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右美托咪定调节miR-199a-5p/G6PD通路诱导铁死亡抑制结直肠癌细胞恶性生物学行为

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摘要 该文探讨右美托咪定(DEX)对结直肠癌细胞增殖、迁移、侵袭及铁死亡的影响, 并阐明miR-199a-5p/葡萄糖-6-磷酸脱氢酶(G6PD)轴在其中的潜在作用机制。选用人结直肠癌细胞系HT29和SW480进行体外实验。CCK-8法检测细胞活力; 克隆形成实验检测细胞增殖能力; 划痕实验和Transwell实验分别检测细胞迁移和侵袭能力; 蛋白质免疫印迹法检测上皮−间质转化(EMT)相关蛋白上皮型钙黏蛋白(E-cadherin)、神经型钙黏蛋白(N-cadherin)和蜗牛家族转录抑制因子1(Snail1)及铁死亡相关蛋白谷胱甘肽过氧化物酶4(GPX4)、溶质载体家族7成员11(SLC7A11)、核因子E2相关因子2(NRF2)和转铁蛋白受体蛋白1(TFRC)的表达水平; 荧光探针法检测细胞内活性氧(ROS)水平; 比色法检测谷胱甘肽(GSH)含量; 荧光探针法检测细胞内Fe^{2+}水平; 铁死亡抑制剂Fer-1(ferrostatin-1)进行干预验证。实时荧光定量PCR检测miR-199a-5p及*G6PD*的mRNA表达水平; 双荧光素酶报告实验验证miR-199a-5p与*G6PD*的靶向关系。细胞分组如下: 对照组(Con)、DEX处理组(100 ng/mL, 24 h)、DEX+miR-199a-5p模拟物组、DEX+miR-199a-5p抑制剂组、DEX+G6PD过表达组及相应的阴性对照转染组。与对照组相比, DEX处理组HT29和SW480细胞的活力、克隆形成数量、迁移及侵袭细胞数均显著降低(均$P<0.01$), E-cadherin蛋白表达水平显著升高($P<0.001$), N-cadherin和Snail1蛋白表达水平显著降低(均$P<0.001$); ROS荧光强度和Fe^{2+}荧光强度均显著升高(均$P<0.001$), GSH含量显著降低($P<0.001$); GPX4、SLC7A11和NRF2蛋白表达水平均显著降低(均$P<0.001$), TFRC蛋白表达水平显著升高($P<0.001$); Fer-1干预可显著逆转DEX诱导的细胞活力下降($P<0.001$)。与对照组相比, DEX处理组miR-199a-5p表达水平显著升高($P<0.001$), *G6PD* mRNA表达水平显著降低($P<0.001$); 双荧光素酶报告实验证实miR-199a-5p可直接靶向结合*G6PD* mRNA的3′-非翻译区。与DEX处理组相比, G6PD过表达组细胞活力、克隆形成数量、迁移及侵袭细胞数均显著升高(均$P<0.01$), E-cadherin蛋白表达水平显著降低($P<0.001$), N-cadherin和Snail1蛋白表达水平均显著升高(均$P<0.001$), ROS荧光强度和Fe^{2+}荧光强度均显著降低(均$P<0.001$), GSH含量显著升高($P<0.001$), GPX4、SLC7A11和NRF2蛋白表达水平均显著升高(均$P<0.001$), TFRC蛋白表达水平显著降低($P<0.001$)。DEX可上调结直肠癌细胞中miR-199a-5p表达, 进而靶向抑制G6PD, 破坏细胞抗氧化防御并诱导铁死亡, 而铁死亡的激活是DEX抑制结直肠癌细胞增殖、迁移和侵袭的关键中介机制。该结果提示miR-199a-5p/G6PD轴是DEX诱导铁死亡并发挥抗结直肠癌作用的潜在调控节点。

关键词 右美托咪定; 铁死亡; 结直肠癌; miR-199a-5p; G6PD

Dexmedetomidine Regulates the miR-199a-5p/G6PD Pathway to Induce Ferroptosis and Suppress Malignant Phenotypes in Colorectal Cancer Cells

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Abstract This work was to investigate the effects of DEX (dexmedetomidine) on proliferation, migration, invasion, and ferroptosis of colorectal cancer cells, and to elucidate the potential mechanisms of the miR-199a-5p/G6PD (glucose-6-phosphate dehydrogenase) axis in this process. Human colorectal cancer cell lines HT29 and SW480 were used for *in vitro* experiments. Cell viability was assessed by CCK-8 assay; colony formation assay was used to evaluate cell proliferation; wound healing and Transwell assays were performed to detect cell migration and invasion, respectively; Western blot was employed to detect the expression levels of EMT (epithelial-mesenchymal transition)-related proteins, including E-cadherin (epithelial cadherin), N-cadherin (neural cadherin), and Snail1 (snail family transcriptional repressor 1), as well as ferroptosis-related proteins, including GPX4 (glutathione peroxidase 4), SLC7A11 (solute carrier family 7 member 11), NRF2 (nuclear factor erythroid 2-related factor 2), and TFRC (transferrin receptor protein 1); intracellular ROS (reactive oxygen species) levels were measured by fluorescence probe method; GSH (glutathione) content was detected by colorimetric method; intracellular Fe^{2+} levels were measured by fluorescence probe method; the ferroptosis inhibitor Fer-1 (ferrostatin-1) was used for intervention validation. The mRNA expression levels of miR-199a-5p and *G6PD* were detected by real-time quantitative PCR; dual-luciferase reporter assay was performed to verify the targeting relationship between miR-199a-5p and *G6PD*. Cell groups were as follows: control group (Con), DEX-treated group (100 ng/mL, 24 h), DEX+miR-199a-5p mimic group, DEX+miR-199a-5p inhibitor group, DEX+G6PD overexpression group, and corresponding negative control transfection groups. Compared with the control group, the DEX-treated group showed significantly decreased cell viability, colony formation number, and numbers of migrating and invading cells in both HT29 and SW480 cells (all $P<0.01$), significantly increased E-cadherin protein expression level ($P<0.001$), and significantly decreased N-cadherin and Snail1 protein expression levels (both $P<0.001$); ROS fluorescence intensity and Fe^{2+} fluorescence intensity were significantly increased (both $P<0.001$), while GSH content was significantly decreased ($P<0.001$); the protein expression levels of GPX4, SLC7A11, and NRF2 were significantly decreased (all $P<0.001$), whereas TFRC protein expression level was significantly increased ($P<0.001$); Fer-1 intervention significantly reversed the DEX-induced decrease in cell viability ($P<0.001$). Compared with the control group, the DEX-treated group exhibited significantly increased miR-199a-5p expression level ($P<0.001$) and significantly decreased *G6PD* mRNA expression level ($P<0.001$); dual-luciferase reporter assay confirmed that miR-199a-5p could directly target the 3′-untranslated region of *G6PD* mRNA. Compared with the DEX-treated group, the G6PD overexpression group showed significantly increased cell viability, colony formation number, and numbers of migrating and invading cells (all $P<0.01$), significantly decreased E-cadherin protein expression level ($P<0.001$), and significantly increased N-cadherin and Snail1 protein expression levels (both $P<0.001$); ROS fluorescence intensity and Fe^{2+} fluorescence intensity were significantly decreased (both $P<0.001$), while GSH content was significantly increased ($P<0.001$); the protein expression levels of GPX4, SLC7A11, and NRF2 were significantly increased (all $P<0.001$), whereas TFRC protein expression level was significantly decreased ($P<0.001$). DEX upregulates miR-199a-5p in colorectal cancer cells, which in turn targets and suppresses G6PD, thereby disrupting cellular antioxidant defense and inducing ferroptosis. Activation of ferroptosis serves as a key mediating mechanism through which DEX inhibits the proliferation, migration, and invasion of colorectal cancer cells. These findings suggest that the miR-199a-5p/G6PD axis is a potential regulatory node underlying the ferroptosis-inducing and anti-colorectal cancer effects of DEX.

