铁过载及铁死亡在心脏疾病中的研究进展

方学贤¹, 蔡昭贤¹, 王浩², 闵军霞¹, 王福倬^{1,2*}

1. 浙江大学医学院公共卫生学院及转化医学研究院, 杭州 310058;

2. 郑州大学公共卫生学院, 郑州 450001

* 联系人, E-mail: fwang@zju.edu.cn

2019-06-27 收稿, 2019-08-15 修回, 2019-08-16 接受, 2019-09-27 网络版发表

国家重点研发计划(2018YFA0507800)、国家自然科学基金(31930057, 31530034, 31970689, 31570791, 31900835)和中国博士后科学基金(2019M650139)资助

摘要 铁作为生命必需的营养元素,在机体组织中的代谢平衡对维持正常的生理功能至关重要。人群流行病学研究和动物实验表明,铁过载在心脏疾病的发生发展过程中发挥重要作用,祛铁可有效缓解心脏疾病进展,但机制尚不清楚。近年来,心脏铁稳态代谢及其分子调控机制获得系列重大进展;铁依赖的新型细胞死亡方式——铁死亡(ferroptosis)概念的提出,为深入理解铁过载与心脏疾病间的关联带来了重要契机。近期,作者团队在国际上率先揭示靶向铁死亡是防控心脏疾病有效措施。本综述系统总结了国内外铁过载与铁死亡对心脏疾病发病的影响及作用机制的最新研究进展,并对未来研究方向进行展望与讨论,旨在为心脏疾病预防和治疗提供新思路与新策略。

关键词 铁, 铁过载, 铁死亡, 心脏疾病

心脏疾病是我国乃至全球范围内致死和致残的主要因素^[1,2],而且心脏疾病负担呈现日渐加重的趋势^[3]。据美国心脏协会(American Heart Association, AHA)统计,美国2013年约有超过370000人因心脏病死亡,带来的直接医疗费用高达2073亿美元^[4]。我国2013年心脏病占居民疾病死因构成在农村为44.8%,在城市为41.9%,居各种疾病之首,高于肿瘤及其他疾病。以冠脉介入(percutaneous coronary intervention, PCI)为例,2001~2011十年间手术量增加21倍,高达208954台/年^[5]。可见,心脏疾病严重影响患者的生活质量及寿命,并给家庭和社会带来沉重的经济负担。至今尚未明确根治心脏疾病的有效方法,因此心脏疾病防治仍是人类亟待解决的科学难题^[6]。

心脏疾病是多种危险因素共同作用的结果,遗传因素发挥重要作用,具有显著的家族聚集性^[7]。尽管单个遗传易感基因对发病风险的影响小,但易感基因的

数量和频率在群体水平分布广泛。环境因素及外界因素也不可忽视,如高血压、血脂异常、糖尿病、吸烟、超重和肥胖、过度饮酒、低体力活动以及不良的膳食方式等。此外,年龄和性别等因素对疾病发生具有明显的影响效应。自从Sullivan^[8]1981在*Lancet*杂志正式提出“铁源性心脏病”假说,越来越多的流行病学和基础医学研究证据表明,铁过载(iron overload)在心脏疾病的发病进程中起着举足轻重的作用。

长期以来,人们对铁过载诱发心脏疾病的分子机制缺乏清晰的认识。2012年,美国哥伦比亚大学的Stockwell课题组^[9]在国际上首次描述了一种铁依赖的既非凋亡又非坏死的新细胞死亡方式,并命名为ferroptosis,中文则普遍将其意译为“铁死亡”。铁死亡概念的提出使得相关研究发生突破性进展,大量研究文献涌现。2019年,本课题组在国际上首次报道铁死亡是导致心脏疾病发生发展的重要机制,系统地阐明了化疗

引用格式: 方学贤, 蔡昭贤, 王浩, 等. 铁过载及铁死亡在心脏疾病中的研究进展. 科学通报, 2019, 64: 2974–2987

Fang X X, Cai Z X, Wang H, et al. Role of iron overload and ferroptosis in heart disease (in Chinese). *Chin Sci Bull*, 2019, 64: 2974–2987, doi: [10.1360/TB-2019-0242](https://doi.org/10.1360/TB-2019-0242)

药物及缺血再灌注等引发的心脏疾病中不但存在铁死亡,而且靶向干预铁死亡能够有效防治心脏疾病的发生发展^[10]。这些重要原创发现为人类靶向铁死亡防控心脏疾病带来无限曙光^[11,12]。本文简要介绍铁稳态代谢概况、系统综述铁过载与铁死亡防控心脏疾病研究的最新进展;同时,运用耳熟能详的我国上古神话“女娲补天”形象演绎心脏铁死亡发生的机制及靶向铁死亡防控心脏疾病的几种有效策略,并以此展开讨论,对未来研究进行了展望与方向引领。

1 铁稳态代谢

铁是机体必需的微量元素之一。它不仅是合成血红蛋白和肌红蛋白的主要原料,也是一系列氧化还原反应过程中许多重要酶的辅助因子,参与电子运输、细胞呼吸及DNA合成等重要生理活动,因此铁在维持机体健康中发挥极为重要的作用。铁缺乏引发的缺铁性贫血至今仍严重威胁全球人类健康;另一方面,过多的铁离子可促进自由基的形成,从而对细胞产生毒性。正是铁离子的“双刃剑”的特性,细胞既需要足量铁满足需求,同时还要避免铁过载造成的危害,因此机体必然存在维持自我平衡的复杂精细的调节系统。近十余年来,铁代谢领域基础研究突飞猛进,维持机体铁代谢稳态的机制逐渐明晰。

1.1 铁的来源与吸收

成年人每天大约需要20~25 mg的铁用来合成红细胞,其中绝大多数来自巨噬细胞通过吞噬衰老的红细胞后回收释放的铁,这个过程称为“铁循环”^[13]。生理情况下,如肠道表皮细胞脱落、皮肤表皮脱落、失血及汗液流失等,人体铁的流失相对较少,所以正常人每天从膳食经小肠吸收的铁只需要补偿机体铁损失,约占5%的机体造血总铁需求量^[14]。

日常饮食中大约含有13~18 mg铁,而正常情况下只有1~2 mg会被吸收。当机体的铁需求增大时(如造血功能增强和怀孕等),机体会增加铁吸收;反之,铁过载时机体就会减少铁吸收^[15]。小肠是人体吸收食物中铁的关键部位,吸收铁的过程开始于十二指肠,食物中的 Fe^{3+} 被还原为 Fe^{2+} ,在胃和十二指肠酸性环境中稳定存在,随后被二价金属离子转运蛋白(divalent metal transporter 1, DMT1)转运至小肠上皮细胞内^[16,17]。膳食中血红素铁(heme iron)的吸收机制目前还不完全清楚,之前曾认为血红素通过HCP1(SLC46A1)通道蛋白吸收^[18],

但是后来发现HCP1其实主要负责运输叶酸^[19]。细胞内血红素在血红素氧化酶1(heme oxygenase 1, Hmox1)的作用下可以将铁释放出来。

1.2 铁的转运、储存与利用

吸收进入小肠上皮的铁离子最终通过位于小肠上皮细胞基底膜的铁泵蛋白(ferroportin 1, FPN1)进入血液循环^[20,21]。FPN1是目前发现的哺乳动物中唯一的细胞铁外排蛋白。有研究认为,这一过程中还需要膜铁转运辅助蛋白Hephaestin(HP)的参与。HP是铜蓝蛋白的同源氧化酶,可以将 Fe^{2+} 氧化成 Fe^{3+} 并使其与转铁蛋白(transferrin, Tf)结合^[22]。与 Fe^{3+} 结合后的Tf(Tf-Fe)是血液循环中铁主要的运输形式,它与细胞表面的转铁蛋白受体(transferrin receptor 1, TfR1)结合后通过内吞方式将铁离子运送到细胞内,供体内骨髓和肝脏等器官组织利用^[23]。

在骨髓中,造血细胞获取的铁会运送至线粒体合成血红素,进而生成血红蛋白(hemoglobin)。如果线粒体吸收的铁没有及时用于合成血红素或者铁硫簇(iron-sulfur clusters),则会引起造血细胞内的铁蓄积,这种线粒体铁蓄积会造成血液中出现高铁红细胞(sideroblast),即经铁染色后形成铁蓄积在细胞核周围的“环状”形态,也称作“铁粒幼细胞性贫血”^[24]。相比之下,肝脏是储存各种营养物质的重要器官。血液循环中的铁,在满足机体代谢所需的铁之后,多余的铁会被肝脏迅速吸收,与铁蛋白(ferritin)结合而储存,避免循环中过多的铁对机体产生氧化损伤^[25]。在机体内需要铁时,这些储存在肝实质细胞中的铁可以被动员出来,运送到血液循环中,以供代谢所需^[26]。

