

Nrf2在心血管疾病中的作用及机制

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摘要 作为一种重要的转录调节因子, 自1994年被发现以来, Nrf2因其抗氧化和解毒作用引起了研究者的广泛兴趣。Nrf2主要通过结合靶基因启动子上的抗氧化应答元件(anti-oxidant response element, ARE), 来转录激活下游靶基因的表达。心血管疾病致死者占全球死亡人数的31%, 严重威胁人类健康。由于在抗氧化应激中的重要作用, Nrf2与心血管疾病发生发展关系密切, 具有潜在治疗价值。该综述主要介绍了Nrf2作为转录因子的结构特点、上游调节机制和下游功能, 并重点对近年来Nrf2在心血管疾病发生发展中的作用研究进展作一介绍。

关键词 Nrf2; 心血管疾病; ROS; 抗氧化应激; 炎症

The Role of Nrf2 in Cardiovascular Diseases and Its Mechanism

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Abstract As an important transcriptional factor, Nrf2 (nuclear factor-erythroid 2-related factor 2) has attracted a lot of interest since its discovery in 1994, owing to its anti-oxidation and detoxification functions. Nrf2 transcriptionally activates the downstream target gene expression primarily by binding to ARE (antioxidant response element) in the promoter region of its target genes. The mortality of cardiovascular diseases accounts for 31% of global deaths, seriously threatening human health. Due to the significant role in anti-oxidative stress, Nrf2 is related to the occurrence and development of cardiovascular disease, with great therapeutic potential. This review mainly introduces the structure characteristics, upstream regulatory signals and downstream functions of Nrf2, also states the research progress of its pathological roles in cardiovascular diseases in recent years.

Keywords Nrf2; cardiovascular diseases; ROS; anti-oxidative stress; inflammation

核因子E2相关因子2(nuclear factor-erythroid 2-related factor 2, Nrf2)蛋白作为转录调节因子, 通过结合靶基因启动子上ARE(antioxidant response element), 在生物体抗氧化、解毒、代谢等多个方面发挥重要的调节作用, 这些生理功能提示其在多种疾病的发生、发展和转归中发挥关键作用, 深入对其结构和功能进行研究有望为临床治疗提供可靠和特异性的靶

点。心血管疾病患病人群基数庞大, 并发症复杂, 严重阻碍“大健康”(One Health)发展。本文就抗氧化应激分子Nrf2与心血管疾病发生发展的关系及研究进展作一综述。

1 Nrf2的分子结构

Nrf2属于帽和领(cap 'n' collar, CNC)碱性亮氨酸

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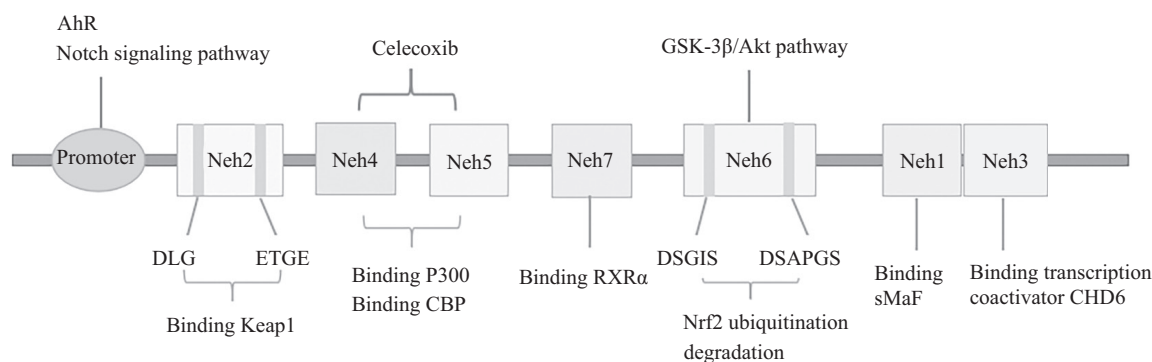


