第620次学术讨论会·强磁场与生命健康: 新条件、新问题、新机遇

铁稳态代谢分子机制及铁磁纳米颗粒研究进展

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摘要 铁是人体必需微量元素, 参与血红蛋白及多种酶的合成, 在氧气运输、免疫调节、核酸合成及基因表达调控等多种生理过程中发挥重要作用. 铁稳态代谢的维持对于机体正常生长发育至关重要, 铁稳态代谢失衡会引发多种疾病. 机体铁稳态代谢的调控由多个环节协调控制; 在分子水平上, 铁调素Hepcidin作为铁稳态代谢的关键调控因子, 通过降解小肠上皮细胞和巨噬细胞上的铁外排蛋白Ferroportin, 调控机体铁稳态. 由此可见, 机体内铁稳态代谢的维持是由多因素、多层次的复杂调控网络协调完成. 铁不仅是必需的营养物质, 还是新型生物材料的重要组分. 铁磁纳米颗粒是一类以铁蛋白或 Fe_3O_4 等作为基础的生物大分子纳米粒子, 因其较强的磁导向性和较好的生物相容性, 已被广泛应用于生物医学领域. 本文围绕我们团队近年在铁稳态代谢领域的系列原创发现, 就铁调素调控铁代谢稳态的分子网络及铁磁纳米颗粒研究国际前沿进展进行系统综述.

关键词 铁稳态, 缺铁性贫血, 铁过载, 血色病, 铁调素, 铁磁纳米颗粒

铁是地球上分布及应用最广的金属元素之一, 约占地壳总量的5%, 在元素丰度表中仅次于氧、硅、铝, 位居第四^[1]. 铁也是人体含量最多的必需微量元素, 在成人体内大约含有3~5 g^[2]. 铁元素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酶类的重要辅因子, 对维持线粒体功能、核酸合成与修复以及氧运输等具有重要意义^[3]. 可见, 铁对于机体生命健康的维持起着至关重要的作用. 机体内铁稳态的维持受到严密的调控, 铁缺乏或铁过载都会严重影响机体健康.

机体铁缺乏多表现为头晕、乏力、烦躁、易怒及免疫力减弱, 严重时可引起儿童认知发育不良, 身体行动能力减弱及不良的妊娠结局^[4]. 而铁元素缺乏主要临床表现为缺铁性贫血, 缺铁性贫血是全球最常

见的营养缺乏性疾病^[5], 全球约有近三分之一的人口患有不同程度的贫血, 多见于儿童和孕妇^[6], 亚洲及非洲等发展中国家更是贫血的高发区^[7]. 据2015年《中国居民营养与慢性病状况报告》指出, 我国6岁及以上居民贫血率为9.7%, 其中6~11岁儿童和孕妇贫血率分别为5.0%和17.2%. 缺铁性贫血是由于机体各种原因导致缺铁而引起的红细胞生成减少性贫血, 血象多呈小细胞低色素性贫血. 缺铁性贫血发生的主要原因是膳食铁摄入不足, 铁需求增加以及铁丢失过多. 此外, 遗传因素也对缺铁性贫血的发生起着重要作用. 我们研究发现*TMPRSS6*基因突变人群血清铁明显降低, 首次揭示*TMPRSS6*是造成人群缺铁性贫血的重要易感基因^[7].

机体铁过载同样影响人类健康. 细胞中铁过量

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蓄积会导致过度的自由基产生,破坏蛋白质、核酸及细胞膜功能,产生细胞毒性。遗传性血色病是一类由于机体过量铁蓄积而引起的疾病,主要累及心脏、肝脏和胰腺,多表现为糖尿病、肝硬化、心脏病及皮肤色素沉着等。临床上主要将遗传性血色病分为四型,最常见的I型血色病是由HFE第282位氨基酸突变引起的,除此之外,HFE上的其他突变位点也逐渐被发现和血色病相关^[8-10]。II型血色病通常早期发病,故又称青少年型血色病,分两个亚型,分别由HJV和HAMP基因突变引起的^[11]。III型血色病由编码转铁蛋白受体2的基因TfR2突变所导致^[12]。以上三型血色病为常染色体隐性遗传病,都参与对铁代谢关键因子铁调素的调控,因此具有相似的铁蓄积表型。而随着研究的深入,一种由铁外排通道基因SLC40A1(也叫FPN1基因,编码Ferroportin蛋白,简称Fpn)突变所导致的常染色体显性遗传性血色病逐渐被发现,该基因不同位点的突变使得Ferroportin铁外排功能丧失或Ferroportin可以抵抗铁调素降解调控,导致机体小肠铁吸收异常及网状内皮细胞组织的巨噬细胞铁释放改变而引起机体铁稳态失衡^[13,14]。SLC40A1突变导致的铁外排功能异常,机体铁代谢紊乱疾病也被统称为铁转运蛋白疾病(Ferroportin disease)^[15]。我们通过对铁代谢异常人群基因组测序,发现中国3个遗传性血色病家系及血色病基因突变位点(HFE H63D; SLC40A1 W158C; SLC40A1 S209L)^[16],丰富了血色病基因突变的人群依据。