Keywords dexmedetomidine; ferroptosis; colorectal cancer; miR-199a-5p; G6PD

结直肠癌是全球常见恶性肿瘤之一, 发病率和死亡率均位居前列, 患者在接受手术、化疗及免疫治疗后仍存在疗效有限、易复发及耐药等问题[1]。因此, 寻找作用于新靶点的潜在药物具有重要的临床意义。铁死亡是一种铁依赖性程序性细胞死亡[2]。肿瘤细胞可通过多种机制逃避铁死亡, 从而促进肿瘤进展和耐药, 而诱导铁死亡可增强肿瘤对治疗的敏感性[3-4]。结直肠癌细胞通常表现出细胞内铁水平升高及脂质代谢紊乱等特征, 使其对铁死亡尤为敏感[5], 提示铁死亡可能成为耐药性结直肠癌治疗中的一个有前景的靶点。

右美托咪定(dexmedetomidine, DEX)是一种高选择性的α2肾上腺素能受体激动剂[6]。近年来研究发现, DEX不仅具有镇静镇痛作用, 还可能在抑制肿瘤中发挥作用, 包括抑制肿瘤细胞的增殖和转移[7-9]。尽管有研究关注DEX与肿瘤细胞死亡的关系, 但其在结直肠癌中是否通过铁死亡发挥作用尚不明确。

微小RNA(microRNA, miRNA)可在转录后水平调控靶基因表达, 参与肿瘤增殖、迁移、侵袭及多种细胞死亡过程[10]。其中有研究表明, miR-199a-5p在结直肠癌组织和细胞中显著下调[11], 但是miR-199a-5p与铁死亡之间的关系目前尚未可知。基于以上背景, 本研究拟探究miR-199a-5p是否通过靶向葡萄糖-6-磷酸脱氢酶(glucose-6-phosphate dehydrogenase, G6PD)参与DEX诱导的结直肠癌细胞铁死亡, 并进一步分析该通路对细胞增殖、迁移和侵袭的影响。G6PD是磷酸戊糖途径的限速酶, 参与NADPH生成和氧化应激防御[12]。在结直肠癌中G6PD可促进细胞增殖、迁移和侵袭[13-14]。然而, miR-199a-5p是否通过靶向G6PD参与结直肠癌的病理进展仍不清楚。

基于此, 本研究旨在探讨DEX对结直肠癌细胞增殖、迁移、侵袭及铁死亡的影响, 并进一步阐明miR-199a-5p及其下游靶基因*G6PD*在其中的调控机制, 为结直肠癌的治疗提供新的理论依据和潜在干预靶点。

1 方法

1.1 试剂与材料

DEX(货号: D407960)购自上海阿拉丁生化科技股份有限公司; 活性氧(reactive oxygen species, ROS)检测试剂盒(货号: S0033S)、谷胱甘肽(glutathione, GSH)检测试剂盒(货号: S0053)、噻吟霉素(货号: ST1007)、胎牛血清(fetal bovine serum, FBS)(货号: C0235)购自上海碧云天生物技术有限公司; Fer-1(ferrostatin-1)(货号: S7243)购自美国Selleck Chemicals公司; 铁离子(Fe^{2+})检测试剂盒(货号: EEA008)购自美国ThermoFisher Scientific公司; TRIzol试剂(货号: 15596026)、Lipofectamine 2000(货号: 11668019)购自美国Invitrogen公司; 逆转录试剂盒(货号: R312-01)购自南京诺唯赞生物科技股份有限公司; CCK-8试剂盒(货号: CK04)购自日本Dojindo Laboratories公司; RPMI-1640培养基(货号: 21870100)购自美国Gibco公司; Transwell小室(货号: PIHP01250)购自美国Millipore公司; 结直肠癌细胞系HT29(货号: HTB-38)、SW480(货号: CCL-228)购自美国典型培养物保藏中心(American Type Culture Collection, ATCC); G5PD(货号: 12263, 1:1 000)、上皮型钙黏蛋白(epithelial cadherin, E-cadherin)(货号: 14472S)购自美国Cell Signaling Technology公司; 核因子E2相关因子2(nuclear factor erythroid 2-related factor 2, NRF2)(货号: AB62352)、转铁蛋白受体蛋白1(transferrin receptor protein 1, TFRC)(货号: AB214039)购自英国Abcam公司; 谷胱甘肽过氧化物酶4(glutathione peroxidase 4, GPX4)(货号: A11243)、溶质载体家族7成员11(solute carrier family 7 member 11, SLC7A11)(货号: A2413)、HRP标记的山羊抗鼠IgG(H+L)(货号: AS003)、HRP标记的山羊抗兔IgG (H+L)(货号: AS014)购自武汉爱博泰克生物科技有限公司; 蜗牛家族转录抑制因子1(snail family transcriptional repressor 1, Snail)(货号: 113099-1-AP)、甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH)(货号: 60004-1-IgG)购自美国Proteintech公司; 神经型钙黏蛋白(neural cadherin, N-cadherin)(货号: sc-59987)购自美国Santa Cruz Biotechnology公司。

1.2 细胞培养、转染与分组

细胞培养: 结直肠癌细胞系HT29和SW480培养于含10% FBS的RPMI-1640培养基中, 并在37 ℃、5% CO_2的恒温培养箱中培养。

细胞转染: 使用含有G6PD的cDNA载体过表达G6PD。将HT29和SW480细胞以1×10^5/孔的密度接种于6孔板中, 当细胞融合度达到70%~80%时, 采用Lipofectamine 2000按照说明书进行转染。转染48 h后, 加入2 μg/mL噻吟霉素进行抗性筛选, 并持续培

