铜死亡在肝细胞癌中的研究进展

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摘要 铜死亡(cuproptosis)是新近发现的一种铜依赖性并与线粒体呼吸相关的程序性细胞死亡形式。过量的铜通过干扰线粒体三羧酸循环(tricarboxylic acid cycle, TCA)和引发蛋白质毒性应激的方式导致细胞死亡。铜代谢和铜死亡与肿瘤的发生发展密切关联, 而肝脏是人体内铜储存和排出的主要器官。在肝癌细胞中诱导铜死亡是一种很有前景的治疗方法, 探究铜死亡与肝细胞癌之间的联系, 有助于寻找新的有效治疗策略。因此, 该文讨论了铜死亡与肝细胞癌发生发展和预后的相关性, 并总结了与铜死亡相关的肝细胞癌治疗方法, 以期为肝细胞癌的诊断、治疗和研究提供参考。

关键词 铜死亡; 铜代谢; 肝细胞癌; 靶向治疗; 预后

Research Progress of Cuproptosis in Hepatocellular Carcinoma

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Abstract Cuproptosis is a newly discovered copper-dependent form of programmed cell death related to mitochondrial respiration. Excessive copper leads to cell death by interfering with mitochondrial tricarboxylic acid cycle and triggering protein toxic stress. Copper metabolism and cuproptosis are closely related to the occurrence and development of tumors. The liver is the main organ in the body where copper is stored and excreted. Inducing cuproptosis in hepatocellular carcinoma cells is a promising therapeutic approach, and exploring the relationship between cuproptosis and hepatocellular carcinoma is helpful to find new and effective therapeutic strategies. Therefore, this paper discusses the correlation between cuproptosis and the occurrence, development and prognosis of hepatocellular carcinoma, and summarizes the therapeutic methods of hepatocellular carcinoma related to cuproptosis, in order to provide references for the diagnosis, treatment and research of hepatocellular carcinoma.

Keywords cuproptosis; copper metabolism; hepatocellular carcinoma; targeted therapy; prognosis

肝癌是我国第四大常见恶性肿瘤和第二大肿瘤相关死亡原因, 肝细胞癌(hepatocellular carcinoma, HCC)是原发性肝癌最主要的类型, 乙型和丙型肝炎是其最常见的致病因素[1-2]。肝脏在铜代谢中起核心作用, 调控铜的生物过程以及合成和分泌铜结合蛋白, 铜代谢的失衡会对肝脏产生不利的影响。细胞内铜的浓度通常保持在相对较低的范围内, 增加铜浓度可引起细胞毒性, 甚至导致细胞死亡[3]。铜是人体必需的微量元素, 维持着酶的活性和转录因子的功能, 铜死亡是一种由细胞内铜的持续积累引起的

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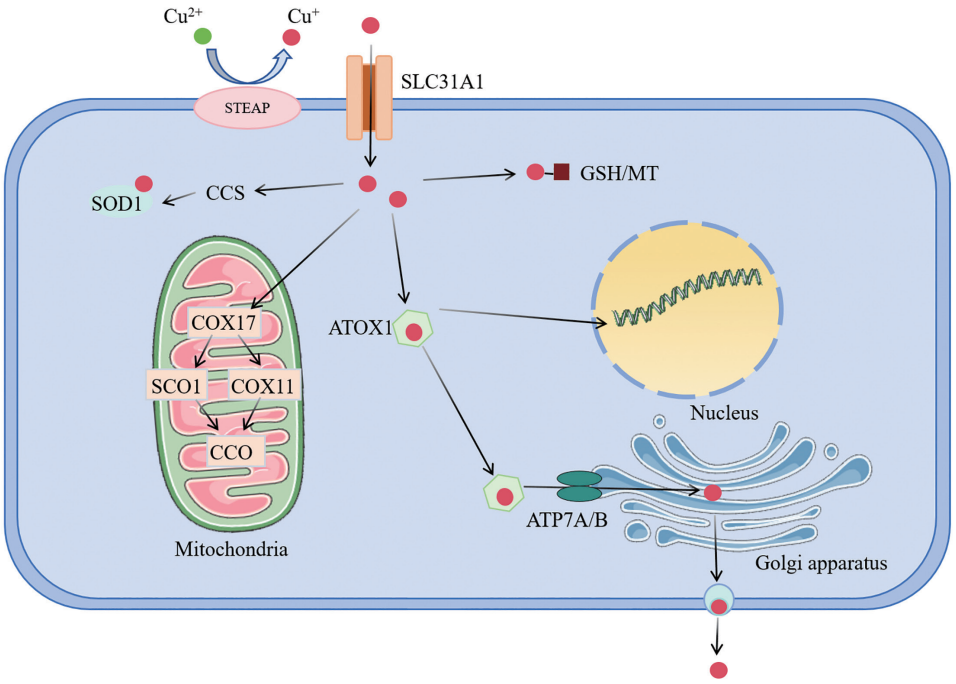
细胞死亡形式，其特征是阻断三羧酸循环和干扰线粒体呼吸[4-5]。铜在HCC中表现出了促癌和抑癌的双重作用，然而铜死亡与HCC的相关性尚需进一步探究，了解铜积累引起细胞毒性的分子机制是开发新的有效疗法的重要一步。本文就铜死亡在HCC的发生发展和治疗预后中的新近研究进展作一综述，旨在进一步探究两者的联系，为开发新疗法、评估治疗效果和构建预后模型提供思路。

