·文献综述·

KEAP1-NRF2 信号通路调控视网膜氧化应激的研究进展

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摘要

氧化应激(OS)是造成机体损伤的重要原因。既往研究显示缺血缺氧、过度光照以及高糖环境等多种因素均可引起视网膜活性氧和自由基增多,从而诱发 OS,造成视网膜损伤并影响正常的视觉功能。Kelch 样环氧氯丙烷相关蛋白 1(KEAP1)与核因子 E2 相关因子 2(NRF2)共同构成机体中主要的抗氧化应激信号通路,通过多种途径调节视网膜能量代谢及细胞增殖凋亡自噬等机制而发挥抗氧化作用,以减轻 OS 所致视网膜损伤。本文将简要综述视网膜中 KEAP1-NRF2 信号通路调控 OS 的作用及机制,以期为后续研究提供思路。

关键词:氧化应激;Kelch 样环氧氯丙烷相关蛋白 1-核因子 E2 相关因子 2 信号通路(KEAP1-NRF2 信号通路);视网膜;机制
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Research progress of Kelch-like ECH-associated protein 1-nuclear factor erythroid 2-related factor 2 signaling pathway regulated retinal oxidative stress

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Abstract

• Oxidative stress (OS) is a major reason for body damage. Studies have shown that a variety of factors, such as ischemia and hypoxia, excessive light and hyperglycemia can cause the increase of reactive oxygen species and free radicals in the retina, thus inducing OS, damaging retina and affecting the normal visual function. Kelch-like ECH-associated protein 1 (KEAP1) and nuclear factor erythroid 2 related factor 2 (NRF2), which together constitute the main antioxidant stress signaling pathway in the body, play an antioxidant role by regulating retinal energy metabolism and cell proliferation, apoptosis and autophagy through various ways, so as to reduce retinal damage caused by OS. In this paper, the role and mechanism of the KEAP1-NRF2 signaling pathway regulation of OS in the retinal are briefly reviewed, aiming to provide ideas for subsequent research.

• KEYWORDS: oxidative stress; Kelch-like ECH-associated protein 1-nuclear factor erythroid 2 related factor 2 signaling pathway (KEAP1-NRF2 signaling pathway); retina; mechanism

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0 引言

氧气是生命活动所必需的化学元素。缺氧等因素可诱使机体活性氧(reactive oxygen species, ROS)和自由基释放增多,从而打破正常的氧代谢平衡并造成细胞膜脂质代谢失调。因此大量 ROS 和自由基得以穿透细胞膜而进入细胞质和细胞核,影响 RNA 及蛋白质的正常表达,最终引起组织结构紊乱和功能异常[1]。视网膜作为眼部最重要的感光元件,也是人体内氧耗量最大的组织之一,因其多不饱和脂肪酸含量丰富且线粒体密度较高,所以对氧代谢变化极为敏感。当各种原因引起氧供失衡后,视网膜极易出现脂质过氧化,并诱发一系列连锁反应导致氧化应激(oxidative stress, OS)损害[2-3]。已有研究表明多种视网

膜疾病均与 OS 有关,而 Kelch 样环氧氯丙烷相关蛋白 1(kelch-like ECH-associated protein 1, KEAP1)与核因子 E2 相关因子 2(nuclear factor erythroid 2-related factor 2, NRF2)共同构成的 KEAP1-NRF2 信号通路在其中扮演了重要角色,因此本文拟对此作一简要综述。

1 KEAP1-NRF2 信号通路

KEAP1 是一种含有 5 个结构域并广泛存在于正常组织细胞质中的结合蛋白,NRF2 是包含 6 个同源且高度保守碱性亮氨酸拉链结构域的抗氧化基因调节蛋白。KEAP1-NRF2 信号通路在调节抗 OS 基因及抵御 ROS 和自由基等有害物质损伤中发挥了重要作用,通过调节下游多种酶类及蛋白表达,以维持机体的氧化还原稳态和细胞存活,因此成为重要的内源性抗 OS 通路之一[4-5]。正常情况下,KEAP1 在细胞质中通过双重甘氨酸重复区与 NRF2 紧密结合,对其进行泛素化修饰并使其降解。组织缺氧诱发 OS 后,酪氨酸激酶催化 KEAP1 与 NRF2 在细胞质解离,随后 KEAP1 在细胞质迅速降解,NRF2 则持续进入细胞核调控其下游的抗氧化反应元件而发挥抗 OS 作用,以保护机体免受过量 ROS 和自由基伤害[6]。