养10~14 d, 以获得稳定过表达G6PD的结直肠癌细胞系。

miR-199a-5p模拟物和miR-199a-5p抑制剂及相应阴性对照(NC)均由上海吉玛基因科技有限公司合成。转染方法同上。

细胞实验分组如下。Con组: 未转染, 加入等体积DMSO; DEX组: 未转染, 加入100 ng/mL DEX处理24 h; DEX+G6PD组: 稳定过表达G6PD后加入100 ng/mL DEX处理24 h; DEX+NC组: 转染阴性对照序列, 加入100 ng/mL DEX处理24 h; DEX+miR-199a-5p组: 转染miR-199a-5p模拟物, 加入100 ng/mL DEX处理24 h; DEX+anti-miR-199a-5p组: 转染miR-199a-5p抑制剂, 加入100 ng/mL DEX处理24 h。

1.3 CCK-8实验

将HT29和SW480细胞(Con组、DEX组、DEX+G6PD组)以1 000个/孔的密度接种于96孔板中[15], 细胞贴壁后给予相应处理。分别在处理1、12和24 h后, 每孔加入10 μL CCK-8试剂, 37 ℃孵育1 h。使用酶标仪在450 nm波长下测定吸光度(*D*)值, 以评估细胞活力。

1.4 克隆形成实验

将HT29和SW480细胞(Con组、DEX组、DEX+G6PD组)以500个/孔的浓度接种在6孔板中[15], 细胞贴壁后给予相应处理。连续培养10~14 d, 直至形成直径≥0.3 mm的克隆。随后用4%多聚甲醛室温固定15 min, 0.1%结晶紫染色20 min, 清水洗涤并晾干。拍照并使用ImageJ软件统计克隆数量。

1.5 ROS生成、GSH活性和Fe^{2+}水平的测定

DCFH-DA荧光探针检测ROS水平: 将各组HT29或SW480细胞培养至适当状态后, 用无血清培养基将DCFH-DA稀释至10 μmol/L, 弃去原培养基后加入探针, 37 ℃孵育20 min, 随后通过酶标仪测定荧光强度。实验结果以对照组荧光强度为100%, 各处理组ROS水平相对对照组的百分比表示(% of Con)。

GSH含量检测: 将各组HT29和SW480细胞以6 000个/孔的密度接种于96孔板中, 细胞贴壁后给予相应处理。次日弃去培养基, 向每个孔加入100 μL 1× GSH-GLO试剂, 并在室温下培养30 min。随后, 每孔加入100 μL重组的Luciferin检测试剂, 轻微混合, 并在室温下培养15 min, 通过酶标仪检测发光强度。

Phen Green™ SK探针检测Fe^{2+}水平: 将各组HT29或SW480细胞培养至适当状态后, 在37 ℃下与Phen Green™ SK溶液一起孵育30 min。收集细胞悬液进行离心(3 000 r/min室温离心10 min), 并用不含血清的培养基洗涤后重悬于PBS中, 通过酶标仪检测发光强度。

1.6 Transwell实验

采用Transwell小室评估结直肠癌细胞的迁移和侵袭能力。在侵袭实验中, 上室预先包被Matrigel, 迁移实验不包被。将HT29和SW480细胞(4×10^4/孔)接种于上室[15], 并在下室中加入含10% FBS的RPMI-1640培养基。细胞培养24 h后, 用4%多聚甲醛室温固定15 min, 并用0.1%结晶紫染色。最后, 在显微镜下计数迁移和侵袭的细胞。

1.7 划痕愈合实验

将HT29和SW480细胞(Con组、DEX组、DEX+G6PD组)以每孔5×10^5个细胞的密度接种于6孔板中[15], 培养至细胞汇合度达到90%。使用无菌200 μL的移液器吸头在孔中央垂直划痕, PBS轻洗去除悬浮细胞, 加入无血清培养基。分别于0 h和48 h拍照记录划痕宽度变化。

1.8 Western blot分析蛋白质表达水平

通过RIPA裂解液提取HT29和SW480细胞的总蛋白。经BCA法定量后, 取30 μg蛋白进行SDS-PAGE电泳分离, 并将蛋白转移至聚偏二氟乙烯膜上。将膜在含5%脱脂奶粉中室温封闭1 h。随后, 将膜与Snail1(1:800)、E-cadherin(1:1 500)、N-cadherin(1:1 500)、GPX4(1:1 500)、SLC7A11(1:1 500)、NRF2(1:1 000)、TRFC(1:1 000)、G6PD(1:1 000)、GAPDH一抗(1:5 000)于4 ℃孵育过夜。用TBST洗涤3次后, 将膜二抗(1:5 000)在室温下孵育1.5 h。滴加ECL发光液进行曝光, 并将GAPDH作为对照, 使用ImageJ软件对图像灰度进行分析。

1.9 实时荧光定量逆转录聚合酶链式反应(quantitative real-time reverse transcription polymerase chain reaction, qRT-PCR)

按照说明书要求, 使用TRIzol试剂提取HT29和SW480细胞(Con组、DEX组、DEX+G6PD组、DEX+NC组、DEX+miR-199a-5p组、DEX+anti-miR-199a-5p组)总RNA, 使用分光光度计检测RNA浓度后进行逆转录。引物序列见表1。mRNA表达水平采用SYBR Green I染料法进行检测。使用$2^{-\Delta\Delta Ct}$法分析数据。*G6PD*的表达以*β-actin*作为内参基因进行归一化分析; miR-199a-5p的表达以*U6*作为内参

表1 qRT-PCR引物序列

Table 1 Sequences of primers for qRT-PCR

基因 Gene	引物序列 Primer sequence
G6PD	Forward: 5′-AAA CGG TCG TAC ACT TCG GG-3′
	Reverse: 5′-GGT AGT GGT CGA TGC GGT AG-3′
miR-199a-5p	Forward: 5′-TCA AGA GCA ATA ACG AAA AAT GT-3′
	Reverse: 5′-GCT GTC AAC GAT ACG CTA CGT-3′
β-actin	Forward: 5′-CAT GTA CGT TGC TAT CCA GGC-3′
	Reverse: 5′-CTC CTT AAT GTC ACG CAC GAT-3′
U6	Forward: 5′-GCT TCG GCA GCA CAT ATA CTA AAA T-3′
	Reverse: 5′-CGC TTC ACG AAT TTG CGT GTC AT-3′

基因进行归一化分析。

1.10 双荧光素酶报告实验

为了进行荧光素酶报告基因检测, 首先将包含miR-199a-5p预测结合位点的*G6PD* 3′-非翻译区(3′-untranslated region, 3′-UTR)片段克隆至pGL3-control荧光素酶报告载体下游, 构建野生型报告质粒(*G6PD*-WT)。随后, 采用QuikChange定点突变试剂盒对*G6PD* 3′-UTR中miR-199a-5p的结合位点进行突变, 构建突变型报告质粒(*G6PD*-MUT)。