1 铜的代谢

1.1 铜的吸收

铜是几乎所有生物体的基本元素，在线粒体呼吸、抗氧化防御和生物化合物合成等一系列生物过程中起着关键的催化辅助因子作用[6]。人体内的铜主要储存在肝脏中，由肠道吸收和胆汁排泄，细胞外的铜以Cu^{2+}的形式存在，这些铜不能直接被细胞利用[7]。在血液中，Cu^{2+}与铜蓝蛋白和白蛋白等血浆蛋白结合后被运输至细胞表面，在金属还原酶STEAP(six-transmembrane epithelial antigen of the prostate)的作用下被还原为Cu^{+}，随后Cu^{+}在铜转运蛋白溶质载体家族31成员1(solute carrier family 31 member 1, SLC31A1)即铜转运蛋白1(copper transporter 1, CTR1)的介导下被转运至细胞内[8]，进入细胞内的Cu^{+}与铜螯合物谷胱甘肽(glutathione, GSH)和金属硫蛋白(metallothionein, MT)螯合储存，或与不同的铜伴侣蛋白相结合而被转运到相应的亚细胞器发挥作用[9]。例如在细胞质中，超氧化物歧化酶铜伴侣(copper chaperone for superoxide dismutase, CCS)激活超氧化物歧化酶1(superoxide dismutase 1, SOD1)，CCS通过调控SOD1在细胞内的分布，保证了体内活性氧(reactive oxygen species, ROS)的平衡，防止了铜过载引起的氧化损伤，SOD1结合Cu^{+}以保护细胞免受自由基的损害，发挥了重要的抗氧化作用；在线粒体中，Cu^{+}与细胞色素C氧化酶(cytochrome c oxidase, CCO)相结合，参与呼吸链和氧化还原途径；在细胞核中，Cu^{+}结合转录因子调控基因表达[10-11](图1)。



Cu^{2+}被还原为Cu^{+}，Cu^{+}在SLC31A1/CTR1的转运下进入细胞，在与铜伴侣结合后被运送到到各种亚细胞器中发挥作用。SLC31A1/CTR1: 溶质载体家族31成员1/铜转运蛋白1; GSH: 谷胱甘肽; MT: 金属硫蛋白; CCS: 超氧化物歧化酶铜伴侣; SOD1: 超氧化物歧化酶1; ATOX1: 抗氧化蛋白1; ATP7A/B: 铜转运ATP酶。

Cu^{2+} is reduced to Cu^{+}, and Cu^{+} is transported into the cell by SLC31A1/CTR1. After binding to the copper partner, it is transported to various suborganelles to function. SLC31A1/CTR1: solute carrier family 31 member 1/copper transporter 1; GSH: glutathione; MT: metallothionein; CCS: copper chaperone for superoxide dismutase; SOD1: superoxide dismutase 1; ATOX1: antioxidant-1; ATP7A/B: copper-transporting ATPases.