图1 Nrf2蛋白的结构

Fig.1 Structure of Nrf2

拉链(basic leucine zipper, bZIP)转录因子家族成员, 共包含605个氨基酸, 根据功能分为Neh1~Neh7 7个结构域^[1]。Neh1包含CNC-bZIP区, 与小Maf蛋白(small masculoaponeurotic fibrosarcoma, sMaf)发生异二聚体化结合, 并与靶基因上的ARE结合, 调控转录^[2-3]。Neh2含有低亲和的DLG(²³LxxQDxDLG³¹)结合基序和高亲和的ETGE(⁷⁷DxETGE⁸²)结合基序^[4], 可与Kelch样环氧氯丙烷相关蛋白-1(epoxy chloropropane Kelch sample related protein-1, Keap1)的Kelch结构域结合, 介导Nrf2的泛素化和降解, 其中Keap1是Nrf2的主要负调节因子^[5]。Neh3结构域依赖VFLVPK基序参与募集辅助蛋白或用于将转录因子桥接至活性转录装置, 对Nrf2的活性发挥重要作用。这可能与Neh3和染色质重构蛋白6(chromodomain helicase DNA-binding protein 6, CHD6)之间的特异性相互作用有关^[6]。Neh4、Neh5共同作用, 介导Nrf2与组蛋白乙酰转移酶P300的结合^[7], 也可协同结合CREB结合蛋白(CREB-binding protein, CBP)^[8], 增强Nrf2/ARE激活途径。塞来昔布是一种选择性环氧氧化酶-2(cyclooxygenase-2, COX-2)抑制剂, 通过激活AMPK-CREB-Nrf2信号传导通路, 引起Nrf2的核转位。Neh6中存在两个重要基序DSGIS³³⁸和DSAPGS³⁷⁸, 其中DSGIS可被糖原合酶激酶-3β(glycogen synthase kinase-3β, GSK-3β)催化磷酸化产生磷酸二酯, 二者均可进一步通过Skp1-Cull1-Rbx1/Roc1 E3泛素连接酶(SCF^{β-TrCP})的识别与结合, 对Nrf2进行泛素化, 可在Keap1失活时支持Nrf2的更新, 从而控制其稳定性和活性^[9]。由于蛋白激酶B(protein kinase B, 也被称为Akt)能够磷酸化GSK-3β并抑制其活性, 磷酸酰肌醇3-激酶(phosphoinositide 3-kinase, PI3K)/Akt信号传导通路可通过抑制GSK-3β来活化Nrf2^[10]。Neh7通过与RXRα结合形成

RXRα-Nrf2的异聚蛋白-蛋白质复合物^[11], 抑制CBP和RNA poll向靶基因启动子的募集^[12]。Nrf2基因上游-650碱基处(从外显子1开始计数)存在一个类似ARE的序列tgccggCgc, 对Nrf2基因表达的正反馈很重要^[13]。芳香烃受体(aromatic hydrocarbon receptor, AhR)在细胞核内二聚化, 作用于Nrf2基因启动子区增加Nrf2的转录^[14]。Notch信号则通过将Notch胞内域转录体募集到Nrf2启动子上保守的Rbpj-κ位点, 直接激活Nrf2信号途径(图1)。

2 Nrf2的活性调节与作用机制

2.1 活性的调节

2.1.1 泛素化降解 泛素化降解是Nrf2蛋白表达和活性调节的重要方式, 泛素分子与Nrf2蛋白共价结合后, 泛素链把Nrf2靶向到蛋白酶体进行降解。Nrf2的泛素化降解主要有Keap1-Nrf2和GSK-3β-Nrf2这两种途径(图2)。

Keap1-Nrf2途径: Keap1作为一种底物衔接蛋白, 募集泛素连接酶骨架蛋白(Cullin3, Cul3)和RING结构域蛋白(RING box protein-1, Rbx1)形成的E3泛素连接酶复合物, 与Nrf2特异性结合, 使细胞质中的Nrf2表达在基础稳态条件下趋于稳定的低水平^[15]。Keap1含有4个功能域: 从N末端开始, 依次是BTB(Broad complex, Tramtrack, and Bric-a-Brac)、IVR(intervening region)、DGR(double glycine repeat)和CTR(C-terminal region)结构域, DGR和CTR被统称为DC结构域^[4]。在E3连接酶的作用下, Keap1同型二聚体的两个Keap1-DC结构域与Nrf2的ETGE和DLG基序结合, 使Nrf2多聚化和泛素化并降解^[13]。在氧化应激条件下, DLG基序从Keap1-Nrf2复合物中分离, Nrf2被释放, 在细胞核中异位, 促进细胞保护基因转录, 清除氧化应