此外,铁在能量代谢中也发挥重要作用,许多参与能量代谢的重要蛋白都需要铁或者血红素作为辅酶或亚基,包括细胞内线粒体氧化呼吸链的复合物和肌红蛋白等^[27]。肌肉,包括骨骼肌、平滑肌和心肌,无疑是利用铁最为活跃的组织器官之一。虽然铁代谢紊乱与能量代谢失衡及肌肉功能密切相关,但相关机制并不清楚,而且许多基础问题尚缺乏明确答案,亟待研究铁代谢在心脏疾病的发生及发展中的作用。

1.3 铁稳态代谢的分子调控机制

人体的铁吸收存在精密的调节系统,而铁调素(hepcidin)在铁稳态的调控中起核心作用。hepcidin是在2000~2001年间被3个实验室先后独立发现,并被证实

主要由肝脏合成分泌(Hep-),具有抗菌特性(-cidin),且在进化上高度保守的25个氨基酸组成的多肽^[28-30]。生理条件下,机体通过调控hepcidin的表达来负反馈调控铁水平。当机体铁过载时,肝脏合成分泌的hepcidin增多,加速FPN1的降解,关闭铁向血液中转运的出口,进而减少小肠上皮细胞和巨噬细胞向血液中输送的铁;当机体铁不足时,hepcidin表达量减少,FPN1通道开放增多,增加小肠对铁的吸收,进而维持机体的铁稳态^[31]。

此外,hepcidin的表达量还受到缺氧、造血需求以及机体炎症因子的直接或间接调控。比如,肝脏中低氧诱导因子HIF1(hypoxia-inducible factor 1)和HIF2可以抑制hepcidin的表达^[32]。而当机体需要增强造血的时候,降低hepcidin表达就会增加铁的吸收。此外,小鼠注射促红细胞生成素(erythropoietin, EPO)后可降低hepcidin水平,并且呈剂量依赖关系,人体试验也证实了这些重要发现^[33]。近期研究发现,当机体严重贫血时,机体反馈性增加造血,上调红细胞调控因子ERFE(erythroid regulator erythroferone, ERFE),抑制hepcidin表达,增加铁吸收^[33]。hepcidin还受到炎症因子的调控,机体在炎症状态下会上调hepcidin表达,机体将铁滞留于细胞内,限制入侵的微生物利用铁,有利于免疫系统杀灭病原菌^[34]。

1.4 心脏铁稳态代谢的维持与调控

在心肌细胞代谢中,铁离子同样发挥重要作用。因为许多参与能量代谢的重要蛋白都需要铁或者血红素作为辅酶或亚基,包括细胞内线粒体氧化呼吸链的复合物和肌红蛋白等。近年,心脏铁代谢研究成果被陆续报道,但相比于肝脏和小肠等器官,目前人们对心脏铁稳态代谢的调控机制还知之甚少。

美国杜克大学Andrews实验室^[35]首次报道转铁蛋白受体(TfR1)心脏特异性敲除小鼠的致死表型,并归因于心肌严重缺铁造成的线粒体功能障碍。TfR1基因的3'非编码区(untranslated region, UTR)存在保守的茎环结构,被称为铁感应元件(iron-responsive element, IRE)^[36]。在心肌细胞铁缺乏时,铁调节蛋白(iron-regulatory proteins, IRPs)与IRE结合,保障TfR1 mRNA的稳定性,进而增加膜上TfR1蛋白表达,有利于促进铁的吸收^[37]。Irp1/2心肌细胞特异性敲除小鼠心脏TfR1表达减少,呈现铁缺乏表型,并对心肌梗死的敏感性增加^[38]。Bayeva等人^[39]则发现, RNA结合蛋白Tristetraprolin(TTP)可以被哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin,

cin, mTOR)抑制,从而减弱其结合并促进心肌细胞TfR1 mRNA降解的功能。Ttp敲除小鼠心脏功能出现异常并伴随铁缺乏,有力地佐证了该机制^[40]。

当机体处于铁过载的疾病状态时,血浆转铁蛋白结合铁的能力达到饱和,非转铁蛋白结合铁(non-transferrin-bound iron, NTBI)开始蓄积^[41]。Oudit等人^[42]研究发现,铁过载小鼠使用治疗剂量的氨氯地平(amlodipine)或维拉帕米(verapamil)后可通过阻滞L型钙通道而抑制心肌细胞NTBI蓄积,从而缓解氧化应激和心功能损伤;心脏特异性过表达L型钙通道的心脏表型则相反。目前,尽管大部分相关研究都集中在L型钙通道上,也有部分研究结果提示T型钙通道也参与了心脏NTBI的转运。T型钙通道在正常成年心脏很难检测到,但在许多病理状态下它的表达会升高^[42]。在存在铁过载的地中海贫血小鼠中,使用T型钙通道阻滞剂依福地平(efonidipine)可有效防治心脏铁水平的持续升高和组织损伤的恶化^[43,44]。此外,有报道提示DMT1也可能参与心肌细胞NTBI的转运^[45,46]。

在心肌细胞铁外排方面,心脏特异性Fpn1基因敲除小鼠表现为心肌铁代谢失衡及心脏功能不全^[47,48]。有趣的是,hepcidin也被发现在心肌细胞存在低水平但持续的表达。与全身敲除导致高铁蓄积不同,心肌细胞特异性敲除hepcidin会导致心脏Fpn1失去调控,并由于铁外排增加而发生致死性铁缺乏^[49]。相反,在心脏特异性敲入(knock-in)不被hepcidin调控的Fpn1 C326Y突变基因,小鼠同样会因为心功能衰竭而死亡^[49]。最近有报道指出,如果在肺动脉平滑肌细胞敲入Fpn1 C326Y突变,小鼠会发生肺动脉高压和右心衰,提示hepcidin/FPN1轴在血管细胞稳态调控中的重要作用^[50]。

1.5 线粒体铁代谢与心脏功能

心脏是人体代谢最为活跃的器官之一,每天消耗的ATP量达到5 kg,约为其自身重量的17倍,而线粒体正是心肌细胞最重要的“能量工厂”^[51]。铁元素对于线粒体执行功能至关重要,特别是用于合成血红素与铁硫簇^[52]。线粒体甚至还具有特殊的线粒体铁蛋白(mitochondrial ferritin)来储存铁离子^[53]。据报道,线粒体铁蛋白敲除小鼠虽然在正常情况下没有展现出异常,但对心脏损伤更加敏感^[54,55]。那么铁离子是如何进入线粒体的呢? 2006年,Shaw等人^[56]在贫血表型的Frascati突变型斑马鱼中首先发现了在造血组织中特异性高表达的mitoferrin-1 (slc25a37)。之后,在各组织中广泛表达的mitoferrin-2

(slc25a38)也被报道^[57]。Mitoferrins定位于线粒体内膜,被认为主要负责将铁从细胞胞质转运入线粒体^[58]。目前, Mitoferrins在心肌细胞中的功能还不明确。

同样, 位于线粒体内膜的ATP结合转运蛋白ABCB10(ATP-binding cassette b10)被发现能够与mitoferrin-1互作并增加其稳定性^[59]。ABCB10杂合子小鼠对心肌缺血再灌注损伤更敏感, 提示该基因可能与相关心脏疾病的耐受相关。与ABCB10不同, ABCB8被认为在线粒体铁外排中发挥重要作用。ABCB8心脏特异性敲除小鼠心肌细胞线粒体出现铁蓄积, 并发展为心肌病^[60]; 而ABCB8过表达则可以显著减轻化疗药物阿霉素与心肌缺血再灌注造成的小鼠心脏损伤^[61,62]。可见, 线粒体铁稳态对于心脏正常功能的维持至关重要。

2 铁过载与心脏疾病

铁过载是指由于铁的供给超过铁的需求, 从而引起体内总铁量过多, 广泛沉积于人体重要器官和组织的实质细胞, 可伴或不伴器官功能障碍。在人体中, 血清中的转铁蛋白饱和度(transferrin saturation)正常约为30%, 当转铁蛋白饱和度在女性体内高于45%或在男性体内高于50%时, 即为铁过载^[63]。当血清转铁蛋白饱和度超过60%时, 非转铁蛋白结合的铁就会在血液循环中积累, 对细胞造成破坏。临床上铁过载分为原发性和继发性两大类, 原发性铁过载是先天性代谢障碍导致体内铁过度蓄积, 即遗传性血色病(hereditary hemochromatosis, HH); 继发性铁过载常因大量输血、长期补充铁剂、摄入含铁饮食或某些血液病等导致。有研究报道, 当中年男性体内血清铁蛋白高于正常范围(>200 mg/L), 则其患心肌梗死的概率是正常人的2.2倍^[64]。

