磷脂过氧化: 神经退行性疾病“易感”的关键因素

林晓敏[1,2], 孙万阳[1,2], 段文君[1,2,3], 龚海标[1,2], 涂龙芳[1,2], 李怡芳[1,2],
栗原博[1,2], 何蓉蓉[1,2,3*]

(1. 暨南大学, 广东省疾病易感性及中医药研发工程技术研究中心, 广东 广州 510632;
2. 暨南大学药学院, 广东 广州 510632;　3. 暨南大学中医学院, 广东 广州 510632)

摘要: 大脑的生化完整性对于维持大脑的正常运转十分重要, 氧化损伤是导致大脑生化完整性破坏的重要原因之一。大脑富含磷脂和多不饱和脂肪酸, 由于高脂质和高耗氧量的特殊性, 大脑比其他器官更容易受到氧化应激的影响, 氧化应激诱导的磷脂过氧化是大脑的氧化−抗氧化系统失衡的结果。当抗氧化系统不足以抵御氧化损伤时, 神经元膜磷脂易受自由基活性氧的攻击发生磷脂过氧化, 进而生成多种毒性氧化产物、细胞膜损伤、线粒体障碍和淀粉样蛋白的急剧累积, 且神经元的大多数蛋白和核酸可以被过氧化产物共价修饰, 导致蛋白原有功能的丧失, 进而引发大脑程序性细胞死亡和神经炎症, 最终导致神经退行性疾病的易感性的增加。本文在总结磷脂过氧化机制的基础上, 重点综述了磷脂过氧化作为关键因素在神经退行性疾病的发生发展中的特点, 为靶向干预磷脂过氧化用于预防神经退行性疾病的潜在策略提供理论依据。

关键词: 磷脂过氧化; 神经退行性疾病; 疾病易感性; 铁死亡; 氧化应激
中图分类号: R964　　**文献标识码**: A　　**文章编号**: 0513-4870(2021)08-2154-10

Phospholipid peroxidation: a key factor in "susceptibility" to neurodegenerative diseases

LIN Xiao-min[1,2], SUN Wan-yang[1,2], DUAN Wen-jun[1,2,3], GONG Hai-biao[1,2], TU Long-fang[1,2],
LI Yi-fang[1,2], KURIHARA Hiroshi[1,2], HE Rong-rong[1,2,3*]

(*1. Guangdong Engineering Research Center of Chinese Medicine and Disease Susceptibility, Jinan University, Guangzhou 510632, China;　2. College of Pharmacy, Jinan University, Guangzhou 510632, China;　3. School of Traditional Chinese Medicine, Jinan University, Guangzhou 510632, China*)

Abstract: The biochemical integrity of the brain is necessary to maintain normal function. Oxidative damage is one of the mortal important reasons leading to the destruction of this integrity. The nervous system is enriched in phospholipid and polyunsaturated fatty acids (PUFAs). Due to the nature of high oxygen-consumption and rich lipids, brain is particularly vulnerable to oxidative damages. Phospholipid peroxidation is one of the results of imbalance in oxidation-antioxidant system. Once the antioxidant system is insufficient to resist oxidative damage, membrane phospholipids will be prone to free radical attack. Phospholipid peroxidation leads to a variety of toxic oxidation products, including membrane damage, mitochondrial dysfunction, rapid accumulation of amyloid, etc. Multiple proteins and nucleic acids can be covalently modified by peroxidation products, resulting in the loss of the protein functions, which eventually triggers programmed cell death and general neuroinflammation in brain, and ends up with an increased susceptibility to neurodegenerative diseases. Based on the knowledge of mechanisms of

收稿日期: 2021-02-02;　修回日期: 2021-03-10.
基金项目: 国家自然科学基金资助项目 (81873209, 81903821); 广东省基础与应用基础研究基金−自然科学基金资助项目 (2019A1515010909); 珠江创新团队 (2017BT01Y036) 和珠江学者计划 (GDUPS2019); 广州市科技计划−基础与应用基础研究资助项目 (907158833068, 201903010062).
*通讯作者 Tel / Fax: 86-20-85221559, E-mail: rongronghe@jnu.edu.cn
DOI: 10.16438/j.0513-4870.2021-0188

phospholipid peroxidation, this review focuses on the characteristics of phospholipid peroxidation as a key factor in the development of neurodegenerative diseases, in order to provide theoretical basis for targeted intervention of phospholipid peroxidation as a potential strategy to prevent neurodegenerative diseases.

Key words: phospholipid peroxidation; neurodegenerative disease; disease susceptibility; ferroptosis; oxidative stress

随着社会的老龄化和平均寿命的延长, 中枢神经系统疾病已成为全球老年人残疾和发病的主要原因, 且每年呈持续性上涨的趋势。这些患者患有严重的行动障碍伴随认知能力障碍, 需要长期的护理, 给家庭和社会带来了巨大的负担[1]。研究表明, 氧化应激是参与不同中枢神经系统疾病的重要毒性机制之一, 而磷脂过氧化是氧化应激的重要结果[2]。自1975年起, 神经退行性疾病 (neurodegenerative disease, ND) 的磷脂过氧化现象就被广泛研究。磷脂 (phospholipids, PLs) 是神经细胞生物膜的主要组成成分, 多不饱和脂肪酸 (polyunsaturated fatty acids, PUFAs) 如花生四烯酸 (arachidonic acid, AA) 酰化在磷脂上形成多不饱和磷脂 (PUFA-PLs) 并参与神经元之间的信号传导。当大脑的氧化–抗氧化系统失去平衡时, PUFA-PLs易受到自由基/活性氧 (reactive oxygen species, ROS) 的攻击而发生氧化链式反应, 产生的过氧化产物不仅能破坏磷脂膜的结构和功能, 同时可以被细胞程序性死亡相关受体如清道夫受体、Toll样受体等识别, 导致神经元发生凋亡、吞噬和铁死亡等程序性细胞死亡过程[3]。本课题组通过长期研究发现情志应激负荷诱导的氧化应激会诱发神经、免疫和代谢等机体多系统生理功能的失衡状态, 增加多种疾病的易感性[4]。大量研究表明, 磷脂过氧化参与了ND的发生和发展进程, 作者据此提出磷脂过氧化是ND易感的关键因素。本文综述了磷脂过氧化参与ND的相关研究, 探索磷脂过氧化增加ND易感的关系, 提出靶向干预磷脂过氧化可用作防治ND的潜在策略。

