Natural Allies for Heart Health: Nrf2 Activation and Cardiovascular Disease Management

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Abstract: The term "cardiovascular diseases" (CVD) refers to various ailments that affect the heart and blood vessels, including myocardial ischemia, congenital heart defects, heart failure, rheumatic heart disease, hypertension, peripheral artery disease, atherosclerosis, and cardiomyopathies. Despite

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significant breakthroughs in preventative measures and treatment choices, CVDs significantly contribute to morbidity and mortality, imposing a considerable financial burden. Oxidative stress (OS) is a fundamental contributor to the development and progression of CVDs, resulting from an inherent disparity in generating reactive oxygen species. The disparity above significantly contributes to the aberrant operation of the cardiovascular system. To tackle this issue, therapeutic intervention primarily emphasizes the nuclear erythroid 2-related factor 2 (Nrf2), a transcription factor crucial in regulating endogenous antioxidant defense systems against OS. The Nrf2 exhibits potential as a promising target for effectively managing CVDs. Significantly, an emerging field of study is around the utilization of natural substances to stimulate the activation of Nrf2, hence facilitating the promotion of cardioprotection. This technique introduces a new pathway for treating CVD. The substances above elicit their advantageous effects by mitigating the impact of OS via initiating Nrf2 signaling. The primary objective of our study is to provide significant insights that can contribute to advancing treatment methods, including natural products. These strategies aim to tackle the obstacles associated with CVDs. (Curr Probl Cardiol 2024;49:102084.)

Introduction

According to the WHO, cardiovascular diseases (CVDs) constitute the leading cause of death worldwide. CVDs constituted approximately 33.3% of worldwide mortality in 2021, leading to an estimated 20.5 million fatalities. Based on the provided reference, the data suggests an average of 56,000 daily fatalities. This figure can be interpreted as 1 death occurring approximately every 1.5 seconds.[1] According to pertinent health data, it is evident that the United States is confronted with a substantial prevalence of CVDs, impacting a total of 92.1 million individuals. Coronary heart disease (CHD) was determined to be the primary cause of deaths linked to CVD in the United States during a specific year. This condition accounted for 54% of CVD cases and resulted in the mortality of 375,476 individuals.[2]

The regions with the highest age-standardized prevalence of ischemic heart diseases (IHD) in 2019 were Central Asia, the Middle East, North Africa, and Eastern Europe.[3] Despite a recent decline in unfavorable outcomes associated with ischemic heart disease (IHD), the overall worldwide burden of IHD remains substantial for both genders.[4] The initiation of coronary atherosclerosis commences after birth and encompasses an ongoing and dynamic progression. The process above commences the accumulation of plaque and the following increase in thickness of the intimal layer. Over the years, the lesion above grows in dimensions and becomes perceptible to the unaided human eye. The clinical manifestations of these diseases generally become evident in patients aged 35 years or older.[5] A robust cardiovascular system is crucial for the survival and overall welfare of mammalian organisms. Regrettably, the incidence of CVD and heart problems significantly impact human morbidity and mortality, with a continuing global increase. A thorough examination of cardiovascular disease patterns over a quarter of a century revealed that in 2015, the worldwide incidence of CVD impacted a population exceeding 400 million individuals, resulting in approximately 18 million deaths. The trend above demonstrates a notable increase of about 5 million deaths since 1990, approximately one-third of the recorded deaths during that particular year.[6] although the diligent events of international health organizations, the imperative task of identifying effective ways to detect and intervene in the early stages of CVD and providing suitable treatment options for patients with concurrent medical conditions continues to be of paramount significance.

The etiology of CVD and other cardiac diseases encompasses complex biological processes that provide difficulties in isolating specific pathophysiological causes. It is frequently exposed to several substances that may harm the cells of the cardiovascular system. Reactive oxygen species (ROS), for example, are endogenous signaling molecules that can be produced from exogenous sources like medicinal drugs. While ROS are essential for maintaining internal equilibrium, an excessive buildup of ROS can result in toxicity.[7] Various intracellular physiological processes have developed to protect and maintain cardiovascular well-being in response to this challenge. Several pharmaceutical compounds currently in clinical trials are designed to target specific pathways to provide therapeutic benefits. For instance, statins are commonly prescribed for the treatment of atherosclerosis,[8] angiotensin-converting enzyme inhibitors are utilized to manage hypertension,[9] and metformin is employed to address cardiovascular problems in individuals with diabetes.[10]

Nevertheless, these pharmaceutical agents effectively manage CVD symptoms. They are typically provided once the problem is clinically apparent, frequently following substantial and occasionally irreparable harm. In contrast, it is possible to employ several approaches to utilize physiological pathways during the early phases of CVD to counteract first harmful stimuli and shield healthy cells and tissues from the progression of the disease.[11] This approach would require enhanced detection techniques and a more proactive stance towards healthcare. A transcription factor that has received significant scientific focus during the past 20 years is the nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2). There exists compelling data indicating that the activation of Nrf2 pathways may provide cellular protection, hence driving more investigation into the cardioprotective properties of NRF2 signaling. In a variety of models of CVDs, including heart malfunction,[12,13] infarction of the myocardium,[14] atherosclerosis,[15] cardiomyopathy,[16] and fibrosis,[17,18] physiological activators of NRF2 have consistently shown the ability to treat disease-like conditions. Despite the lack of application of Nrf2 stimulation as a cardioprotective mechanism in patients, the growing body of research suggests that its clinical translation may be expected shortly.