将HT29和SW480细胞分别接种于24孔板中, 当细胞融合度达到70%~80%时, 使用Lipofectamine 2000按照说明书进行共转染。转染分组如下。HT29细胞, NC组: 转染阴性对照序列, 同时转染*G6PD*-WT或*G6PD*-MUT报告质粒; miR-199a-5p组: 转染miR-199a-5p模拟物, 同时转染*G6PD*-WT或*G6PD*-MUT报告质粒。SW480细胞, NC组: 转染阴性对照序列, 同时转染*G6PD*-WT或*G6PD*-MUT报告质粒; anti-miR-199a-5p组: 转染miR-199a-5p抑制剂, 同时转染*G6PD*-WT或*G6PD*-MUT报告质粒。每组均共转染Renilla荧光素酶载体作为内参, 用于校正转染效率差异。

转染48 h后, 收集细胞并使用双荧光素酶报告基因检测系统按照说明书进行检测。分别测定萤火虫荧光素酶和Renilla荧光素酶活性, 并以萤火虫荧光素酶活性与Renilla荧光素酶活性的比值作为相对荧光素酶活性。

1.11 统计数据

所有实验至少重复3次。数据以均值±标准差($\bar{x}\pm s$)表示。采用GraphPad Prism 8.0软件进行统计分析。两组间比较采用独立样本Student's *t*检验; 多组间总体比较采用单因素方差分析(One-Way ANOVA), 组间两两比较采用Tukey事后检验。$P<0.05$认为差异具有统计学意义。

2 结果

2.1 DEX对结直肠癌细胞增殖、迁移和侵袭的影响

DEX的化学结构如图1A所示。CCK-8结果显示, 与对照组相比, 1 ng/mL DEX处理HT29和SW480细胞1 h、12 h、24 h后, 细胞活力无显著差异。10 ng/mL DEX处理24 h后, HT29和SW480细胞活力[HT29: (85.11±7.21)%; SW480: (89.23±3.22)%]显著降低($P<0.01$)。然而, 当100 ng/mL DEX处理24 h后, HT29和SW480细胞活力[HT29: (71.15±3.28)%; SW480: (60.13±10.12)%]极显著降低($P<0.001$); 因此, 在后续研究中采用100 ng/mL DEX和24 h作为DEX的处理条件(图1B)。

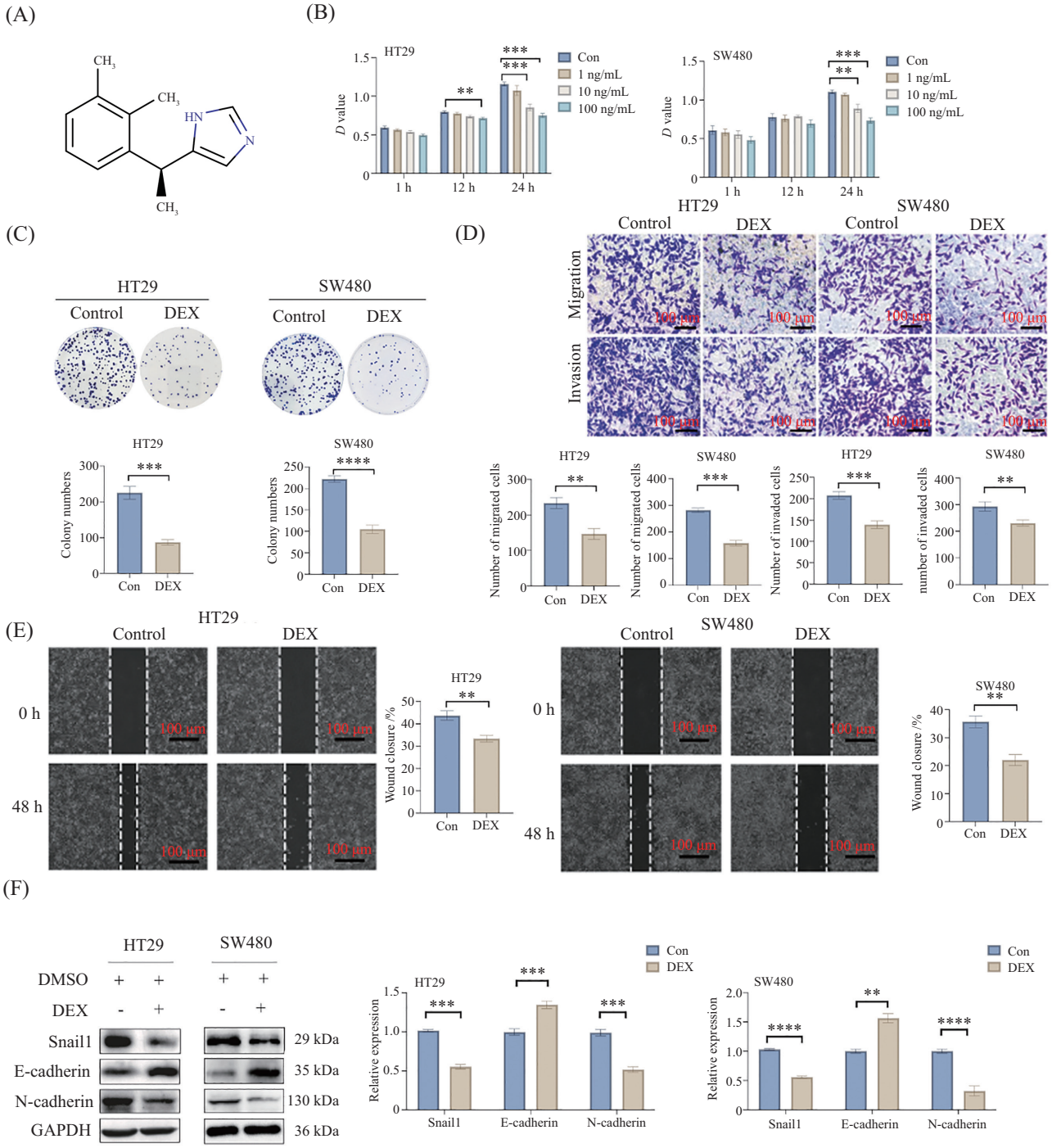
克隆形成实验(图1C)证明, 与对照组相比, DEX处理极显著降低了克隆形成[HT29: (81.45±8.29)%; SW480: (101.45±5.12)%]的数量($P<0.001$)。

此外, Transwell迁移/侵袭实验和划痕实验表明, 与对照组相比, DEX处理后HT29(侵袭: 141.67±9.33; 迁移: 137.29±7.67)和SW480细胞(侵袭: 201.45±4.51; 迁移: 133.25±4.17)的迁移和侵袭能力显著降低($P<0.01$, 图1D和图1E)。

