

心肌细胞铁死亡及其检测方法

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【摘要】铁死亡是一种新型程序性细胞死亡方式。近年来研究发现,铁死亡在心脏疾病的发生发展中发挥了重要作用。随着心肌细胞铁死亡领域的深入研究,人们对心肌细胞铁死亡检测的精确性和可重复性的关注度日益增加,揭示心肌细胞铁死亡的详细分子机制及开发精确的铁死亡检测方法具有极其重要的指导意义与临床价值。现对目前研究中识别、测量和评估心肌细胞铁死亡的最佳方法加以综述。

【关键词】心肌细胞;铁死亡;细胞死亡;检测方法

【DOI】10.16806/j.cnki.issn.1004-3934.2023.02.016

Methods for Detection of Ferroptosis in Cardiomyocytes

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【Abstract】Ferroptosis is a new way of programmed cell death. In recent years, it has been found that ferroptosis plays an important role in the occurrence and development of heart disease. Following the deep research in the field of cardiomyocytes ferroptosis, people pay more attention to the accuracy and repeatability of the detection of myocardial cell ferroptosis, which is of great significance to the research on the mechanism and detection method of cardiomyocyte ferroptosis. In this article, we summarize the best methods to identify, measure and evaluate ferroptosis in cardiomyocytes.

【Key words】Cardiomyocytes; Ferroptosis; Cell death; Detection method

铁死亡是一种新型程序性细胞死亡模式,当细胞内铁离子代谢异常时,细胞内氧化还原稳态失衡,脂质活性氧(reactive oxygen species, ROS)堆积,导致细胞死亡^[1]。与其他细胞死亡形式不同,铁死亡具有铁依赖性、谷胱甘肽(glutathione, GSH)与谷胱甘肽过氧化物酶4(glutathione peroxidase 4, GPX4)介导的氧化还原平衡紊乱及脂质过氧化等特征(图1)^[2]。自铁死亡发现以来,研究者们发现其参与了癌症^[3]、神经退行性疾病^[4]、肝病^[5]及肾衰竭^[6]等多种疾病的发生发展。而铁死亡在心脏组织中的调控功能及分子机制,是近年来心血管疾病研究的热点内容。研究表明,通过诱导/抑制心脏铁死亡的发生,能调控不同心血管疾病的发展进程,挽救患者生命^[7-12]。

1 铁死亡及其在心脏疾病中的作用

铁是人体内多种分子和酶中必不可少的辅助因

子,也是铁死亡发生的必需元素。细胞通过转铁蛋白/铁蛋白作用产生具有氧化还原活性的 Fe^{2+} ,形成氧化还原活性不稳定铁池(labile iron pool, LIP),当LIP中铁含量增加时, Fe^{2+} 通过芬顿反应催化ROS生成,导致细胞发生脂质过氧化,诱导细胞发生铁死亡^[2,10,13-14];而通过铁螯合剂等方式减少LIP中的铁会抑制铁死亡发生^[1,15]。

细胞中的铁在线粒体中发挥重要功能^[16-17]。而线粒体是为心脏组织持续活动和收缩提供持续能量的供应站,在心肌细胞中含量丰富,这也说明铁在心脏功能中的重要性^[18]。研究表明,铁元素含量异常会导致多种心血管疾病的发生,缺铁会使线粒体功能降低,心肌细胞收缩力减弱,导致心脏功能受损^[19],50%的慢性心力衰竭患者存在铁缺乏^[20]。而铁过载可导致心脏组织和心肌细胞摄取和积聚过量的铁,ROS产

基金项目:国家自然科学基金青年科学基金(82000381);黑龙江省博士后基金面上项目(LBH-Z19188)