1 大脑磷脂构成的特殊性

磷脂也称磷脂类或磷脂质, 通常由磷酸基团构成的亲水头和脂肪酸构成的疏水尾通过甘油主链连接组成: 磷酸基团可以被甘油、胆碱、乙醇胺和丝氨酸等有机小分子修饰; 脂肪酸通过酯或醚键连接在主链甘油基团的第一个和第二个碳原子, 分别表示为*sn*-1和*sn*-2位酰化脂肪链[5,6]。磷脂具有两性特点, 其分子亲水端相互靠近, 疏水端相互靠近, 能够同时与蛋白质、糖脂、胆固醇等共同构成磷脂双分子层, 构成生物膜结构[7]。常见的甘油磷脂有磷脂酸 (PA)、磷脂酰胆碱 (PC)、磷脂酰乙醇胺 (PE)、磷脂酰丝氨酸 (PS)、磷脂酰肌醇 (PI)、磷脂酰甘油 (PG)、心磷脂 (CL) 等。

地球早期生命脂肪酸组成主要是单不饱和脂肪酸 (monounsaturated fatty acids, MUFAs), 但是由于MUFAs没有疏水性基团而导致膜缺乏流动性[8]。在漫长的进化过程中, 富含的双烯丙基碳结构的PUFAs参与脂质代谢并被整合到磷脂层中形成PUFA-PLs[9], 这一变化改善了生物膜的流动性, 也是完成物质运输、信息传递、能量转换和细胞免疫等正常细胞功能的重要保障[10]。PUFAs中丰富的双烯丙基亚甲基单元对ROS高度敏感, 导致PUFA-PLs容易发生过氧化反应[9,11]。不饱和程度越高的磷脂对氧化应激越敏感, 如大脑中多数磷脂在*sn*-2位置含有PUFAs, 其中一些在*sn*-1位置也含有PUFAs[12]。不饱和磷脂的氧化对磷脂的生物活性的影响巨大, 可以减少细胞膜中PUFAs含量, 破坏磷脂的结构, 从而改变生物膜的流动性和渗透性, 以及膜上蛋白的酶活性和受体功能等[13]。此外, 磷脂过氧化产物可能被细胞相关受体识别而改变细胞进程, 并诱导细胞发生凋亡、吞噬或铁死亡等程序性细胞死亡, 或者启动/调节先天性和适应性免疫反应[3,14], 在相应疾病中发挥作用[15]。

哺乳动物大脑的脂质含量高于其他器官[16], 并且大脑的脂质组成与非神经组织的脂质组成相比显示出明显的多样化[17]。脂质占大脑干重的50%～60%, 其中包括约50%的磷脂、40%的糖脂、10%的胆固醇和胆固醇酯, 以及微量的甘油三酯[18]。人类大脑含有的甘油磷脂中, 占比最高的是PE (35.6%) 和PC (32.8%), 其他包括PS (16.6%)、PI (2.6%)、PA (2.0%) 和少量CL和PG[19]。大脑也富含PUFAs, 约占脂肪酸的25%～30%, 主要由二十二碳六烯酸 (docosahexaenoic acid, DHA)、AA和少量亚油酸 (linoleic acid, LA) 组成[19,20]。PUFAs在长链脂肪酸–辅酶A合成酶4 (acyl-CoA synthetase long-chain family member 4, ACSL4) 和溶血磷脂酰基转移酶 (lysophospholipid acyltransferases, LPLATs), 如溶血卵磷脂酰基转移酶3 (lysophosphatidylcholine acyltransferase 3, LPCAT3) 的作用下与磷脂酯化形成PUFA-PLs, 维持大脑神经元、胶质细胞和内皮细胞的结构和功能, 如膜的通透性和流动性, 并促进内吞和胞吐作用、离子通道活性以及包括神经递质受体在内的

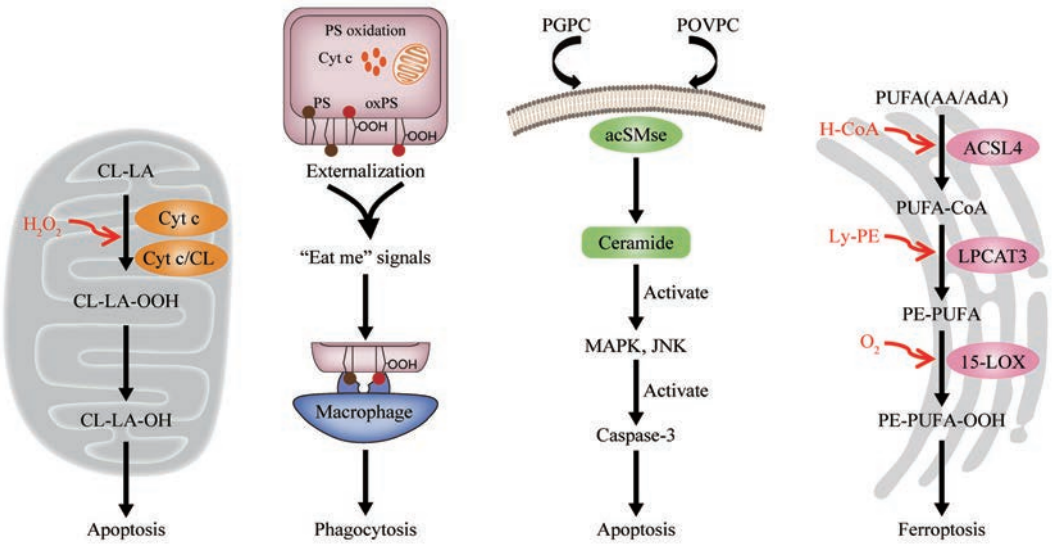

Figure 1 Different signaling pathways of phospholipid peroxidation. Mono-oxygenated species of CL-LA (CL-LA-OHs), which are formed in mitochondria *via* the Cyt c/CL-driven reaction, are identified as apoptotic signals. Oxidatively modified PS on the surface of apoptotic cells plays an essential "eat-me" role for macrophage phagocytosis. The oxidized phospholipid POVPC and PGPC are potent inducers of apoptosis, regulated by the activation of acSMse and caspase-3 through MAPK and JNK pathway. PUFAs containing AA and AdA are acylated into phospholipids through remodeling mechanism mediated by *e.g.* ACSL4 and LPCAT3. Hydroperoxy-arachidonoyl/adrenoyl PE species (PE-PUFA-OOHs), known as iconic ferroptosis signals, are mainly formed in endoplasmic reticulum pushed by 15-LOX, which are capable of oxygenating AA/AdA to 12-HpETE and 15-HpETE in variable amounts. CL-LA: Cardiolipin-linoleic acid; POVPC: 1-Palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine; PGPC: 1-Palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine; acSMse: Acid sphingomyelinase; MAPK: Mitogen-activated protein kinase; JNK: c-Jun NH_2-terminal protein kinase; PUFAs: Polyunsaturated fatty acids; AA: Arachidonic acid; AdA: Adrenic acid; LyPE: Lysophatidylethanolamine; ACSL4: Acyl-CoA synthetase long-chain family member 4; LPCAT3: Lysophosphatidylcholine acyltransferase 3; 15-LOX: 15-Lipoxygenase