图1 铜的代谢机制图

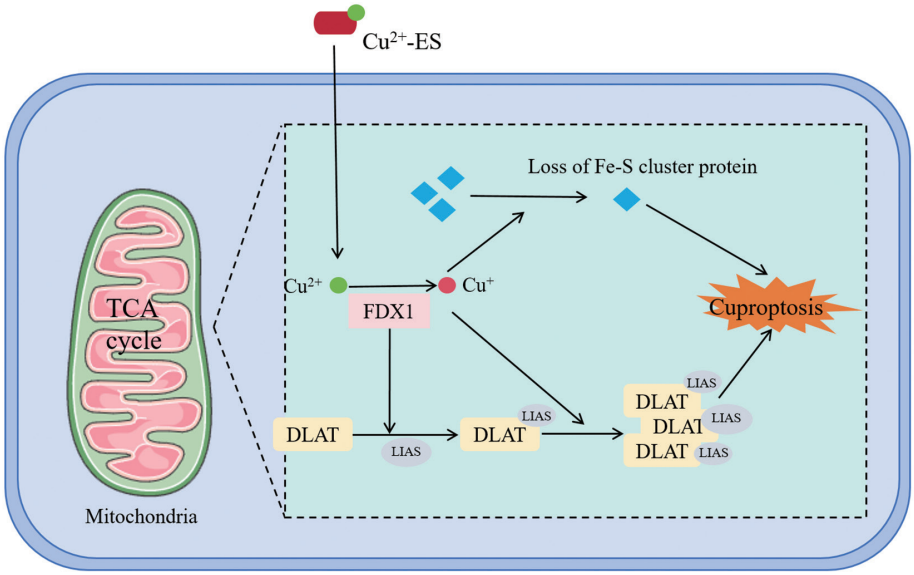
Fig.1 Mechanism diagram of copper metabolism

1.2 铜的排出

铜的排出是防止铜的持续积累从而产生细胞毒性的重要过程，抗氧化蛋白1(antioxidant-1, ATOX1)介导Cu^+从胞质转运到高尔基体反面网络(*trans* Golgi network, TGN)腔内，然后Cu^+与铜转运ATP酶(copper-transporting ATPases, ATP7A/B)相结合，以促进细胞内过量Cu^+的排出，ATP7A/B在铜排出的过程中起核心作用，Cu^+通过ATP7A/B从细胞质转运到TGN的管腔，当细胞内Cu^+的浓度增加或超过阈值时，ATP7A/B蛋白从TGN转移到质膜上，将Cu^+排出细胞[12]，这些蛋白质共同协调铜的吸收、排出以及细胞内利用，以控制细胞内铜的水平保持在特定的范围内。肝脏是体内铜储存和排出的主要器官，ATP7A在几乎所有细胞中都有表达，而ATP7B主要存在于肝脏中，肝脏通过ATP7B发挥功能将过量的铜排泄到胆汁中并使其最终离开体内，未被吸收的铜则从粪便中排出。ATP7A和ATP7B的功能障碍可分别导致机体出现铜缺乏与铜过载，引起严重的多系统疾病，如Menkes病[13]和Wilson病[14]。ATP7B将Cu^+从肝脏泵回血液中后，Cu^+再次与可溶性伴侣结合并转运至特定的组织和器官中。在到达靶组织后，铜催化广泛的生理反应，包括线粒体能量产生、神经递质代谢和细胞外基质重塑等[9]，铜的吸收、排出和利用共同维持机体的铜稳态。

2 铜死亡的发生机制

铜死亡是由于铜稳态的破坏而导致细胞出现铜过载后发生的一种程序性细胞死亡，TSVETKOV等[5]的研究证明了铜离子载体能诱导细胞死亡，其原因主要在于细胞内铜浓度的增加，铜离子载体如伊利司莫(elesclomol, ES)可以直接结合细胞外的Cu^{2+}并将其运输到细胞内，Cu^{2+}在铜死亡的中枢调节因子铁氧还蛋白1(ferredoxin 1, FDX1)编码的还原酶作用下被还原成Cu^+，细胞内过量的Cu^+在三羧酸循环中与脂酰化的线粒体蛋白(如DLAT)相结合，引起蛋白质的寡聚和Fe-S簇丢失，并产生ROS诱导氧化应激，这些异常过程共同作用最终导致细胞发生铜死亡(图2)。ES的直接靶点是FDX1，FDX1的缺失会增加细胞对铜死亡的抗性，Cu^{2+}-ES复合物能结合FDX1并抑制Fe-S簇的形成[15]，FDX1/LIAS是DLAT等线粒体蛋白质脂酰化的上游调节因子，能促进DLAT的脂酰化，脂酰化的DLAT在高铜水平的环境下显著增加，从而引发铜死亡[16]。细胞内铜的积累超过限度是引起铜死亡的直接原因，通过利用铜离子载体直接将Cu^{2+}送入细胞内、过表达铜转运蛋白SLC31A1/CTR1、抑制铜螯合物GSH的合成以及下