此外,铁代谢可能的修饰基因如Mon1a, CYBRD1及GNPAT基因多态性也提示与机体高铁蓄积相关^[17-19],但还需体内模型进一步探究分子机制。

铁死亡(Ferroptosis)是一种铁依赖性的不同于凋亡、坏死及自噬的新型细胞死亡模式^[20],铁死亡与凋亡的区别如表1。研究显示铁死亡参与到多种疾病的发病过程中,如缺血再灌注急性肾损伤,亨廷顿舞蹈症,癌症及神经退行性病变^[21]。研究发现Slc7a11基因敲除鼠可促进铁死亡发生,这为抑制铁死亡治疗血色病相关损伤提供了潜在的靶点^[22]。

在铁死亡的发生过程中,已发现活性氧自由基(reactive oxygen species, ROS)可以使生物膜发生氧化损伤导致细胞死亡。谷胱甘肽过氧化物酶4(GPX4)可抑制脂质过氧化过程而减少脂质活性氧的形成^[23]。P53可下调SLC7A11的mRNA及蛋白水平^[24]从而抑制胱氨酸/谷氨酸转运体systemX_c⁻吸收胱氨酸,谷胱甘

肽合成减少而降低谷胱甘肽过氧化物酶GPX活性^[25],活性氧自由基增加。ACSL4通过氧化的细胞膜磷脂堆积引发细胞铁死亡^[26]。

核受体辅激活因子NCOA4是一种选择性的运载受体,可介导铁蛋白的选择性自噬(Ferritinophagy)。细胞内的铁蛋白通过储存和释放铁来维持机体内铁平衡。每个球形的铁蛋白分子由24个亚基构成,最多可储存4500个三价铁离子。沉默NCOA4的核酸表达,可抑制铁蛋白降解而抑制铁死亡。相反,过表达NCOA4可增加铁蛋白降解,导致细胞内铁过载并促发铁死亡^[27]。

小分子抑制剂爱拉斯汀(Erastin)可以通过抑制胱氨酸/谷氨酸转运体吸收胱氨酸导致脂质过氧化增多而发生铁死亡,Erastin引发的铁死亡是铁依赖性的,铁螯合剂去铁胺(deferoxamine, DFO)、环吡酮(Ciclopirox, CPX)可以抑制Erastin诱发的铁死亡^[20]。铁过载可以直接促进铁死亡发生^[22]。铁离子主要通过转铁蛋白/转铁蛋白受体系统进入细胞,敲低转铁蛋白受体基因TFRC表达可以减少进入细胞的铁离子而抑制Erastin诱发的铁死亡^[28]。由此可见铁对于铁死亡发生的必要性。

此外,某些铁代谢调控蛋白也能影响铁死亡的发生进展。FBXL5(F-box and leucine rich repeat protein 5)通过铁反应元件结合蛋白IRP2可以稳定转铁蛋白和转铁蛋白受体的mRNA水平^[29]。然而,铁在细胞死亡过程中发挥的具体功能仍不清楚,可能是动员出来的游离铁促进了脂质过氧化损伤,或者是影响了铁依赖相关酶的功能。

在铁死亡的研究进程中,逐渐发现可以用于检

表1 铁死亡与凋亡的主要区别

Table 1 The major distinction between ferroptosis and apoptosis

特征	铁死亡	凋亡
铁依赖	是	否
Caspase激活	否	是
是否能被zVAD等Caspase抑制剂阻断	否	是
依赖脂质过氧化	是	否
能被ferrostatin-1等抗氧化剂阻断	是	否
形态学特征	线粒体变小,密度增大	细胞皱缩、染色质凝集
引起炎症或免疫反应	是	否

测铁死亡的生物标志物,包括ACSL4水平、脂质过氧化水平、PTGS2表达水平及NADPH含量等^[30~32]。但铁死亡生物标志物的特异性及生理意义都还存在诸多未知,有待深入的研究;特别是铁死亡直观特异的检测指标还亟待研究和建立。

随着老龄化社会到来,肿瘤、阿兹海默症、心血管疾病、肥胖、糖尿病等重大慢性疾病对我国老龄化社会的威胁日益严峻。其中,铁代谢失衡是引发这些慢性疾病的重要风险因素。铁过载引发肿瘤发生的关键分子机制,铁过载人群罹患肿瘤的风险比正常人高几十倍,研究发现铁代谢中间产物血红素结合抑癌蛋白p53改变蛋白构象,干扰p53启动下游抑癌基因的转录与表达,并降低p53蛋白稳定性,从而促发肿瘤的分子机制;并揭示了“铁螯合剂”等降铁药物是肿瘤个性化治疗和药物筛选的新策略^[33]。人群流行病学荟萃分析和病例-对照研究表明,膳食血红素铁摄入会增加心血管疾病发病风险,并且呈现剂量依赖性^[34];人体超重和肥胖与机体铁缺乏显著相关,揭示了早期监测并纠正铁缺乏对预防肥胖的重要性^[35],而且通过难治性贫血模型*TMPRSS6*敲除小鼠发现缺铁能抑制高脂膳食诱导的肥胖^[36]。我们研究发现汉族人群中*TMPRSS6*多态性与2型糖尿病发病相关,其发生可能是通过血清铁蛋白介导的^[37]。机体铁过载与2型糖尿病呈现显著正相关^[38],胰岛 β 细胞的生理功能受细胞铁水平调控^[39]。此外,有研究发现阿兹海默症患者血清铁偏低,且常伴有(轻度)缺铁性贫血,而特定脑区存在高铁蓄积,从根本上颠覆了“机体铁过载是引发阿兹海默症病因”的传统观点,揭示阿兹海默症需要降低脑铁蓄积和升高机体铁(防贫血)并举的防治新策略^[40]。这些研究成果表明,纠正机体铁代谢紊乱是肿瘤、心血管疾病、代谢综合征等重大慢病防治的核心环节。