膜结合蛋白的活性[20,21] (图1)。

2 神经元磷脂过氧化发生机制及氧化产物

大脑中丰富的PUFA-PLs可能是神经元对氧化应激尤为敏感的原因之一[1]。磷脂过氧化主要有酶促和非酶促两种途径。酶促氧化途径由多种氧化酶, 如脂氧合酶12-LOX (12-lipoxygenase) 和15-LOX (15-lipoxygenase)、细胞色素P450 (CYP) 和细胞色素c (Cyto c) 介导, 通常对定位于酶催化位点的氧化底物进行氧化; 非酶促氧化由自由基或非自由基ROS引发, 其氧化特点具有随机性。酶促和非酶促氧化过程都经历起始、传播和终止3个阶段的链式反应[22]。两者的区别仅在于起始阶段启动反应不同: 在起始阶段, 芬顿反应产生原始自由基, 自由基 (非酶) 或磷脂过氧化相关酶 (酶促) 攻击PUFA-PLs, 从双烯丙基亚甲基中提取氢形成底物自由基。在传播阶段, 底物自由基和氧分子快速反应, 生成过氧自由基 (LOO•), 并伴随产生烷基 (L•)、环氧烷基 (OL•)、羟基烷基 (HOL•)、烷氧基 (LO•) 和环氧过氧基 (OLOO•) 等初级氧化产物; 过氧自由基进一步和其他PUFA-PLs中的双烯丙基亚甲基单元反应, 将过氧自由基转化为非自由基氢过氧化物 (LOOH), 产生新的碳中心自由基引发过氧化循环; LOOH能够在铁或铜等金属离子的催化作用下发生Fenton反应, 生成活泼烷氧基和羟基自由基[3,23]。在终止阶段, 两个LOO•自由基反应形成一个非自由基和氧气, 氧气持续参与反应形成一个循环[22]。在终止阶段主要通过亲脂性“断链”抗氧化剂终止反应, 如*β*-胡萝卜素或维生素E[24], 或谷胱甘肽过氧化物酶4 (glutathione peroxidase 4, GPX4)[25]、过氧化物酶VI[26]和谷胱甘肽转移酶 (glutathione transferase, GST)[27]等终止链式反应 (图2)。

酶促和非酶促氧化反应能够产生相同的初级氧化产物, 即过氧自由基和氢过氧化物。初级氧化产物可以经过3个途径进一步氧化生成次级氧化产物。途径一是过氧自由基和氢过氧化物引入额外氧原子生成具有官能团的各种氧化磷脂 (oxPLs), 如羟基产物 (hydroxy-PLs)、氢过氧基产物 (hydroperoxy-PLs)、酮基产物 (keto-PLs) 和环氧产物 (epoxy-PLs); 途径二是过氧自由基环化产生环状过氧化物, 其经历内环化和重排产生双环内过氧化物 (isoPGs)、异前列腺素 (isoPs)、异丁烷 (isoTx) 和异戊二烯 (isoLGs), 或氧化引入另

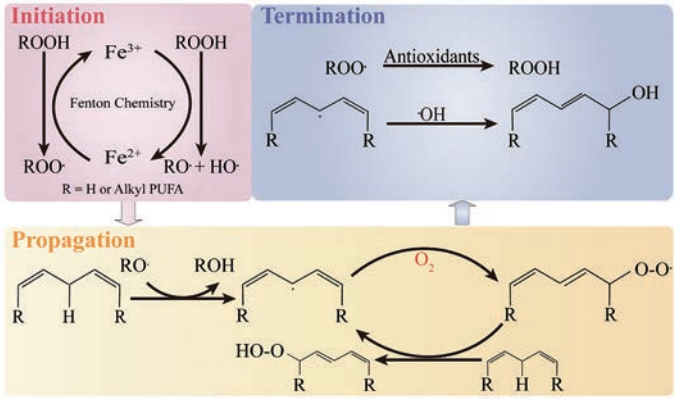

Figure 2 The three steps of phospholipid oxidation. In the initiation step, the key event is the formation of free radical by Fenton chemistry. In the propagation step, free radicals attack PUFAs as substrates and produce new radicals. The propagation step will continue until the termination step. In the termination step, radicals are bound by antioxidants or reacting with non-radical products

外的非环状或环状过氧化物基团分子的重排, 并进一步氧化产生异呋喃 (isofuran); 途径三是氢过氧化物经氧化/裂解或聚合/裂解产生被截短的末端带醛基的γ-羟基-α,β-不饱和oxPLs、带有末端呋喃基团的不饱和oxPLs和带有末端羰基的饱和oxPLs。途径一和二产生非断裂的oxPLs, 途径三产生断裂的oxPLs[3]。次级氧化产物经后续氧化能够生成氧化终产物, 如丙二醛 (malondialdehyde, MDA) 和4-羟基壬烯醛 (4-hydroxynonenal, 4-HNE), MDA和4-HNE的含量被用作标记脂质过氧化的程度。

MDA可以被酶代谢, 也可以与细胞、组织蛋白和DNA反应形成加合物, 导致蛋白质合成减少、DNA损伤和突变。MDA对亲核试剂如碱性氨基酸残基 (赖氨酸、组氨酸或精氨酸) 具有强反应性, 与游离氨基酸或蛋白质的初始反应能够产生席夫碱加合物, 也称为高级脂质过氧化终产物 (advanced lipid peroxidation end products, ALE)[28]。ALE能够通过促进分子内或分子间的蛋白质、DNA交联参与二次有害反应导致蛋白质和DNA的生化特性改变[28-31]。MDA可导致蛋白质发生修饰、聚集以及酶失活[32], 并参与诱导海马神经元的退化[33]。此外, 有体外研究也证明丙烯醛对不同类型神经细胞系有毒性作用, 提示MDA可直接诱导中枢神经系统神经变性和损伤[34]。4-HNE的神经毒性已在神经退行性疾病中得到验证, 4-HNE可以扩散到不同的细胞区室并与不同的底物相互作用, 如与半胱氨酸、组氨酸和赖氨酸残基的共价结合, 4-HNE可以通过修饰膜相关的葡萄糖、谷氨酸转运蛋白、离子动力ATP酶、参与淀粉样蛋白代谢的酶和细胞骨架蛋白等导致酶活性的损失或降低以及蛋白质结构的改变来引起神经元功能障碍和变性[35]。已有研究表明, 在阿尔茨海默症 (Alzheimer's disease, AD) 和肌萎缩性侧索硬化症 (amyotrophic lateral sclerosis, ALS) 中, 发现与能量代谢相关的α-烯醇酶和ATP-合酶, 以及与抗氧化防御相关的超氧化物歧化酶被4-HNE氧化修饰, 抗氧化和代谢功能的紊乱可能导致神经元易受兴奋性毒性和细胞死亡的影响[36]。β-淀粉样蛋白 (β-amyloid protein, Aβ) 具有可与4-HNE反应的亲核侧链, 4-HNE通过添加1,4-缀合物共价修饰Aβ, 并增强Aβ聚集和毒性, 进而引起氧化应激导致更多的4-HNE和毒性更高的Aβ低聚物形成[37]。