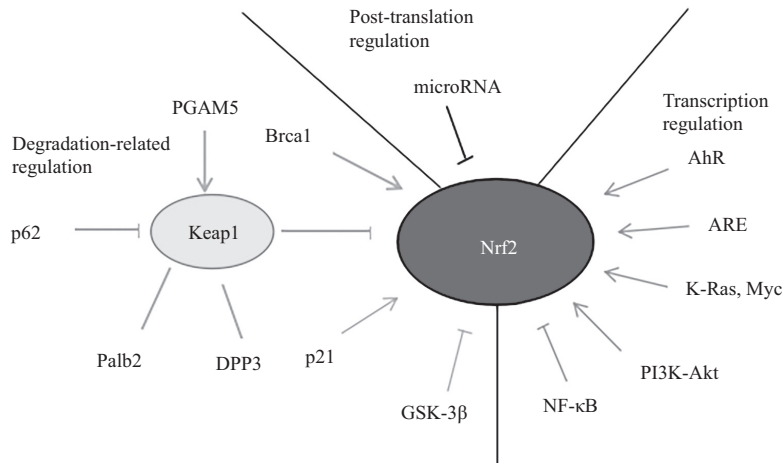


图2 Nrf2的转录、翻译后和降解相关调节

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

激。另外有文献表明,在氧化应激条件下,Cul3与Keap1-Cul3复合物分离,使Nrf2从蛋白酶体降解中逃脱,并导致其后的核转位^[16-17]。

在细胞中,有许多分子如p62^[18]、乳腺癌易感蛋白1(breast cancer susceptibility 1, Brca1)^[19]、线粒体丝氨酸/苏氨酸蛋白磷酸酶5(phosphoglycerate mutase 5, PGAM5)^[20]、二肽基肽酶3(dipeptidyl-peptidase 3, DPP3)^[21]、p21^[22]、乳腺癌易感基因相关蛋白2(partner and localizer of BRCA2, Palb2)^[23]等,通过干扰Keap1和Nrf2的相互作用,对Nrf2的泛素化进行调节。

GSK-3β-Nrf2途径: GSK-3β是一种丝氨酸/苏氨酸蛋白激酶,可通过PI3K/Akt途径或WNT信号通路途径激活和调节^[24]。GSK-3β可磷酸化Neh6结构域中的特定丝氨酸残基DSGIS,被泛素连接酶衔接子β-TrCP识别,并通过Cullin1/Rbx1复合物激发蛋白酶体降解^[24-25]。根据“双通量控制器”模型,GSK-3β-TrCP可将Nrf2水平微调至满足细胞的瞬时代谢需求,而Keap1则通过快速增加Nrf2水平,来感知和响应环境侵害^[25]。

2.1.2 转录相关 Nrf2转录受到表观遗传修饰机制的调控,包括其启动子区域的高甲基化或单核苷酸多态性(single nucleotide polymorphism, SNP)等,导致Nrf2表达降低^[26]。在转录起始阶段,Nrf2受到多个转录因子和信号通路的调节,包括芳烃受体(aryl hydrocarbon receptor, AhR)、NF-κB、Kras、B-Raf、Myc、PI3K-Akt、Notch等^[10,27-29]。另外,Nrf2基因的启动子中含有类似ARE的序列,导致Nrf2扩增的正反馈^[29](图2)。

2.2 Nrf2的下游基因与功能

Nrf2调控的下游靶基因数量众多、功能强大,下文将选取Nrf2的抗氧化和解毒、代谢调控、线粒体功能调控、自噬和凋亡调控等作用作一简单介绍。

2.2.1 抗氧化与解毒 Nrf2通过调控各种抗氧化和II期解毒基因的转录,来保护细胞免受各种有害物质的损伤,包括活性氧、辐射、环境毒素和食物中的异生素等^[30]。

