]	
Statins	+/−
Low-dose aspirin	+/−
Novel drugs	
ACE-I/ARB: Enalapril [68]	+
Statins: Pravastatin [69]	+
Insulin sensitizer drugs: Pioglitazone [70]	+
Antioxidant drugs	
Vitamin C, Vitamin E, Beta Carotene, Allopurinol [71]	+
Estrogen drugs [72]	+*
Gene-based treatment	
Vascular Endothelial Growth Factor (VEGF) [73]	+
Endothelial Nitric Oxide Synthase (eNOS) [74]	+
Superoxide dismutase (SOD) [75]	+
Other drugs	
5-Methyltetrahydrofolate (5-MTHF) [76]	+/−
L-arginine [77]	+
Tetrahydrobiopterin (BH4) [78]	+/−

+, benefit; +/−, mixed benefit and no benefit; *, benefit but risk; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

and damage the function of vascular endothelial cells [63]. In recent years, it has also been discovered that the classic inflammasome NOD-like receptor protein 3 (NLRP3) signaling pathway is also involved in the damage of CMEC. Under the stimulation of ischemia–reperfusion, the expression level of NLRP3 in CMEC increased, the activity of caspase-1 (caspase-1) increased, and the levels of downstream interleukin (IL)-1β and IL-18 were also

significant rise, causing local inflammation, and ultimately damage endothelial cell function. Further studies have shown that this may be related to thioredoxin interaction protein (TXNIP) binding and activating NLRP3 inflammasome during ischemia–reperfusion. Moreover, after adding a ROS scavenger to the same system, the interaction between TXNIP and NLRP3 was lifted, and the downstream signal was significantly inhibited, suggesting that ROS may be the upstream signal for NLRP3 inflammasome activation [64]. Interestingly, studies have found that NLRP3 protein is almost not expressed in cardiomyocytes, but expressed in CMEC, which further suggests that NLRP3 inflammasomes play an important role in the pathophysiological process of CMEC [64–66].

7. Pharmacotherapy of CMVD

The pharmacotherapy of CMVD are mainly divided into conventional treatments (including anti-angina treatments and anti-atherosclerosis treatments) and novel treatments that target the improvement of CMEC's dysfunction mentioned above [67]. Given that conventional anti-angina drugs (such as nitrates, calcium channel antagonists, β-receptor blockers) and anti-atherosclerotic drugs (such as statins, aspirin) have not significant clinical treatment effects on CMVD. Therefore, this has prompted people to explore novel drugs for the treatment of CMVD. Among them, new drugs targeting the improvement of coronary microvascular endothelial cell dysfunction are the research hotspots (Table 1). At present, there are no large-sample randomized clinical trials with CMVD as the research objective and cardiovascular events as the observation endpoint in the literature. Therefore, which treatment can reduce the cardiovascular event rate of CMVD is still unclear. Nevertheless, there have been small randomized clinical studies or non-randomized observational studies with CMVD as the research object and coronary microvascular function as the endpoint in the literature [79,80]. With the development of new drugs, we believe that more new drugs will be put into clinical use in the future.
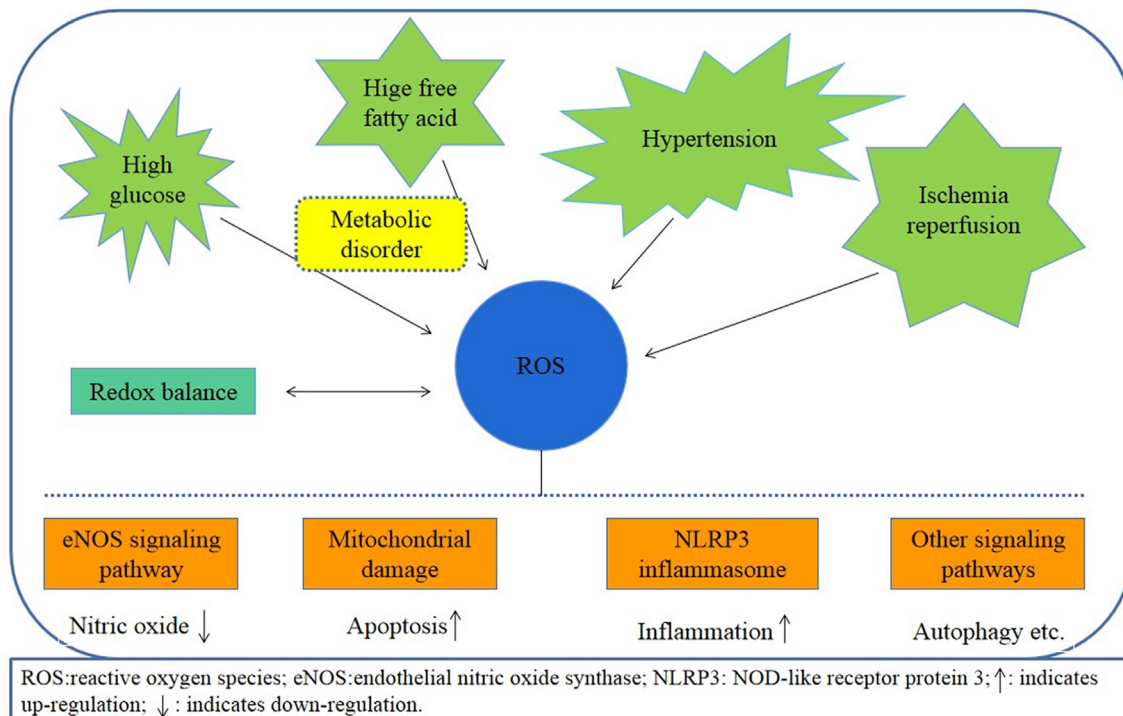