伊利司莫(ES)将Cu^{2+}运输到细胞内，Cu^{2+}在FDX1的作用下被还原为Cu^+，过量的Cu^+与脂酰化的线粒体蛋白相结合，阻断三羧酸循环，导致细胞铜死亡。FDX1: 铁氧还蛋白1; DLAT: 二氢硫辛酸转乙酰基酶; LIAS: 硫辛酸合成酶。

ES (elesclomol) tranships Cu^{2+} into the cell, Cu^{2+} is reduced to Cu^+ under the action of FDX1, and excess Cu^+ binds to fatty acylated mitochondrial proteins, blocking the TCA (tricarboxylic acid) cycle and causing cuproptosis. FDX1: ferredoxin 1; DLAT: dihydrolipoic acid transacetylase; LIAS: lipoic acid synthase.

图2 铜死亡的分子机制图

Fig.2 Molecular mechanism diagram of cuproptosis

调ATP7A/B蛋白的表达等方式提高细胞内Cu^{+}的浓度可诱导细胞铜死亡[17]。

3 铜死亡与肝细胞癌

3.1 铜死亡与肝细胞癌的发生发展

铜在HCC的发生发展中具有两面性。一方面，铜通过多种途径发挥促癌作用，细胞外较高的铜浓度通过调节MYC/CTR1轴促进HCC细胞的生长、迁移和侵袭[18]，敲低CTR1或使用铜螯合剂能减少糖酵解来阻碍HCC的发生，表明铜能够以影响糖代谢重编程的方式来调节肝癌的进展[19]。一项实验证明，*SOD1*基因敲除的小鼠由于肝细胞的氧化损伤而导致HCC的发生率升高，而铜浓度的升高可以刺激ROS产生从而增强肝细胞的氧化损伤，提示铜可能通过ROS依赖的机制促进HCC的发生[20]。HCC的发生和发展还依赖于再生血管提供的能量和氧气，铜转运蛋白CTR1促进血管内皮生长因子受体2型(vascular endothelial growth factor receptor type 2, VEGFR2)的信号转导，ATP7A限制VEGFR2降解，从而加快了HCC的新血管生成的进度[21-22]。缺氧诱导因子-1α(hypoxia inducible factor-1α, HIF-1α)与HCC的发病机制相关，HCC患者的血清HIF-1α水平与铜水平显著升高，且两者呈正相关[23]，铜通过影响HIF-1α的表达进一步促进HCC的血管生成，同样，铜在乳腺癌等肿瘤中亦表现出了促进血管生成的作用[24]。此外，Cu^{2+}介导的CD147的自结合激活了PI3K/Akt信号通路，还增强了HCC细胞刺激邻近的成纤维细胞基质金属蛋白酶2(matrix metalloproteinase 2, MMP-2)表达的能力，增加了HCC细胞的侵袭性，从而促进了肿瘤的转移[25]；肿瘤内铜水平会影响癌细胞中程序性死亡–配体1(programmed cell death-ligand 1, PD-L1)表达，补充铜可以增强肿瘤细胞PD-L1的表达，并调节PD-L1驱动的肿瘤免疫逃逸的关键信号通路[26]，从而促进免疫逃逸。另一方面，当铜持续积累超过机体的耐受阈值时，可导致各种形式的细胞死亡，包括细胞凋亡、焦亡、铁死亡和铜死亡等，从而发挥抑癌作用[5,27-29]。铜水平的升高已被证实可以促进肿瘤的发生和发展[30-31]，肝豆状核变性患者HCC发病率的增加提示铜过载会促进恶性转化[32-33]。临床证据表明，HCC患者的血清铜水平明显高于慢性肝炎患者，且铜浓度与疾病的进展和发病率相关[34]。研究发现，铜转运蛋白ATP7A/B、SLC31A1和SLC31A2的表达在肝癌样本中有显著改变，与HCC组织和细胞中铜水平的升高有关[19]，并且与患者较差的生存率相联系，这些证据提示铜在HCC的发病机制中可能具有致癌的作用(表1)。