醌氧化还原酶1[NAD(P)H: quinone oxidoreductase-1, NQO1]是一种细胞内普遍存在的黄素酶,以二聚体的形式发挥作用。每个单体结合一个黄素腺嘌呤二核苷酸(flavin adenine dinucleotide, FAD),利用“NAD(P)H+a醌→NAD(P)+氢醌”反应,从生物系统中除去醌,发挥解毒作用^[31]。NQO1还可还原被氧化的维生素K或与氧化循环相关酶竞争性结合,保护细胞免受氧化应激。

血红素氧合酶-1(heme oxygenase-1, HO-1)通路:在氧化应激条件下,游离的Nrf2易位至细胞核,与sMaf二聚化并与HO-1基因的ARE元件结合,促进其转录。HO-1的产物如CO、胆红素在氧化应激和细胞损伤过程中发挥强大的抗氧化作用^[32]。此外,HO-1也可直接抑制促炎细胞因子、激活抗炎细胞因子,维持炎症过程的平衡^[10]。

谷胱甘肽(glutathione, GSH)可还原蛋白质内形成的二硫键,同时转化为谷胱甘肽二硫化物(glutathione disulfide, GSSG),防止ROS对细胞的损伤。Nrf2的直接转录靶点——Xc⁻系统中的xCT亚基可以将GSH合成原料胱氨酸导入细胞^[33]。Nrf2还可促

进多种含ARE的GSH相关基因的表达,快速响应氧化应激并使细胞保持平衡的氧化还原状态^[34]。另外,Nrf2可作用于谷胱甘肽还原酶的转录,维持还原型谷胱甘肽水平。

2.2.2 物质代谢 Nrf2可促进参与磷酸戊糖途径(pentose phosphate pathway, PPP)、嘌呤核苷酸从头合成(*de novo synthesis*)等的相关酶转录。代谢组学分析表明,在活性PI3K-Akt信号传导的情况下,Nrf2促进嘌呤核苷酸合成和谷氨酰胺代谢^[10]。Nrf2还可转录调控细胞中苹果酸酶1(malic enzyme1, ME1)等产生NADPH的酶活性^[35]。Nrf2对这些代谢基因的激活,促进了糖酵解及氨基酸、核苷酸和NADPH的合成,对细胞代谢过程和氧化还原平衡有重要作用。

2.2.3 线粒体功能 Nrf2缺乏时,线粒体复合物I的活性受损,ROS生成增多。当Nrf2被激活时,氧化磷酸化效率更高,增加了线粒体内膜的质子传导,减少了超氧化物的产生。而且,Nrf2可增强三羧酸循环的活性,促进线粒体脂肪酸氧化,提高线粒体呼吸的底物利用以及氧化磷酸化中的ATP产生,这对于氧化应激条件下的细胞保护尤为重要。研究发现,Nrf2增加核苷酸合成,对维持线粒体在氧化和炎症应激条件下的完整性有关键作用^[33]。

2.2.4 自噬和凋亡 Nrf2下游基因还调控细胞的凋亡和自噬过程。在氧化应激条件下,Nrf2诱导p62/SQSTM1和核点蛋白52(nuclear dot protein 52, NDP52)的表达,促使受损细胞器通过自噬途径降解^[36]。此外,Nrf2/Keap1通路的激活可保护细胞在神经变性时免于凋亡^[37]。

3 Nrf2与各类心血管疾病的关系

在人群中,Nrf2基因启动子呈现出多态性,影响启动子中的ARE活性,多项队列研究发现,这些启动子多态性的人群数据与Nrf2表达减少以及心血管疾病发病率增加相一致^[38-39]。在动物实验中也证实,Nrf2敲除小鼠较正常小鼠心血管事件发生率增加,提示Nrf2表达与各种心血管疾病的发生发展有着重要联系,这引起了研究者的广泛兴趣和对相关机制的探索。

3.1 动脉粥样硬化

动脉粥样硬化(atherosclerosis, AS)是一种进行性疾病,是冠心病、脑梗死、外周血管病形成的主要原因^[40]。研究发现,Nrf2在AS的发展过程中起到