由此可见,铁稳态的维持对机体生命健康起着至关重要的作用。

1 铁稳态维持的调控机制

哺乳动物细胞铁稳态的维持存在着非常复杂的调控网络(图1)。机体内维持铁稳态主要通过小肠铁的吸收及外排,血液的运输,组织的利用,肝脏的储存以及巨噬细胞的吞噬循环这5个环节来实现的^[41~43],每个环节都受到机体严密的调控。

首先,十二指肠上皮细胞吸收膳食中的铁元素

(包括血红素铁和非血红素铁)来补偿皮肤、黏膜细胞脱落及失血等造成的铁流失,吸收的铁通过位于小肠上皮细胞基底膜上的铁外排蛋白Ferroportin释放进入血液,随后铁与位于血液中的转铁蛋白Transferrin(以下简称Tf)结合经由血液循环运送到如骨髓造血系统等各个需铁的组织器官。

结合铁的Tf称作Holo-Tf, Holo-Tf可以与广泛表达于细胞膜表面的转铁蛋白受体Transferrin receptor1(以下简称TfR1)结合,形成的复合物一起被内吞形成内体,Sec151在其中发挥重要作用^[44]。随后铁与Tf解离,解离后的三价铁经Steap3还原为二价铁^[42],通过二价金属转运体Divalent Metal Transporter 1(以下简称Dmt1)或离子通道蛋白Trpm11释放到细胞质中供细胞合成利用,而不含铁的Apo-Tf/TfR1重新回到细胞膜上。随后Tf与TfR1解离,Tf回到血液循环中继续下一次的运铁行动,而TfR1留在细胞膜上等待下一次与Holo-Tf结合^[2]。

机体所需要的铁只有5%~10%是来自小肠对于膳食铁的吸收,其余大部分还是来源于巨噬细胞吞噬衰老或损伤的红细胞。巨噬细胞通过吞噬并在溶酶体中裂解红细胞,经过血红素加氧酶HO催化血红素分解释放出其中的铁,释放出的三价铁(Fe^{3+})被Steap3还原为二价铁(Fe^{2+}),经由Dmt1或Trpm11从溶酶体中释放到胞质中^[45]。随后通过Ferroportin将铁运送出巨噬细胞^[41]供机体利用。但当机体铁水平过高时,肝脏将会把多余的铁以铁蛋白Ferritin的形式暂存起来待有需要时由Ferroportin运出利用。但是机体铁再循环发生障碍时,十二指肠膳食铁的吸收却可补偿铁再循环失衡所引起的铁动员障碍^[43]。最新研究发现肾脏中敲除Ferroportin导致近曲小管铁滞留而血清铁及肝铁的降低,近曲小管Ferroportin缺失却可减轻肾脏急性缺血性损伤,这提示Ferroportin在调控肾脏铁再循环及急性缺血性损伤等病理情况下发挥重要作用^[46]。

机体内部铁代谢受到严密的调控,铁稳态代谢调控网络的5个环节相辅相成,互相依赖,某些重要环节出现问题将严重破坏铁代谢平衡,导致多种铁代谢失衡相关疾病的发生。

2 Hepcidin-Ferroportin轴研究进展及Hepcidin代谢调控通路

肝脏作为体内最大的储铁器官在维持机体铁代

图1 (网络版彩色)细胞铁代谢模式图。十二指肠上皮细胞外的二价铁可经DMT1进入细胞。胞外转铁蛋白分子可结合2个三价铁通过转铁蛋白/转铁蛋白受体复合体一起被内吞形成内体, Sec151参与内体循环的过程。被内吞入溶酶体中的三价铁在Steap3作用下还原为二价铁经DMT1或TRPML1释放到胞质中。铁蛋白Ferritin可结合胞质中的三价铁。胞质中的二价铁可经铁外排蛋白Ferroportin(Fpn)运出细胞外, Mon1A参与Fpn的铁外排过程。Hepcidin可介导Fpn内化降解。BMP信号与位于细胞膜上的BMP受体结合引发SMAD1/5/8磷酸化, 磷酸化的SMAD1/5/8与SMAD4形成异源复合物, 作为转录因子进入细胞核调控Hepcidin表达。TMPS6可以剪切HJV下调Hepcidin, SMAD7通过抑制SMAD1/5/8和SMAD4复合物抑制Hepcidin

Figure 1 (Color online) Scheme map of cell iron metabolism. Ferrous ions could be directly absorbed via DMT1 which located on the plasma membrane. Holo-transferrin(Holo-Tf, contains two ferric ions) binds to transferrin receptor1(TfR1), and translocated into endosome, which is facilitated by Sec151. When fused to lysosome, Iron/Tf/TfR1 complexes are dissociated, ferric ions are reduced to ferrous ions by Steap3 metalloredutase, and undergo efflux into cytosol by DMT1 and/or TRPML1. Labile iron could be captured by ferritin cage, oxidized into ferric ions, and be stored via mineralization. Ferrous ions could be exported from cytosol by ferroportin, Mon1A is involved in this process. Hepcidin binds to Fpn and triggers the latter internalization and degradation. Hepcidin transcription is dominated by BMP signaling. BMP ligands bind to their receptors including the indispensable co-receptor—hemojuvelin (HJV), and subsequently phosphorylate R-SMADS (SMAD1, SMAD5, SMAD8), and promote the formation of the complexes of SMAD1/5/8 and SMAD4 and their translocation from cytosol to nucleus, and eventually upregulate Hepcidin expression. Metriptase 2 (encoding gene: *TMPS6*) cleaves HJV while SMAD7 inhibits Bmp signaling to downregulate Hepcidin transcription