2.1 遗传性血色病与心脏疾病

遗传性血色病是一种组织大量铁沉积并导致功能障碍为主要特征的常染色体遗传疾病, 其发病遍及全球, 最常见于北欧日耳曼和高加索人种中^[65]。人类6号染色体上的HFE基因突变是导致HH的主要原因, 其中错义突变C282Y纯合突变频率约占HH患者的80%~85%^[66]。非HFE血色病分别由HJV(HFE2), HAMP1, TFR2和FPN1四种基因的突变导致, 其发病率显著低于HFE血色病, 而且没有地区和种族差异^[67]。这5种铁代谢调节基因突变导致肝脏分泌hepcidin减少或hepcidin抵抗, 引起小肠铁吸收增加及巨噬细胞铁释放增多, 大量的铁离子沉积在肝脏、胰腺及心脏等敏感的实质细

胞内, 造成组织结构损伤, 导致脏器病变, 引发肝硬化、糖尿病和心衰等病症。器官受累程度与血清铁超负荷的速度和时间有关, 这主要由基因突变类型决定^[68]。

根据Olson等人^[69]的报道, 大概有三分之一的HH患者死于心脏病变。铁离子最初沉积于心外膜, 继而透过整个心室壁, 心室肌最常受累, 但较少沉积于心房, 心脏传导系统也可能被波及。Gaenger等人^[70]研究发现, HH病人中血管内皮中层组织呈损伤性增厚, 同时功能受损, 采用祛铁治疗后血管内皮组织功能得到恢复, 并且心脏疾病的发病率也有所降低。Failla等人^[71]对HH患者进行祛铁治疗后也发现桡动脉壁的硬化情况较治疗前有较大改善。还有许多学者从遗传学角度研究两者的关系, 认为HFE基因突变与心脏疾病风险存在正相关^[72-75]。近年的一项荟萃分析显示, HFE基因H63D错义突变会显著升高冠心病风险, 但C282Y突变则没有类似的关联^[76]。

2.2 弗里德赖希共济失调及线粒体铁过载与心力衰竭

弗里德赖希共济失调(Friedreich's ataxia, FRDA)是一种罕见的常染色体隐性遗传疾病, 由德国医学家弗里德赖希^[77]在1863年首先报道。但直到100多年后, 其病因才被现代分子遗传学解开, 即编码Frxataxin (FXN)基因的第三个内含子上的GAA三联密码子重复扩展突变, 导致FXN蛋白的表达严重减少^[78]。患者发病时间一般为5~10岁左右, 大多在25岁之前。GAA重复越多, 发病越早, 严重患者在幼儿时期就出现症状。虽然FRDA以慢性进行性小脑共济失调为主要病理特征, 但其最主要的死因却是充血性心力衰竭^[79]。

人类FXN基因位于第9号染色体长臂, 可产生7个可变剪接转录本, 其中最长功能性转录本由5个外显子组成。成人体内FXN在心脏和脊髓的表达最多, 其次是肝脏、骨骼肌和胰腺。FXN是一种主要位于线粒体基质的蛋白, 其功能与线粒体内铁硫簇的合成密切相关, 被认为有储存铁、铁伴侣分子、铁感应负性调节因子和表达调节性代谢开关四大功能^[80-82]。如上所述, 心脏是主要的耗氧器官之一, 线粒体数目多且功能活跃, 因此成为易感受累器官之一。FXN敲除小鼠模型和FRDA患者, 均出现心肌细胞胞质内铁缺失和线粒体铁积累的表型。同时, 铁硫簇含量和依赖于铁硫簇的酶活性均显著减少, 线粒体功能发生障碍^[83]。此外, 有研究发现, FXN在器官脂质代谢的过程中也发挥着重要作

用^[84]. FXN缺失或低表达均可导致细胞脂滴积聚与脂质过氧化, 这些变化也参与了FRDA的发生发展^[85,86]. Raman等人^[87]采集了FRDA患者的血清, 分析发现存在明显的血脂异常, 并与心脏功能的损伤程度呈正相关.

2.3 继发性铁过载与心脏疾病

遗传性或获得性贫血, 如 β 地中海贫血(β -Thalassemia major, β -TM)、镰刀细胞贫血(sickle cell disease, SCD)和骨髓增生异常综合征(myelodysplastic syndromes, MDS)等, 患者需要长期反复的输血治疗, 很容易造成机体内铁的逐渐累积^[88]. 目前, 以 β -TM的相关研究最多. 据统计, β -TM的死亡患者中大概有2/3为心源性, 且心脏铁过载的概率在男性患者比女性要多两倍以上^[89]. Aessopos等人^[90,91]收集大量病例分析研究后认为, 慢性铁过载诱导的左心室功能障碍及肺动脉高压(pulmonary hypertension)是 β -TM患者死亡的首要原因. 超微电子显微镜显示过量的铁沉积于患者心肌细胞溶酶体内, 同时可观察到心肌细胞肌纤维的缺失^[92]. 2000年, 一项来自英国的全国性研究显示, 约50%的 β -TM患者在35岁以前死亡, 主要问题是祛铁治疗的顺应性太差^[93]. 近年来, 随着磁共振成像(T2*)检测脏器铁沉积技术的引进, 祛铁治疗的普及与加强和其他临床治疗策略的改进, 心脏铁过载造成的 β -TM患者死亡已呈现减少的趋势^[94].

与 β -TM相类似, 大部分MDS患者有贫血症状且进行性加重, 导致最终出现输血依赖^[95]. 但是许多患者在接受输血治疗前就已经出现了铁过载现象, 这显然与输注红细胞无关. 目前认为无效红细胞生成引起的铁吸收增加是铁过载的另一个主要原因^[96]. 上述两种机制相互作用, 逐渐形成恶性循环, 从而促进了铁过载的发生. Jaeger等人^[97]回顾性分析了239例MDS患者, 其中46例进展为继发性铁过载, 这些患者中心力衰竭的发生率超过40%, 并导致14例患者死亡.

心脏继发性铁过载的另一大原因是蒽环类抗生素(anthracycline)的使用. 蒽环类抗生素, 尤其阿霉素(doxorubicin)是临床上用于抗恶性肿瘤治疗的广谱化疗药物. 自20世纪60年代问世起, 阿霉素与其他化疗药物及分子靶向药物联合应用, 一直是临床抗肿瘤治疗的一线标准方案. 阿霉素抗肿瘤疗效显著, 且应用广泛, 但其引起的心脏毒性呈进展性及不可逆转, 严重限制了其临床应用^[98]. 虽然人们从未停止过对于阿霉素引起

的心脏毒性机制的探索, 但目前尚缺乏公认的分子机制^[99]. 近年来, 越来越多的证据提示, 使用阿霉素导致的心脏铁过载在其心脏毒性的发生发展中起着极其重要的作用^[100].

阿霉素可直接与心肌细胞中的 Fe^{3+} 反应生成复合物, 促进 Fe^{2+} 向 Fe^{3+} 的氧化反应, 并产生大量的活性氧簇(reactive oxygen species, ROS)^[101]. Miranda等人^[102]利用Hfe敲除小鼠作为血色病模型, 发现其对阿霉素的心脏毒性更加敏感. Panjraht等人^[103]用高铁膳食饲喂大鼠后给予阿霉素, 相较于正常膳食组表现出了更严重的心脏损伤. Ichikawa等人^[62]的研究表明, 使用阿霉素后铁主要蓄积于小鼠心肌细胞的线粒体中并影响有氧呼吸, 在过表达线粒体铁外排通道蛋白ABCB8后心脏毒性即得到缓解.

2.4 膳食铁摄入与心脏疾病

人体每天通过消化道尤其是小肠吸收膳食中的铁, 以此来补充由于肠道细胞脱落等原因丢失的铁, 保障机体铁水平的稳态平衡. 随着社会经济水平的不断提高和物质文明的快速发展, 许多发达国家与地区中铁营养的核心问题已经从铁缺乏转变为铁过剩, 并由此导致了包括心脏疾病和2型糖尿病等在内的诸多慢性疾病^[104].

基于“卫生专业人员随访研究”(health professionals follow-up study, HPFS)的数据, 来自哈佛大学公共卫生学院的Ascherio等人^[105]在全球范围内率先报道了膳食铁和冠心病的关联. 在校正了其他危险因素后, 他们发现摄入血红素铁较多的男性更有可能发生心肌梗死. 5年之后, Klipstein-Grobusch等人^[106]在荷兰人群(Rotterdam study)中报道了类似的结果, 而且指出这种正相关在因心梗死亡的病例中更显著. 来自“护士健康研究”(nurses' health study, NHS)和“爱荷华州妇女健康研究”(Iowa women's health study, IWHS)的数据则提示, 膳食血红素铁会增加2型糖尿病和酗酒的女性罹患心血管并发症的风险^[107,108].

基于已发表的队列研究数据, 我们运用荟萃分析发现膳食血红素铁的摄入与心脏疾病风险显著相关, 且呈现出明显的剂量反应关系, 即血红素铁的膳食摄入每天增加1 mg, 发生心脏疾病的风险能够增加7%; 而膳食非血红素铁摄入与心脏疾病风险却没有关联. 进一步亚组分析揭示这种正相关在北美人群更显著, 这可能与红肉摄入量更高有关^[109].