了双重作用。

一方面,Nrf2能减弱或阻止AS发展。AS的形成关键环节在于氧化低密度脂蛋白(oxidized low density lipoprotein, ox-LDL)的产生。活性氧诱导LDL氧化成ox-LDL,当巨噬细胞吸收脂蛋白衍生的胆固醇多于其排泄时,细胞内的游离胆固醇会转化为胆固醇酯,并堆积在脂质滴中,导致动脉壁中泡沫细胞形成,加剧AS斑块的形成^[41]。巨噬细胞的抗氧化反应是降低ROS水平并保护线粒体和其他细胞器及以蛋白质和核酸免受氧化损伤的关键,但是在斑块巨噬细胞中,抗氧化基因的转录和抗氧化GSH的线粒体运输受到抑制,会放大动脉壁的炎症^[41]。有实验表明,LDL受体缺陷的小鼠Nrf2缺失会加剧AS病变并增加促炎基因的表达^[42]。同时,Nrf2可上调巨噬细胞中的过氧化物酶1(peroxiredoxin 1, Prdx1)表达,防止巨噬细胞受ox-LDL诱导形成泡沫细胞,从而阻止AS的形成^[40,43]。总之,已证明Nrf2是有效的抗氧化分子,调控各种抗氧化和解毒基因的转录,保护细胞免受包括活性氧在内的各种有害物质的损伤,提示Nrf2的抗氧化及抗炎作用,均在一定程度上影响AS斑块的形成,有助于抵抗AS的发展。

另一方面,Nrf2也可能加剧AS演变,但其机制有多方面的解释。有实验证明,高胆固醇血症载脂蛋白E(apolipoprotein E, ApoE)缺失小鼠易于发生AS,而部分ApoE缺失小鼠在敲除Nrf2的情况下,斑块沉积减少并保留血管壁弹性^[44-45],也就是说Nrf2的缺失对ApoE缺陷小鼠的AS起到了保护作用。这可能是由于Nrf2可上调清道夫受体CD36的表达,增强了巨噬细胞中oxLDL的摄取和泡沫细胞的形成,促进了AS的发展^[46]。同时,在对胆固醇晶体激活炎症小体与AS的实验中发现,胆固醇晶体能够活化Nrf2,Nrf2作为炎症小体的正调节剂,增强IL-1介导的血管炎症,加重AS^[47]。此外,骨髓移植实验显示,骨髓来源的巨噬细胞(bone-marrow-derived macrophage, BMDM)中的Nrf2活性增加,也加剧了AS的形成。已知斑块形成前期多为M2巨噬细胞,晚期多为M1巨噬细胞,Nrf2对AS的调节可能是通过M1、M2巨噬细胞的数量或表型变化实现的^[48]。基于Nrf2在AS发展中功能的重要性和复杂性,在未来靶向Nrf2治疗AS的过程中,应考虑到其他合并疾病、服用药物、饮食习惯等其他因素的综合影响,并在这些方面进行更深入的机制研究探索。

3.2 缺血再灌注损伤与心肌梗死

在细胞水平上, 局部缺血的最初损伤会导致ROS的增加^[13]。而挽救缺血性损伤的心肌组织, 必须要恢复代谢并充分去除有害代谢物, 因此血液的再灌注是必不可少的。血液流向缺血区产生大量ROS, 其对生物分子产生快速和严重的损害, 被称为心肌再灌注损伤(myocardial reperfusion injury, MRI)^[49]。缺血再灌注(ischemia/reperfusion, I/R)引起的炎症反应与氧化应激是导致心肌缺血再灌注损伤的重要因素。在炎症过程中, TNF- α 、IL-6和IL-8等细胞因子被释放, 巨噬细胞与嗜中性粒细胞向心肌组织浸润^[50]。

据报道, Nrf2通过增加解毒途径和抗氧化电位来保护心脏成纤维细胞和心肌细胞免受氧化应激^[51]。I/R导致Nrf2与Keap1分离, 促进Nrf2易位至细胞核, 与ARE结合从而激活II期解毒和抗氧化基因。Nrf2/ARE途径通过凋亡蛋白[例如, B淋巴细胞瘤-2(B-cell lymphoma-2, Bcl-2)和Bax(Bcl2-associated X)]和II期解毒酶(例如HO-1)等多种物质影响细胞生命活动^[50]。