3.2 铜死亡与肝细胞癌的治疗

铜死亡主要发生在利用氧化磷酸化作为首要供能途径的细胞中，而Warburg效应是肝癌发生发展中的标志性事件，即使在氧气充足条件下，肝癌细胞也更倾向于利用糖酵解而不是氧化磷酸化来产生中间代谢物和能量，从而能起到抵抗铜死亡的效果[35-36]。p53作为肿瘤抑制因子，可以抑制Warburg效应并在肝癌细胞中驱动糖酵解向氧化磷酸化的转换，同时p53调控铜离子转化途径中两个关键组分Fe-S簇和铜螯合物GSH的生成，在铜离子的转化过程中发挥作用[37]，

表1 铜在肝细胞癌发生发展中的双重作用

Table 1 The dual role of copper in the occurrence and development of hepatocellular carcinoma

功能 Function	相关因素 Related factors	方式 Approach	参考文献 References
Carcinogenic effect	MYC/CTR1	Promote tumor growth, migration and invasion	[18]
Carcinogenic effect	CTR1	Inhibit glycolysis	[19]
Carcinogenic effect	ROS	Lead to oxidative damage	[20]
Carcinogenic effect	VEGFR2	Promote angiogenesis	[21-22]
Carcinogenic effect	HIF-1α	Promote angiogenesis	[23]
Carcinogenic effect	PI3K/Akt	Increase invasiveness	[25]
Carcinogenic effect	PD-L1	Promote immune escape	[26]
Anticancer effect	Apoptosis, pyroptosis, ferroptosis, cuproptosis	Cause tumor cell death	[5,27-29]

CTR1: 铜转运蛋白1; ROS: 活性氧; VEGFR2: 血管内皮生长因子受体2型; HIF-1α: 缺氧诱导因子-1α; PI3K/Akt: 磷脂酰肌醇3-激酶/蛋白激酶B; PD-L1: 程序性死亡–配体1。

CTR1: copper transporter 1; ROS: reactive oxygen species; VEGFR2: vascular endothelial growth factor receptor type 2; HIF-1α: hypoxia inducible factor-1α; PI3K/Akt: phosphatidylinositol 3-kinase/protein kinase B; PD-L1: programmed cell death-ligand 1.

利用p53协同诱导铜死亡或许是一种有前途的根除肝癌细胞的策略，同时，驱动肝癌细胞的糖酵解向氧化磷酸化的转换也是配合铜死亡治疗的一项有效措施。

铜死亡相关基因(cuprotoisis-related genes, CRGs)在HCC等多种肿瘤中均有显著表达，参与和影响肝癌的进程，介导免疫逃逸[38]，具有成为HCC早期诊断、精确治疗和预后评估的重要标志物和新靶点的潜力。一项研究表明，在30个临床HCC标本中铜死亡相关基因*PDXK*的表达上调，敲除*PDXK*能削弱肝癌细胞的增殖和侵袭能力，沉默*PDXK*可增加肝癌细胞对铜死亡的敏感性[39]，另一研究基于3种铜死亡相关基因(*GCSH*、*LIPT1*和*CDKN2A*)建立了HCC预后模型，验证了*LIPT1*促进HCC细胞的生长、侵袭和迁移，敲低*LIPT1*的表达可抑制HCC细胞的增殖迁移[40]，证明了靶向铜死亡相关基因是有潜力的疗法之一。此外，铜死亡和铁死亡调控因子之间存在强烈的相互作用与联系，探寻两者之间的潜在联系和相关分子机制或许能发现更有效的HCC治疗策略[41]，铁死亡诱导剂通过抑制FDX1的降解增加线粒体蛋白的脂酰化，减少细胞内铜螯合物GSH的合成，并增加脂酰化蛋白的寡聚从而促进肝癌细胞发生铜死亡，铁死亡诱导剂联合铜离子载体是一种可行的HCC治疗方案[42]，同时，研究发现一种新型水溶性铜和葡萄糖酸盐复合物能通过触发细胞凋亡和铁死亡相关机制来抑制癌细胞生长[43]。利用铜与其他细胞死亡方式之间的协同作用诱导肿瘤细胞死亡或许也是开发抗癌疗法的一种有前景的策略。