Several pharmacological treatments targeting Nrf2 have been subjected to clinical trials for various disorders, including nephropathy, inflammation, malignancies, myopathies, and respiratory diseases.[19] The treatment of multiple sclerosis and psoriasis with dimethyl fumarate, a chemical molecule with electrophilic properties that increase the integrity and nuclear translocation of Nrf2,[20] has received FDA approval. Patients with type 2 diabetic mellitus (T2DM) and chronic kidney disease were administered 2-cyano-3, 12-dioxoolean1, 9-dien-28oic acid-methyl ester (CDDO-Me), an electrophilic Nrf2 activator. This improvement in glomerular filtration rates lasted for an entire year after the treatment was finished.[21] There is a robust association between Nrf2 and heart toxicity or disease mitigation. In this scenario, many activators exhibiting this characteristic could be reutilized to augment cardiovascular efficacy. This study aims to synthesize the current understanding of cellular mechanisms contributing to cardiac problems and CVD development, emphasizing the potential of modulating Nrf2 signaling pathways to provide physiological protection. The study will focus on the crucial role of Nrf2 in mitigating the early pathophysiological changes that occur as a response to harmful events preceding CVD.

The identification of Nrf2 emerged from experimental investigations by the Kan research group, with the primary objective of assessing the regulatory mechanisms governing the β-globin gene. The *NFE2L2* gene

is responsible for encoding the Nrf2 protein.[22] Subsequently, in 1997, Itoh and colleagues conducted a study to describe Nrf2 as a protein involved in the antioxidant response, with the ability to stimulate the function of phase II detoxifying enzymes. The homozygous knockout of Nrf2 in mice resulted in a significant reduction in the expression of NQO1 (NAD(P)H quinone oxidoreductase 1).[23] Similarly, animals with a defect in Nrf2 exhibited a diminished ability to efficiently orchestrate an antioxidant response when confronted with various pro-oxidative chemicals. Since then, Nrf2′s importance in controlling these antioxidant responses has been extensively recognized.

However, the functions of Nrf2 expand beyond this specific job. The Nrf2 protein directly impacts over 1% of the human genome, activating around 240 genes involved in various biological processes.[24-26] The entities mentioned in this context have diverse functions across multiple domains, encompassing bioenergetics, carbohydrate and lipid metabolism, iron regulation, and the facilitation of other transcription factors.[27]

NFE2L Family Members

NRF2, NFE2, NRF1, and NRF3 are all transcription factors that belong to the cap n' collar (CNC) bZIP subfamily.[28] NRF2 has been the focus of more thorough research and characterization, even though all 3 NRF protein family members have shown cytoprotective qualities. As a result, there has been a notable focus on Nrf2 in scientific investigation, leading to a comparatively restricted understanding of the functions and behaviors of NRF1 and NRF3.[29] Growing empirical evidence suggests that NRF1 may be crucial in regulating normal physiological activities. The potential roles of these systems encompass the facilitation of proteasomal reactions, the control of chaperone gene expression, and the preservation of protein quality control mechanisms.[30] NRF3 has received limited attention in the context of its specific family, and there is a substantial need for further investigation to elucidate its various physiological functions. The limited understanding of the subject can be partially attributed to the apparent inefficiency of phenotypic alterations resulting from the removal of the NFE2L3 gene in organisms, despite the notable vulnerability to oxidative stress (OS) observed in both NRF1 and NRF2 knockout models of mice.[31] The significance of NRF3 in regulating cellular proliferation has been extensively acknowledged, rendering it a topic of considerable significance in cancer research.[32,33] The evidence indicates that NRF1 notably impacts cardiovascular physiological processes, specifically regulating redox balance, providing cellular protection, and

promoting cardiac regeneration in neonates.[34] The forthcoming progress in NRF1 transcriptomics research and its potential ramifications in preventing or treating heart disease or cardiotoxicity is anticipated. Given the extensive body of data connecting Nrf2 to these functional characteristics thus far and the ongoing improvements in the development of pharmacological interventions for modulating Nrf2 in clinical contexts, this protein will serve as the primary subject of investigation in this review.

Molecular Structure of Nrf2

Nrf2, a transcription factor that falls under the cap 'n' collar (CNC) basic leucine zipper (bZIP) family, comprises 605 amino acids. It is categorized into 7 distinct structures, NEH1 through NEH7, which are assigned based on functional roles.[27] The CNC-bZIP domain of Neh1 forms a compound with the small musculoaponeurotic fibrosarcoma (sMaf) protein, binding to the antioxidant response element (ARE) on the target gene.[22,23] The Neh2 domain possesses a binding motif consisting of the high-affinity ETGE (77DxETGE82) and the low-affinity DLG (23LxxQDxDLG31).[35] The ubiquitination and subsequent degradation of Nrf2 is facilitated by its attachment to the Kelch domain of Keap1, a protein that shares similarity with the KELch-like epichlopropane Kelch motif. Keap1 has been identified as the primary component responsible for suppressing Nrf2.[36] The presence of the VFLVPK motif is essential for facilitating the recruitment of auxiliary proteins by the Neh3 domain and establishing a connection between transcription factors and active transcription machinery. The proper functioning of Nrf2 is of utmost importance. The potential linkage between Neh3 and the DNA-binding protein 6 (CHD6) interaction has been suggested.[37] To enhance the activation pathway of Nrf2/ARE, Neh4, and Neh5 work together to enable the binding of Nrf2 to histone acetyltransferase P300, as well as to bind CREB-binding protein (CBP) synergistically.[38,39] The translocation of Nrf2 into the nucleus is induced by the activation of the AMPK-CREB-Nrf2 signaling cascade, initiated by the selective COX-2 inhibitor celecoxib—Nehemiah 6 features 2 significant motifs, namely DSGIS338 and DSAPGS378. The catalysis of DSGIS can be facilitated through the phosphorylation of glycogen synthase kinase-3β (GSK-3β), producing phosphodiester. Both individuals can ubiquitinate Nrf2 by recognizing and binding to the Skp1-Cul1-Rbx1/Roc1E3 ubiquitin ligase (SCFβ-TrCP). This interaction facilitates the renewal of Nrf2 in the absence of Keap1, ultimately regulating its stability and activity.[40] Protein kinase B, commonly called Akt, phosphorylates glycogen synthase kinase-3β (GSK-

3β) and hinders its activity. The phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is responsible for activating nuclear factor erythroid 2-related factor 2 (Nrf2) by suppressing GSK-3β.[41] The binding of Neh7 to RXRα results in the formation of a heteromeric protein-protein complex known as RXRα-Nrf2.[42] This complex effectively hinders the recruitment of CBP and RNA poll to the promoters of target genes.[43] A sequence, namely tgccggCgc, resembles the ARE motif and is located around 650 bases upstream of the Nrf2 gene when measured from exon 1. This region plays a crucial role in facilitating the positive feedback mechanism of Nrf2 gene expression, as indicated by previous research.[44] The AhR, a receptor for aromatic hydrocarbons, forms dimers within the nucleus and exerts its influence on the promoter region of the Nrf2 gene, resulting in an upregulation of Nrf2 transcription.[45] To activate the Nrf2 signaling pathway, the Notch signaling pathway facilitates the recruitment of transcription from the Notch internal domain to the conserved rBPJ- location on the Nrf2 promoter (Fig 1).

