Ferroptosis的信号通路及调控

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摘要 Ferroptosis是近年发现的调控细胞死亡的方式, 在生化、基因和形态学等方面与其他形式的细胞死亡有显著的差异。Ferroptosis过程受严格而复杂的调控机制控制, 其特点是, 脂质过氧化物和致死的活性氧(reactive oxygen species, ROS)积累。但Ferroptosis可被铁螯合剂和脂质过氧化反应抑制剂所抑制。Ferroptosis的错误调控与多种生理和病理过程有关, 包括肿瘤细胞的死亡、神经退行性疾病、急性肾功能衰竭以及肝和心脏缺血再灌注损伤等。

关键词 铁代谢; 活性氧; Ferroptosis; GPX4; 脂质过氧化

Signal Pathway and Regulation of Ferroptosis

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Abstract Ferroptosis, found in recent years, is a kind of regulation of cell death. There are significant differences in biochemistry, gene and morphology from other forms of cell death. The Ferroptosis process is controlled by strict and complex regulatory mechanisms, characterized by lipid peroxidation and lethal reactive oxygen species (ROS) accumulation. And the Ferroptosis process can be inhibited by iron chelators and lipid peroxidation inhibitors. The regulation of Ferroptosis is associated with a variety of physiological and pathological processes, including the death of tumor cells, neurodegenerative diseases, acute renal failure and liver and heart ischemia reperfusion injury.

Keywords iron metabolism; reactive oxygen species; Ferroptosis; GPX4; lipid peroxidation

Ferroptosis是近年来发现的调控细胞死亡的方式, 由Dixon等[1]于2012年提出并命名。 Ferroptosis的主要特点是脂质过氧化产物和铁代谢等产生的活性氧(reactive oxygen species, ROS)积累。这种铁依赖的细胞死亡形式在基因、生化和生理特征等方面与凋亡、自噬、坏死、程序性坏死等细胞死亡方式不同[2]。本文主要阐述Ferroptosis的特征、信号通路、调控及其在一些疾病中的作用。

1 Ferroptosis的特征

Ferroptosis在形态学上与凋亡、程序性坏死和自噬等细胞死亡方式有显著的差异(表1)。Ferroptosis的最典型形态学特征有: 细胞膜完整、质膜起泡、细胞膜聚缩、线粒体膜密度凝聚、线粒体嵴减少或消失以及线粒体外膜破裂等[3]。在成纤维细胞和肾组织中通过调节GPX4(glutathione peroxidase 4)失活而诱导Ferroptosis, 电镜观察发现, 线粒体外膜破裂[4]。Erastin处理的癌细胞细胞核完整且没有核固缩、染色质着边等现象[1]。

2 Ferroptosis的信号通路

Ferroptosis信号通路最显著的信号特征是活性氧(ROS)的生成(主要由铁代谢产生), 它将Ferroptosis分成上、下游两个部分, 即上游ROS的产生过程和下游ROS执行细胞死亡的过程(图1)。

一定浓度的ROS对生物体的生长、发育、繁殖

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表1 Ferroptosis、凋亡、程序性坏死和自噬等细胞死亡方式的比较(根据参考文献[2]修改)

Table 1 Thecomparation of Ferroptosis, apoptosis, necroptosis and autophagy (modified from reference [2])