2.5 祛铁治疗可有效防控心脏疾病

目前的实验室研究与临床实践发现, 通过有效的祛铁治疗可延缓铁过载的发生, 减轻铁过载的危害, 显著改善相关心脏疾病的预后。尤其是对于已经合并心功能不全的原发性或继发性铁过载患者, 不能耐受传统的放血疗法, 使用铁螯合剂治疗是更合适的方案^[110]。铁螯合剂可与血浆或组织内的铁离子结合并促进其通过尿液或者胆汁排出, 从而降低体内的铁含量。目前常用的铁螯合剂主要包括祛铁胺(deferoxamine)、祛铁酮(deferiprone)、地拉罗司(deferasirox)和右丙亚胺(dexrazoxane); 此外, deferitritin和desferriethiocin等新一代铁螯合剂也正在积极开展临床前试验^[111]。

临床试验结果表明, 对合并心衰的重症地中海贫血患者使用祛铁胺, 可显著降低心脏铁水平, 改善心脏功能与远期预后^[112,113]。祛铁酮虽然稳定性与临床效果略差于祛铁胺^[114], 但其和祛铁胺联合使用能达到更好的心脏保护效果^[115]。地拉罗司是批准上市的新一代口服祛铁剂, 其药物动力学实验与临床试验均表明, 地拉罗司半效期长、生物利用度高, 对于铁过载相关心脏病具有良好的治疗效果^[116,117]。

右丙亚胺则是临床上治疗蒽环类药物心脏毒性的唯一特效药^[118]。多中心随机对照的临床研究结果显示, 右丙亚胺对接受蒽环类药物化疗的癌症患者具有显著的心脏保护作用, 并且不影响抗肿瘤治疗的效果^[119,120]。值得注意的是, 其他类型的铁螯合剂对化疗病人并没有类似的心脏保护作用。究其原因, 目前认为, 右丙亚胺可特异性地被转运进入心脏, 通过降低心肌细胞尤其是线粒体内的铁含量来达到保护心脏的效果^[10,62]。

尽管铁螯合剂的临床作用已被越来越广泛地接受和认可, 但其毒副作用较大限制其广泛应用。例如, 祛铁胺会导致视网膜病变、听力受损、骨骼畸形和生长停滞等系列不良反应^[121]。单纯的祛铁治疗显然不能满足临床需求, 发现铁过载导致心脏损伤的精细分子机制, 并以此作为靶点研发新型疗法将是未来的发展方向。

3 铁死亡及其在心脏疾病中的作用

3.1 铁死亡是铁依赖性非凋亡性的新型细胞死亡方式

细胞死亡不仅是所有细胞的最终命运, 而且它与细胞分裂和增殖一样, 在整个机体的正常运转以及病理生理过程中具有不可替代的作用^[122]。长期以来, 细

胞死亡被简单地分为凋亡(apoptosis)和坏死(necrosis)两大类型。然而, 随着对细胞死亡机制的深入研究, 程序性坏死(necroptosis)、自噬(autophagy)和焦亡(pyroptosis)等多种新的程序性细胞死亡方式被相继发现。

美国哥伦比亚大学的研究人员在研究小分子erastin杀死RAS突变的肿瘤细胞的机制时发现了一种新的程序性细胞死亡方式。经过深入研究, 证实铁离子螯合剂可有效抑制该类型细胞死亡, 而凋亡、坏死、自噬抑制剂没有效果, 表明铁离子在这种新型的细胞死亡过程中发挥了重要作用, 并由此将这种铁依赖(iron-dependent)的死亡方式命名为ferroptosis, 即铁死亡^[9]。铁死亡在细胞形态学、遗传学以及生物化学等方面与已知的其他细胞死亡方式均有明显的不同, 其主要特征表现为细胞死亡过程中伴随着铁离子依赖性的大量脂质过氧化物累积^[123]。

3.2 铁死亡的调控机制

铁死亡的发生及可能的调控机制见图1。细胞内的游离铁离子通过芬顿反应(Fenton reaction)与过氧化氢相互作用, 从而导致组成生物膜的多不饱和脂肪酸(polyunsaturated fatty acids, PUFAs)发生脂质过氧化, 这是目前已知的铁死亡启动的基础机制^[123]。谷胱甘肽过氧化物酶4(glutathione peroxidase 4, GPX4)是一种硒蛋白, 它可特异并高效地清除磷脂过氧化氢, 从而抑制铁死亡的发生^[124]。无论是使用RSL3等小分子抑制GPX4的活性, 还是直接敲除GPX4基因, 都会导致磷脂过氧化水平急剧增加和启动铁死亡^[125,126]。Gpx4全身敲除会导致小鼠胚胎在7.5天死亡^[127,128]。可见, GPX4

图1 (网络版彩色)铁死亡发生及调控分子机制示意图
Figure 1 (Color online) Current understanding of regulatory pathways in ferroptosis

是铁死亡过程中的重要调控因子。

GPX4完成抗氧化作用需要消耗谷胱甘肽(glu-tathione), 后者由谷氨酸、半胱氨酸及甘氨酸组成^[129]。其中, 半胱氨酸的来源胱氨酸的吸收被认为是谷胱甘肽合成的限速步骤。谷氨酸/胱氨酸反向转运体(system x_c^-)由跨膜转运蛋白SLC7A11和跨膜调节蛋白SLC3A2组成, 在摄取一分子胱氨酸的同时外排一分子谷氨酸^[130]。前述的小分子Erastin就是通过抑制system x_c^- , 导致肿瘤细胞摄取胱氨酸与合成谷胱甘肽障碍而发生铁死亡^[9]。此外, *Slc7a11*基因敲除后, 小鼠对铁过载导致的肝脏损伤敏感性也显著增加^[131]。

另一重要的铁死亡负向调控因子则是前文有所提及的铁蛋白(ferritin)^[132]。铁蛋白由重链和轻链两种亚基构成球壳空腔结构, 是细胞内最主要的储铁蛋白。其中重链具有亚铁氧化酶活性, 可将 Fe^{2+} 氧化为 Fe^{3+} , 进而储存在球壳状的空腔内。胞质中未被利用或排出细胞的铁离子被储存在铁蛋白中, 从而维持细胞内铁稳态, 减少芬顿反应导致的氧化应激, 达到保护细胞的目的。当铁蛋白的表达异常时, 细胞内铁离子的稳态也被打破^[25]。核受体共同活化子4(nuclear receptor coactivator 4, NCOA4)介导的自噬过程可选择性地降解铁蛋白, 造成细胞内游离铁水平升高, 并诱发铁死亡^[133,134]。与GPX4和SLC7A11相比, 铁蛋白在铁死亡中的研究进展尚局限于体外细胞培养体系中, 它在体内调控铁死亡的生理学及病理生理学作用还有待进一步深入研究。

3.3 铁死亡是心脏疾病防治的重要靶点

作为一种终末分化细胞, 心肌细胞在出生后就不能分裂增生, 其死亡将导致心肌细胞数量的减少, 从而引起心脏结构和功能上的缺陷, 加剧心力衰竭。因此, 心肌细胞死亡的调控通路具有重大的研究价值与转化意义, 抑制心肌细胞死亡是保护心脏功能、防治心脏疾病的关键策略。

在已知的程序性细胞死亡方式中, 半胱氨酸-天冬氨酸蛋白水解酶(caspase)依赖的细胞凋亡通路是最早被发现参与心脏疾病的^[135]。随着研究的推进, 其他死亡方式在心脏疾病中的作用被逐渐认识。例如Lu等人^[136]发现, 细胞自噬抑制剂可在大鼠阿霉素心肌病模型中减轻心脏损伤程度。Zhang等人^[137]研究揭示, RIP3-CaMKII通路介导的新型程序性坏死机制在多种心脏疾病中扮演的重要角色。近期, 多项研究提示gasdermin

介导的细胞焦亡也参与糖尿病心肌病和缺血性心脏病等的发生发展^[138,139]。可见, 心肌病理生理学过程中存在多种细胞死亡方式以及复杂的调控网络。

为深入研究心脏疾病中细胞死亡的作用, 我们利用多种细胞死亡抑制剂干预并结合细胞死亡通路基因敲除小鼠展开研究, 发现铁死亡特异抑制剂ferrostatin-1(Fer-1)可显著降低阿霉素导致的心脏毒性并提高小鼠的存活率^[10]。与之前报道的*Ripk3*基因敲除小鼠相比, 注射Fer-1效果相似, 且两者的保护效应可以叠加, 说明心脏中铁死亡与程序性坏死独立并存。值得关注的是, 通过药物抑制CaMKII(被认为是RIP3下游分子), 并没有在阿霉素模型小鼠上展示出之前报道的保护效果^[10,137]。这提示心脏程序性坏死的下游通路及关键靶点分子还有待进一步研究和确认。