Regulation and Mechanism of Nrf2 Activity

Regulation of Activity

Ubiquitination Degradation is Nrf2 Protein Expression. As an essential mode of activity regulation, ubiquitin molecules covalently bind to Nrf2 proteins, and the ubiquitin chain targets Nrf2 to the proteasome for degradation. Two pathways, Keap1-Nrf2 and GSK-3β-Nrf2, are the main ways of ubiquitination degradation of Nrf2 (Fig 2). Keap1-Nrf2 pathway: As a substrate adaptor protein, Keap1 recruits ubiquitin ligase skeleton protein (Cullin3, Cul3) and Ring domain protein (RING box protein-1, Rbx1) to form the E3 ubiquitin ligase complex, which binds specifically to Nrf2. The expression of Nrf2 in the cytoplasm tends to be stable at a low level under basal homeostasis.[46] There are 4 functional areas in Keap1: DGR (double glycine repeat) begins at the C-terminal area of the N, BTB (Broad complex, Tramtrack, and Bric-a-Brac), IVR (intervening region), and DGR domains. The CTR domain is collectively called the DC domain. Two KEap1-DC domains of the Keap1 homo-dimer engage with the ETGE and DLG motifs of Nrf2, which causes Nrf2 to polymerize, be ubiquitinated, and then be degraded. This occurs as a result of E3 ligase activity. As a result of the DLG motif being liberated from the Keap1-Nrf2 complex under OS, Nrf2, which is ectopically located in the nucleus, is released, encouraging the transcription of genes that protect

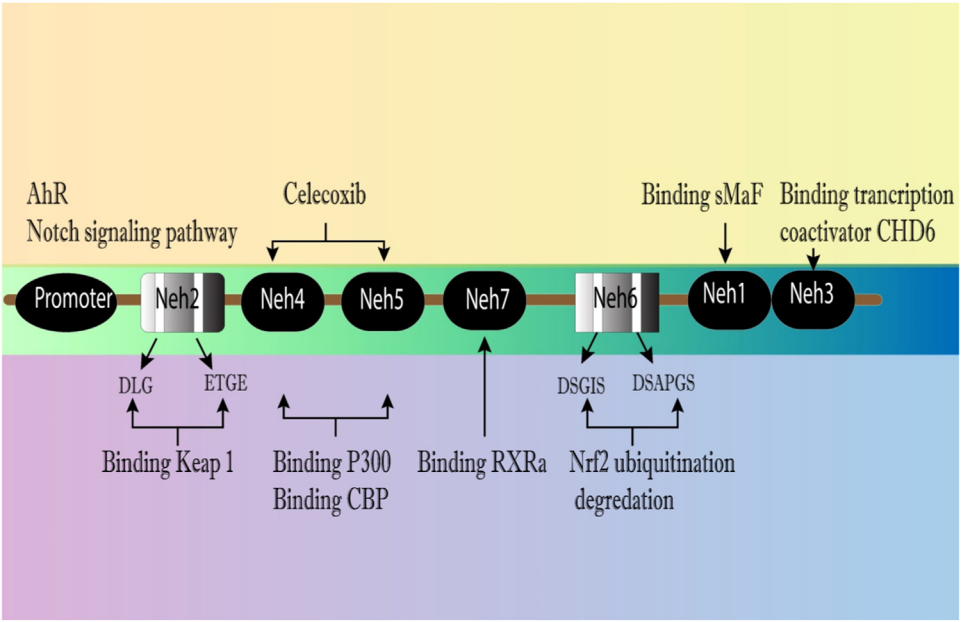

FIG 1. Structure of Nrf2.

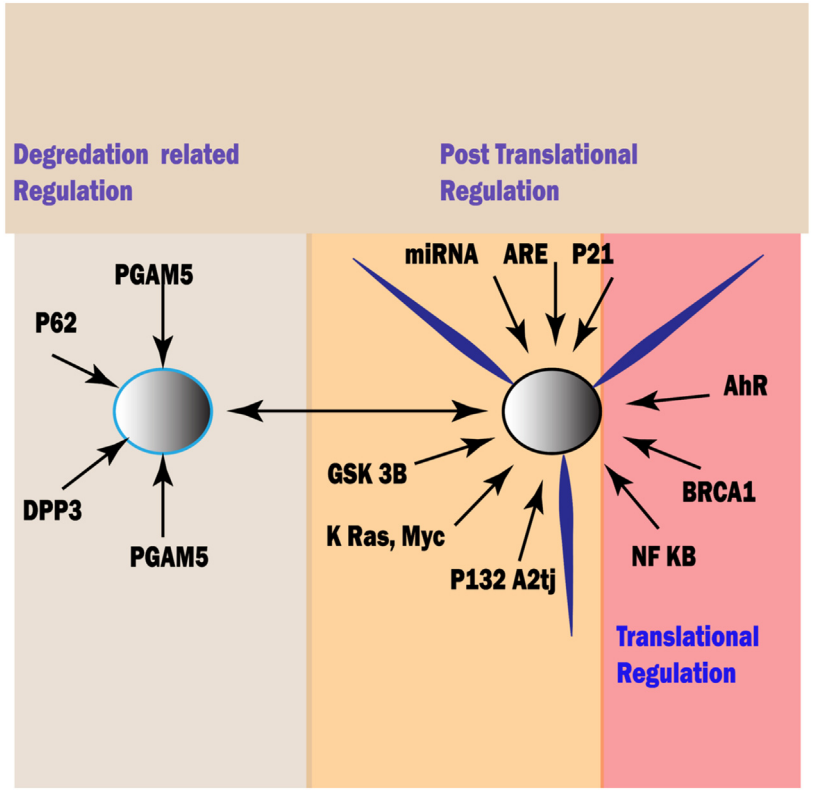

FIG 2. Transcription, post-translation, and degradation-related regulation of Nrf2.