	Ferroptosis	凋亡 Apoptosis	程序性坏死 Necroptosis	自噬 Autophagy
Cell membrane	Lack of rupture and blebbing of the plasma membrane; rounding-up of the cell	Plasma membrane blebbing; rounding-up of the cell	Rupture of plasma membrane	Lack of change
Cytoplasm	Small mitochondria with condensed mitochondrial membrane densities, reduction or vanishing of mitochondria crista, as well as outer mitochondrial membrane rupture	Retraction of pseudopods; reduction of cellular volume	Cytoplasmic swelling (oncosis); swelling of cytoplasmic organelles	Accumulation of double-membraned autophagic vacuoles
Nucleus	Normal nuclear size and lack of chromatin condensation	Reduction of nuclear volume; nuclear fragmentation; chromatin condensation	Moderate chromatin condensation	Lack of chromatin condensation
Inflammatory reaction	Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory
Core regulators	Positive: VDAC2/3, Ras, NOXTFR1, p53, CARS Negative: GPX4, SLC7A11 HSPB1, NRF2	Positive: p53, Bax, Bak, other pro-apoptotic Bcl-2 family proteins Negative: Bcl-2, Bcl-xL, other anti-apoptotic Bcl-2 family proteins	Positive: RIP1, RIP3MLKL	Positive: ATG5, ATG7, Beclin1, other ATG family proteins
Inducement of death	Ironmetabolism disorder, antioxidation overload	Physiological or pathological factors induced slight stimulation, such as lack of growth factors	Pathologicalstimulus induced factors, such as hypoxia, infection, poisoning	Lack of nutrition and other adverse conditions induce

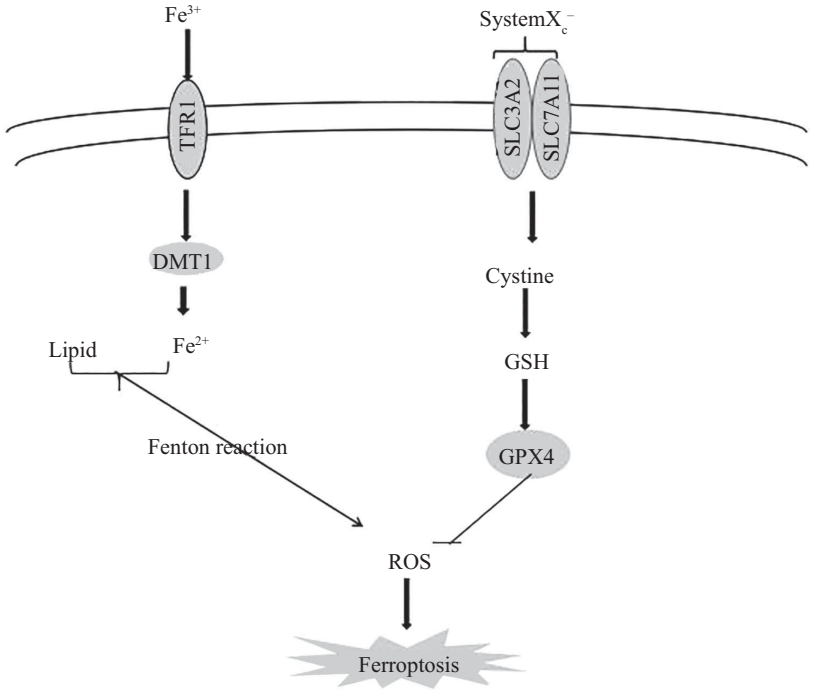

图1 铁死亡的调控单元(根据参考文献[2]修改)

Fig.1 Regulatory unit of Ferroptosis (modified from reference [2])