结合心脏功能系列实验结果, 铁死亡被认定在阿霉素心肌病的发生发展中发挥了关键作用。为寻找该模型中铁死亡发生过程的关键调控因子, 我们进行了全转录组测序, 发现血红素加氧酶-1(Hmox1)显著上调^[10]。使用Hmox1抑制剂可显著遏制阿霉素小鼠心脏铁死亡的发生发展并保护心脏功能; 而通过加载血红素诱导Hmox1过表达则效果相反。Hmox1是铁卟啉化合物血红素分解代谢过程中的限速酶, 可将血红素分解为一氧化碳、胆绿素和 Fe^{2+} , 而心肌中含有丰富的血红素用来合成肌红蛋白、细胞色素等。阿霉素注射后, 小鼠表现出血红素降低, 铁和胆绿素(胆绿素的氧化产物)水平升高; 而抑制Hmox1表达或者敲除上游调控分子Nrf2均可减轻心肌细胞铁蓄积。因此, Hmox1的激活介导了血红素中铁离子的释放, 铁离子蓄积在心肌细胞从而诱发了铁死亡。

铁死亡被认为是通过细胞膜或者细胞器膜发生脂质过氧化损伤来杀伤细胞, 但具体的亚细胞定位一直存在争议^[140]。我们通过对阿霉素处理小鼠心脏组织进行透射电子显微镜观察, 发现阿霉素小鼠的线粒体形态发生明显变化, 后续实验证实ATP生成与线粒体膜电位均显著下降; 而这些变化均能被Fer-1所恢复。尽管之前有文献提示铁死亡可能发生于细胞膜^[141,142], 但分离心肌线粒体后, 我们发现铁蓄积和脂质过氧化在阿霉素作用后主要发生在心肌细胞的线粒体而不是细胞质基质中。与传统的抗氧化剂TEMPO相比, 经改造而特异富集于线粒体的MitoTEMPO可有效抑制铁死亡, 保护心脏功能, 从而更加明确了线粒体损伤与心脏损伤在阿霉素模型中的因果关系^[10]。

在铁死亡相关疾病中,脏器的缺血再灌注(ischemia/reperfusion, I/R)损伤受到广泛关注. 2014年,德国Linkermann等人^[143]率先在国际上报道了铁死亡参与肾脏缺血再灌注损伤的发生. 几乎同时,德国Marcus Conrad课题组^[126]则发现抑制铁死亡还可以显著改善肝脏的缺血再灌注损伤. Gao等人^[144]建立了离体(*ex vivo*)的小鼠心脏缺血再灌注模型,发现通过抑制谷氨酰胺代谢从而抑制铁死亡可以减轻损伤. 为进一步明确铁死亡在其中的作用,我们以小鼠为实验对象建立在体(*in vivo*)心肌缺血再灌注模型,发现给予铁死亡抑制剂可明显减轻缺血再灌注导致的急性和慢性心脏损伤,为防治相关疾病提供了前景光明的新思路和新策略^[10,11]. 阿霉素心肌病及心肌缺血再灌注损伤引发心肌细胞铁过载、铁死亡发生以及相关治疗靶点的模式图见图2.

美国华盛顿大学的研究团队^[145]揭示,心脏移植手术后发生的中性粒细胞募集(neutrophil recruitment)现象是由铁死亡调控的. 供体心脏在移植后由于缺血缺氧等原因可诱导发生铁死亡,细胞内容物将释放并通过TLR4/Trif/Type 1 IFN 通路募集中性粒细胞,造成坏死性炎症(necroinflammation). 因此,铁死亡抑制疗法有望改善临床心脏移植手术的预后.

前文所述的祛铁治疗,无疑是通过直接减轻铁负荷发挥作用的,其内在本质很可能是抑制铁死亡的发生. 有研究结果证实,铁螯合剂在心脏和肝脏等重要器

官的疾病与损伤中可以发挥铁死亡抑制剂的作用^[10,131]. 因此,以铁死亡为靶点的转化医学研究,将成为心脏疾病防治领域富有潜力的新方向.

4 总结

铁是最早被人类认识的生命必需微量元素,而铁死亡作为最“年轻”的程序性细胞死亡类型,相关研究刚刚起步. 无疑,铁代谢失衡及铁死亡极大地参与了心脏疾病的发生发展,而线粒体是影响心脏铁死亡的主要细胞器. 铁死亡抑制剂、铁螯合剂、线粒体特异性抗氧化剂、Hmox1抑制剂以及低铁膳食等5种不同途径如同“女娲补天”传说中的“五色石”一般,挽狂澜于既倒,扶大厦之将倾,成功缓解了阿霉素导致的心脏损伤,

图2 阿霉素心肌病与心肌缺血再灌注损伤中心肌细胞铁死亡调控模式图

Figure 2 Schematic model depicts the role of cardiac ferroptosis in cardiomyopathy induced by doxorubicin or ischemia/reperfusion

图3 “女娲补天”诠释靶向铁死亡是防控心脏疾病关键靶点. 阿霉素或缺血再灌注等有害因素促发心肌细胞线粒体铁离子(红颗粒)蓄积及脂质过氧化(黄点)产生,诱发心脏(天空)损伤. 功能研究提示铁死亡抑制剂(Fer-1)、铁离子络合剂(DXZ)、线粒体还原剂(Mito-TEMPO)、卟啉抑制剂(ZnPP)以及低铁膳食(low iron diet)等5种策略均可有效防治铁死亡介导的心脏疾病

Figure 3 The ancient Chinese myth “Nüwa Mends the Heavens” illustrates ferroptosis as a key target for treating heart diseases. Both the anticancer drug doxorubicin and cardiac ischemia/reperfusion induce ferroptosis-mediated cardiomyopathy (depicted as a gaping hole in the sky) via mitochondrial iron overload and lipid peroxidation

这指明了将来的研究方向并使我们对铁死亡研究的临床转化充满信心。铁过载以及铁死亡是心脏疾病的关键机制,而靶向铁过载和铁死亡,无疑给心脏疾病的防控带来无限曙光(图3)。

总之,铁死亡自2012年被发现以来,日益引起人们的重视,相关研究不断增加。目前普遍认为,靶向铁死亡不仅可以防治脏器损伤,而且可以治疗肿瘤。然而,迄今为止,铁死亡研究领域的一些基础的关键科学问

题尚待回答,比如铁死亡过程中是否存在关键执行分子?生理过程中是否存在铁死亡?尽管如此,铁死亡研究领域持续拓展,其活跃程度令人瞩目,不断涌现铁死亡相关的新靶点和新机制的报道。总之,铁死亡在心脏疾病等重大慢病发生发展中的重要作用业已引起广泛关注,相信更多深入的研究将会揭示铁死亡的精细分子机制,从而为靶向铁死亡防治心脏疾病的临床应用提供更加充分的科学依据。

参考文献

- Zhou M, Wang H, Zhu J, et al. Cause-specific mortality for 240 causes in China during 1990–2013: A systematic subnational analysis for the Global Burden of Disease Study 2013. *Lancet*, 2015, 387: 251–272
- Abubakar II, Tillmann T, Banerjee A. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, 2015, 385: 117–171
- Yang Z J, Liu J, Ge J P, et al. Prevalence of cardiovascular disease risk factor in the Chinese population: The 2007–2008 China National Diabetes and Metabolic Disorders Study. *Eur Heart J*, 2012, 33: 213–220
- Mozaffarian D, Benjamin E J, Go A S, et al. Heart disease and stroke statistics-2016 update: A report from the American Heart Association. *Circulation*, 2016, 133: e38–e360
- Zheng X, Curtis J P, Hu S, et al. Coronary catheterization and percutaneous coronary intervention in China. *JAMA Intern Med*, 2016, 176: 512–521
- Benjamin E J, Virani S S, Callaway C W, et al. Heart disease and stroke statistics—2018 update: A report from the American Heart Association. *Circulation*, 2018, 137: e67
- Ueshima H, Sekikawa A, Miura K, et al. Cardiovascular disease and risk factors in Asia. *Circulation*, 2008, 118: 2702–2709
- Sullivan J L. Iron and the sex difference in heart disease risk. *Lancet*, 1981, 1: 1293–1294
- Dixon S J, Lemberg K M, Lamprecht M R, et al. Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell*, 2012, 149: 1060–1072
- Fang X, Wang H, Han D, et al. Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci USA*, 2019, 116: 2672–2680
- Conrad M, Proneth B. Broken hearts: Iron overload, ferroptosis and cardiomyopathy. *Cell Res*, 2019, 29: 263–264
- Hampton T. From the literature. *Circulation*, 2019, 139: 2278–2279
- Hentze M W, Muckenthaler M U, Andrews N C. Balancing acts. *Cell*, 2004, 117: 285–297
- Andrews N C. Forging a field: The golden age of iron biology. *Blood*, 2008, 112: 219–230
- Muckenthaler M U, Rivella S, Hentze M W, et al. A red carpet for iron metabolism. *Cell*, 2017, 168: 344–361
- Gunshin H, Mackenzie B, Berger U V, et al. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature*, 1997, 388: 482–488
- Fleming M D, Trenor C C, Su M A, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet*, 1997, 16: 383–386
- Shayeghi M, Latunde-Dada G O, Oakhill J S, et al. Identification of an intestinal heme transporter. *Cell*, 2005, 122: 789–801
- Qiu A, Jansen M, Sakaris A, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell*, 2006, 127: 917–928
- Donovan A, Brownlie A, Zhou Y, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature*, 2000, 403: 776–781
- Donovan A, Lima C A, Pinkus J L, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. *Cell Metab*, 2005, 1: 191–200
- Chen H, Attieh Z K, Su T, et al. Hephaestin is a ferroxidase that maintains partial activity in sex-linked anemia mice. *Blood*, 2004, 103: 3933–3939
- Levy J E, Jin O, Fujiwara Y, et al. Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat Genet*, 1999, 21: 396–399
- Ohba R, Furuyama K, Yoshida K, et al. Clinical and genetic characteristics of congenital sideroblastic anemia: Comparison with myelodysplastic syndrome with ring sideroblast (MDS-RS). *Ann Hematol*, 2013, 92: 1–9
- Darshan D, Vanoaica L, Richman L, et al. Conditional deletion of ferritin H in mice induces loss of iron storage and liver damage. *Hepatology*,