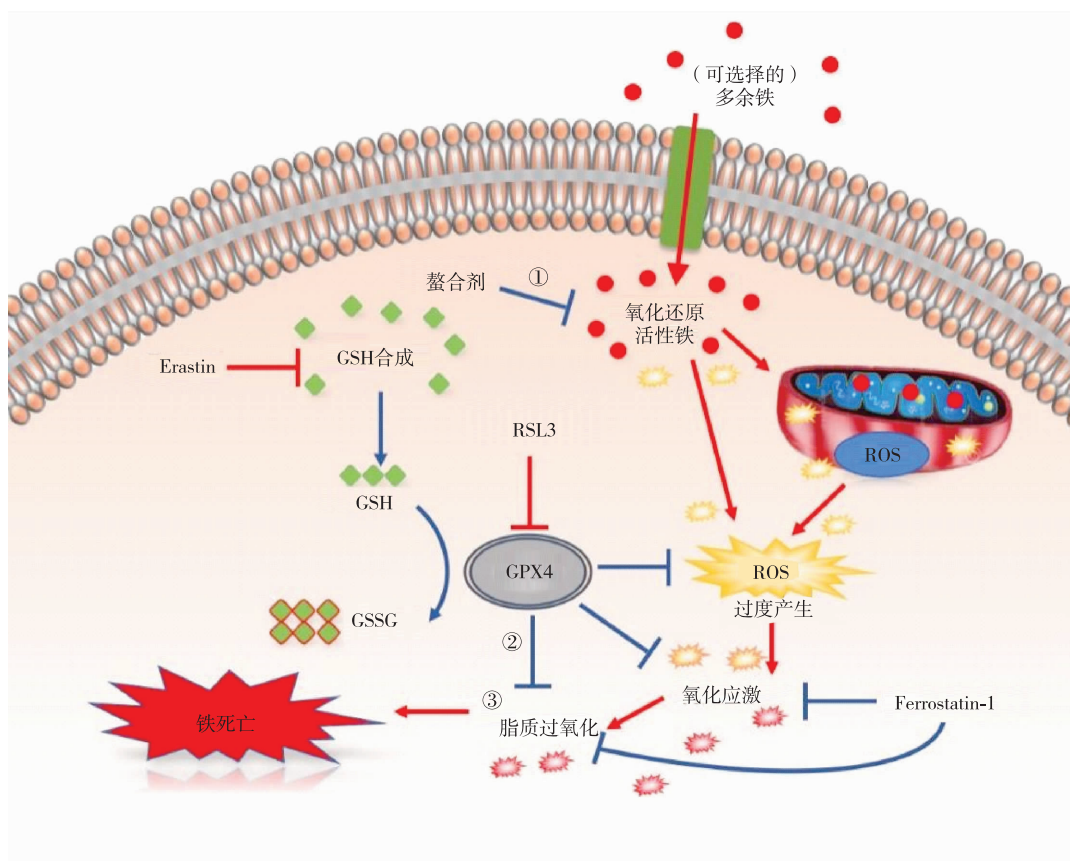
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量增加,心肌细胞更易发生氧化应激^[21-24]。此外,过量的铁也会被线粒体吸收,导致线粒体内 ROS 过量产生,并可能导致线粒体形态的改变,进而造成血管功能受损,加剧动脉粥样硬化、心律失常和心力衰竭的发展^[1,25-29]。

研究^[30]表明,ROS 产生的增加与动脉粥样硬化、高血压和充血性心力衰竭等多种心血管疾病相关。而细胞内氧化还原稳态能保护心肌细胞免受线粒体中恒定的氧化还原活性和 ROS 产生所造成的有害影响,对于维持心脏组织的正常功能至关重要^[29]。当细胞发生氧化应激时,膜双层脂质的氧化修饰,特别是脂质过氧化,已成为细胞命运的重要调节者^[31]。2019 年,王福倬教授课题组首次明确了抗肿瘤药物阿霉素引起的铁死亡在心脏疾病中发挥重要作用,并提出由阿霉素引发的铁储存与脂质过氧化主要发生于心肌细胞线粒体中,明确了线粒体膜的脂质过氧化是心肌细胞发生铁死亡的关键机制^[9]。GPX4 是铁死亡中重要的抗氧化酶,以 GSH 为有效还原剂和辅助因子。研究^[32-37]表明,维持 GPX4 活性能抑制 ROS 的累