cells and reversing the effects of OS. In addition, it has been shown that Cul3 separates from the Keap1-Cul3 complex under OS, allowing Nrf2 to escape from proteasome degradation and resulting in subsequent nuclear translocation.[47,48]

In cells, there are many molecules such as p62,[49] breast cancer susceptibility 1(Brca1),[50] linear serine/threonine protein mutase5, phosphoglycerate mutase5, PGAM5),[51] dipeptidyl-peptidase 3(DPP3),[52] p21,[53] partner and localizer of BRCA2, Palb2,[54] etc., By preventing Keap1 and Nrf2 from interacting, Nrf2′s ubiquitination can be controlled. The GSK-3-Nrf2 pathway The PI3K/Akt pathway or the WNT signaling pathway can activate and control the serine/threonine protein kinase GSK-3.[55] The ubiquitin ligase adaptor -TrCP recognizes GSK-3, which then phosphorylates a particular serine residue in the Neh6 domain called DSGIS. This promotes proteasomal degradation through the Cullin1/Rbx1 complex.[26] According to the "dual flux controller" model, GSK-3β-TrCP fine-tunes Nrf2 levels to meet the transient metabolic needs of cells, while Keap1

senses and responds to environmental aggression by rapidly increasing Nrf2 levels .

Transcription-related Nrf2 transcription is regulated by epigenetic modification mechanisms, including hypermethylation of its promoter region or single nucleotide polymorphism (SNP), reducing Nrf2 expression.[56] During transcription initiation, Nrf2 is regulated by several transcription factors and signaling pathways, including the aryl hydrocarbon receptor, AhR), NF-κB, Kras, B-Raf, Myc, PI3K-Akt, Notch, etc.[57-59] In addition, the promoter of the Nrf2 gene contains sequences similar to ARE, resulting in positive feedback of Nrf2 amplification (Fig 2).

Downstream Genes and Functions of Nrf2

Nrf2 regulates many downstream target genes, and their functions are powerful. The roles of Nrf2 in antioxidant and detoxification, metabolic regulation, mitochondrial function regulation, autophagy, and apoptosis regulation will be briefly introduced in the following paragraphs.

Detoxification and antioxidant By controlling the transcription of several antioxidant and phase II detoxification genes, such as ROS, radiation, environmental toxins, and probiotics in food, Nrf2 shields cells from various hazardous chemicals.[60] Quinone oxidoreductase-1, or NQO1, is a widely distributed flavase that is a dimer. Each monomer interacts with a flavin adenine dinucleotide (FAD) to remove quinone from the body and have a detoxifying effect using the reaction "NAD(P)H+ a quinone NAD (P)+ hydroquinone.[61] NQO1 can also reduce oxidized vitamin K or competitively bind to enzymes associated with the oxidation cycle, protecting cells from OS.

Heme oxygenase-1 (HO-1) pathway: When subjected to OS, free Nrf2 moves into the nucleus, forms a dimer with sMaf, and then bonds to the ARE element of the HO-1 gene to encourage transcription. A significant antioxidant role is played in OS and cell injury by HO-1 products such as CO and bilirubin.[62] HO-1 can also directly suppress pro-inflammatory cytokines and activate anti-inflammatory cytokines to keep the balance of inflammatory processes.[10] Glutathione (GSH) can reduce the disulfide bond formed in the protein and, at the same time, convert it to glutathione disulfide (GSSG), preventing ROS damage to cells. The xCT subunit in the Xc− system, the direct transcription target of Nfr2, can introduce cystine, the raw material for GSH synthesis, into cells.[63] Nrf2 can also promote the expression of various GSH-related genes containing ARE, rapidly respond to OS, and maintain a balanced REDOX state of cells.[64]

In addition, Nrf2 can act on the transcription of GSH reductase and maintain the reduced GSH level.

Substance Metabolism Nrf2 can promote the transcription of enzymes involved in the pentose phosphate pathway (PPP) and de novo synthesis of purine nucleotides. Metabolomics analysis has shown that Nrf2 promotes purine nucleotide synthesis and glutamine metabolism in the case of active PI3K-Akt signaling. Nrf2 can also transcriptionally regulate the enzyme-producing activity of malic enzyme1 (ME1) in enzyme1 to produce NADPH.[65] The activation of these metabolic genes by Nrf2 promotes glycolysis and the synthesis of amino acids, nucleotides, and NADPH, which plays an essential role in cell metabolism and REDOX balance.

When mitochondrial function Nrf2 is deficient, the activity of mitochondrial complex I is impaired, and the production of ROS is increased. When Nrf2 is activated, oxidative phosphorylation is more efficient, increasing proton conduction in the mitochondrial inner membrane and reducing superoxide production. Moreover, Nrf2 can enhance the activity of the tricarboxylic acid cycle, promote mitochondrial fatty acid oxidation, and improve substrate utilization of mitochondrial respiration and ATP production during oxidative phosphorylation, which is particularly important for cell protection under OS conditions. Studies have found that Nrf2 increases nucleotide synthesis and maintains mitochondrial integrity under oxidative and inflammatory stress conditions.[66]

The downstream Nrf2 gene also regulates apoptosis and Autophagy. Under OS, Nrf2 induces the expression of p62/SQSTM1 and nuclear dot protein 52(NDP52), which promotes the degradation of damaged organelles through autophagy. In addition, activation of the Nrf2/Keap1 pathway protects cells from apoptosis during neurodegeneration.[67]