- 2009, 50: 852–860
- 26 Zhang Z, Zhang F, Guo X, et al. Ferroportin1 in hepatocytes and macrophages is required for the efficient mobilization of body iron stores in mice. *Hepatology*, 2012, 56: 961–971
 - 27 Beard J L. Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr*, 2001, 131: 568–579
 - 28 Park C H, Valore E V, Waring A J, et al. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem*, 2001, 276: 7806–7810
 - 29 Krause A, Neitz S, Mägert H J, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. *FEBS Lett*, 2000, 480: 147–150
 - 30 Pigeon C, Ilyin G, Courselaud B, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *J Biol Chem*, 2001, 276: 7811–7819
 - 31 Nemeth E, Tuttle M S, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, 2004, 306: 2090–2093
 - 32 Peyssonnaud C, Zinkernagel A S, Schuepbach R A, et al. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *J Clin Invest*, 2007, 117: 1926–1932
 - 33 Robach P, Recalcati S, Girelli D, et al. Alterations of systemic and muscle iron metabolism in human subjects treated with low-dose recombinant erythropoietin. *Blood*, 2009, 113: 6707–6715
 - 34 Wrighting D M, Andrews N C. Interleukin-6 induces hepcidin expression through STAT3. *Blood*, 2006, 108: 3204–3209
 - 35 Xu W, Barrientos T, Mao L, et al. Lethal cardiomyopathy in mice lacking transferrin receptor in the heart. *Cell Rep*, 2015, 13: 533–545
 - 36 Wilkinson N, Pantopoulos K. The IRP/IRE system *in vivo*: Insights from mouse models. *Front Pharmacol*, 2014, 5: 15
 - 37 Casey J L, Koeller D M, Ramin V C, et al. Iron regulation of transferrin receptor mRNA levels requires iron-responsive elements and a rapid turnover determinant in the 3' untranslated region of the mRNA. *EMBO J*, 1989, 8: 3693–3699
 - 38 Haddad S, Wang Y, Galy B, et al. Iron-regulatory proteins secure iron availability in cardiomyocytes to prevent heart failure. *Eur Heart J*, 2017, 38: 362–372
 - 39 Bayeva M, Khechaduri A, Puig S, et al. mTOR regulates cellular iron homeostasis through tristetraprolin. *Cell Metab*, 2012, 16: 645–657
 - 40 Sato T, Chang H C, Bayeva M, et al. mRNA-binding protein tristetraprolin is essential for cardiac response to iron deficiency by regulating mitochondrial function. *Proc Natl Acad Sci USA*, 2018, 115: e6291–e6300
 - 41 Brissot P, Ropert M, Le Lan C, et al. Non-transferrin bound iron: A key role in iron overload and iron toxicity. *Biochim Biophys Acta*, 2012, 1820: 403–410
 - 42 Oudit G Y, Sun H, Trivieri M G, et al. L-type Ca^{2+} channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. *Nat Med*, 2003, 9: 1187–1194
 - 43 Kumfu S, Chattipakorn S, Chinda K, et al. T-type calcium channel blockade improves survival and cardiovascular function in thalassemic mice. *Eur J Haematol*, 2012, 88: 535–548
 - 44 Khamsekaew J, Kumfu S, Wongjaikam S, et al. Effects of iron overload, an iron chelator and a T-Type calcium channel blocker on cardiac mitochondrial biogenesis and mitochondrial dynamics in thalassemic mice. *Eur J Pharmacol*, 2017, 799: 118–127
 - 45 Davis M T, Bartfay W J. Ebselen decreases oxygen free radical production and iron concentrations in the hearts of chronically iron-overloaded mice. *Biol Res Nurs*, 2004, 6: 37–45
 - 46 Wetli H A, Buckett P D, Wessling-Resnick M. Small-molecule screening identifies the selanazol drug ebselen as a potent inhibitor of DMT1-mediated iron uptake. *Chem Biol*, 2006, 13: 965–972
 - 47 Lakhal-Littleton S, Wolna M, Carr C A, et al. Cardiac ferroportin regulates cellular iron homeostasis and is important for cardiac function. *Proc Natl Acad Sci USA*, 2015, 112: 3164–3169
 - 48 Fang X, Wang H, An P, et al. Cardiomyocyte-specific deletion of ferroportin using MCK-Cre has no apparent effect on cardiac iron homeostasis. *Int J Cardiol*, 2015, 201: 90–92
 - 49 Lakhal-Littleton S, Wolna M, Chung Y J, et al. An essential cell-autonomous role for hepcidin in cardiac iron homeostasis. *eLife*, 2016, 5: e19804
 - 50 Lakhal-Littleton S, Crosby A, Frise M C, et al. Intracellular iron deficiency in pulmonary arterial smooth muscle cells induces pulmonary arterial hypertension in mice. *Proc Natl Acad Sci USA*, 2019, 116: 13122–13130
 - 51 Taegtmeyer H. Energy metabolism of the heart: From basic concepts to clinical applications. *Curr Probl Cardiol*, 1994, 19: 59–113
 - 52 Paul B T, Manz D H, Torti F M, et al. Mitochondria and Iron: Current questions. *Expert Rev Hematol*, 2017, 10: 65–79
 - 53 Drysdale J, Arosio P, Invernizzi R, et al. Mitochondrial ferritin: A new player in iron metabolism. *Blood Cells Molecules Dis*, 2002, 29: 376–383
 - 54 Maccarinelli F, Gammella E, Asperti M, et al. Mice lacking mitochondrial ferritin are more sensitive to doxorubicin-mediated cardiotoxicity. *J Mol Med*, 2014, 92: 859–869
 - 55 Wu W, Chang S, Wu Q, et al. Mitochondrial ferritin protects the murine myocardium from acute exhaustive exercise injury. *Cell Death Dis*, 2016, 7: e2475