使用Western blot检测EMT相关蛋白的表达变化, 结果显示DEX处理极显著下调了Snail1(HT29: 0.61±0.16; SW480: 0.56±0.11)和N-cadherin(HT29: 0.55±0.15; SW480: 0.32±0.07)的表达, 同时上调了E-cadherin(HT29: 1.31±0.28; SW480: 1.63±0.18)的表达($P<0.001$, 图1F)。

2.2 DEX对结直肠癌细胞铁死亡的影响

结果显示, DEX处理极显著增加了HT29



A: DEX的化学结构示意图; B: CCK-8实验检测HT29和SW480细胞经不同浓度DEX(1、10、100 ng/mL)处理1、12或24 h后的细胞活力; C: 克隆形成实验检测DEX处理对HT29和SW480细胞克隆的影响; D: Transwell迁移/侵袭实验检测DEX处理对HT29和SW480细胞迁移侵袭能力的影响; E: 伤口愈合实验显示DEX处理对HT29和SW480细胞迁移能力的影响; F: Western blot检测DEX处理后EMT相关蛋白的变化。**P<0.01, ***P<0.001, ****P<0.000 1。n=3。

A: schematic diagram of the chemical structure of DEX; B: CCK-8 assay was used to detect cell viability of HT29 and SW480 cells treated with different concentrations of DEX (1, 10, 100 ng/mL) for 1, 12 or 24 h; C: colony formation assay was performed to detect the effect of DEX treatment on colony formation of HT29 and SW480 cells; D: Transwell migration and invasion assays were used to evaluate the effect of DEX treatment on migration and invasion abilities of HT29 and SW480 cells; E: wound healing assay showing the effect of DEX treatment on the migration capacity of HT29 and SW480 cells; F: Western blot analysis of the changes in EMT-related proteins after DEX treatment. **P<0.01, ***P<0.001, ****P<0.000 1. n=3.

图1 DEX抑制结直肠癌细胞的增殖、迁移和侵袭

Fig.1 DEX inhibits proliferation, migration and invasion of colorectal cancer cells

(213.22±3.26)和SW480(219.45±10.29)细胞中的ROS水平(P<0.001, 图2A), 同时极显著降低了细胞内GSH(HT29: 52.33±4.22; SW480: 45.33±3.44)活性(P<0.001, 图2B)。

进一步检测了细胞内Fe^{2+}水平, 结果表明, DEX处理极显著促进了HT29(195.33±1.22)和SW480(171.45±3.67)细胞内Fe^{2+}的积累(P<0.001, 图2C)。

蛋白质免疫印迹(Western blot)进一步显示, DEX处理极显著下调了抗铁死亡相关蛋白GPX4(HT29: 0.21±0.03; SW480: 0.44±0.07)、SLC7A11(HT29: 0.33±0.05; SW480: 0.26±0.04)和NRF2(HT29: 0.61±0.04; SW480: 0.47±0.08)的表达, 而促铁死亡相关蛋白转铁蛋白受体TFRC(HT29: 2.33±0.33; SW480: 2.61±0.17)的表达则极显著上调(P<0.001, 图2D)。

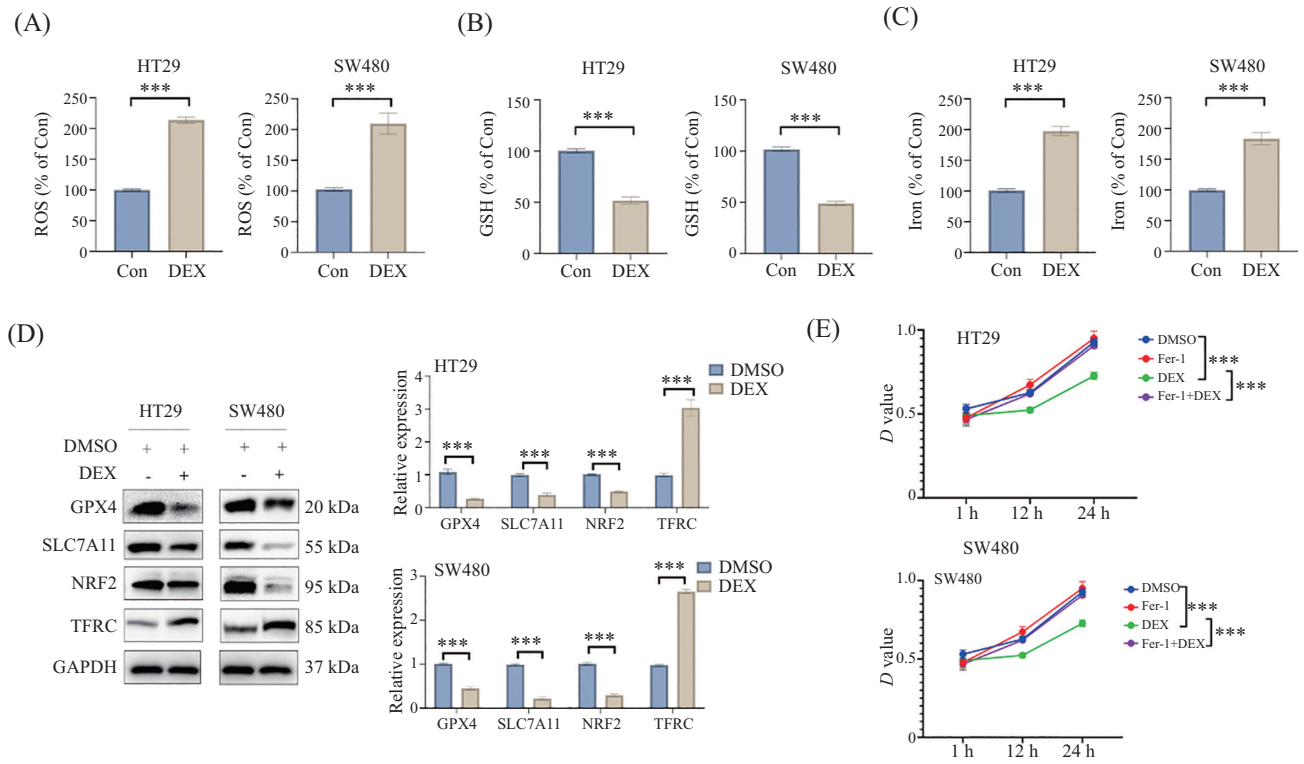
为进一步验证DEX诱导的细胞死亡是否依赖铁死亡途径, 使用铁死亡抑制剂Fer-1进行干预。结果显示, Fer-1处理(HT29: 81.56±2.22; SW480: 89.31±4.66)可极显著逆转DEX(HT29: 65.11±4.18; SW480: 62.31±3.22)诱导的细胞活力下降, 提高结直肠癌细胞的存活率(P<0.001, 图2E)。