The Canonical Control Mechanisms Governing the Regulation of Nrf2

Nrf2′s intracellular activity can be controlled by various methods, with post-translational alterations and degradation pathways receiving much attention.[68] The principal mechanism by which NRF2 is regulated is through KEAP1-mediated proteasomal degradation. The KEAP1 homodimers exhibit the ability to interact with both the Neh2 domain of NRF2 and serve as a mediator for CULLIN3 (CUL3) and RING box protein (RBX) E3 ubiquitin ligases, (Fig 3).[69,70] The complicated complex then engages in polyubiquitination of NRF2, thereby facilitating its targeting for proteasomal destruction. This process results in a steady turnover of basal NRF2 and

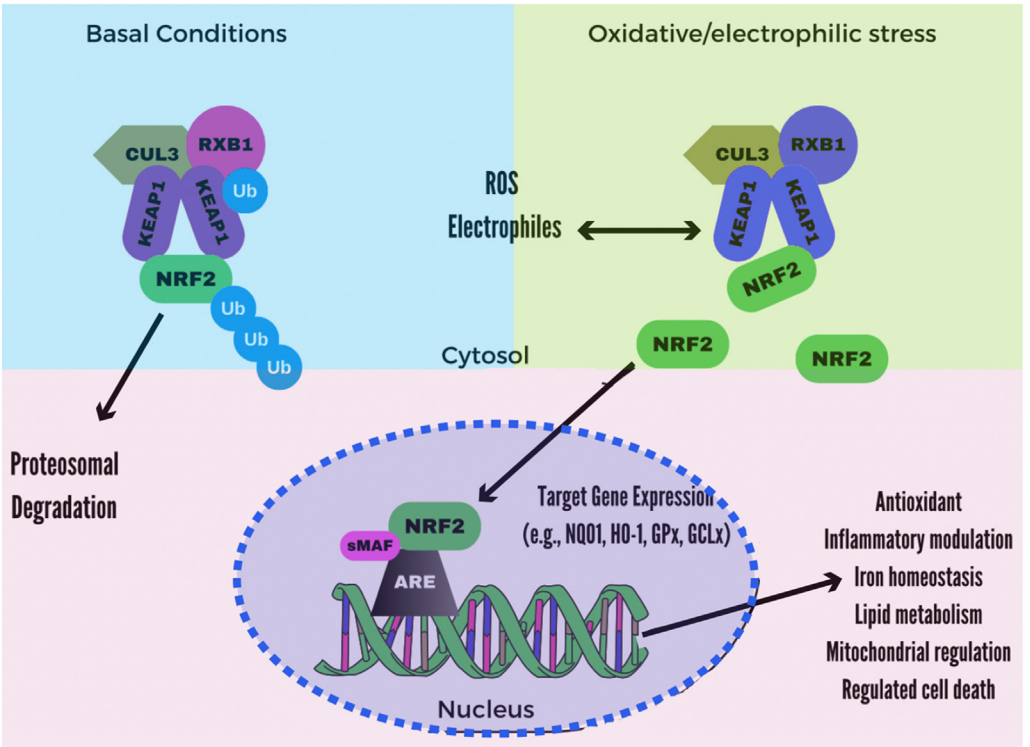

FIG 3. KEAP1-mediated regulations affect NRF2 function in normal and stressed cells. Homodimer binding keeps Nrf2 in the cytoplasm in basal KEAP1. CUL3-RBX1 and KEAP1 create an E3 ubiquitin ligase complex to ubiquitinate Nrf2. This change prepares NRF2 for proteolysis. Increased ROS or electrophilic cysteine residues in KEAP1 disrupt NRF2–KEAP1 interaction. This permits freshly generated NRF2 to enter the nucleus, heterodimerize with sMAF proteins, and bind AREs. This sequence activates antioxidant and protective genes.

restricts its abundance.[71,72] As a result, the duration of NRF2′s half-life is relatively brief, often ranging from 10 to 30 minutes.[73,74]

The research conducted by the Yamamoto team showcased the ability to prolong the half-life in macrophages by administering the electrophile diethylmaleate (DEM). This intervention effectively stimulated the activation of NRF2 target genes for 2 hours. Nevertheless, the impact above is attained by post-transcriptional regulation, as there is no simultaneous elevation in NRF2 mRNA. The KEAP1 protein has many thiol groups, including many cysteine residues. These cysteines can interact with electrophilic substances such as DEM, disrupting the connection between NRF2 and KEAP1. Extensive investigation has been conducted by the Dinkova-Kostova group on the particular cysteine groups present in KEAP1 and their influence on the binding of NRF2. It is worth noting that Cys151 has been identified as a residue of considerable importance in its response to stress, as indicated by previous research.[75]

The link between KEAP1 and CUL3 is stable, and NRF2 remains attached to the KEAP1 dimer despite changes in Cys151 and other residues induced by outside stimulation or internal signaling molecules such as ROS.[76] Regardless of the potency of the stimulus, it has been observed that NRF2-activating drugs can decouple KEAP1-DLG contacts. However, it is important to note that the essential "latch" ETGE mechanism remains unchanged.[77] In these particular situations, it is noteworthy that NRF2 effectively occupies the binding sites of KEAP1, enabling the titration process through newly produced NRF2 molecules. This step facilitates the translocation of these molecules to the nucleus, where they participate in subsequent transcriptional regulatory actions, (Fig 3). The ability to finely regulate the degradation of NRF2 and modify KEAP1 to activate NRF2 provides the cellular system with a highly responsive and adaptable mechanism to cope with changes in the intracellular environment. In brief, NRF2 is a crucial stress-responsive transcription component with a wide range of functions with significant implications.

Relationship Between Nrf2 and Various CVDs

The variability of the Nrf2 gene promoter in the population impacts ARE activity in the promoter. According to several cohort studies, the population statistics of these promoter polymorphisms are compatible with the decline in Nrf2 expression and the rise in the incidence of cardiovascular illnesses.[78,79] Additionally, it has been demonstrated in animal studies that Nrf2 knockout mice have a higher incidence of heart attack and stroke than normal mice, indicating that Nrf2 expression is crucial

for both the development and occurrence of various CVDs. This has piqued the interest of many researchers now looking into associated mechanisms.