等生命过程是必需的。但当机体内的抗氧化机制失衡, 机体产生过量ROS时, 会对组织细胞造成严重的损伤。ROS主要损伤生物膜、蛋白质和核酸等。

2.1 Ferroptosis过程中ROS的产生

铁代谢紊乱和谷胱甘肽过氧化物酶4(GPX4)活性损失是ROS(特别是脂质过氧化物)产生的主要原因。机体内循环铁通常与转铁蛋白结合, 以Fe^{3+}的形式存在。Fe^{3+}通过膜蛋白转铁蛋白受体1(transferrin receptor 1, TFR1)进入细胞, 并存在于内体(endosome)中。在内体中, Fe^{3+}被铁氧化还原酶(iron oxide reductase, also termed STEAP3)还原为Fe^{2+}。最后, Fe^{2+}转运体1(divalent metal transporter 1, DMT1)介导Fe^{2+}从内体中释放到细胞质中的动态铁库中。过量的铁储存在铁蛋白(ferritin)中, 铁储蛋白复合体(iron storage protein complex)由铁蛋白轻链(ferritin light chain, FTL)和铁蛋白重链(ferritin heavy chain 1, FTH1)组成[6]。铁输出由膜蛋白铁转运蛋白(membrane protein ferroportin, also termed SLC11A3)介导。当组织细胞内的铁稳态被破坏时, 过量的铁通过Fenton反应将H_2O_2和脂质过氧化物转化为ROS(图2)。*Ras*突变的Ferroptosis敏感性细胞与*Ras*未突变的Ferroptosis不敏感性细胞相比, TFR1的表达量上升而铁储蛋白亚基FTL和FTH1的表达量下降。这表明, 增加铁摄入和减少铁储存可能导致铁过载, 进而引起Ferroptosis; 同时, 通过铁螯合剂(deferoxamine、desferrioxamine mesylate、ciclopirox olamine)降低铁过载能够抑制erastin诱导的Ferroptosis[7]。GPX4的作用刚好相反, 在还原型谷胱甘肽(glutathione, GSH)辅助下将H_2O_2和脂质过氧化物分别转化为H_2O和相应的醇[8](图2)。

GPX4的活性受到谷胱甘肽(GSH)的控制, 因此GSH的调控也是Ferroptosis过程中ROS产生的关键步骤。首先, 细胞膜上的谷氨酸/胱氨酸反向转运体$SystemX_c^-$(由SLC7A11和SLC3A2两个亚基组成)将胱氨酸转入细胞, 然后胱氨酸转化为半胱氨酸并合成GSH。研究表明, 用水杨酸偶氮磺胺吡啶(sulfasalazine)抑制$SystemX_c^-$的活性能引起Ferroptosis, 而通过β-巯基乙醇增加细胞胱氨酸的摄入可以抑制erastin诱导的HT1080细胞死亡[1]。

当然, 其他来源的ROS也能引起Ferroptosis。Ferroptosis诱导剂erastin可直接与线粒体膜上的电压依赖(pentose phosphate pathway, PPP)产生NADPH和戊糖, NADPH氧化酶(NADPH oxidase, NOX)能够转移电子穿过生物膜, 并将氧转化为过氧化物。研究发现, 在Calu-1和HT1080细胞中典型的NOX抑制剂diphenyleneiodonium能够部分抑制erastin诱导的Ferroptosis[1]。

2.2 Ferroptosis中ROS的作用

Ferroptosis过程中已发现ROS的主要作用是生物膜氧化损伤。膜脂富含多不饱和脂肪酸(polyunsaturatedfatty acids, PUFAs), PUFAs与ROS有很高的亲和性。因此, 铁代谢产生的ROS可以使生物膜氧化损伤, 引起细胞死亡。

研究显示, 在KBM7细胞中脂质代谢的关键酶溶血磷脂酰胆碱酰基转移酶3(lysophosphatidylcholine acyltransferase 3, LPCAT3)和长链酰基-CoA合成酶家族成员4(acyl-CoA synthetase long-chainfamily member 4, ACSL4)表达的上调能够提高Ras选择性致死的小分子3(Ras-selective lethal smallmolecul 3, RSL3)和DPI7(铁死亡诱导剂)诱导的Ferroptosis[9]。ACSL4酰化物花生四烯酸(arachidonic acid, AA)和LPCAT3催化酰化AA进入膜磷脂, 因此, 抑制ACSL4和LPCAT3

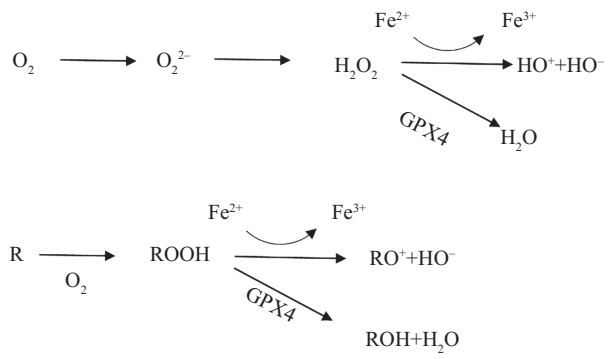

图2 铁的Fenton和GPX4的作用[5]