2.3 DEX对miR-199a-5p和G6PD表达的影响

qRT-PCR结果表明, DEX处理极显著上调了HT29(1.51±0.17)和SW480(1.75±0.36)细胞中miR-199a-5p的表达水平(P<0.001, 图3A)。

为了进一步研究miR-199a-5p在结直肠癌细胞中的作用机制, 将miR-199a-5p模拟物和anti-miR-199a-5p转染到经100 ng/mL DEX处理的结直肠癌细胞HT29和SW480中, qRT-PCR结果显示转染成功(P<0.001, 图3B)。

结果显示, 与NC组相比, miR-199a-5p过表达极显著降低了*G6PD*-WT报告基因(HT29: 0.33±0.11)的荧光素酶活性(P<0.001), 而miR-199a-5p抑制剂则(2.11±0.26)极显著增强其活性(P<0.001); 然而, 在*G6PD*-MUT组中, 各处理组之间荧光素酶活性无显



A: 检测DEX处理后HT29和SW480细胞内的ROS水平变化; B: 检测DEX处理后HT29和SW480细胞内的GSH水平变化; C: 检测DEX处理后HT29和SW480细胞内的Fe^{2+}水平变化; D: Western blot检测DEX处理后对铁死亡相关蛋白表达的影响; E: CCK-8检测Fer-1对DEX诱导的细胞死亡的影响。***P<0.001。n=3。

A: detection of intracellular ROS levels in HT29 and SW480 cells after DEX treatment; B: detection of intracellular GSH levels in HT29 and SW480 cells after DEX treatment; C: detection of intracellular Fe^{2+} levels in HT29 and SW480 cells after DEX treatment; D: Western blot analysis of the effect of DEX treatment on the expression of ferroptosis-related proteins; E: CCK-8 assay to detect the effect of Fer-1 on DEX-induced cell death. ***P<0.001. n=3.

图2 DEX诱导结直肠癌细胞发生铁死亡

Fig.2 DEX induces ferroptosis in colorectal cancer cells

著差异(图3D)。此外, miR-199a-5p可极显著降低G6PD(0.26±0.14)表达水平, 而敲低miR-199a-5p可极显著提高G6PD(1.48±0.15)的表达水平(P<0.001, 图3E), 说明miR-199a-5p抑制G6PD的表达。

同时, DEX处理也极显著降低了HT29 (0.51±0.12)和SW480(0.41±0.15)细胞中G6PD的表达水平(P<0.001, 图3F)。

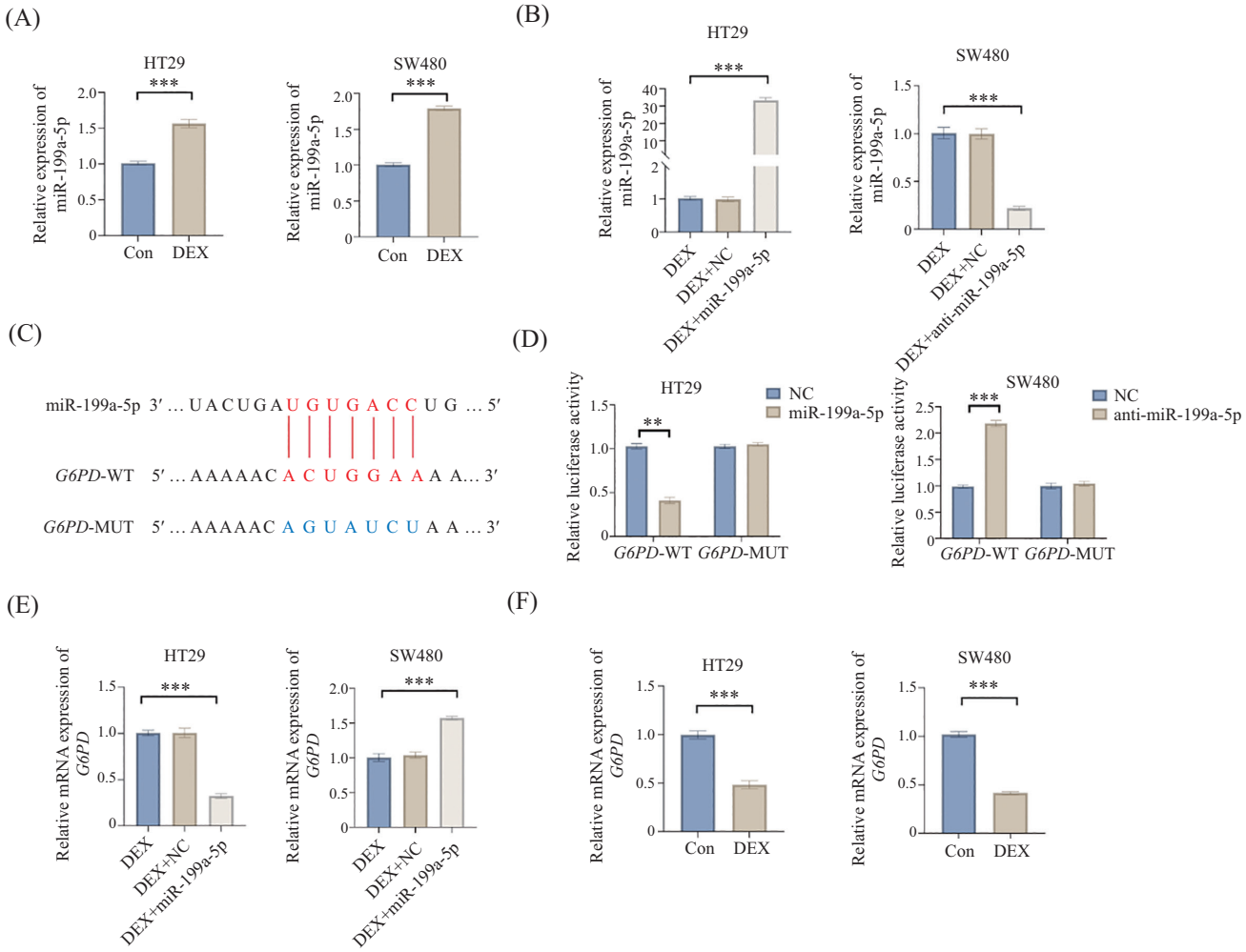
2.4 G6PD过表达对DEX诱导的铁死亡的影响

qRT-PCR结果显示, G6PD过表达恢复了*G6PD* mRNA的表达(HT29: 1.23±0.11; SW480: 1.05±0.06)(P<0.001, 图4A)。

功能实验显示, G6PD过表达(HT29: 97.22±1.45; SW480: 95.33±2.31)极显著逆转了DEX诱导的细胞活力下降(P<0.001, 图4B)。