3 磷脂过氧化增加神经退行性疾病的易感性

大脑除了高脂质的特殊结构外, 还具有高耗氧量的特性。大脑不间断的工作需要大量的氧气供给, 消耗呼气中约20%的氧气, 产生大量的活性氧代谢产物, 如超氧化物、羟基自由基和过氧化氢等[38]。然而, 大脑的神经元缺乏再生能力, 同时膜表面积和细胞质体积比率高、氧化还原过渡金属较多[39]、过氧化氢酶和谷胱甘肽过氧化物酶较低[40]。大脑PUFA-PLs中丰富的双烯丙基结构对自由基具有高度氧化敏感性, 因此大脑易发生磷脂过氧化, 生成初级、次级和最终氧化产物及毒性的蛋白共价修饰复合物[41]。因此, 磷脂过氧化导致的氧化损伤和神经炎症等给神经元带来致命一击, 是凋亡、坏死或铁死亡的细胞程序性死亡的开启, 是增加ND易感的关键因素 (图3)。

3.1 磷脂过氧化增加AD的易感性 AD是一种以记忆障碍伴随语言和行为障碍为特征的神经退行性疾病[42]。Aβ沉积形成老年斑和tau蛋白过度磷酸化形成神经元纤维缠结是AD的两个病理特征[43,44]。

脂质过氧化被报道是AD发病的重要因素之一[44]。AD脑中高铁和高ROS水平的氧化应激环境会诱导磷脂发生过氧化, 已有研究显示, AD患者大脑和血浆中磷脂谱显著降低, 海马中PE、PI和总游离脂肪

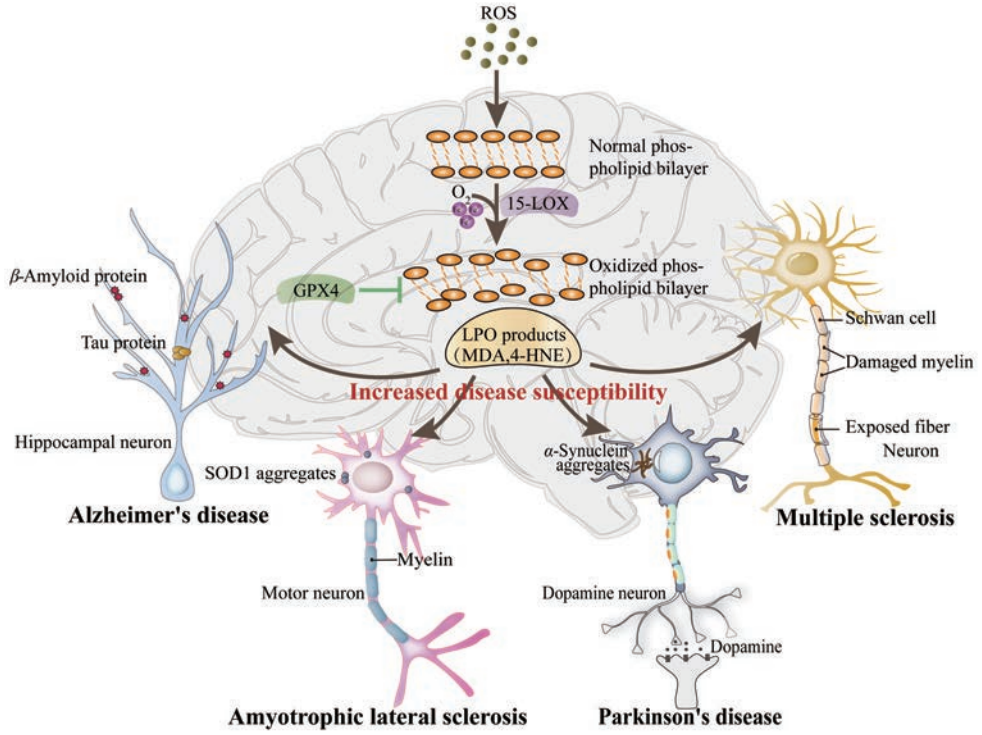

Figure 3 Phospholipid peroxidation increases the susceptibilities of neurodegenerative diseases. ROS attacks normal phospholipid bilayer of neurons in the O_2 and 15-LOX-driven reaction. Oxidative damage of phospholipids gives rise to the formation of LPO products, such as MDA and 4-HNE, which can be reduced by GPX4. In Alzheimer's disease, LPO products lead to the deposition of *β*-amyloid protein and the formation of tangles by tau protein in hippocampal neurons. In Parkinson's disease, LPO induces the misfolding and aggregation of *α*-synuclein and aggravates the disease progress. In amyotrophic lateral sclerosis (ALS), LPO increases the vulnerabilities of SOD1 misfolding and loss of motor neuron, supporting the view that oxidative stress is the main mechanism of ALS. In multiple sclerosis, phospholipid peroxidation is closely related to demyelination and neuronal damage. ROS: Reactive oxygen species; LPO: Lipid peroxidation; MDA: Malondialdehyde; 4-HNE: 4-Hydroxynonenal; GPX4: Glutathione peroxidase 4; SOD1: Superoxide dismutase 1

酸水平显著降低[45]。在AD患者的杏仁核、海马和海马旁回等脑区和脑室脑脊液中可检测到高浓度的游离4-HNE及其蛋白复合物[46-48]。4-HNE能够靶向干扰能量代谢和抗氧化相关蛋白质，引起细胞重要功能的紊乱，进而导致神经元细胞的死亡[49]。此外，有数据显示AD患者大脑的MDA水平明显高于健康受试者[50]。在大脑中累积的MDA可与多种蛋白质共价结合并促进异常蛋白质加合物的形成，后者将严重扰乱多个大脑区域的功能，如额叶、颞叶、枕叶和海马的结构和神经信号[51]。

Ademowo等[52]发现PC的氧化产物之一——1-棕榈酰基-2-(5-氧代戊酰基)-*sn*-甘油-3-磷酸胆碱在AD患者中浓度较高，并且与患者的认知能力呈负相关，可视作早期AD和轻度认知障碍诊断生物标志物。AD小鼠模型以及AD患者死后的大脑样本中都可观察到PS-OOHs和PI-OOHs的特异性蓄积[53]，而在AD和轻度认知障碍(MCI)受试者大脑的突触小体外小叶上发现有明显PS外翻[54]，提示PS-OOH参与AD早期的神经元的丢失，即持续的脂质过氧化会导致毒性氧化产物的累积，进而触发严重的神经元损伤，增加AD的易感性或使AD恶化。