积,进而保护心肌细胞免受氧化应激和脂质过氧化伤害。

近年来随着对铁死亡的深入研究及高通量测序技术的发展,新一代铁死亡调控蛋白被揭示,铁死亡抑制蛋白 1 (ferroptosis suppressor protein 1, FSP1)、二氢乳清酸脱氢酶 (dihydroorotate dehydrogenase, DHODH) 与 GPX4 成为并重的三个铁死亡关键调控分子,在各种疾病的发生发展中发挥了重要作用^[38-40]。然而,目前 FSP1 与 DHODH 在铁死亡中的详细分子机制尚不明确,GPX4 仍是铁死亡调控的经典研究对象,并且目前大部分的铁死亡诱导剂与抑制剂,靶向目标仍为 GSH 或 GPX4。如最早已知的铁死亡诱导剂之一——Erastin, Erastin 能抑制胱氨酸/谷氨酸逆向转运蛋白 (Xc-系统),使得细胞胱氨酸无法被还原为半胱氨酸并因此不能合成 GSH,从而抑制 GPX4 降低细胞中 ROS 的能力^[32] (图 1)。另一种铁死亡诱导剂 RSL3 的机制相对简单,直接抑制 GPX4 活性,从而使细胞的氧化还原平衡倾向于 ROS 积累,进而发生氧化应激和铁死亡^[2,41] (图 1)。



注:铁死亡的特征:①铁依赖性,②GSH 和 GPX4 介导的氧化还原平衡失调,③脂质过氧化。尖箭头表示激活,钝箭头表示抑制,红色箭头表示促进铁死亡的过程,蓝色箭头表示抑制铁死亡的过程。GSSG,谷胱甘肽二硫酸盐;RSL3, Ras 选择性致死化合物 3。

图 1 心肌细胞中铁蛋白沉积的信号通路

2 铁死亡的鉴定

2.1 GSH/GPX4

由上述内容可知,铁死亡在包括心血管疾病在内的人类多种疾病中发挥重要功能,因此,铁死亡的鉴定对各种疾病的诊断及治疗具有重要价值。目前研究认为,铁死亡的必要条件、氧化还原机制和副产物可作为检测铁死亡的基础(表 1)。就机制而言,GSH 和 GPX4 活性失衡是铁死亡的独特特征,且并不是已知的心脏中其他类型细胞死亡的主要途径,因此,可

针对 GSH 及 GPX4 活性的变化情况判断铁死亡的发生。以 5,5'-二硫代双-2-硝基苯甲酸作为检测试剂,氧化型谷胱甘肽(谷胱甘肽二硫酸盐)作为标准显色来测量 GSH 和总谷胱甘肽的降低^[42]。总谷胱甘肽过氧化物酶活性可通过测量由 GSH 和烟酰胺腺嘌呤二核苷酸磷酸催化的叔丁基氢过氧化物的还原速率来定量^[43]。Western Blot 也能检测 GPX 的活性,以确定铁死亡降低是否是由于直接抑制 GPX4 蛋白本身的酶活性或下调表达(表 1)^[44]。

表 1 心肌细胞铁死亡的检测方法

特异性元素	在铁死亡中的功能	检测方法	局限性	参考文献
GPX4 和 GSH	GPX4 活性只在铁死亡发生时降低,在其他细胞死亡形式中未有明显改变;铁死亡也会导致 GSH/总谷胱甘肽比值降低和/或总谷胱甘肽降低	(1) 比色测定法评估 GPX4 活性; (2) GPX4 本身可通过抗 GPX4 的抗体检测	(1) GPX4 活性持续时间一般较短,因此实验必须对时间敏感; (2) 抗体检测不能准确反映 GPX4 的活性	[12,42]
ROS 与脂质过氧化	脂质 ROS 对铁死亡具有高特异性,并且由于 GPX4 活性的特异性降低使得其积累量远高于其他细胞死亡形式;脂质过氧化是氧化应激的下游结果,也是铁死亡的另一个明显特征	(1) 特异性荧光探针(如 Liperflu)进行定性和半定量分析; (2) ELISA 和 TBAR 检测脂质 ROS 下游副产物(如 4-HNE 蛋白质加合物和 MDA); (3) 抑制剂:利用特异性抗氧化性铁死亡抑制剂,如 Ferrostatin-1 协同分析	下游产物如 4-HNE 通常很快随着时间的推移而稀释,因此对细胞的固定和检测时间具有较高要求	[1,7,9,45-47]
铁依赖性	铁死亡需铁,但不需异常高水平的铁。游离 Fe ²⁺ 水平升高可能是细胞正在发生铁死亡的标志。如果发生铁死亡,螯合剂或其他减少细胞内铁的方法应该可显著减少细胞死亡和脂质 ROS,恢复 GPX4 和 GSH。而其他非铁依赖性细胞死亡形式在通过螯合剂或其他技术去除铁时则不受影响	(1) 可通过使用例如非洛嗪等化合物的比色反应来检测铁离子水平,该化合物与铁结合时能显色; (2) 影响铁离子浓度的螯合剂或基因操纵(如转铁蛋白受体、铁蛋白和线粒体铁蛋白)也会影响铁死亡。因此,可利用该手段检测铁依赖性	游离 Fe ²⁺ 水平可能不代表铁死亡的水平,需另一种实验与之配对进行验证	[9-10,48-49]
线粒体检测	虽然单纯的线粒体断裂与铁死亡并不能完全区别开,但若同时合并大量脂质 ROS 出现在线粒体膜上即可诊断为铁死亡	(1) 观测线粒体发生铁死亡特征性形态改变:线粒体膜密度增加,嵴减少; (2) GPX4 功能降低后几个小时会有细胞碎裂的现象。可使用线粒体标记物如 Mito Tracker 检测线粒体的形态	线粒体形态可能因为细胞种类不同而出现差异,因此必须使用成像软件进行定量分析	[1,50]