- 56 Shaw G C, Cope J J, Li L, et al. Mitoferrin is essential for erythroid iron assimilation. *Nature*, 2006, 440: 96–100
- 57 Paradkar P N, Zumbrennen K B, Paw B H, et al. Regulation of mitochondrial iron import through differential turnover of mitoferrin 1 and mitoferrin 2. *Mol Cell Biol*, 2009, 29: 1007–1016
- 58 Richardson D R, Lane D J R, Becker E M, et al. Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. *Proc Natl Acad Sci USA*, 2010, 107: 10775–10782
- 59 Chen W, Paradkar P N, Li L, et al. Abcb10 physically interacts with mitoferrin-1 (Slc25a37) to enhance its stability and function in the erythroid mitochondria. *Proc Natl Acad Sci USA*, 2009, 106: 16263–16268
- 60 Ichikawa Y, Bayeva M, Ghanefar M, et al. Disruption of ATP-binding cassette B8 in mice leads to cardiomyopathy through a decrease in mitochondrial iron export. *Proc Natl Acad Sci USA*, 2012, 109: 4152–4157
- 61 Chang H C, Wu R, Shang M, et al. Reduction in mitochondrial iron alleviates cardiac damage during injury. *EMBO Mol Med*, 2016, 8: 247–267
- 62 Ichikawa Y, Ghanefar M, Bayeva M, et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest*, 2014, 124: 617–630
- 63 Fleming R E, Ponka P. Iron overload in human disease. *N Engl J Med*, 2012, 366: 348–359
- 64 Salonen J T, Nyyssönen K, Korpela H, et al. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation*, 1992, 86: 803–811
- 65 Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. *Gastroenterology*, 2010, 139: 393–408.e2
- 66 Distant S, Robson K J H, Graham-Campbell J, et al. The origin and spread of the HFE-C282Y haemochromatosis mutation. *Hum Genet*, 2004, 115: 269–279
- 67 Franchini M. Hereditary iron overload: Update on pathophysiology, diagnosis, and treatment. *Am J Hematol*, 2006, 81: 202–209
- 68 Pietrangelo A. Hereditary hemochromatosis — A new look at an old disease. *N Engl J Med*, 2004, 350: 2383–2397
- 69 Olson L J, Edwards W D, McCall J T, et al. Cardiac iron deposition in idiopathic hemochromatosis: Histologic and analytic assessment of 14 hearts from autopsy. *J Am College Cardiol*, 1987, 10: 1239–1243
- 70 Gaenzler H, Marschang P, Sturm W, et al. Association between increased iron stores and impaired endothelial function in patients with hereditary hemochromatosis. *J Am College Cardiol*, 2002, 40: 2189–2194
- 71 Failla M, Giannattasio C, Piperno A, et al. Radial artery wall alterations in genetic hemochromatosis before and after iron depletion therapy. *Hepatology*, 2000, 32: 569–573
- 72 Waalen J, Felitti V, Gelbart T, et al. Prevalence of coronary heart disease associated with HFE mutations in adults attending a health appraisal center. *Am J Med*, 2002, 113: 472–479
- 73 Campbell S, George D K, Robb S D, et al. The prevalence of haemochromatosis gene mutations in the West of Scotland and their relation to ischaemic heart disease. *Heart*, 2003, 89: 1023–1026
- 74 Candore G, Balistreri C R, Lio D, et al. Association between HFE mutations and acute myocardial infarction: A study in patients from Northern and Southern Italy. *Blood Cells Mol Dis*, 2003, 31: 57–62
- 75 Rasmussen M L, Folsom A R, Catellier D J, et al. A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: The Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis*, 2001, 154: 739–746
- 76 Lian J, Xu L, Huang Y, et al. Meta-analyses of HFE variants in coronary heart disease. *Gene*, 2013, 527: 167–173
- 77 Koeppen A H. Friedreich's ataxia: Pathology, pathogenesis, and molecular genetics. *J Neurol Sci*, 2011, 303: 1–12
- 78 Campuzano V, Montermini L, Moltò M D, et al. Friedreich's ataxia: Autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science*, 1996, 271: 1423–1427
- 79 Weidemann F, Störk S, Liu D, et al. Cardiomyopathy of Friedreich ataxia. *J Neurochem*, 2013, 126: 88–93
- 80 Adamec J, Rusnak F, Owen W G, et al. Iron-dependent self-assembly of recombinant yeast frataxin: Implications for Friedreich ataxia. *Am J Human Genet*, 2000, 67: 549–562
- 81 Yoon T, Cowan J A. Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis. *J Biol Chem*, 2004, 279: 25943–25946
- 82 Adinolfi S, Iannuzzi C, Prisci F, et al. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. *Nat Struct Mol Biol*, 2009, 16: 390–396
- 83 Koeppen A H, Ramirez R L, Becker A B, et al. The pathogenesis of cardiomyopathy in Friedreich ataxia. *PLoS One*, 2015, 10: e0116396
- 84 Tamarit J, Obis È, Ros J. Oxidative stress and altered lipid metabolism in Friedreich ataxia. *Free Radical Biol Med*, 2016, 100: 138–146
- 85 Navarro J A, Ohmann E, Sanchez D, et al. Altered lipid metabolism in a Drosophila model of Friedreich's ataxia. *Human Mol Genet*, 2010, 19: 2828–2840
- 86 Abeti R, Parkinson M H, Hargreaves I P, et al. Mitochondrial energy imbalance and lipid peroxidation cause cell death in Friedreich's ataxia. *Cell Death Dis*, 2016, 7: e2237

- 87 Raman S V, Phatak K, Hoyle J C, et al. Impaired myocardial perfusion reserve and fibrosis in Friedreich ataxia: A mitochondrial cardiomyopathy with metabolic syndrome. *Eur Heart J*, 2011, 32: 561–567
- 88 Wood J C. Cardiac iron across different transfusion-dependent diseases. *Blood Rev*, 2008, 22(Suppl 2): 14–21
- 89 Borgna-Pignatti C, Cappellini M D, De Stefano P, et al. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. *Blood*, 2006, 107: 3733–3737
- 90 Aessopos A, Farmakis D, Deffereos S, et al. Thalassemia heart disease. *Chest*, 2005, 127: 1523–1530
- 91 Aessopos A, Kati M, Farmakis D. Heart disease in thalassemia intermedia: A review of the underlying pathophysiology. *Haematologica*, 2007, 92: 658–665
- 92 Kolnagou A, Michaelides Y, Kontos C, et al. Myocyte damage and loss of myofibers is the potential mechanism of iron overload toxicity in congestive cardiac failure in thalassemia: Complete reversal of the cardiomyopathy and normalization of iron load by deferiprone. *Hemoglobin*, 2008, 32: 17–28
- 93 Modell B, Khan M, Darlison M. Survival in β -thalassaemia major in the UK: Data from the UK Thalassaemia Register. *Lancet*, 2000, 355: 2051–2052
- 94 Modell B, Khan M, Darlison M, et al. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*, 2008, 10: 42
- 95 Roy N B A, Myerson S, Schuh A H, et al. Cardiac iron overload in transfusion-dependent patients with myelodysplastic syndromes. *British J Haematol*, 2011, 154: 521–524
- 96 Tanno T, Miller J L. Iron loading and overloading due to ineffective erythropoiesis. *Adv Hematol*, 2010, 2010: 358283–358290
- 97 Jaeger M, Aul C, Sohngen D, et al. Secondary hemochromatosis in polytransfused patients with myelodysplastic syndromes. *Beitr Infusionsther*, 1992, 30: 464–468
- 98 Singal P K, Iliskovic N. Doxorubicin-induced cardiomyopathy. *N Engl J Med*, 1998, 339: 900–905
- 99 Cai F, Luis M A F, Lin X, et al. Anthracycline-induced cardiotoxicity in the chemotherapy treatment of breast cancer: Preventive strategies and treatment. *Mol Clin Oncol*, 2019, 11: 15–23
- 100 Gammella E, Maccarinelli F, Buratti P, et al. The role of iron in anthracycline cardiotoxicity. *Front Pharmacol*, 2014, 5: 1–6
- 101 Xu X, Persson H L, Richardson D R. Molecular pharmacology of the interaction of anthracyclines with iron. *Mol Pharmacol*, 2005, 68: 261–271
- 102 Miranda C J, Makui H, Soares R J, et al. Hfe deficiency increases susceptibility to cardiotoxicity and exacerbates changes in iron metabolism induced by doxorubicin. *Blood*, 2003, 102: 2574–2580
- 103 Panjraht G S, Patel V, Valdiviezo C I, et al. Potentiation of Doxorubicin cardiotoxicity by iron loading in a rodent model. *J Am College Cardiol*, 2007, 49: 2457–2464
- 104 Hallberg L. Advantages and disadvantages of an iron-rich diet. *Eur J Clin Nutr*, 2002, 56(Suppl 1): 12–18
- 105 Ascherio A, Willett W C, Rimm E B, et al. Dietary iron intake and risk of coronary disease among men. *Circulation*, 1994, 89: 969–974
- 106 Klipstein-Grobusch K, Grobbee D E, den Breeijen J H, et al. Dietary iron and risk of myocardial infarction in the Rotterdam Study. *Am J Epidemiol*, 1999, 149: 421–428
- 107 Qi L, van Dam R M, Rexrode K, et al. Heme iron from diet as a risk factor for coronary heart disease in women with type 2 diabetes. *Diabetes Care*, 2007, 30: 101–106
- 108 Lee D H, Folsom A R, Jacobs Jr D R. Iron, zinc, and alcohol consumption and mortality from cardiovascular diseases: The Iowa Women's Health Study. *Am J Clin Nutr*, 2005, 81: 787–791
- 109 Fang X, An P, Wang H, et al. Dietary intake of heme iron and risk of cardiovascular disease: A dose-response meta-analysis of prospective cohort studies. *Nutr Metab Cardiovasc Dis*, 2015, 25: 24–35
- 110 Fabio G, Minonzio F, Delbini P, et al. Reversal of cardiac complications by deferiprone and deferoxamine combination therapy in a patient affected by a severe type of juvenile hemochromatosis (JH). *Blood*, 2007, 109: 362–364
- 111 Bulbake U, Singh A, Domb A J, et al. Therapeutic macromolecular iron chelators. *Curr Med Chem*, 2019, 26: 323–334
- 112 Anderson L J, Westwood M A, Holden S, et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: A prospective study using T2* cardiovascular magnetic resonance. *Br J Haematol*, 2004, 127: 348–355
- 113 Marcus R E, Davies S C, Bantock H M, et al. Desferrioxamine to improve cardiac function in iron-overloaded patients with thalassaemia major. *Lancet*, 1984, 323: 392–393
- 114 Maggio A, D'Amico G, Morabito A, et al. Deferiprone versus deferoxamine in patients with thalassemia major: A randomized clinical trial. *Blood Cells Molecules Dis*, 2002, 28: 196–208
- 115 Tanner M A, Galanello R, Dessi C, et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. *Circulation*, 2007, 115: 1876–1884