谢平衡的过程中发挥了不可或缺的作用^[2]。铁转运蛋白Ferroportin是目前唯一已知的铁外排通道蛋白, 广泛分布于小肠上皮、巨噬细胞及肝实质细胞中^[41,43]。铁稳态维持的多个环节都需要Ferroportin的参与, 而肝实质细胞可以通过分泌铁调素来调控Ferroportin。铁调素是系统铁稳态最主要的调控因子(图2), 它最初是在尿液中发现的一种由25个氨基酸组成的抗菌多肽^[47], 可以结合位于细胞膜表面的Ferroportin, 促使其内吞、泛素化最终被降解, 从而抑制小肠铁的吸收、巨噬细胞铁的外排^[48]。前文已经提到, 铁调素分泌减少或功能缺失是引起人类遗传性血色病的主要原因, 而铁调素分泌过多则会造成慢性贫血和难治性贫血^[49]。当贫血和缺氧时肝脏铁调素的表达被抑制而提高机体可利用的铁水平以供造血需求^[50]。相反, 当机体铁过载时铁调素表达上调而抑制膳食铁的进一步吸收及巨噬细胞铁的释放^[51]。同时铁调素的表达也可以被炎症因子、造血需求等因素所调

控^[52]。肝脏铁调素的分泌调控具体是如何实现的? 目前的研究表明, 机体主要通过以下几个信号通路对铁调素的分泌进行严密的调控。

2.1 机体铁水平对Hepcidin的调控

骨形态发生蛋白Bone morphogenetic protein/SMAD蛋白*Drosophila* mothers against decapentaplegic protein(以下简称BMP/SMAD)信号通路响应肝铁水平调控肝脏铁调素的表达作为最主要的铁调素表达调控通路已经逐渐被明确^[53]。膳食铁过量吸收及肝脏铁含量升高会导致BMP6表达增加^[54,55], 促使其受体BMPR及辅助受体HJV复合物结合, 进而引发SMAD1/5/8蛋白磷酸化, 磷酸化的SMAD1/5/8可以与SMAD4形成异源复合物^[56], 作为转录因子进入细胞核, 结合铁调素的启动子区域中BMP/SMAD响应元件, 从而激活铁调素的转录^[57]。BMP/SMAD通路存在负向调控机制。研究表明, 在前体蛋白转化酶

图2 (网络版彩色) 机体铁稳态调控模式图。膳食铁经小肠上皮细胞吸收被铁外排蛋白运输到血浆中。巨噬细胞通过吞噬衰老损伤的红细胞释放其中的铁。血浆中的铁与转铁蛋白结合被运输到需铁的细胞中利用(主要是有核红细胞)。过多的铁被肝细胞吸收以铁蛋白的形式存储。肝细胞还可以分泌铁调素, 铁调素通过降解铁外排蛋白抑制小肠铁的吸收、巨噬细胞及肝细胞铁的释放进而调控系统铁稳态。多种因素可以调控铁调素。高铁及炎症刺激铁调素表达而造血缺氧等可以下调铁调素表达

Figure 2 (Color online) Proposed model of systemic iron metabolism. Dietary iron is absorbed via duodenum epithelial cells and exported into circulation by Ferroportin. Labile iron soon binds to transferrin in blood, and is transport to tissues (mostly to reticulocytes) via Tf-TfR1 pathway. Senescent erythrocytes are phagocytized by splenic red pulp macrophage, iron ions are released and exported back to the circulation. Excess iron is stored by ferritin in hepatocytes. Hepcidin is secreted by hepatocytes, and it maintains systemic iron homeostasis by degrading Ferroportin to inhibit iron absorption and release. Various factors contributes to hepcidin regulation. Iron overload and inflammatory stimuli upregulate hepcidin whereas erythropoiesis and hypoxia stress suppress hepcidin expression

Furin的作用下, HJV可从细胞膜上脱落, 产生小片段的可溶性s-HJV^[58]。当s-HJV被加到肝癌细胞系中时, 能够竞争结合BMPs, 抑制细胞中铁调素表达^[59], 和膜上锚定的HJV对铁调素的调控作用相反。此外, *TMPRSS6*基因编码的II型跨膜丝氨酸蛋白酶Matriptase2, 可通过剪切HJV而抑制铁调素的表达^[60], 因此*TMPRSS6*突变的人群及*TMPRSS6*敲除小鼠都表现为铁调素表达显著上调^[61,62], 从而导致难治性缺铁性贫血的发生。BMP/SMAD信号通路抑制型SMAD家族成员SMAD7, 通过抑制SMAD下游信号通路下调铁调素的表达^[63]。我们研究发现, 在SMAD7肝脏敲除小鼠模型中, SMAD6, BAMBI和Follistatin通过阻断BMP/SMAD信号下调铁调素表达, 丰富了铁调素调控的代谢网络^[64]。

正常生理情况下, 机体铁调素对机体铁水平起到负反馈调节的作用。Hfe是非典型主要组织相容性复合体I类分子, 可以分别与TfR1和Transferrin receptor2(以下简称TfR2)结合形成复合物^[65]。在Hfe及TfR2基因敲除的小鼠中, 不能响应血液循环中高转