研究表明, 4-羟基壬烯酸可以通过形成加合物直接诱导Keap1的构象变化, 或通过增加线粒体ROS的产生间接诱导Keap1的构象变化, 激活Nrf2, 在活化抗氧化酶的基础上, 刺激GSH生物合成, 进一步保护心脏, 但4-羟基壬烯酸诱导Nrf2核聚集的机制仍有待阐明^[52]; 表皮生长因子(epidermal growth factor, EGF)预处理可以增加心脏中Nrf2表达, 诱导Nrf2下游靶基因*NQO-1*和*HO-1*高表达的同时, 使炎症因子如TNF- α 和IL-6表达降低^[53], 减少了I/R诱导的ROS产生和细胞凋亡。除此之外, 多种外来或体内自身物质可通过激活Nrf2-ARE系统, 在I/R过程中对心脏起到保护作用, 这提示可利用这些物质开展对缺血再灌注的临床治疗。

心肌梗死是长时间缺血导致的心肌细胞死亡, 急性心肌梗死(acute myocardial infarction, AMI)是全世界死亡的主要原因, 急性心肌缺血是心梗的最常见原因。心肌大梗死将导致心源性休克, 致命性心律失常和急性心脏衰竭, 因此在医疗救治中控制梗死面积是首要的^[13]。有实验发现, 在心梗模型中Nrf2缺乏小鼠梗死面积增加^[54], 提示在心肌缺血中Nrf2可有效地控制心肌梗死面积。保护心脏组织免受缺血或再灌注损伤的最有效方法是预处理或后处理。有实验显示, 缺血预处理促使La蛋白与Nrf2

mRNA的5'UTR结合增加^[55], 通过Nrf2的从头翻译心肌中Nrf2蛋白水平升高, 控制抗氧化和解毒基因簇的转录, 从而快速激活内源性防御^[56], 使梗死面积减少约50%。也有实验证明, 缺血后处理诱导心肌激活信号转导与转录激活因子3(signal transducer and activator of transcription3, STAT3)从而调节Nrf2的核易位, *HO-1*和超氧化物歧化酶(superoxide dismutase, SOD)等抗氧化基因的表达, 起到减少梗死面积, 防止血管功能障碍和中性粒细胞积聚的作用^[57]。然而在临床治疗中, 面对的病人通常合并多种慢性病, 导致前处理与后处理治疗无效果, 并且可能会造成对动脉的重复性损伤^[57]。因此还需要进一步探索机体多种疾病合并发展的机制, 寻找合适的治疗方案。总之, Nrf2可以通过激活相关下游靶细胞的转录, 减少缺血再灌注后的心肌梗死面积、心肌细胞凋亡和I/R损伤炎症, 从而部分保留心脏功能。

3.3 心肌肥大与心力衰竭

心肌肥大是对由心梗等疾病引起的心脏功能减弱的代偿, 当心肌肥大不足以代偿心脏功能的减弱时, 就会发生心力衰竭。心力衰竭和许多心力衰竭的危险因素与氧化应激有关^[58], 因此, Nrf2水平与心力衰竭的严重程度相关^[59]。

对比正常小鼠, Nrf2敲除小鼠在主动脉缩窄处理后, 继发于病理压力超负荷心脏的中氧化应激大幅增加, 出现心肌增厚、纤维化和细胞凋亡, 明显增加了心力衰竭的发生^[60]。同时, Nrf2缺失小鼠会出现左心室舒张功能障碍, 其特征是左心室肥大和肌浆网Ca²⁺ ATP酶表达的下调、心肌舒张能力受损以及心脏中Ca²⁺处理能力下降^[61]。因此, Nrf2在心肌肥大和心力衰竭中对心脏有保护作用。