3.2 磷脂过氧化增加帕金森病的易感性 帕金森病(Parkinson's disease, PD)也是一种进行性神经退行性疾病，临床表现为不可控震颤、动作迟缓、肌肉僵直和体位不稳等运动症状，病理表现为黑质多巴胺能(dopamine, DA)神经元缺失、DA含量下降和*α*-突触核蛋白(*α*-synuclein, *α*-Syn)聚积，形成路易小体[55]。PD中神经变性是一个多因素过程，可能涉及DA代谢异常、铁水平增加、过氧化氢生成、线粒体异常、蛋白酶体功能和小胶质细胞增生等。越来越多的证据显示，磷脂及其氧化产物介导的氧化应激和炎症在PD的发病机制中起到重要作用[56,57]。

临床研究报道了PD患者大脑中MDA累积，促进脂质过氧化的启动和氧化应激，显著升高4-HNE的水平[58]。另外，MDA能修饰DA神经元中的*α*-Syn并形成加合物，导致线粒体功能障碍以及ROS介导的神经

元凋亡, 这在临床患者大脑中得到验证[59]。与体内研究的结果一致, MDA在大鼠脑黑质纹状体DA系统中能够充当PD神经毒素的作用, 激活星形胶质细胞和小胶质细胞的促炎作用, 导致α-Syn的聚集和程序性细胞死亡[34]。

PD死亡患者的大脑皮层和路易体中检测到4-HNE的免疫定位[60], 4-HNE修饰α-Syn导致构象变化和低聚物形成, 且修饰的α-Syn低聚物具有毒性, 可导致神经元的退化[61]。此外, 研究发现4-HNE会改变DA转运和损失, 前者含量与PD的严重程度成正比[62]。因此, 脂质过氧化修饰蛋白质的蓄积和DA神经元的丢失可能会增加PD的易感性以及加剧PD患者的运动功能和认知功能障碍。

3.3 磷脂过氧化增加ALS的易感性 ALS是最常见的运动神经元疾病, 其特征是大脑和脊髓中运动神经元的逐渐退化, 导致肌肉萎缩、麻痹和死亡[63]。兴奋性毒性、线粒体功能障碍、内质网应激、神经炎症和氧化应激等因素都与ALS的发病相关。ALS患者的尿液、脑脊液、血液和个体组织中均发现了MDA修饰蛋白、DNA氧化标记物8-羟基-2-脱氧鸟苷 (8-OHdG)[64]。

在ALS患者的脊髓运动神经元中观察到4-HNE及其修饰蛋白的水平升高, 血清和脊髓液中4-HNE的水平显著升高, 与疾病的程度呈正相关[65]。4-HNE可以修饰与轴突的发育、传输和细胞外信号调节相关的3种关键蛋白—二氢嘧啶酶相关蛋白2 (DRP-2)、热休克蛋白70和α-烯醇化酶, 导致运动神经元功能的丧失。这一结果支持了氧化应激和磷脂过氧化作为ALS潜在发病机制的观点[66]。

3.4 磷脂过氧化增加多发性硬化症 (multiple sclerosis, MS) 的易感性 MS是以中枢神经系统脱髓鞘和局灶性病变为特征的炎症性疾病[67]。Haider等[68]在MS患者大脑硬化斑块中发现氧化的脂质和DNA呈现高度富集, 在少突胶质细胞和星形胶质细胞的细胞质中观察到MDA和氧化磷脂表位, 轴突球体以及灰质病灶内的神经元也有大量氧化磷脂聚集, 并具有变性迹象, 表明氧化磷脂的产生和多发性硬化症轴突或神经元的损伤密切相关。MS患者的红细胞和血小板的长链脂肪酸特别是PUFAs减少, 且LA浓度明显较低[69]; LA-PC和AA-PC分子总量显著减少[70]。EO6单克隆抗体的蛋白质印迹分析证实了MS患者大脑内存在oxPC, oxPC被认为是MS病变中神经炎症的标志物[71]。上述证据提示, 磷脂过氧化可能是增加MS易感的关键因素。

4 铁死亡可能是磷脂过氧化增加神经退行性疾病“易感”的重要机制

铁死亡是铁和磷脂过氧化依赖的程序性细胞死亡方式, 属于一种代谢性细胞坏死[72]。ND的发生发展中, 普遍存在铁积累和脂质过氧化, 并伴随着谷胱甘肽 (glutathione, GSH)、GPX4水平的降低、ROS产生、脂氧合酶的活化和钙内流等氧化应激现象, 与2012年发现的这种铁死亡现象高度相似[73]。目前已有研究发现, 铁死亡诱导的神经元的死亡与多种ND如AD、PD和ALS均密切相关, 铁死亡可能是相关疾病神经元丢失的重要调控方式[74-76]。

磷脂过氧化是铁死亡发生的基础条件, 铁死亡一般通过氨基酸代谢、铁代谢和脂质代谢等3种调控机制进行调节[77,78], 其最上游可追溯到GPX4-GSH-半胱氨酸轴的代谢调节, 在*Gpx4*敲低的小鼠中会导致年龄相关的神经退行性改变和神经元丢失, 且饮食中维生素E缺乏会加剧这种情况[79], 提示铁死亡抑制蛋白GPX4是PD治疗的潜在靶标。此外, FSP1-CoQ(10)-NAD(P)H (ferroptosis suppressor protein 1-coenzyme Q10-nicotinamide adenine dinucleotide phosphate) 是完全独立于GPX4-GSH-半胱氨酸轴的信号系统, 可能抑制铁死亡的执行[80]。

在铁死亡未被发现之前, 谷氨酸诱导的神经元死亡一直被认为是谷氨酸的兴奋毒性引起的, 之后才发现谷氨酸通过竞争性抑制胱氨酸摄入的能力, 导致谷胱甘肽耗竭而诱导神经元发生铁死亡, 揭示了谷氨酸毒性的新机制[81]。Kagan等[82]通过氧化磷脂组学等分析方法发现诱导铁死亡的重要“充氧中心”是内质网, 在内质网相关隔室中, AA和AdA特异性酰化的磷脂被氧化成PE-AA-OOH和PE-AdA-OOH, 且这两种氧化磷脂是发生铁死亡的重要标志信号。

关于ND易感性与磷脂过氧化的紧密关系这一点, 本课题组采用多个模型获得了最新数据。首先, 在经典的PD模型*SNCA*^{A53T}转基因小鼠中发现磷脂氧化产物15-氢过氧 (Hp)-花生四烯酰基-磷脂酰乙醇胺 (15-HpETE-PE) 的累积[83]; 另外, 采用铁死亡诱导剂sorafenib和PD诱导剂6-OHDA负荷构建了负荷脂质过氧化的PD易感性模型, 结果表明该易感因素可加速并加剧PD样行为学症状和DA神经元丢失, 而脂质过氧化抑制剂trolox显示出对以上症状的缓解作用[84], 因此认为磷脂过氧化可协同α-Syn低聚物增加DA神经元对铁死亡敏感性, 磷脂过氧化是PD发病的潜在关键机制。除此以外, 磷脂重塑途径相关酶可能也参与PD进展, 如与磷脂重塑相关的钙非依赖磷脂酶iPLA$_2\beta$ (Ca^{2+}-independent phospholipase A_2 beta, 由*PLA2G6/PNPLA9*基因编码) 的基因突变会显著削弱其切割*sn*-2上oxPUFAs的能力, 引起氧化磷脂15-HpETE-PE的不断蓄积[85]。据此, 课题组利用CRISPR-Cas9基因编辑