Fig. 1. Signaling pathways of coronary microvascular endothelial cell damage.

8. Conculations and outlook

Research on CMEC is in the ascendant, and its pathophysiological molecular mechanisms are gradually revealed. This article reviews the latest advances in the study of the molecular mechanisms of CMEC dysfunction, including ROS, NOS, metabolism, inflammation, aging and other multi-level signal transduction, and the molecular events involved still need further study. However, according to current research, ROS should be at the core of CMEC damage and in the upstream link. In the damage caused by ischemia, high glucose, high free fatty acid, and high blood pressure, although it triggers different signal pathways, it leads to apoptosis, NOS activity decreased, mitochondrial damage, metabolic disorders, cell senescence and many other cell phenotypes, but tracing the origin, the excessive accumulation of ROS all play an important role (Fig. 1). At present, the signal pathways related to ROS and CMEC damage have been partly clarified, but the causality and specific mechanisms need to be further studied. It is certain that ROS, as an important molecular messenger in cells, has many functions in CMEC.

Coronary microvascular have their particularities, and CMEC is more susceptible to changes in the microenvironment. Under the stimulation of pathological factors, CMEC has priority over myocardial cells to undergo pathological changes, and such changes are more concealed and difficult to detect. Positron emission tomography (PET) myocardial perfusion imaging combined with CFR (<2.0) method has been established to diagnose CMVD, but it has a few limitations. Although some results have been achieved in clinical and basic research on CMVD and CMEC, there is still a lack of studies on early specific serum markers of CMEC damage and cohort studies on the correlation between CMEC injury-specific genes and the prognosis of CMVD.

In terms of treatment, conventional treatments have not alleviated the progression of CMVD. Data shows that more than 50% of CMVD patients will develop angiographic coronary artery disease in the next 10 years. Although there have been some basic studies on drugs that protect the function of CMEC, few intervention drugs that directly target CMEC have been transformed into clinical applications. It is hoped that through the study of pathophysiological mechanisms for targeted treatment of CMEC dysfunction, effective treatment strategies for CMVD can be further explored.

The etiology of CMVD involves a variety of cells and tissues. In addition to CMEC, vascular smooth muscle cells, cardiomyocytes, and immune cells (such as macrophages, neutrophils) are all involved in the occurrence and development of CMVD. This article only reviews the molecular mechanism of CMEC damage. To systematically understand the pathophysiological mechanism of CMVD, it is necessary to focus on the overall situation and analyze the signal transduction of various types of cells and the cross-talk between cell tissues. With the change of molecular biology technology and the vigorous development of multi-omics and big data research, it is believed that the scientific problems surrounding CMEC will be solved one by one.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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