2 KEAP1-NRF2 调控视网膜氧化应激的机制

视网膜是体内氧耗量最高的组织之一,含有大量不饱和脂肪酸且具有极高的线粒体密度,因此对氧含量变化高度敏感,极易受到 OS 损伤。ROS 和自由基异常增多是诱发 OS 的中心环节,缺血缺氧、过度光照以及高糖环境均是促进视网膜 ROS 和自由基增多,并引起 OS 而导致损伤的重要原因。

2.1 KEAP1-NRF2 与视网膜缺血缺氧所致氧化应激 缺血缺氧是促进视网膜 ROS 与自由基异常增多而引起 OS 的首要因素[7]。血流供应减少使视网膜出现相对缺氧,并诱导 ROS 异常累积后,大量消耗了超氧化物歧化酶(superoxide dismutase, SOD)及过氧化氢酶,因此削弱了组织的抗氧化能力,致使构成血-视网膜屏障的视网膜内皮细胞(retinal endothelial cell, REC)和视网膜色素上皮层代谢异常[8-10];继而在肿瘤坏死因子(tumor necrosis factor, TNF)、核苷酸结合寡聚化结构域受体蛋白 3(NOD-like receptor protein 3, NLRP3)等炎性因子作用下引起血管渗漏和新生血管形成[11-12],最终造成视觉功能损伤。增强 KEAP1-NRF2 信号通路活性并上调抗氧化基因 SOD 等的表达,可抑制 TNF 和 NLRP3 小体异常蓄积,并逆转视网膜中内皮-间质转化以及上皮-间质转化[11-12],改善上述病理变化。此外,参与炎症反应的烟酰胺腺嘌呤二核苷酸磷酸氧化酶调节体内 ROS 表达,并影响视网膜中 NRF2 的抗氧化能力[13-14]。干预 KEAP1-NRF2 信号通路在对抗 OS 所致损伤方面具有重要作用,有望成为治疗相关疾病的靶点。另有研究发现,激活 NRF2 刺激 REC 旁分泌可促进血运重建并抑制病理性新生血管形成[15],KEAP1-NRF2 在视网膜中抗 OS 作用机制的多样性值得进一步研究。

2.2 KEAP1-NRF2 调控光照所致视网膜氧化应激 除缺血缺氧外,过度光照是破坏视网膜微环境并诱导 OS 而损伤视网膜的另一重要因素。适当的光照刺激可被视网膜神经元感知并传递至大脑产生正常视觉信号。然而随着光照时间延长及光照强度提高,可使视网膜上的感光细胞吸收能量增多并导致 ROS 累积,同时诱使自由基与细胞膜上的多不饱和脂肪酸相互作用生成丙二醛等脂质过氧化产物,对细胞膜正常结构和功能造成破坏,继而促进细胞凋亡并导致其出现不可逆损伤[16-18]。过量释放的 ROS 可引起视网膜转录因子肌细胞增强因子 2 氧化还原修饰异常,并抑制 NRF2 转录,进而引起视网膜对光诱导 OS 反应增强,并出现感光细胞层厚度变薄、感光细胞死亡、视紫红质减少等形态变化及功能障碍等病理改变[19]。增强 NRF2 通路活性并促进 SOD 和血红素加氧酶-1(heme oxygenases-1, HO-1)等内源性抗氧化物表达后,降低了 ROS 含量,光致 OS 引起的视网膜损伤也得以缓解[20]。但 Liang 等[21]指出 NRF2 抑制光致 OS 损伤的功效仅限于视网膜轻度损伤,对于严重损害的保护作用似乎不甚明显。因此,KEAP1-NRF2 信号通路对于光致 OS 损伤的保护作用还有待深入研究。

2.3 KEAP1-NRF2 调控高糖所致视网膜氧化应激 高糖环境可通过促进脂质代谢并增加游离脂肪酸含量,使细胞膜出现脂质过氧化,造成细胞及线粒体异常凋亡而诱发 OS[22]。此外,高糖还可中断氧化呼吸链的电子传递,引起细胞膜电位下降,继而诱使 ROS 和自由基等超氧化物过度生成,从而造成组织损伤[23-24]。激活 NRF2 可恢复降低的视网膜细胞膜电位水平,并促进其下游的抗氧化物表达而缓解高糖所致 OS[25]。半胱氨酸残基修饰变化可以调控 KEAP1 与 NRF2 解离速度而影响 NRF2 核易位,并与抗氧化反应元件结合,因此在保护视网膜免受高糖所致 OS 中同样发挥重要作用[26]。这些结果提示了 KEAP1-NRF2 缓解高糖所致 OS 损伤作用机制的复杂性。