Fig.2 The role of iron in Fenton and GPX4[5]

能够减少膜中的一些敏感脂肪酸的氧化。ACSL4使细胞膜富含多不饱和脂肪酸, 在一个Ferroptosis小鼠模型中用药物抑制ACSL4活性可以缓解组织灭亡[10]。脂肪酸氧化酶(lipoxygenases, LOX)能够把PUFAS氧化成它们的过氧羟基中间物, 但对Ferroptosis而言只有5-LOX是必需的[11]。最近有研究发现, Ferroptosis涉及高度组织化的氧化中心, 其中内质网相关的氧化发生在磷脂酰乙醇胺库中, 抑制花生四烯酸和肾上腺色素缩氨脲(adrenoyl, AdA)酯化为磷脂酰乙醇胺(phosphatidylethanolamine, PE)或抑制ACSL4能够抵抗Ferroptosis[12]。ROS在Ferroptosis过程中是否还参与其他的氧化损伤有待更进一步的研究。

3 Ferroptosis的调控

根据Ferroptosis的信号通路, 其调控可以在ROS生成和ROS作用两方面进行。但是由于ROS在Ferroptosis中的作用机制还不十分清楚, 所以本文主要介绍Ferroptosis在ROS生成水平上的调控并将其分为正调控和负调控两部分。

3.1 Ferroptosis的正调控

通过调控铁代谢过载, 调控GSH耗尽, 进而使GPX4失活或直接下调GPX4都能使ROS过量, 引起Ferroptosis。研究发现, GPX4$^{-/-}$ T细胞能迅速积累脂质过氧化物并发生Ferroptosis[13]。Ferroptosis诱导剂erastin能直接与线粒体膜上的线粒体电压依赖阴离子通道(voltage-dependent anion channel, VDAC)作用产生ROS[3]。此外, erastin也能直接抑制SystemX_c^-的活性, 减少GSH的生成, 抑制GPX4的活性进而加速ROS的产生[14]。诱导剂RSL5直接与VDAC2/3作用产生ROS。RSL3则直接与GPX4结合, 抑制GPX4的活性, 诱导ROS产生[15]。诱导剂丁磺氨酸(buthionine sulfoximine, BSO)在*Ras*突变细胞中不可逆地抑制γ-谷氨酸半胱氨酸合成酶(GSH合成的限速酶)的活性, 减少GSH的合成, 进而降低GPX4活性[15]。铁死亡诱导剂(Ferroptosis-inducing agents, FIN)根据作用机制分为两类, 第一类FIN(如DP12)通过耗尽GSH抑制GPX4的活性, 而第二类FIN则直接抑制GPX4的活性[15]。水杨酸偶氮磺胺吡啶通过抑制SystemX_c^-的活性, 降低GSH的水平, 进而使GPX4失活[16]。

在一些肿瘤细胞中, Ferroptosis发生需要p53的参与, 这个过程是依赖于p53对SLC7A11合成(SystemX_c^-的组成单元)的抑制实现的[17]。半胱氨酰-tRNA合成酶(cysteinyl-tRNA synthetase, CARS)能够剥夺胱氨酸, 减少GSH的合成, 使GPX4失活[18]。敲低*CARS*能够抑制erastin诱导的Ferroptosis, 而CARS的过表达则能增强一些癌细胞的erastin的敏感性[18]。细胞外过量的谷氨酸或谷氨酸盐(glutamate)能够抑制SystemX_c^-的活性, 最终诱导Ferroptosis[19]。