技术构建了 *Pnpla9*$^{R748W/R748W}$ 点突变小鼠, 发现这种突变不但能造成小鼠出现 PD 样的运动障碍, 还能诱导中脑产生 15-HpETE-PE 的累积, 并发现多巴胺能神经元标记物酪氨酸羟化酶 (tyrosine hydroxylase, TH) 的水平显著降低。以上结果提示, iPLA$_2\beta$ 可能是一个新的铁死亡调控蛋白, iPLA$_2\beta$ 突变是一种潜在的 PD 发病机制, 并由此提出, 多巴胺能神经元可能对铁死亡尤其敏感, 磷脂过氧化的堆积在此发展过程中至关重要[83]。综上, 磷脂过氧化是机体对以 PD 为例的 ND 相关疾病易感的关键因素, 抑制磷脂过氧化或干预抗氧化酶可作为 ND 的潜在防治策略。

5 结语及展望

ND 的病理生理过程是一个复杂而动态的过程, 涉及多种信号传导、细胞炎症、凋亡吞噬和铁死亡等过程, 随着基于 LC-MS 的氧化脂质分析技术的发展, 磷脂过氧化已被发现与许多 ND 的发生发展过程息息相关, 其涉及的酶促或非酶促的复杂磷脂氧化信号在不同疾病的发病机制中发挥复杂的生理或病理效应。尽管以往的研究报道称 ND 与遗传缺陷相关, 但本课题组发现磷脂过氧化是机体对多种 ND 更加易感的重要原因, 可加剧疾病表型的发生, 因此可作为 ND 的重要防治策略。目前 ND 的疗法旨在缓解症状, 没有解决根本的病理学问题, 随着疾病的进展, 药物治疗的功效也逐渐降低。基因疗法可通过直接实现神经保护、神经修复和症状控制等效果来发挥最终纠正潜在的致病机制的作用[86,87]。此外, 中药疗法由于药性平和、毒副作用小, 在 ND 治疗中也占据天然优势。一些中药具有良好的抗氧化作用, 如人参、天麻能清除自由基, 使神经元免受氧化损伤[88]。本实验室验证了中药复方天麻钩藤颗粒通过抑制 15-LOX 介导的脂质过氧化作用而降低小鼠和大鼠对 PD 的易感性[84]。虽然关于脂质过氧化与 ND 相关的基础研究已取得一定的成果, 但远不足以阐明 ND 发病的具体机制。由于磷脂氧化产物的复杂性与多样性, 其在 ND 中的作用机制和继发性损伤机制等方面仍面临较大的挑战。因此, 深入挖掘磷脂过氧化增加 ND 易感的分子机制将有助于疾病机制的揭示以及治疗靶标的探索。

作者贡献: 林晓敏完成文献的查阅和整理、起草文稿; 孙万阳和段文君完善内容和修改稿件; 龚海标和涂龙芳负责完成综述部分内容; 李怡芳和栗原博提供了改进建议; 何蓉蓉为文章提供总指导和思路。所有作者均对本文有所贡献。

利益冲突: 所有作者均声明不存在利益冲突。

References

[1] Petrovic S, Arsic A, Ristic-Medic D, et al. Lipid peroxidation and antioxidant supplementation in neurodegenerative diseases: a review of human studies [J]. Antioxidants (Basel), 2020, 9: 1128.

[2] Salim S. Oxidative stress and the central nervous system [J]. J Pharmacol Exp Ther, 2017, 360: 201-205.

[3] Bochkov VN, Oskolkova OV, Birukov KG, et al. Generation and biological activities of oxidized phospholipids [J]. Antioxid Redox Signal, 2010, 12: 1009-1059.

[4] Zhu SR, Luo X, Li YF, et al. Emotional stress-induced Shanghuo syndrome increases disease susceptibility [J]. China J Chin Mater Med (中国中药杂志), 2018, 43: 1529-1535.

[5] Niebylski CD, Salem NJ. A calorimetric investigation of a series of mixed-chain polyunsaturated phosphatidylcholines: effect of *sn*-2 chain length and degree of unsaturation [J]. Biophys J, 1994, 67: 2387-2393.

[6] Zerouga M, Jenski LJ, Stillwell W. Comparison of phosphatidylcholines containing one or two docosahexaenoic acyl chains on properties of phospholipid monolayers and bilayers [J]. Biochim Biophys Acta, 1995, 1236: 266-272.

[7] Berg JM, Tymoczko JL, Stryer L. Biochemistry [M]. New York: W H Freeman, 2002: 488-527.

[8] Lombard J, Lopez-Garcia P, Moreira D. The early evolution of lipid membranes and the three domains of life [J]. Nat Rev Microbiol, 2012, 10: 507-515.

[9] Hulbert AJ, Kelly MA, Abbott SK. Polyunsaturated fats, membrane lipids and animal longevity [J]. J Comp Physiol B, 2014, 184: 149-166.

[10] Zhang L, Sun SQ, Shen XJ, et al. Membrane fluidity and atherosclerosis [J]. Med Recapit (医学综述), 2001, 7: 56-58.

[11] Pratt DA, Tallman KA, Porter NA. Free radical oxidation of polyunsaturated lipids: new mechanistic insights and the development of peroxyl radical clocks [J]. Acc Chem Res, 2011, 44: 458-467.

[12] Sparvero LJ, Amoscato AA, Kochanek PM, et al. Mass-spectrometry based oxidative lipidomics and lipid imaging: applications in traumatic brain injury [J]. J Neurochem, 2010, 115: 1322-1336.

[13] Farooqui AA, Horrocks LA. Lipid peroxides in the free radical pathophysiology of brain diseases [J]. Cell Mol Neurobiol, 1998, 18: 599-608.

[14] Kagan VE, Tyurina YY, Sun WY, et al. Redox phospholipidomics of enzymatically generated oxygenated phospholipids as specific signals of programmed cell death [J]. Free Radic Biol Med, 2020, 147: 231-241.

[15] Anthonymuthu TS, Kenny EM, Lamade AM, et al. Oxidized phospholipid signaling in traumatic brain injury [J]. Free Radic Biol Med, 2018, 124: 493-503.

[16] White DA. The phospholipid composition of mammalian tissues [M]// Ansell GB, Dawson RMC, Hawthorne HN. Form and Function of Phospholipids. Amsterdam: Elsevier, 1973: 441-482.