铁蛋白饱和度的刺激而失去对铁调素的调控作用, 但仍能感应肝铁的增加而上调铁调素的表达, 说明Hfe和TfR2主要通过感应循环中holo-Tf来调控铁调素的表达^[66]。

有研究表明, 在TfR1上存在Hfe与Tf的共同作用位点, 而Hfe可以与Holo-Tf竞争结合TfR1^[67]。当受到血液循环中高转铁蛋白饱和度的刺激时, Holo-Tf竞争性结合TfR1, 而Hfe则与TfR1脱离进而和TfR2结合调控铁调素的表达^[68]。虽然TfR2也可以结合Holo-Tf来介导铁的吸收, 但结合效率极低^[69], 所以目前主要认为TfR2是通过调控肝脏铁调素的表达来调控机体铁稳态。有研究表明, Hfe, TfR2和HJV三者可以在肝细胞膜上形成复合物^[70], Hfe和TfR2双敲后发现胞外信号调控激酶Erk1/2及SMAD1/5/8的磷酸化水平都显著下调, 铁调素蛋白水平显著下降, 说明BMP/SMAD和胞外信号调控激酶Erk/细胞分裂酶原蛋白激酶Mapk(以下简称Erk/Mapk)信号通路都参与了Hfe/TfR2介导的铁调素调控过程^[71]。另也有体外研究提示Hfe与TfR2可以通过激活ERK/MAPK通路

来促进铁调素的转录^[72],但此条代谢调控途径的具体调控过程仍有待进一步研究确证。

2.2 炎症对Hepcidin的调控

铁调素最早被作为一种抗菌多肽发现^[47]。机体发生炎症反应时,肝脏可以分泌铁调素来降低外周铁水平^[73]。目前认为,分泌铁调素的目的是为抵抗病原菌的侵袭,减少血液循环中可被细菌利用的游离铁离子,让病原菌得不到所需的铁而生长繁殖受限。当机体由于感染或其他原因(免疫系统紊乱、败血症、炎性肠病、肿瘤等)而产生慢性炎症时,会激活前炎症因子如干扰素IFN- γ 、白介素1(IL-1)、白介素6(IL-6)等的表达增加,从而增强铁调素的表达^[52]。机体炎症情况下,刺激炎症响应分子IL-6的产生,IL-6与其受体结合后激活胞内JAK/STAT(Janus kinase/Signal Transducer and Activator of Transcription)信号通路,从而导致STAT3发生磷酸化,而磷酸化的STAT3可以进入细胞核并结合到铁调素启动子区附近的结合位点从而促进其表达^[74]。研究发现BMP/SMAD和JAK/STAT两个通路之间还存在交互作用共同调控铁调素^[2]。

脂多糖(Lipopolysaccharide, LPS)是革兰氏阴性菌细胞壁的主要成分,感染状态下,脂多糖可以结合Toll样受体(Toll-like Receptor 4, TLR4),通过MyD88依赖和非依赖途径激活NF- κ B表达,NF- κ B作为一种转录调控分子进一步调控前炎症因子转录^[75]。IL-1 β 和Activin B都可通过BMP/SMAD通路上调铁调素的表达^[76-78]。也有研究显示IL-1 β 可促进Activin B二聚体的形成^[79],小鼠肝脏中IL-1 β 可以显著增强Activin B的mRNA水平^[76],但具体的调控机制还需进一步研究。

2.3 造血及缺氧对Hepcidin的调控

铁是合成血红蛋白重要原料之一,机体造血与铁循环之间存在精密的调控关系。当机体处于生理或病理性失血、各种毒素或理化因素引起的溶血、 β 球蛋白基因突变引起的 β 地中海贫血等情况时,造血增加而需要更多的铁作为原料,为了补偿正常代谢所需,小肠上皮吸收的膳食铁及巨噬细胞释放的铁增加以满足造血需求。造血活动主要通过抑制铁调素增加循环铁^[80,81]。肾脏分泌的促红细胞生成素Erythropoietin(以下简称EPO)是造血过程中的关键调控分子,主要促进红细胞生成^[82,83]。EPO也被发现可

以介导铁调素的表达下调^[50],但不能抑制辐照后小鼠的铁调素^[84],说明EPO对铁调素的调控依赖造血系统本身,其中还存在中间的关键因子。近年来随着对造血研究的逐步深入,一种由前体红细胞分泌的激素Erythroferrone(ERFE)被发现在造血调控铁调素途径中发挥关键作用。当造血增加时,机体的EPO分泌增加,与其受体EPOR结合后可以激活下游JAK2/STAT5调控通路,因为ERFE的启动子区含有STAT5的结合位点,STAT5可以直接调控ERFE的转录^[85]。有研究表明,在*TMPRSS6*敲除小鼠中,EPO可以增加Erfe的转录却不能下调铁调素表达,*BMP/SMAD*信号通路高表达时,EPO对铁调素的抑制作用减弱,提示Erfe介导*TMPRSS6*-HJV-BMP/SMAD通路抑制铁调素水平^[86]。此外,生长分化因子15(growth differentiation factor15, GDF15)和扭转原肠胚形成蛋白1(twisted gastrulation-1, TWSG1)也被认为参与了造血调控铁调素表达的过程^[87,88]。但这些分子的具体调控铁调素的机制还缺乏充足的体内实验证据。