在心肌肥大的早期, Keap1释放Nrf2, 其易位至细胞核内, 启动许多抗氧化基因, 例如SOD、CAT和GPx等的转录, 对心脏中的病理性氧化应激进行广泛的细胞防御; 然而, 到晚期阶段, 前期Nrf2的过度激活导致Nrf2表达下调, 无法维持心肌细胞内氧化还原稳态。因此, 心脏持续的氧化应激将诱导心脏重塑, 并最终导致心力衰竭^[58]。另一研究也发现, 心梗后6周, 虽然Nrf2的转录增加, 但心梗诱导的富含microRNAs的外泌体抑制了Nrf2的翻译, 导致慢性心力衰竭中Nrf2靶向抗氧化酶的减少并促进氧化应激^[62]。因此, Nrf2在早期对心肌肥大和心力衰竭有一定的抵抗作用, 但在长期病理过程中Nrf2的表达

减少,最终导致其无法维持心脏保护作用。

p27kip1蛋白,一种细胞周期调节蛋白,可与细胞周期蛋白CDK2和CDK4相互作用,抑制G₁期的细胞周期进程。有研究显示,Nrf2可以通过使其上调抑制血管紧张素II诱导的心肌细胞肥大^[63]。因此,Nrf2是在持续血管紧张素II刺激的情况下,维持心脏结构和功能完整性的关键调节因子。

Nrf2是心力衰竭发展过程中心脏蛋白酶体表达和功能的关键调节剂^[64]。研究证实,慢性亚硝酸盐治疗可防止缺血诱导的心力衰竭,激活H₂S合成酶与Nrf2,增加H₂S生物利用度,抑制心肌氧化应激^[65],并且,H₂S通过多种方式激活Nrf2:Keap1的硫酸化修饰、Nrf2的磷酸化以及从细胞核中去除Nrf2抑制物Bach1,导致Nrf2的释放和核易位,增加了Nrf2的核积累以及Nrf2的ARE结合活性,引起心脏蛋白酶体β1(半胱氨酸蛋白酶样)和β5(胰凝乳蛋白酶样)亚基活性增强。因此,增强Nrf2信号传导可增强心脏蛋白酶体的功能,在缺血性心力衰竭发作后减轻蛋白酶体功能不全的发展^[64]。

3.4 糖尿病性心肌病

糖尿病是葡萄糖代谢功能障碍的慢性疾病。糖尿病可导致心血管并发症和心脏重塑,最初表现为心肌肥大和细胞凋亡,然后发展为左心室舒张功能不全,严重时可导致心力衰竭。代谢紊乱、钙稳态调节异常、心脏自主神经病变、胰岛素抵抗、心肌肥大和纤维化是糖尿病性心肌病(diabetic cardiomyopathy, DCM)的特征,其中心肌肥大和纤维化尤为重要^[66]。证据表明,高血糖诱导的氧化应激以及随后的炎症和硝化应激在糖尿病性心肌病的发生和发展中起关键作用^[67]。

有实验结果显示,在糖尿病早期阶段心肌Nrf2反应性表达上调,其下游基因*NQO1*、*HO-1*和*GST*的mRNA水平也相应上调,以克服糖尿病患者的早期氧化损伤,保护心肌细胞免受高水平葡萄糖造成的死亡^[68-69]。*HO-1*已被证明是细胞中Nrf2抗氧化损伤和抗心肌肥大的防御保护机制中的重要介体^[70],一般情况下,染色质重塑酶Brg1(Brahma-related gene 1, Brg1)可协助Nrf2激活*HO-1*以增加心肌抗氧化能力,应对氧化应激^[66]。但在糖尿病晚期,心脏的抗氧化功能进一步受损,在产生过量的ROS/活性氮(reactive nitrogen species, RNS)基础上使Nrf2和Brg1失活,心脏Nrf2表达显著下降,导致*HO-1*产生减少,加剧

心肌肥大和细胞凋亡,并且在此阶段观察到心脏中糖代谢水平明显降低,糖尿病心肌病恶化加快^[66,68]。另外,氧化应激可导致成人心肌细胞胰岛素抵抗,使胰岛素诱导的葡萄糖摄取受抑制。Nrf2的激活可抑制氧化应激诱导的细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK)活性,逆转氧化应激诱导的胰岛素抗性,刺激成人心肌细胞摄取葡萄糖^[68]。