Atherosclerosis

Coronary heart disease, stroke, and peripheral vascular disease are all primarily caused by atherosclerosis (AS), a progressive disease.[80] According to the study, Nrf2 has 2 functions in the emergence of AS.

On the one hand, Nrf2 can inhibit or stop the growth of AS. Oxidized low-density lipoprotein (ox-LDL) synthesis is a crucial component in the development of AS. Oxidation of LDL to ox-LDL is induced by ROS. Macrophages absorb more lipoprotein-derived cholesterol than is released, which causes the intracellular free cholesterol to be transformed into cholesterol esters and stored in lipid droplets, causing foam cells to form in the arterial wall and aggravating the development of AS plaques.[81] The antioxidant response of macrophages is vital to reducing ROS levels and protecting mitochondria, other organelles, proteins, and nucleic acids from oxidative damage. However, in plaque macrophages, the transcription of antioxidant genes and mitochondrial transport of Antioxidant GSH are inhibited, which magnifies the inflammation of the artery wall. Experiments have shown that the loss of Nrf2 in mice with defective LDL receptors can exacerbate AS lesions and increase the expression of pro-inflammatory genes.[82] At the same time, Nrf2 can up-regulate the expression of peroxiredoxin 1(Prdx1) in macrophages and prevent macrophages from forming foam cells induced by ox-LDL, thus preventing the formation of AS.[83]

Moreover, Nrf2 has been proven to be an effective antioxidant molecule, regulating the transcription of various antioxidant and detoxification genes and protecting cells from the damage of various harmful substances, including ROS, suggesting that the Antioxidant and anti-inflammatory effects of Nrf2 affect the formation of AS plaques to a certain extent and contribute to the development of resistance to AS. On the other hand, Nrf2 may also exacerbate the evolution of AS, but its mechanism has many explanations. Experiments have shown that mice with hypercholesterolemia apolipoprotein E (ApoE) deletion are prone to AS, and some ApoE deletion mice can reduce plaque deposition and retain the elasticity of the blood vessel wall when Nrf2 is knocked out.[84] In other words, in ApoE-deficient mice, Nrf2 deficiency protects against AS. This may be so because Nrf2 can boost ox-LDL absorption in macrophages, accelerate the growth of foam cells, and up-regulate the expression of the scavenger

receptor CD36.[85,86] Additionally, it was discovered that cholesterol crystals might activate Nrf2, a positive regulator of the inflammasome, which increased IL-1-mediated vascular inflammation and exacerbated AS in the experiment on stimulating the inflammasome and AS by cholesterol crystals.[87] Additionally, bone marrow transplantation studies have demonstrated that AS development is made worse by increased Nrf2 activity in bone marrow-derived macrophages (BMDM). The early stage of plaque formation is known to be predominately occupied by M2 macrophages, while M1 macrophages predominately occupy the late stage. Changes in the quantity or function of M1 and M2 macrophages may allow Nrf2 to control AS.[88] Based on the importance and complexity of the function of Nrf2 in the development of AS, in the process of targeting Nrf2 for the treatment of AS in the future, the combined influence of other co-existing diseases, taking drugs, diet and other factors should be taken into account. More in-depth mechanism research should be conducted in these aspects.[89]

Heart Attack: The Role of Ischemia-Reperfusion Injury

The initial ischemia-induced cell injury causes an increase in ROS. Blood reperfusion is crucial for the survival of ischemic myocardium because it is required to remove toxic compounds and restore metabolism fully. Myocardial reperfusion injury (MRI), caused by quick and severe damage to biomolecules due to blood flow to an ischemic region, is known as. Myocardial ischemia-reperfusion damage is significantly influenced by OS and inflammation brought on by ischemia/reperfusion (I/R). TNF-, IL-6 , and IL-8 are released throughout the inflammatory phase, and macrophages and neutrophils penetrate the cardiac tissue.[90] By enhancing the detoxification route and antioxidant capability, Nrf2 has been shown to protect cardiac fibroblasts and heart cells from OS.[91] I/R stimulates the translocation of Nrf2 to the nucleus, causes Nrf2 to become separated from Keap1, and binds to ARE to trigger phase II detoxifying and antioxidant genes. Apoptotic proteins, such as B-cell lymphoma-2 (Bcl-2) and Bax (Bcl2-associated X), and phase II enzymes for detoxification, such as HO-1, are some examples of the substances that have an impact on cell survival through the Nrf2/ARE pathway. Studies have shown that 4-hydroxynonenoic acid can directly induce the conformational change of Keap1 by forming adduces or indirectly induce the conformational change of Keap1 by increasing the production of mitochondrial ROS, activate Nrf2, stimulate the biosynthesis of GSH based on activating antioxidant enzymes, and further protect the heart.

However, the mechanism of Nrf2 nucleus aggregation induced by 4-hydroxynonenoic acid remains elucidated.[92] Increased Nrf2 expression in the heart is the result of pretreatment with epidermal growth factor (EGF), which also induces an elevated expression of Nrf2 downstream target genes such as NQO-1 and HO-1 and suppresses the expression of inflammatory factors like TNF- and IL-6.[93] I/R reduced the production of ROS and the apoptosis it produced. Additionally, several exogenous or endogenous compounds have been shown to protect the heart during ischemia-reperfusion by stimulating the Nrf2-ARE system. This suggests that these drugs may be used to treat ischemia-reperfusion clinically.