在缺氧条件下,缺氧诱导因子(HIF1, HIF2)表达增强,而铁调素启动子区含有HIFs的结合位点,HIFs可以直接与铁调素启动子区结合抑制其转录水平^[89]。不仅如此,缺氧还可以促进EPO的表达,通过造血途径抑制铁调素表达^[90,91]。

2.4 Hepcidin的表观遗传调控机制

铁调素作为调控机体铁稳态的核心因子,存在着多种平衡调控的分子机制,除了上述几个经典的铁调素调控通路以外,还存在着其他的调控机制。

近年来随着表观遗传调控研究的兴起,也为铁代谢机制研究带来了新的思路和契机。越来越多的表观遗传调控分子被发现参与到铁调素表达调控与铁稳态代谢中。甲基化CpG结合域蛋白MBD5可通过组蛋白乙酰化作用调控小肠铁蛋白重链而调控铁代谢^[92],去乙酰化酶Sirtuin 2可通过对转录因子NRF2的去乙酰化作用调控铁代谢^[93]。

我们的研究表明,组蛋白去乙酰化酶HDAC1可以不依赖于其去乙酰化酶的活性调控铁调素,而是可以与SMAD4蛋白组合形成复合物,随即复合物入核结合到铁调素的启动子区抑制铁调素的转录^[94]。另一种组蛋白去乙酰化酶HDAC3需要与辅抑制因子NCOR1和NCOR2组成复合物才能发挥去乙酰化酶的活性,HDAC3可以结合到铁调素的启动子区抑制

铁调素的转录^[95].

3 铁稳态失衡的防治

机体内的循环铁含量调控是个动态平衡的过程, 正常情况下机体可以通过代偿等作用维持体内铁稳态. 但在很多病理情况下, 体内外各种不利因素超过了机体自身的代偿能力, 体内铁稳态遭到破坏, 就需要外源手段介入辅助机体维持体内的铁代谢平衡.

缺铁性贫血是严重威胁我国居民的健康问题之一. 临床上除了饮食疗法, 常用各种补充铁剂, 包括硫酸亚铁、葡萄糖酸亚铁、琥珀酸亚铁和右旋糖苷铁等.

遗传性血色病目前临床上治疗的基本原则是减少体内的铁, 常用的治疗手段是放血疗法及铁螯合剂治疗, 其他的措施包括限制膳食铁及酒精摄入, 对于已经发展成肝癌的终末期血色病患者, 肝脏移植则是潜在的治疗途径^[96]. 临床上也有利用质子泵抑制剂减少非血红素铁的吸收^[97].

铁调素是铁稳态代谢的核心调控因子, 因此近年来将铁调素作为治疗铁失衡的靶点越来越受到科研人员的关注. 随着对铁代谢研究的逐步深入, 对铁调素调控网络认识的不断深化, 多种代谢调控通路越来越明晰, 根据不同疾病的发病机制, 逐渐开发出针对铁调素代谢网络的不同调控分子的靶向治疗策略. Mini-hepcidin是一类铁调素的模拟短肽小分子, 在动物模型中对由于铁调素降低引起的铁过载有很好的疗效^[98]. 小RNA抑制*TMPRSS6*可增强体内铁调素的水平^[99]. 我们发现腺嘌呤(维生素B₄)可以激活cAMP-PKA通路, 介导BMP/SMAD通路上调铁调素^[100]. 靶向铁调素调控信号通路上多种小分子抑制剂及传统中医药小分子提取物的运用为临床治疗缺铁性贫血带来了新的思路. 黑豆种皮提取物及传统中医药如鸡血藤和当归等可以通过调控BMP/SMAD信号通路来抑制铁调素的表达^[101]. 这对于缺铁的人群来说是很有前景的治疗策略.

4 铁磁纳米颗粒在生物医学领域的应用

铁除了是一种营养物质, 还可以用于制备新型高分子生物材料. 随着纳米技术的飞速发展及多学科间的交叉融合, 磁性铁纳米颗粒应运而生, 近年来更是被深入研究并越来越多地应用到生物医学领域, 在磁靶向药物、疾病诊断和基因靶向治疗等方面具有

广泛应用市场. 基于铁稳态代谢重要分子铁蛋白及转铁蛋白等的生理特性而开发的铁磁纳米颗粒为肿瘤及重大疾病的诊断与治疗带来福音.

磁性铁蛋白纳米颗粒(magnetoferritin nanoparticles)由于大小均一、低毒及低免疫反应性、蛋白质外壳易于修饰等优点在肿瘤检测及靶向治疗方面具有广阔的应用前景^[102]. 研究人员利用可降解的淀粉纤维天然还原剂和抗氧化纳米载体, 制备得到一种稳定不易氧化、不易聚集的铁纳米颗粒, 具有较高的体内铁生物利用率, 为治疗缺铁性贫血带来新的希望^[103].

金属铂(Pt)有清除氧化自由基的活性, 但临床给药途径一直是难题. 研究者利用铁蛋白笼状结构特点, 合成了高度稳定且具有过氧化物酶活性的“铂-铁蛋白纳米颗粒”, 解决了铂给药难题, 并观察到了良好的抗氧化功能, 为靶向治疗氧化损伤提供了新思路^[104]. 此外, 研究者还成功制备了能靶向肿瘤的铁蛋白荧光探针, 能特异性被肿瘤成纤维细胞表面的丝氨酸蛋白酶FAP- α (fibroblast activation protein- α)识别并剪切发光, 实现肿瘤原位荧光成像, 为肿瘤诊断提供了新方法^[105]. 磁共振成像是肿瘤诊断最佳手段之一, 但传统的造影剂具有低弛豫时间和高毒的缺点. 有研究者提出利用铁蛋白搭载造影剂钆的螯合物与放射性同位素实现肿瘤诊断的多态成像^[106,107].