- 116 Porter J B. Deferasirox: An update. *Hemoglobin*, 2009, 33(Suppl 1): 70–75
- 117 Pennell D J, Porter J B, Cappellini M D, et al. Efficacy of deferasirox in reducing and preventing cardiac iron overload in β -thalassemia. *Blood*, 2010, 115: 2364–2371
- 118 Cvetkovi R S, Scott L J. Dexrazoxane. *Drugs*, 2005, 65: 1005–1024
- 119 Marty M, Espié M, Llombart A, et al. Multicenter randomized phase III study of the cardioprotective effect of dexrazoxane (Cardioxane®) in advanced/metastatic breast cancer patients treated with anthracycline-based chemotherapy. *Ann Oncol*, 2006, 17: 614–622
- 120 Venturini M, Michelotti A, Del Mastro L, et al. Multicenter randomized controlled clinical trial to evaluate cardioprotection of dexrazoxane versus no cardioprotection in women receiving epirubicin chemotherapy for advanced breast cancer. *J Clin Oncol*, 1996, 14: 3112–3120
- 121 Ryals B, Westbrook E, Schacht J. Morphological evidence of ototoxicity of the iron chelator deferoxamine. *Hearing Res*, 1997, 112: 44–48
- 122 Green D R. The coming decade of cell death research: Five riddles. *Cell*, 2019, 177: 1094–1107
- 123 Stockwell B R, Friedmann Angeli J P, Bayir H, et al. Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*, 2017, 171: 273–285
- 124 Friedmann Angeli J P, Conrad M. Selenium and GPX4, a vital symbiosis. *Free Rad Biol Med*, 2018, 127: 153–159
- 125 Yang W S, SriRamaratnam R, Welsch M E, et al. Regulation of ferroptotic cancer cell death by GPX4. *Cell*, 2014, 156: 317–331
- 126 Friedmann Angeli J P, Schneider M, Proneth B, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. *Nat Cell Biol*, 2014, 16: 1180–1191
- 127 Imai H, Hirao F, Sakamoto T, et al. Early embryonic lethality caused by targeted disruption of the mouse PHGPx gene. *Biochem Biophys Res Commun*, 2003, 305: 278–286
- 128 Yant L J, Ran Q, Rao L, et al. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Rad Biol Med*, 2003, 34: 496–502
- 129 Maiorino M, Conrad M, Ursini F. GPx4, Lipid peroxidation, and cell death: Discoveries, rediscoveries, and open issues. *Antioxid Redox Signal*, 2018, 29: 61–74
- 130 Lu L, Hope B T, Shaham Y. The cysteine-glutamate transporter in the accumbens: A novel role in cocaine relapse. *Trends Neurosci*, 2004, 27: 74–76
- 131 Wang H, An P, Xie E, et al. Characterization of ferroptosis in murine models of hemochromatosis. *Hepatology*, 2017, 66: 449–465
- 132 Sun X, Ou Z, Chen R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. *Hepatology*, 2016, 63: 173–184
- 133 Hou W, Xie Y, Song X, et al. Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy*, 2016, 12: 1425–1428
- 134 Gao M, Monian P, Pan Q, et al. Ferroptosis is an autophagic cell death process. *Cell Res*, 2016, 26: 1021–1032
- 135 Takemura G, Kanoh M, Minatoguchi S, et al. Cardiomyocyte apoptosis in the failing heart—A critical review from definition and classification of cell death. *Int J Cardiol*, 2013, 167: 2373–2386
- 136 Lu L, Wu W, Yan J, et al. Adriamycin-induced autophagic cardiomyocyte death plays a pathogenic role in a rat model of heart failure. *Int J Cardiol*, 2009, 134: 82–90
- 137 Zhang T, Zhang Y, Cui M, et al. CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. *Nat Med*, 2016, 22: 175–182
- 138 Toldo S, Mauro A G, Cutter Z, et al. Inflammasome, pyroptosis, and cytokines in myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*, 2018, 315: 1553–1568
- 139 Yang F, Qin Y, Wang Y, et al. LncRNA KCNQ1OT1 mediates pyroptosis in diabetic cardiomyopathy. *Cell Physiol Biochem*, 2018, 50: 1230–1244
- 140 Feng H, Stockwell B R. Unsolved mysteries: How does lipid peroxidation cause ferroptosis? *PLoS Biol*, 2018, 16: e2006203–e2006217
- 141 Kagan V E, Mao G, Qu F, et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol*, 2017, 13: 81–90
- 142 Wenzel S E, Tyurina Y Y, Zhao J, et al. PEBP1 warden ferroptosis by enabling lipoxygenase generation of lipid death signals. *Cell*, 2017, 171: 628–641.e26
- 143 Linkermann A, Skouta R, Himmerkus N, et al. Synchronized renal tubular cell death involves ferroptosis. *Proc Natl Acad Sci USA*, 2014, 111: 16836–16841
- 144 Gao M, Monian P, Quadri N, et al. Glutaminolysis and transferrin regulate ferroptosis. *Mol Cell*, 2015, 59: 298–308
- 145 Li W, Feng G, Gauthier J M, et al. Ferroptotic cell death and TLR4/Trif signaling initiate neutrophil recruitment after heart transplantation. *J Clin Invest*, 2019, 129: 2293–2304

Summary for “铁过载及铁死亡在心脏疾病中的研究进展”

Role of iron overload and ferroptosis in heart disease

Xuexian Fang¹, Zhaoxian Cai¹, Hao Wang², Junxia Min¹ & Fudi Wang^{1,2*}

¹ School of Public Health & Institute for Translational Medicine, Zhejiang University School of Medicine, Hangzhou 310058, China;

² College of Public Health, Zhengzhou University, Zhengzhou 450001, China

* Corresponding author, E-mail: fwang@zju.edu.cn

The essential trace element iron is a key component of hemoglobin, playing a role in erythropoiesis and oxygen transport. In addition, iron plays a key role in several enzymatic and metabolic processes. Iron deficiency causes anemia, whereas iron overload causes organ-related diseases such as heart failure, liver cirrhosis, and diabetes mellitus. Therefore, maintaining iron homeostasis is essential for the survival of nearly all living organisms.

Iron overload-related cardiomyopathy is a secondary form of heart disease resulting from iron accumulation in the myocardium and is the leading cause of morbidity in hereditary hemochromatosis, thalassemia major, and other conditions associated with secondary iron overload. A growing body of epidemiological and molecular evidence supports the notion that iron overload is pathogenic and that iron chelation therapy can be clinically beneficial in treating many forms of heart disease. However, the precise mechanisms by which iron overload leads to heart disease are poorly understood.

In recent years, significant progress has been made toward understanding the regulatory mechanisms that underlie iron overload in many diseases. In particular, ferroptosis, a newly identified iron-dependent form of cell death, has drawn considerable attention. Ferroptosis has morphological, biochemical, and genetic features that are distinct from other forms of cell death such as apoptosis, necrosis, and autophagy, and mounting evidence suggests that ferroptosis participates in the initiation and progression of many diseases and conditions, including tumorigenesis, ischemia/reperfusion injury, kidney failure, and diseases affecting the nervous system and the hematological system.

Although the death of terminally differentiated cardiomyocytes is a crucial pathogenic factor in the development of cardiomyopathy, the underlying mechanism remains unknown. Recently, we reported that inhibiting ferroptosis in mice can protect against both chemotherapy-induced cardiomyopathy and ischemia/reperfusion-induced cardiomyopathy. With respect to the underlying mechanism, we found that excess accumulation of free iron in the mitochondria of cardiomyocytes causes membrane lipid peroxidation, providing new insights into the pathogenic mechanisms that underlie iron overload-associated cardiomyopathy.

In this review, we briefly introduce the molecular mechanisms that regulate cardiac iron homeostasis. In addition, we provide an overview of studies regarding the role that iron overload-associated cardiomyopathy and ferroptosis play in the pathogenesis and progression of heart disease. Finally, we discuss the role of targeting ferroptosis as a promising new therapeutic strategy for myocardial chemotherapeutic and ischemia/reperfusion injury.

iron, iron overload, ferroptosis, heart disease

doi: [10.1360/TB-2019-0242](https://doi.org/10.1360/TB-2019-0242)