Acute myocardial infarction (AMI) is the world's top cause of death and the most prevalent cause of myocardial infarction. Myocardial infarction is the loss of heart muscle cells brought on by persistent ischemia. Controlling the infarct size in medical care is crucial since a sizeable myocardial infarction can result in cardiogenic shock, lethal arrhythmia, and severe heart failure.[13] Experiments have found that the infarct size of NRF2-deficient mice increased in myocardial infarction models, suggesting that Nrf2 can effectively control myocardial infarction size during myocardial ischemia.[94] Pretreatment or posttreatment is the most efficient strategy to prevent ischemia or reperfusion injury to cardiac tissue. Experiments have demonstrated that ischemic preconditioning increases La protein binding to the 5′UTR of Nrf2mRNA and that this increases the level of Nrf2 protein in the myocardium, which controls transcription of antioxidants and detoxification gene clusters, rapidly activating endogenous defense and reducing infarct size by about 50%.[95,96]

Additionally, studies have demonstrated that ischemia post-processing caused cardiac STAT3 (signal transducer and activator of transcription-3) activation to control Nrf2 nuclear translocation. Reducing the size of infarcts, minimizing vascular dysfunction, and inhibiting neutrophil buildup were all achieved by increasing the production of antioxidant genes, including HO-1 and superoxide dismutase (SOD).[97] However, in clinical treatment, patients are usually complicated with multiple chronic diseases, leading to ineffective pretreatment and post-treatment and may cause repetitive injury to arteries. Therefore, it is necessary to explore further the mechanism of the combined development of various diseases in the body and find suitable treatment plans. In conclusion, Nrf2 can reduce myocardial infarction size, myocardial cell apoptosis, and I/R injury inflammation after ischemia-reperfusion by activating transcription of relevant downstream target cells, thereby partially preserving cardiac function.

Myocardial Hypertrophy-Related Congestive Heart Failure

Cardiac hypertrophy is compensation for the weakened heart function caused by diseases such as myocardial infarction, and heart failure occurs when cardiac hypertrophy is insufficient to compensate for the weakened heart function. Heart failure and many risk factors for heart failure are associated with OS, so Nrf2 levels are associated with the severity of heart failure.[12,98] Compared with normal mice, Nrf2 knockout mice significantly increased OS secondary to pathologically stressed hearts after aortic coarctation treatment. They showed myocardial thickening, fibrosis, and apoptosis, which significantly increased the occurrence of heart failure.[99] Meanwhile, Nrf2 deficient mice develop left ventricular diastolic dysfunction, characterized by left ventricular hypertrophy and downregulation of sarcoplasmic reticulum Ca2+ ATPase expression, impaired myocardial diastolic capacity, and decreased Ca2+ processing capacity in the heart.[100] Therefore, Nrf2 has a protective effect on the heart in cardiac hypertrophy and heart failure. In the early stage of cardiac hypertrophy, Keap1 releases Nrf2, which translocations into the nucleus and initiates the transcription of many antioxidant genes, such as SOD, CAT, and GPx, to provide extensive cellular defense against pathological OS in the heart. However, by the late stage, the overactivation of Nrf2 in the early stage leads to the downregulation of Nrf2 expression and the inability to maintain REDOX homeostasis in cardiomyocytes. Therefore, continuous OS on the heart will induce cardiac remodeling, eventually leading to heart failure. Another study also found that 6 weeks after myocardial infarction. However, Nrf2 transcription was increased, and microRNAs rich exosomes induced by myocardial infarction inhibited Nrf2 translation, decreasing Nrf2-targeted antioxidant enzymes and promoting OS in chronic heart failure.[101] Therefore, Nrf2 has a specific resistance to myocardial hypertrophy and heart failure in the early stage. However, the expression of Nrf2 is reduced in the long-term pathological process, which ultimately leads to its inability to maintain cardiac protection.

The p27kip1 protein, a cell cycle regulator, interacts with the cyclins CDK2 and CDK4 to inhibit cell cycle progression in the G1 phase. Studies have shown that up-regulating Nrf2 can inhibit angiotensin II-induced cardiomyocyte hypertrophy.[102] Therefore, Nrf2 is a crucial regulator in maintaining the structural and functional integrity of the heart in the presence of continuous angiotensin II stimulation. Nrf2 is a crucial regulator of cardiac proteasome expression and function in the development of heart failure.[103] Studies have confirmed that chronic nitrite treatment can

prevent ischemia-induced heart failure, activate H2S synthetase and Nrf2, increase H2S bioavailability, inhibit myocardial OS,[104] and H2S activates Nrf2 in a variety of ways: sulfation modification of Keap1, phosphorylation of Nrf2 and removal of Nrf2 inhibitor Bach1 from the nucleus resulting in the release and nuclear translocation of Nrf2 increasing the nuclear accumulation of Nrf2 and the ARE binding activity of Nrf2. The activity of cardiac proteasome β1(cysteine-like) and β5(chymotrypsin) subunits was enhanced. Therefore, enhanced Nrf2 signaling can enhance the function of the cardiac proteasome and reduce the development of proteasome dysfunction after the onset of ischemic heart failure.

Diabetic Cardiovascular Complications

Diabetes is a chronic disease of glucose metabolism dysfunction. Diabetes can lead to cardiovascular complications and heart remodeling that initially manifest as myocardial hypertrophy and apoptosis, then progress to left ventricular diastolic dysfunction, which in severe cases can lead to heart failure. Metabolic disorders, dysregulation of calcium homeostasis, cardiac autonomic neuropathy, insulin resistance, myocardial hypertrophy, and fibrosis are the characteristics of diabetic cardiomyopathy (DCM), of which myocardial hypertrophy and fibrosis are significant.[105] Evidence suggests that OS induced by hyperglycemia and subsequent inflammatory and nitrifying stress play a crucial role in the occurrence and development of diabetic cardiomyopathy.[106]