吉非替尼(Gefitinib)、阿霉素(Doxorubicin)、顺铂(Cisplatin)等是临床常用的抗肿瘤化疗药物, 但由于系统毒性及低生物利用率的缺点限制了药物的效果. 研究者在pH控制下通过聚合解聚作用将阿霉素搭载铁蛋白^[108], 实现靶向给药及精准化疗, 在避免副作用的同时, 极大提高抗肿瘤效果. 以类似的手段也可以将顺铂装载入铁蛋白, 极大提高药物利用率^[109]. 以铁蛋白为载体的生物纳米材料, 具有组织相容性高、易吸收、代谢性好等优点, 为肿瘤和氧化损伤等疾病的诊断和治疗提供了颠覆性新策略.

铁蛋白纳米粒子不仅在疾病的诊断与治疗方面有着极大的优势, 在预防医学领域也表现出巨大的应用潜力. 铁蛋白纳米粒子由于其独特的空间构象及耐高温等特点给疫苗的开发提供新的思路. 研究人员利用铁蛋白的笼状纳米结构, 将来源于卵清蛋白的OT-1和OT-2肽段搭载铁蛋白纳米颗粒中呈递于树突状细胞, 并成功诱导抗原特异性CD4或CD8阳性T细胞增殖^[110]. 也有研究人员将流感病毒血凝素HA融合入幽门螺旋杆菌铁蛋白亚基N端中, 自组装

形成融合蛋白,用这种改造的纳米铁蛋白免疫动物可以产生中和性抗体,具有流感疫苗的作用.同时他们还发现这种改造的新型疫苗与免疫佐剂联合使用时比传统的流感疫苗产生的抗体滴度更高,效力更强,免疫效应更广阔^[111].这对新疫苗的研发具有很好的借鉴作用.

超顺磁性 Fe_3O_4 磁性纳米粒子是常见的一种磁性纳米颗粒,因其独特的磁靶向性、良好的生物相容性及长半衰期等特性在生物医学领域受到广泛关注.将阿霉素结合到 Fe_3O_4 纳米药物载体系统中,在磁场控制下实现靶向药物输送^[112],这种新型的给药方式为精准医学发展提供可行模式. Fe_3O_4 磁性纳米粒子可以在外磁场作用下提高基因复合物转染细胞的效率^[113]而在基因治疗方面具有明显优势.将 Fe_3O_4 纳米粒子表面修饰钆可以制备T1-T2双模式磁共振造影剂,极大提高了肿瘤诊断的精确性,拓宽了磁共振在分子成像的应用^[114].将磁性 Fe_3O_4 纳米颗粒置于交变的磁场中时,由于自旋产热可以使肿瘤局部升温而破坏肿瘤细胞.利用这一特性,研究人员将磁性 Fe_3O_4 纳米粒子导入肿瘤组织中,在外源性交变磁场的作用下,不仅可以获得肿瘤磁共振成像,同时使得肿瘤组织局部升温达到有效温度可杀伤肿瘤细胞,而不损伤正常组织,实现诊断性治疗的目的^[115].

研究发现转铁蛋白结合纳米颗粒可有效识别并特异性杀伤拉莫斯癌细胞,而无需搭载其他抗肿瘤药物,为癌症的靶向治疗提供新途径^[116].青蒿素(Dihydroartemisinin, DHA)是一种特异性抗疟疾药物,近年来更是被用于肿瘤治疗.研究人员基于纳米氧化石墨烯(GO)双向装载青蒿素DHA和转铁蛋白(Transferrin, Tf),研制出一种新型纳米药物DHA-GO-Tf. Tf既可以作为三价铁的载体,也可将纳米药物靶向运送到高表达转铁蛋白受体(TfR)的肿瘤组织中.三价铁被还原为二价铁后可以与DHA反应而增强其靶向作用能力及对肿瘤细胞的杀伤作用^[117].钆纳米粒子因具有良好的生物相容性及易代谢性在肿瘤的光热疗法方面具有一定优势.利用转铁蛋白修饰钆纳米粒子合成纳米药物Tf-RuNPs,这种新型纳米药物具有更强的光热特性,具有更高的细胞药物吸收率及更好的肿瘤消融作用^[118],为肿瘤的治疗提供新的策略.

Polo样蛋白激酶Polo-like kinase1(PLK1)的小干扰RNA(siPLK1)可以穿过血脑屏障治疗神经胶质母细胞

瘤.为了增强其治疗效果,利用转铁蛋白修饰的纳米颗粒结合siPLK1合成Tf-PEG-PLL/MNP@siPLK1.这种新型化合物极大提高了穿透血脑屏障的效率,可以显著抑制神经胶质母细胞瘤生长,延长小鼠生存时间^[119].

转铁蛋白纳米颗粒不仅可以作为药物搭载系统靶向肿瘤治疗,也可以应用于磁共振造影剂.以转铁蛋白修饰传统造影剂钆形成新型纳米磁共振造影剂,对比传统造影剂,这种新型纳米造影剂具有更强的对比度,更少的钆用量,更强的穿透血脑屏障作用,是脑部核磁共振潜在的新型造影剂^[120].