克隆形成实验显示, 恢复G6PD表达(HT29: 316.33±6.78; SW480: 337.22±5.23)极显著增加了DEX处理后HT29和SW480细胞的克隆数量和大小(P<0.001, 图4C)。

此外, Transwell迁移、侵袭实验和划痕实验进一步证实, G6PD过表达(侵袭HT29: 285.65±5.11, SW480: 271.78±2.32; 迁移HT29: 211.23±3.27, SW480: 271.56±5.19; 划痕HT29: 61.45±4.29; SW480: 63.28±3.17)显著恢复了DEX抑制的结直肠癌细胞侵袭和迁移能力(P<0.01, 图4D和4E)。

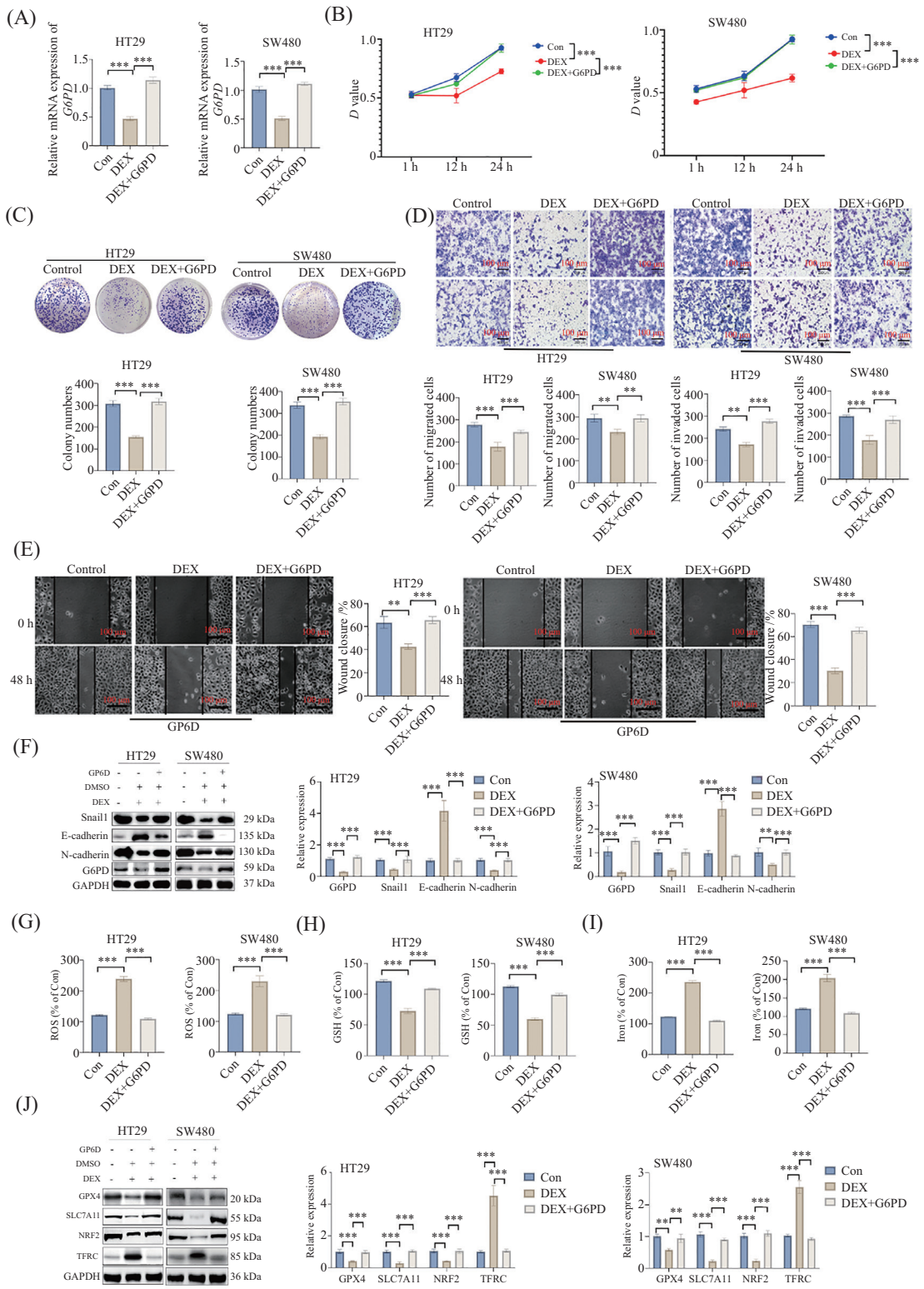


A: DEX处理后miR-199a-5p的表达; B: 转染效率; C: *G6PD*和miR-199a-5p靶向结合位点, 其中红色为靶向位点, 蓝色为突变位点; D: 双荧光素酶报告实验结果; E、F: *G6PD* mRNA表达; **P<0.01, ***P<0.001。n=3。

A: miR-199a-5p expression after DEX treatment; B: transfection efficiency; C: predicted binding sites between *G6PD* and miR-199a-5p, the red sequences indicate target sites, and the blue sequences indicate mutant sites; D: results of dual-luciferase reporter assay; E,F: mRNA expression of *G6PD*. **P<0.01, ***P<0.001. n=3.

图3 DEX调控miR-199a-5p/G6PD通路

Fig.3 DEX regulates the miR-199a-5p/G6PD pathway



A: *G6PD* mRNA的表达; B: CCK-8实验检测不同组的细胞活力; C: 克隆形成实验检测不同组的细胞克隆数量和大小; D、E: Transwell迁移/侵袭实验及伤口愈合实验检测不同组的细胞迁移及侵袭能力; F: Western blot检测不同组的G6PD和EMT相关蛋白表达情况; G~I: ROS、GSH和Fe^{2+}水平; J: Western blot检测不同组的铁死亡相关蛋白表达。**P<0.01, ***P<0.001。n=3。

A: mRNA expression of *G6PD*; B: CCK-8 assay for cell viability in different groups; C: colony formation assay detecting the number and size of cell colonies in different groups; D,E: Transwell migration and invasion assays and wound healing assay for detecting cell migration and invasion abilities in different groups; F: Western blot analysis of the expression of G6PD and EMT-related proteins in different groups; G-I: levels of ROS, GSH and Fe^{2+}; J: Western blot analysis of the expression of ferroptosis-related proteins in different groups. **P<0.01, ***P<0.001. n=3.