铜是人体必不可少的微量元素之一，除了作为辅助因子维持细胞的正常功能以及在细胞内持续积累导致铜死亡以外，铜与部分药物的联合使用也表现出了对肿瘤具有积极的治疗效果。例如，曲克芦丁(troxerutin, TXER)单独使用对肝细胞癌Huh-7细胞没有显著的抗癌作用，而TXER与铜联合能通过产生自由基诱导Huh-7细胞广泛的细胞死亡，且对正常的肝细胞没有毒性作用[44]。双硫仑联合铜(DSF/Cu)抑制了PD-L1表达上调所引起的免疫抑制从而提高了抗肿瘤疗效，可有效抑制HCC细胞的迁移、侵袭和血管生成[45]，DSF/Cu会损害线粒体稳态导致细胞铁死亡，并增加HCC细胞对DSF/Cu诱导的铁死亡的敏感性，同时，DSF/Cu可以协同索拉非尼共同发挥细胞毒性作用[29]，阻止肿瘤生长。此外，铜螯合剂通过减少细胞内的可利用铜来抑制HCC的增殖和血管生成，例如，曲恩汀通过减少白细胞介素-8(interleukin-8, IL-8)的分泌阻止铜作为辅助因子在血管生成中发挥作用[46]，从而减少新血管的生成，阻碍癌症的进展。这表明抑制新血管的生成也是HCC的有效疗法之一。

3.3 铜死亡与肝细胞癌的预后

铜死亡相关基因的表达与HCC的预后密切相关[47-49]。铜离子载体ES通过促进DLAT的寡聚诱导铜死亡作为抗癌药物，DLAT的高表达伴随着PD-L1的高表达，与不良预后相关[50-51]，下调DLAT的表达可有效抑制铜死亡，DLAT与HCC的临床分期和分级呈正相关，且在HCC组织中高表达，可以帮助确定肝癌的预后，是有前途的预后标志物[52]。一项研究构建了由铜死亡相关基因*CDKN2A*、*DLAT*、*DLST*、*GLS*和*PDHA1*组成的预后模型，该模型用于预测HCC患者的生存可能性，在HCC细胞系中这5种与预后关联基因的表达量显著升高，与预后不良有关[53]。采用铜死亡风险模型对患者进行评分，发现高危组和低危组患者的预后出现显著差异，表明铜死亡风险模型评分可以作为HCC患者的独立预后生物标志物[54]。此外，WEI等[55]的研究发现IRSp53家族成员BAIAP2L2蛋白在HCC中过表达，并促进HCC细胞的侵袭迁移，是新型的HCC预后标志物，可能是潜在的治疗靶点，通过调节铜死亡来影响HCC的预后。利用铜死亡相关基因构建HCC预后模型是评估预后的可行手段，铜死亡及其相关基因与HCC的联系诸多，其具体机制尚需进一步发掘。

长链非编码RNA(long non-coding RNA, lncRNA)通过调节癌细胞的生物学行为参与HCC的发生和进展，然而铜死亡相关的lncRNA的临床意义尚不清楚。 基于4个参与铜死亡发展的预后特征lncRNA(AL590705.3、LINC02870、KDM4A-AS1和MKLN1-AS)构建模型，根据风险评分分组，低危组和高危组之间1、3、5年生存率出现差别，且在免疫细胞亚群和免疫检查点基因的表达等方面均存在显著差异，该模型为具有不同风险的个体提供了不同的治疗建议[56]。基于铜死亡相关的lncRNA构建的HCC预后模型展示出了良好的准确性，表现出了评估HCC患者预后的能力[57-58]，可用于预后预测和免疫评估，为免疫治疗提供参考[59]。铜死亡相关的lncRNA与HCC的肿瘤免疫微环境、肿瘤免疫治疗的疗效以及关键免疫检查点的表达有关，并且可以预测临床免疫治疗的

反应和帮助筛选HCC的潜在敏感药物[60]。利用铜死亡相关lncRNA或许能构建出更精准的预后评估模型，推动临床个体化医疗的发展。

4 总结与展望

人体内的铜稳态是由多个维度协调的，铜稳态的失衡会引起各种相关疾病的发生。铜死亡与肝癌的发生发展以及治疗、预后密切相关，靶向铜死亡相关基因亦是探索有效疗法的新途径，铜与部分治疗药物的联合使用已表现出对肿瘤良好的疗效，是有前景的用药策略。目前HCC的治疗已广泛采用手术、放疗等多种治疗方法，但患者的5年生存率和生活质量并未得到明显改善。铜死亡在HCC中的研究任重而道远，未来可尝试关联其他基因以构建更准确的治疗和预后模型，开发效果更佳的铜协同药物以及推出个体化治疗方案，继续深入探究铜死亡与HCC之间的联系和作用，挖掘潜在的分子机制，为更多HCC患者带来福音。

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