5 展望

近年来,随着对铁代谢研究的不断深入,铁稳态代谢调控网络逐渐明晰,铁代谢领域的许多重大科学问题得到解决,铁元素在多种组织器官的生理意义及在神经退行性疾病、炎症及肿瘤等不同病理模型中的作用将逐渐被揭示.而铁代谢调控网络的深化研究也为铁稳态失衡相关疾病的药物研发奠定了理论基础.随着现代科技及生物医学检测技术的发展,越来越多铁代谢领域的创新性成果将不断涌现;平衡铁调素调控网络将成为分子靶向铁稳态失衡相关疾病诊治的重要策略.而基于铁稳态代谢重要分子研发的新型铁磁纳米材料也将在生命科学领域展示更广阔的应用前景.

展望铁代谢的未来研究方向,将更多集中于铁离子在脑、心脏、肾脏及胰腺等重要器官的生理与病理意义及作用分子机制研究.铁离子的亚细胞器精确定位与调控机制也是领域亟待解决的重大科学问题.同时,探索铁离子与其它离子间相互作用与调控网络,也将为铁代谢与人类健康提供全新思路与突破.

21世纪以来,随着社会经济的发展与医疗卫生事业的进步,人群健康数据库逐渐建立并越来越完善.基于人群健康信息的医学大数据统计分析 with 基础研究相结合已然成为未来医学科研发展的必然趋势;近年来大热的人工智能(artificial intelligence)为医学科研注入了新的生机与活力.铁稳态代谢研究与大数据分析及人工智能相结合,推动铁代谢科研从人群到基础再回到人群的研究模式是必然的未来方向.可以预见,铁代谢的研究必将随着大数据及人工智能时代的到来而进入一个崭新的阶段,将为精准医学快速发展提供重要科学依据.

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Summary for “铁稳态代谢分子机制及铁磁纳米颗粒研究进展”

Advances in iron homeostasis and ferromagnetic nanoparticles

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The essential trace element iron is a major structural component required for the synthesis and function of hemoglobin and various enzymes, and is therefore involved in many key physiological processes, including oxygen transport, nucleic acid synthesis, gene expression, and immune regulation. Iron homeostasis is therefore essential to maintaining health. For example, iron deficiency leads to anemia, whereas iron overload can lead to the formation of free radicals, which cause lipid peroxidation, DNA damage, and changes in other bioactive molecules. Thus, iron overload has been implicated in a wide range of diseases, including hemochromatosis, diabetes, cardiovascular disease, neurodegeneration, and cancer. Systemically, iron homeostasis is tightly regulated by several processes, including iron absorption in the intestine, iron circulation in the blood, iron utilization in various tissues, iron storage in the liver, and iron recycling in splenic red pulp macrophages. In recent years, new genetic animal models and cutting-edge technologies have led to considerable progress regarding the molecular mechanisms that underlie iron metabolism.

Ferroptosis, a recently identified iron-dependent form of cell death, has been implicated in several disease conditions, including acute kidney failure following ischemia/reperfusion, Huntington's disease, cancer, and neurodegeneration. Although no definitive biomarkers have been identified, lipid peroxidation, PTGS2 expression, and NADPH levels have been suggested as possible biomarkers of ferroptosis. Nevertheless, the regulatory mechanisms underlying ferroptosis are fairly complex and poorly understood.

Recently, our understanding of the pathways that regulate hepcidin, the master regulator of iron homeostasis, has increased considerably. Hepcidin regulates systemic iron levels by facilitating degradation of the sole iron exporter, Ferroportin, thereby controlling the absorption, utilization, and storage of iron. Hepcidin also cooperatively regulates iron homeostasis in response to various stimuli; specifically, iron overload and inflammation upregulate hepcidin expression, whereas erythropoiesis and hypoxic stress downregulate hepcidin expression. In addition, several epigenetic processes regulate hepcidin expression, thereby regulating iron metabolism. New studies are continually revealing novel molecular mechanisms related to the complex networks that regulate hepcidin, providing exciting new possibilities for treating iron-related diseases.

Iron-containing biological materials have also been studied for their clinical potential. For example, ferromagnetic nanoparticles, which are ferritin- or Fe₃O₄-containing biomacromolecules, have been applied in a wide variety of biomedical fields due to their superior magnetic properties and biocompatibility, making them ideal drug carriers. Importantly, nanoparticles readily penetrate the blood-brain barrier and enter the central nervous system, improving the bioavailability and therapeutic efficacy of drugs. Alternatively, an external magnetic field can be used to direct ferromagnetic nanoparticles to specific lesions. For example, a drug-containing transferrin nanoparticle complex could be used to target specific tumor cells due to their increased expression of the transferrin receptor compared to healthy tissue. Superparamagnetic iron oxide nanoparticles may also be a promising tool for destroying cancer cells using a rapidly alternating external magnetic field. Thanks to their wide range of properties and features, ferromagnetic nanoparticles offer a wealth of promising biomedical applications.

Here, we review the molecular mechanisms that regulate iron homeostasis, focusing on groundbreaking discoveries in hepcidin regulation and recent progress regarding biomedical applications for ferromagnetic nanoparticles.

iron homeostasis, iron-deficient anemia, iron overload, hemochromatosis, hepcidin, ferromagnetic nanoparticles

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