以上结果表明,Nrf2在调节心脏胰岛素敏感性中发挥关键作用,其抗氧化作用在预防糖尿病和早期糖尿病代偿反应中发挥重要作用,但在晚期其调节功能受限,提示如果能够在晚期逆转Nrf2的表达减少和活性降低,可有效抑制糖尿病性心肌病的发生和发展。靶向Nrf2可能为治疗心脏胰岛素抵抗和糖尿病性心肌病提供一种新的治疗方法。

4 药物靶点与展望

Nrf2可作为包括心血管疾病在内的多种疾病的潜在治疗靶点,因此探索开发调控Nrf2活性或者作用途径的药物,将成为未来临床治疗心血管疾病的重要方法。体内一些信号通路或活性物质可以通过激活Nrf2通路或促进Nrf2的表达,起到保护心脏的作用。活化的PI3K-Akt信号既能使Nrf2功能性扩增,核可用性增强,激活代谢基因,又能调节Nrf2介导的抗氧化基因的表达^[10,28]。因此推测,神经调节蛋白1(neuregulin 1, NRG1)和异丙酚等物质或许能通过激活PI3K-Akt信号通路起到心脏保护作用。Notch信号可直接激活Nrf2应激反应途径,增加Nrf2及其靶基因的表达水平^[71],可起到心脏细胞保护作用。IL-17、Delta样配体1(delta-like ligand 1, DLL1)因激活Notch信号从而成为潜在治疗药物。塞来昔布是一种选择性COX-2抑制剂,通过激活AMPK-CREB-Nrf2信号传导通路,引起Nrf2的核转位。CREB和Nrf2通路共同上调抗氧化和抗炎基因如*HO-1*和H-铁蛋白(H-ferritin, *FHC*)的表达,从而改善内皮功能,最大限度地降低患者的心血管风险^[72]。

另外,体外的一些天然产物或人工合成物质也被证明可通过调节Nrf2活性,来缓解氧化应激诱发的心血管疾病。在心肌缺血再灌注过程中,芬太尼(fentanyl)与布托啡诺(butorphanol)协同作用,可以激活Nrf2-ARE途径,增加下游基因*NQO1*和*HO-1*的表达,减少氧化应激,降低心脏缺血再灌注损伤^[73]。雷公藤内酯醇通过诱导Nrf2/*HO-1*防御通路的激活,减

轻大鼠心肌I/R损伤^[74]。 α -硫辛酸、 H_2S 、白藜芦醇等几种天然分子^[75-77]以及阿托伐他汀预处理,可在I/R过程中直接激活Nrf2-ARE系统^[50]。丹参可通过激活Akt和ERK1/2来介导Nrf2信号通路^[78],糖皮质激素也可以通过激活脂蛋白型前列腺素D合成酶(lipocalin-type prostaglandin D synthase, L-PGDS)促进合成前列腺素D2(prostaglandin D2, PGD2)^[79], PGD2主要通过结合前列腺素F_{2 α} (prostaglandin F_{2 α} , PGF_{2 α})受体激活Nrf2。Nrf2信号通路增强了内源性抗氧化酶的活性,抑制I/R损伤诱导的氧化应激,发挥心脏保护作用。丹皮酚和丹参素联合应用通过激活Nrf2/HO-1信号传导,抑制氧化应激,减轻心肌梗死大鼠的细胞凋亡^[80]。另外,蛋白酶体抑制剂MG-132,可通过增加心脏中Nrf2及其下游抗氧化基因的表达,治疗糖尿病性心脏病^[81]。萝卜硫素上调Nrf2表达和转录活性,导致Nrf2核积累和磷酸化,以及Nrf2下游抗氧化基因的表达增加,可预防糖尿病引起的高血压和心功能不全^[82]。杨梅素激活Nrf2介导的抗氧化信号传导,促进Nrf2介导的抗氧化酶的表达并减轻糖尿病心肌炎症^[83]。

总之, Nrf2作为一种重要的转录因子,在心血管疾病发生和发展过程中至关重要,虽然目前尚处于集中研究Nrf2在心血管疾病中的作用机制阶段,但相信研制出靶向Nrf2的临床药物,治疗心血管疾病未来可期。

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