2.4 KEAP1-NRF2 对视网膜细胞自噬的调节 线粒体是进行有氧呼吸并产生能量的主要场所,对氧含量变化高度敏感,因此是 ROS 的主要作用靶点。另外,线粒体也是调节 OS 及细胞自噬的关键环节。研究表明 OS 引起胞质蛋白、线粒体和内质网等结构破坏后,自噬机制开始清除受损的细胞器,并通过自噬受体蛋白 p62 诱导 KEAP1 降解,同时促使 NRF2 从细胞质转移至细胞核中积聚而参与调控 DNA 损伤反应,最终使 NRF2 与抗氧化基因结合而维持细胞稳态并发挥保护作用[27-28]。剧烈的 OS 可抑制视网膜上皮细胞 NRF2 表达并降低抗氧化物 HO-1 水平,导致自噬功能障碍并引起损伤。使用 NRF2 激活剂瞬时上调 p62 可抑制自噬体降解,自噬反应的保护作用也因此增强[29-31]。提示时相变化可能影响 NRF2 对自噬机制的调节。由于自噬机制既可发挥正面作用,也可产生不良影响;因此 NRF2 改善 OS 所致自噬异常而减轻视网膜损伤的调节机制尚需更多研究来深入探讨。

3 KEAP1-NRF2 与其他通路相互作用

KEAP1-NRF2 信号通路作为调控 OS 的中心环节,多种信号通路均能与其相互影响而发挥抗 OS 作用。腺苷酸激活蛋白激酶(AMP-activated protein kinase, AMPK)信

号通路是参与细胞能量代谢的重要因子。激活 AMPK 可与 NRF2 共同作用而减弱视网膜中由 OS 所致细胞内脂质异常蓄积,从而避免组织损伤[32]。丝氨酸/苏氨酸激酶(phosphoinositide kinase - 3/serine - threonine - protein kinase, PI3K/AKT)信号通路通过调节细胞代谢、生长、增殖和凋亡,以维持视网膜的正常功能。OS 可使视网膜中的 PI3K/AKT 表达下降,继而抑制细胞增殖并促进凋亡,最终引起损伤。激活 PI3K/AKT 可提高 NRF2 磷酸化及核易位水平,视网膜细胞抗 OS 能力也得以增强,并通过抑制视网膜细胞上皮间质转化而缓解组织功能异常[33-35]。NRF2 还可与 JNK 信号通路相互作用而激活其下游 HO-1、NQO-1 等二相抗氧化基因来减轻 OS 所致视网膜损伤[36]。

丝裂原活化蛋白激酶(mitogen - activated protein kinase, MAPK)作为调节细胞膜到细胞核信号转导的重要因子,对减轻 OS 所致视网膜炎症有重要意义。有报道称,降低 MAPK 磷酸化及活化水平可引起 NRF2 表达增加,并抑制炎性通路 NF-kb 的磷酸化水平而使其失活,进而抑制细胞凋亡以减轻视网膜炎症反应[37]。然而另有研究报道激活 MAPK/ERK 信号通路以促进 NRF2 表达而抑制 OS 所致细胞凋亡的作用仅在激活早期出现,MAPK/ERK 激活晚期反而促进视网膜的细胞凋亡,提示 NRF2 对细胞凋亡的调控作用与时相有关,MAPK 与 NRF2 在不同时相的反馈机制存在差异[38]。由此可见,NRF2 对 OS 所致炎性因子的调节作用机制极为复杂,如何对其进行调控而减轻 OS 所致视网膜损伤值得进一步研究。

4 总结与展望

综上所述,调控 KEAP1-NRF2 信号通路与其上下游多种信号通路的相互作用,是增强视网膜抗氧化能力,清除异常蓄积的 ROS 和自由基,并减轻脂质过氧化而缓解 OS 所致损伤的重要途径,提示 KEAP1-NRF2 信号通路在治疗相关疾病方面具有强大潜力,为治疗 OS 所致视网膜损伤提供了新思路和靶点。

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