[17] Bozek K, Wei Y, Yan Z, et al. Organization and evolution of brain lipidome revealed by large-scale analysis of human, chimpanzee, macaque, and mouse tissues [J]. Neuron, 2015, 85: 695-702.

[18] Qi K, Hall M, Deckelbaum RJ. Long-chain polyunsaturated fatty acid accretion in brain [J]. Curr Opin Clin Nutr Metab Care, 2002, 5: 133-138.

[19] Sastry PS. Lipids of nervous tissue: composition and metabolism [J]. Prog Lipid Res, 1985, 24: 69-176.

[20] Bazinet RP, Layé S. Polyunsaturated fatty acids and their metabolites in brain function and disease [J]. Nat Rev Neurosci, 2014, 15: 771-785.

[21] Bos DJ, van Montfort SJ, Oranje B, et al. Effects of omega-3 polyunsaturated fatty acids on human brain morphology and function: what is the evidence? [J]. Eur Neuropsychopharmacol, 2016, 26: 546-561.

[22] Yin H, Xu L, Porter NA. Free radical lipid peroxidation: mechanisms and analysis [J]. Chem Rev, 2011, 111: 5944-5972.

[23] Reis A, Spickett CM. Chemistry of phospholipid oxidation [J]. Biochim Biophys Acta, 2012, 1818: 2374-2387.

[24] Grune T, Schröder P, Biesalski HK. Low Molecular Weight Antioxidants [M]. Berlin: Springer, 2005: 77-90.

[25] Savaskan NE, Ufer C, Kühn H, et al. Molecular biology of glutathione peroxidase 4: from genomic structure to developmental expression and neural function [J]. Biol Chem, 2007, 388: 1007-1017.

[26] Manevich Y, Fisher AB. Peroxiredoxin 6, a 1-cys peroxiredoxin, functions in antioxidant defense and lung phospholipid metabolism [J]. Free Radic Biol Med, 2005, 38: 1422-1432.

[27] Yang Y, Cheng JZ, Singhal SS, et al. Role of glutathione *s*-transferases in protection against lipid peroxidation. Overexpression of HGSTA2-2 in K562 cells protects against hydrogen peroxide-induced apoptosis and inhibits JNK and caspase 3 activation [J]. J Biol Chem, 2001, 276: 19220-19230.

[28] Pizzimenti S, Ciamporcero E, Daga M, et al. Interaction of aldehydes derived from lipid peroxidation and membrane proteins [J]. Front Physiol, 2013, 4: 242.

[29] Traverso N, Menini S, Maineri EP, et al. Malondialdehyde, a lipoperoxidation-derived aldehyde, can bring about secondary oxidative damage to proteins [J]. J Gerontol A Biol Sci Med Sci, 2004, 59: B890-B895.

[30] Negre-Salvayre A, Coatrieux C, Ingueneau C, et al. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors [J]. Br J Pharmacol, 2008, 153: 6-20.

[31] Cheng J, Wang F, Yu DF, et al. The cytotoxic mechanism of malondialdehyde and protective effect of carnosine *via* protein cross-linking/mitochondrial dysfunction/reactive oxygen species/MAPK pathway in neurons [J]. Eur J Pharmacol, 2011, 650: 184-194.

[32] Taso OV, Philippou A, Moustogiannis A, et al. Lipid peroxidation products and their role in neurodegenerative diseases [J]. Ann Res Hosp, 2019, 3: 2.

[33] Erejuwa OO, Sulaiman SA, Ab Wahab MS. Evidence in support of potential applications of lipid peroxidation products in cancer treatment [J]. Oxid Med Cell Longev, 2013, 2013: 931251.

[34] Wang YT, Lin HC, Zhao WZ, et al. Acrolein acts as a neurotoxin in the nigrostriatal dopaminergic system of rat: involvement of α-synuclein aggregation and programmed cell death [J]. Sci Rep, 2017, 7: 45741.

[35] Mattson MP. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders [J]. Exp Gerontol, 2009, 44: 625-633.

[36] Perluigi M, Coccia R, Butterfield DA. 4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: a toxic combination illuminated by redox proteomics studies [J]. Antioxid Redox Signal, 2012, 17: 1590-1609.

[37] Siegel SJ, Bieschke J, Powers ET, et al. The oxidative stress metabolite 4-hydroxynonenal promotes alzheimer protofibril formation [J]. Biochemistry, 2007, 46: 1503-1510.

[38] Anthonymuthu TS, Kenny EM, Bayır H. Therapies targeting lipid peroxidation in traumatic brain injury [J]. Brain Res, 2016, 1640: 57-76.

[39] Halliwell B. Reactive oxygen species and the central nervous system [J]. J Neurochem, 1992, 59: 1609-1623.

[40] Evans PH. Free radicals in brain metabolism and pathology [J]. Br Med Bull, 1993, 49: 577-587.

[41] Frankel EN. Lipid oxidation [J]. Prog Lipid Res, 1980, 19: 1-22.

[42] Scheltens P, Blennow K, Breteler MM, et al. Alzheimer's disease [J]. Lancet, 2016, 388: 505-517.

[43] Mattson MP. Pathways towards and away from Alzheimer's disease [J]. Nature, 2004, 430: 631-639.

[44] Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein [J]. J Alzheimers Dis, 2001, 3: 75-80.

[45] Prasad MR, Lovell MA, Yatin M, et al. Regional membrane phospholipid alterations in Alzheimer's disease [J]. Neurochem Res, 1998, 23: 81-88.

[46] Markesbery WR, Lovell MA. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease [J]. Neurobiol Aging, 1998, 19: 33-36.

[47] Sayre LM, Zelasko DA, Harris PL, et al. 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease [J]. J Neurochem, 1997, 68: 2092-2097.

[48] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes [J]. Free Radic Biol Med, 1991, 11: 81-128.

[49] Di Domenico F, Tramutola A, Butterfield DA. Role of 4-hydroxy-2-nonenal (hne) in the pathogenesis of Alzheimer disease and

other selected age-related neurodegenerative disorders [J]. Free Radic Biol Med, 2017, 111: 253-261.

[50] Dare LR, Garcia A, Soares CB, et al. The reversal of memory deficits in an Alzheimer's disease model using physical and cognitive exercise [J]. Front Behav Neurosci, 2020, 14: 152.

[51] López-Riquelme N, Alom-Poveda J, Viciano-Morote N, et al. Apolipoprotein eε4 allele and malondialdehyde level are independent risk factors for Alzheimer's disease [J]. SAGE Open Med, 2016, 4: 2050312115626731.

[52] Ademowo OS, Dias HKI, Milic I, et al. Phospholipid oxidation and carotenoid supplementation in Alzheimer's disease patients [J]. Free Radic Biol Med, 2017, 108: 77-85.

[53] Maki RA, Tyurin VA, Lyon RC, et al. Aberrant expression of myeloperoxidase in astrocytes promotes phospholipid oxidation and memory deficits in a mouse model of Alzheimer disease [J]. J Biol Chem, 2009, 284: 3158-3169.