Experimental results have shown that in the early stage of diabetes, the reactive expression of myocardial Nrf2 is up-regulated. The mRNA levels of its downstream genes NQO1, HO-1, and GST are also up-regulated accordingly to overcome the early oxidative damage of diabetic patients and protect cardiomyocytes from death caused by high glucose levels.[107,108] HO-1 has been proven to be an essential mediator in the cellular defense and protection mechanism of Nrf2 against oxidative damage and myocardial hypertrophy.[109] Brahma-related gene1 (Brg1), a chromatin remodeling enzyme, can assist Nrf2 in activating HO-1 to increase myocardial antioxidant capacity. However, in the advanced stage of diabetes, the antioxidant function of the heart is further impaired. Based on excessive ROS/ reactive nitrogen species (RNS), Nrf2 and Brg1 are inactivated, and the expression of cardiac Nrf2 is significantly decreased, resulting in reduced production of HO-1. Myocardial hypertrophy and apoptosis were aggravated, significantly reduced glucose metabolism in the heart was observed at this stage, and the deterioration of diabetic cardiomyopathy was accelerated. In addition, OS can lead to insulin

resistance in adult cardiomyocytes and inhibit insulin-induced glucose uptake. The activation of Nrf2 can inhibit OS-induced extracellular regulated protein kinases (ERK) activity, reverse OS-induced insulin resistance, and stimulate glucose uptake by adult cardiomyocytes. These results indicate that Nrf2 plays a crucial role in regulating cardiac insulin sensitivity, and its antioxidant effect is essential in preventing diabetes and the compensatory response in early diabetes. However, its regulatory function is limited in the late stage, suggesting that if the reduced expression and activity of Nrf2 can be reversed in the late stage, the reduction of NRF2 expression and activity can be reversed. It can effectively inhibit the occurrence and development of diabetic cardiomyopathy. Targeting Nrf2 may provide a new therapeutic approach for treating cardiac insulin resistance and diabetic cardiomyopathy.

Potential Outcomes and Drug Targets

Since Nrf2 is a potential therapeutic target for many diseases, including CVDs, investigating and developing medicines that affect Nrf2 activity or action pathways will become a crucial strategy for the clinical treatment of cardiovascular disorders. The Nrf2 pathway or Nrf2 expression can be activated or promoted by certain signaling pathways or active chemicals in the body, which can protect the heart. A PI3K-Akt signal that is activated can increase Nrf2 functionality, improve nuclear availability, activate metabolic genes, and control the expression of antioxidant genes through Nrf2. Neuregulin 1 (NRG1) and propofol function as cardioprotective agents by triggering the PI3K-Akt signaling pathway. The Nrf2 stress response pathway can be directly activated by notch signaling, raising the expression of Nrf2 and its target genes, which helps protect cardiac cells. Because they stimulate Notch signaling, IL-17 and delta-like ligand 1 (DLL1) are possible therapeutic medicines.[110] As a selective COX-2 inhibitor, celecoxib activates the AMPK-CREBNrf2 signaling pathway to cause Nrf2 to translocate into the nucleus. Increasing the expression of antioxidant and anti-inflammatory genes like HO-1 and H-ferritin (FHC), CREB, and Nrf2 pathways help patients' endothelial function and lowers their risk of cardiovascular disease.[111]

Additionally, it has been demonstrated in vitro that several natural or synthetic medicines can reduce cardiovascular illnesses brought on by OS by controlling Nrf2 activity. Fentanyl and butorphanol together activate the Nrf2-ARE pathway during myocardial ischemia-reperfusion, boost the expression of subsequent genes NQO1 and HO-1, lessen OS, and lessen cardiac ischemia-reperfusion injury.[112] Triptolide can reduce

myocardial I/R injury by causing the Nrf2/HO-1 defense mechanism to become activated.[113] The NRF2-ARE system can be directly activated during I/R process by atorvastatin, H2S, resveratrol, and other endogenous compounds,[114-116] and pretreatment. Aside from glucocorticoids stimulating lipocalin-type prostaglandin D synthase, Salvia miltiorosa can modulate the Nrf2 signaling pathway by stimulating Akt and ERK1/2.[117] L-PGDS encourages prostaglandin D2 (PGD2) synthesis, and PGD2 primarily activates Nrf2 by attaching to the prostaglandin F2 (PGF2) receptor.[118] The Nrf2 signaling pathway promotes endogenous antioxidant enzyme activity, prevents OS brought on by I/R injury, and provides cardioprotection. In rats with myocardial infarction, combining phenol and Danshosin can decrease OS by increasing Nrf2/HO-1 signaling and reducing apoptosis.[119] Furthermore, by boosting the production of Nrf2 and its downstream antioxidant-related genes in the heart, the proteasome inhibitor MG-132 can alleviate diabetic cardiomyopathy.[120] As a result of sulforaphane's upregulation of Nrf2 expression and transcriptional activity, Nrf2 nuclei accumulate and become phosphorylated. Additionally, Nrf2-downstream antioxidant genes are expressed more frequently, which can help prevent diabetes-related hypertension and cardiac dysfunction.[121] Myricetin decreases diabetic cardiac inflammation by promoting Nrf2-mediated antioxidant oxidase production and activating Nrf2-mediated antioxidant signalling.[122]

In summary, Nrf2, an important transcription factor, is critical in the onset and progression of cardiovascular illnesses. Although research on the role of Nrf2 in cardiovascular disorders is still ongoing, it is anticipated that clinical medications that target Nrf2 will one day be created to treat CVDs.

Conclusions and Future Prospective

An optimal cardiovascular system that functions effectively necessitates continuous and adaptable responses to various stimuli. When the regulation of these processes is compromised, it can disrupt the body's homeostasis and potentially give rise to numerous disorders. The likelihood of experiencing adverse cardiovascular events and comorbidities like Type 2 Diabetes Mellitus (T2DM), as well as the harmful effects of some medications such as anthracycline chemotherapy, is further influenced by aging. It is commonly accepted that the transcription factor Nrf2 is crucial in controlling stress responses. Researchers have been conducting investigations on the activation of this particular strategy as an alternative therapy to achieve cardioprotection. The deregulation of

Nrf2 signaling often precedes or worsens the progression of CVDs. An increasing body of scientific literature provides evidence in favor of the concept that enhancing Nrf2 levels, particularly during the initial phases of cellular damage, can reduce cell vulnerability and promote the maintenance of cardiovascular system health. In the upcoming years, there exists the possibility of substantial progress in comprehending the Nrf2 gene regulation network, perhaps resulting in the identification of innovative therapeutic strategies for the management of CVDs.

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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