注:4-HNE,4-羟基壬烯醛;MDA,丙二醛;ELISA,酶联免疫吸附试验;TBAR,硫代巴比妥酸。

2.2 ROS

ROS 的产生是铁死亡发生的关键环节,目前可通过荧光探针如 2',7'-二氯二氢荧光素二乙酸酯或其他细胞器特异性探针 MitoSOX 等很容易地检测到细胞内 ROS 的产量^[7,51]。然而,由于 ROS 的分子机制较为复杂,在细胞坏死和坏死性凋亡中也扮演着重要角色,单纯的 ROS 检测不能单独用于表明心肌细胞发生铁死亡,需更具有特异性的检测方法进一步区分铁死亡中 ROS 与心脏中其他形式的细胞死亡所产生的 ROS。

2.3 脂质过氧化

脂质过氧化是铁死亡的另一个明显特征,也是氧

化应激的下游结果。细胞脂质过氧化物水平可通过免疫染色法、比色法或流式细胞术测定法进行测定^[52]。亲脂性荧光探针 Liperflu 和 C11-BODIPY 在被脂质过氧化物氧化时可与脂质双层结合并发出荧光,进而利用流式细胞仪测定活细胞中脂质过氧化物的水平^[45,52-53]。ELISA 和免疫组织化学可用来检测特定的脂质过氧化物如 4-羟基壬烯醛^[46-47]。将硫代巴比妥酸作为检测试剂通过比色法可测定丙二醛含量。

2.4 铁元素

虽然具有氧化还原活性的铁是发生铁死亡的必需元素,但需注意的是,当细胞内铁浓度正常时仍可

能发生铁死亡,而细胞可溶性、氧化还原活性铁的丢失可抑制铁死亡^[1,7,9]。在细胞或组织裂解物样品中检测铁的总量可确定铁死亡的发生是否由于过量铁的积累。Ferrozine(菲洛嗪)是一种铁检测化合物,当与亚铁离子结合时形成红色复合物,可用于检测和量化纳摩尔级的铁总量^[48]。向红菲咯啉和呋喃三嗪二钠盐也可使用比色测定法检测样品中的总铁,后者更敏感并且仅需更小的样品体积^[54]。此外,可使用 T2 和 T2* 核磁共振成像在机体水平进行铁检测,观察心肌和其他器官和组织中的铁沉积^[55]。

2.5 抑制剂类

除了上述铁死亡检测方法和实验外,铁死亡抑制剂如 Ferrostatin-1、Liproxstatin-1 和维生素 E 等通过清除自由基降低细胞内 ROS 与脂质过氧化总量的脂溶性抗氧化剂也可用于确定是否发生铁死亡^[7,9,11,56]。如怀疑在实验中发生铁死亡,可加入 Ferrostatin-1 或类似的铁死亡抑制剂观察是否会减少细胞脂质过氧化和细胞死亡。

3 总结与展望

铁死亡作为新型程序性细胞死亡方式,近年来在心脏疾病中的重要作用逐渐被揭示,但详细分子机制仍有待研究。精确、可重复的心肌细胞铁死亡的检测技术能直观地反映心脏损伤形式,准确的铁死亡生物标志物可为心脏疾病提供新的靶向治疗模式,具有重大的临床转化价值。因此,未来的挑战在于进一步揭示铁死亡在心脏疾病中的详细调控机制,开发新的铁死亡特异性标志物检测技术,为心脏疾病患者带来最大的福音。

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收稿日期:2022-08-07

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收稿日期:2022-07-25