[54] Bader Lange ML, Cenini G, Piroddi M, et al. Loss of phospholipid asymmetry and elevated brain apoptotic protein levels in subjects with amnestic mild cognitive impairment and Alzheimer disease [J]. Neurobiol Dis, 2008, 29: 456-464.

[55] Reich SG, Savitt JM. Parkinson's disease [J]. Med Clin North Am, 2019, 103: 337-350.

[56] Farooqui T, Farooqui AA. Lipid-mediated oxidative stress and inflammation in the pathogenesis of Parkinson's disease [J]. Parkinsons Dis, 2011, 2011: 247467.

[57] Chang KH, Chen CM. The role of oxidative stress in Parkinson's disease [J]. Antioxidants (Basel), 2020, 9: 597.

[58] Schaur RJ, Siems W, Bresgen N, et al. 4-Hydroxy-nonenal-a bioactive lipid peroxidation product [J]. Biomolecules, 2015, 5: 2247-2337.

[59] Shamoto-Nagai M, Maruyama W, Hashizume Y, et al. In Parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid-peroxidation product, and accumulates in the dopamine neurons with inhibition of proteasome activity [J]. J Neural Transm (Vienna), 2007, 114: 1559-1567.

[60] Castellani RJ, Perry G, Siedlak SL, et al. Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem lewy bodies in humans [J]. Neurosci Lett, 2002, 319: 25-28.

[61] Qin L, Wu X, Block ML, et al. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration [J]. Glia, 2007, 55: 453-462.

[62] Fedorova TN, Logvinenko AA, Poleshchuk VV, et al. Lipid peroxidation products in the blood plasma of patients with Parkinson's disease as possible biomarkers of different stages of the disease [J]. Neurochem J, 2019, 13: 391-395.

[63] van Es MA, Hardiman O, Chio A, et al. Amyotrophic lateral sclerosis [J]. Lancet, 2017, 390: 2084-2098.

[64] Singh A, Kukreti R, Saso L, et al. Oxidative stress: a key modulator in neurodegenerative diseases [J]. Molecules, 2019, 24: 1583.

[65] Pedersen WA, Fu W, Keller JN, et al. Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients [J]. Ann Neurol, 1998, 44: 819-824.

[66] Perluigi M, Fai Poon H, Hensley K, et al. Proteomic analysis of 4-hydroxy-2-nonenal-modified proteins in g93a-sod1 transgenic mice--a model of familial amyotrophic lateral sclerosis [J]. Free Radic Biol Med, 2005, 38: 960-968.

[67] Courtney AM, Treadaway K, Remington G, et al. Multiple sclerosis [J]. Med Clin North Am, 2009, 93: 451-476.

[68] Haider L, Fischer MT, Frischer JM, et al. Oxidative damage in multiple sclerosis lesions [J]. Brain, 2011, 134: 1914-1924.

[69] Gul S, Smith AD, Thompson RH, et al. Fatty acid composition of phospholipids from platelets and erythrocytes in multiple sclerosis [J]. J Neurol Neurosurg Psychiatry, 1970, 33: 506-510.

[70] Navarro X, Segura R. Red blood cell fatty acids in multiple sclerosis [J]. Acta Neurol Scand, 1989, 79: 32-37.

[71] Qin J, Goswami R, Balabanov R, et al. Oxidized phosphatidylcholine is a marker for neuroinflammation in multiple sclerosis brain [J]. J Neurosci Res, 2007, 85: 977-984.

[72] Conrad M, Pratt DA. The chemical basis of ferroptosis [J]. Nat Chem Biol, 2019, 15: 1137-1147.

[73] Lewerenz J, Ates G, Methner A, et al. Oxytosis/ferroptosis-(re-) emerging roles for oxidative stress-dependent non-apoptotic cell death in diseases of the central nervous system [J]. Front Neurosci, 2018, 12: 214.

[74] Lane DJR, Ayton S, Bush AI. Iron and Alzheimer's disease: an update on emerging mechanisms [J]. J Alzheimers Dis, 2018, 64: S379-S395.

[75] Do Van B, Gouel F, Jonneaux A, et al. Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC [J]. Neurobiol Dis, 2016, 94: 169-178.

[76] Conrad M, Kagan VE, Bayir H, et al. Regulation of lipid peroxidation and ferroptosis in diverse species [J]. Genes Dev, 2018, 32: 602-619.

[77] Li J, Cao F, Yin H, et al. Ferroptosis: past, present and future [J]. Cell Death Dis, 2020, 11: 88.

[78] Zheng J, Conrad M. The metabolic underpinnings of ferroptosis [J]. Cell Metab, 2020, 32: 920-937.

[79] Hambright WS, Fonseca RS, Chen L, et al. Ablation of ferroptosis regulator glutathione peroxidase 4 in forebrain neurons promotes cognitive impairment and neurodegeneration [J]. Redox Biol, 2017, 12: 8-17.

[80] Doll S, Freitas FP, Shah R, et al. Fsp1 is a glutathione-independent ferroptosis suppressor [J]. Nature, 2019, 575: 693-698.

[81] Ratan RR. The chemical biology of ferroptosis in the central nervous system [J]. Cell Chem Biol, 2020, 27: 479-498.

[82] Kagan VE, Mao G, Qu F, et al. Oxidized arachidonic and adrenic

PEs navigate cells to ferroptosis [J]. Nat Chem Biol, 2017, 13: 81-90.

[83] Sun WY, Tyurin VA, Mikulska-Ruminska K, et al. Phospholipase iPLA(2)β averts ferroptosis by eliminating a redox lipid death signal [J]. Nat Chem Biol, 2021, 17: 465-476.

[84] Jiang YN, Guo YZ, Lu DH, et al. Tianma gouteng granules decreases the susceptibility of Parkinson's disease by inhibiting ALOX15-mediated lipid peroxidation [J]. J Ethnopharmacol, 2020, 256: 112824.

[85] Liu GY, Moon SH, Jenkins CM, et al. The phospholipase iPLA(2)γ is a major mediator releasing oxidized aliphatic chains from cardiolipin, integrating mitochondrial bioenergetics and signaling [J]. J Biol Chem, 2017, 292: 10672-10684.

[86] Kaufmann KB, Büning H, Galy A, et al. Gene therapy on the move [J]. EMBO Mol Med, 2013, 5: 1642-1661.

[87] Chen W, Hu Y, Ju D. Gene therapy for neurodegenerative disorders: advances, insights and prospects [J]. Acta Pharm Sin (药学学报), 2020, 55: 1347-1359.

[88] Xu L, Chen WF, Wong MS. Ginsenoside Rg1 protects dopaminergic neurons in a rat model of Parkinson's disease through the IGF-I receptor signalling pathway [J]. Br J Pharmacol, 2009, 158: 738-748.