3.2 Ferroptosis的负调控

调节细胞内铁代谢正常进行, 提高GSH水平进而活化GPX4, 直接活化GPX4和直接抑制ROS产生等都能阻止细胞发生Ferroptosis。HSPB1(heat shock protein beta 1)在肌动蛋白动力过程和铁摄取过程中均起到重要作用。抑制热休克因子转录因子-1(heat shock factor-1, HSF-1)依赖的HSPB1的表达能够抑制erastin诱导的Ferroptosis[20]。在BJeLR细胞中通过ShRNA下调转铁蛋白受体1(transferrin receptor 1, TFR1)能抑制erastin诱导的细胞死亡, 表明抑制铁摄取可以防止Ferroptosis[21]。第一代Ferroptosis抑制剂Ferrostatin-1在HT1080细胞中抑制erastin和RSL3诱导的Ferroptosis, 其活性依赖于芳香胺, 芳香胺能特异性地抑制脂质氧化反应产生的ROS的积累[1]。

转录因子NF-E2相关因子2(nuclear factor erythroid 2-related factor 2, NRF2)在肝癌细胞抗Ferroptosis过程中起重要作用, 上调NRF2能够启动抗氧化蛋白基因和铁代谢蛋白基因的转录, 进而阻止Ferroptosis[22]。在HT22细胞中, Ferroptosis抑制剂Zileuton主要通过抑制5-LOX的活性降低ROS的产生, 进而抑制谷氨酸(glutamate)和erastin诱导的Ferroptosis[23]。

最终, Ferroptosis是否发生主要看在各种Ferroptosis正负调控因素的共同作用下细胞ROS是否过载。

4 Ferroptosis与疾病的联系

Ferroptosis的错误调控与多种生理和病理过程有关, 包括肿瘤细胞的死亡、神经退行性疾病、急性肾功能衰竭以及肝和心脏缺血再灌注损伤等。最近, 研究人员在117癌细胞中检测到了Ferroptosis诱导剂erastin的抗癌活性, 并发现*Ras*(原癌基因)突变与erastin的剂量间没有联系[21]。FDA批准诱导Ferroptosis的药物sorafenib、sulfasalazine和artesunate在癌症治疗方面有巨大的潜力[24]。双氢青蒿素

(dihydroartemisinin)通过诱导Ferroptosis使头颈癌细胞的细胞周期阻滞[25]。然而, Ferroptosis在肿瘤发生中的作用仍不清楚。研究发现, 在大鼠海马脑片培养模型中, 通过Ferrostatin-1抑制细胞死亡有助于防止谷氨酸诱导的神经毒性[1]。Kang等[26]研究表明, FMS样酪氨酸激酶-3(FMS-like tyrosine kinase-3, FLT-3)的有效抑制剂能抑制ROS的产生和脂质过氧化反应, 进而阻止神经元发生Ferroptosis。通过调节GPX4活性诱导前脑神经元发生Ferroptosis能够引起认知功能障碍和神经退行性疾病[27]。这些研究表明, Ferroptosis引起的神经细胞死亡可能是神经退行性疾病的重要成因。

在新鲜分离的肾小管急性损伤模型中, Ferrostatin-1对其有保护作用, 表明在急性肾功能衰竭(acute kidney failure, AKF)中Ferroptosis调节细胞死亡[28]。用SRS16-86(第三代ferrostatin)抑制Ferroptosis能阻止急性缺血再灌注损伤和草酸肾病相关的急性肾功能衰竭[29]。对乙酰氨基酚(acetaminophen)过量是目前急性肝功能衰竭的最常见原因, 对乙酰氨基酚已被证明能诱导原代肝细胞发生Ferroptosis, 并且Ferroptosis抑制剂能抑制对乙酰氨基酚诱导的细胞死亡[30]。

5 总结与展望

Ferroptosis是近年新发现的细胞死亡的一种形式。Ferroptosis信号通路的研究中, 研究人员对其上游过程即ROS的产生已经有了一定的成果和较为系统的认识, 但是对其下游ROS执行杀死细胞功能的了解甚少, 值得深入研究。Ferroptosis过程为无菌炎症反应, 所以深入研究并精确控制Ferroptosis在癌症、神经退行性疾病、心肝肾损伤等疾病的治疗方面有着巨大的潜力。

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