图4 G6PD过表达逆转DEX对结直肠癌细胞的抑制作用

Fig.4 Overexpression of G6PD reverses the inhibitory effect of DEX on colorectal cancer cells

Western blot证明, DEX对EMT标志物的抑制作用可被G6PD过表达(Snail1, HT29: 1.21±0.32, SW480: 1.28±0.28; E-cadherin, HT29: 1.11±0.18, SW480: 0.89±0.09; N-cadherin, HT29: 1.06±0.11, SW480: 1.06±0.14)极显著逆转(P<0.001, 图4F)。

在铁死亡相关指标检测中, G6PD过表达极显著降低了DEX诱导的ROS表达水平(HT29: 105.56±1.33; SW480: 112.48±1.27)和减轻了Fe^{2+}过载(HT29: 89.33±2.23; SW480: 104.33±2.19), 并部分恢复了细胞内GSH(HT29: 106.43±3.34; SW480: 91.45±2.67)水平(P<0.001, 图4G~图4I)。

Western blot分析进一步显示, DEX诱导的GPX4(HT29: 1.07±0.18; SW480: 1.15±0.32)、SLC7A11(HT29: 0.87±0.11; SW480: 1.05±0.12)和NRF2(HT29: 1.17±0.16; SW480: 1.17±0.16)表达下调在G6PD过表达后被极显著逆转, 而TFRC(HT29: 1.05±0.14; SW480: 0.78±0.16)的上调趋势亦被极显著削弱(P<0.001, 图4J)。

3 讨论

DEX是一种在临床围手术期广泛应用的受体激动剂, 既往研究表明, DEX在围手术期可通过减轻应激反应、抑制炎症因子释放以及保护免疫功能, 对肿瘤患者的短期和长期预后产生潜在有益影响[6]。近年来, 越来越多的基础研究提示DEX可能直接参与肿瘤细胞生物学行为的调控, 如DEX可通过抑制Smad2/3磷酸化直接阻断TGF-β信号通路, 从而抑制非小细胞肺癌细胞的增殖与迁移, 且该效应呈现显著的浓度依赖性特征[16], 在缺氧诱导的肺癌细胞和结直肠癌中DEX通过调节HIF-1α信号通路促进癌细胞增殖[17]。但是, 该条件是缺氧条件, 与本研究存在本质区别, 这种差异可能来自于缺氧条件下的肿瘤微环境的改变。

本研究所用100 ng/mL浓度在体外实验中展现出显著抗肿瘤效应, 但该浓度远高于临床镇静治疗中的常用血药浓度[6]。然而, 肿瘤局部组织中药物浓度可能因组织灌注、药物分布特性以及给药方式的优化而高于血药浓度。此外, 体外实验采用的药物浓度往往用于机制验证而非直接模拟临床用药条件。

肿瘤细胞对程序性细胞死亡的逃逸是导致肿瘤进展和治疗失败的重要机制之一[18], 铁死亡是一种由铁依赖性脂质过氧化累积所介导的调节性细胞死亡形式, 近年来已成为靶向克服肿瘤耐药的新兴治疗策略方向[3-4]。越来越多的研究开始关注DEX与肿瘤铁死亡之间的关系[19]。与坏死、自噬和凋亡不同, 铁死亡以细胞内Fe^{2+}积累、脂质过氧化增强及抗氧化系统失衡为主要特征, 最终导致ROS过量生成和细胞损伤[20]。结直肠癌细胞普遍存在氧化应激水平升高及铁代谢紊乱的特征, 使其对铁死亡尤为敏感[5]。

在本研究中, 我们发现DEX处理可显著诱导结直肠癌细胞内Fe^{2+}积累和ROS生成, 并伴随GSH明显耗竭以及铁死亡关键调控蛋白GPX4和SLC7A11表达下调, 提示DEX能有效激活铁死亡通路。此外, 铁死亡抑制剂Fer-1可明显逆转DEX诱导的细胞死亡, 进一步证实DEX介导的细胞死亡依赖于铁死亡机制。上述结果与Huang等[21]关于DEX通过调控铁输入和输出维持铁稳态的研究结果相一致。Wang等[22]的研究也支持DEX在铁代谢调控中的作用, 并且表明DEX可通过调节铁死亡途径减轻心肌缺血再灌注损伤亡。因此, 结合本研究结果, 我们认为DEX在结直肠癌中的抗肿瘤作用可能部分依赖于铁死亡的激活。

miRNA在肿瘤发生发展及细胞死亡调控中发挥重要作用。既往研究表明, DEX可通过调控miRNA的表达参与调节肿瘤进程[23]。miR-199a-5p的过表达被证明可显著抑制结直肠癌细胞的生长和转移[24]。因此, 我们推测DEX可能通过调节miR-199a-5p的表达发挥其抗肿瘤作用。本研究发现, DEX显著上调结直肠癌细胞中miR-199a-5p的表达。进一步通过生物信息学预测结合双荧光素酶报告实验, 我们鉴定出*G6PD*是miR-199a-5p的直接下游靶基因。然而, DEX如何实现miR-199a-5p的上调, 其上游调控机制尚不明确。推测可能存在以下调控路径: 其一, DEX可能通过激活α2肾上腺素能受体下游的转录因子(如NF-κB、AP-1等)直接或间接增强miR-199a-5p的转录活性; 其二, DEX可能通过改变miR-199a-5p基因启动子区的表观遗传修饰来解除其转录抑制; 其三, DEX也可能通过影响miRNA加工成熟过程中的关键酶活性, 来调控成熟miR-199a-5p的生成。未来研究可进一步通过染色质免疫共沉淀、DNA甲基化测序及RNA免疫沉淀等手段, 深入揭示DEX调控miR-199a-5p表达的分子基础。

G6PD作为磷酸戊糖途径的关键限速酶, 在维持NADPH生成和细胞氧化还原稳态中至关重要。有研究表明, 其转录活性的激活被证明能够促进结直肠癌核酸和脂质的生物合成, 同时降低细胞内活性氧水平[12,25], 促进肿瘤发生发展, 这和本研究结果一致。本研究通过构建G6PD的过表达质粒进行功能回复实验发现, G6PD过表达可显著逆转DEX诱导的结直肠癌细胞存活、增殖、迁移和侵袭抑制效应, 并恢复EMT表型及抑制铁死亡的发生, 提示miR-199a-5p/G6PD轴在DEX介导的铁死亡及抗结直肠癌作用中发挥核心调控作用。

综上所述, 本研究表明DEX可能通过激活miR-199a-5p/G6PD信号轴, 破坏结直肠癌细胞的抗氧化防御体系, 诱导铁死亡的发生, 从而抑制肿瘤细胞的增殖、迁移及侵袭。研究不仅拓展了DEX在肿瘤生物学领域中的作用认识, 也为结直肠癌的治疗策略提供了新的潜在靶点及理论依据。尽管本研究揭示了DEX在结直肠癌细胞中的潜在抗肿瘤作用及其分子机制, 但仍存在一定局限性。本研究主要基于体外细胞实验, 尚缺乏动物模型和临床样本的验证, DEX在体内对结直肠癌生长、转移及铁死亡调控作用仍需进一步明确。后续研究有必要通过构建结直肠癌动物模型, 系统评估DEX在体内的抗肿瘤效应及其潜在作用机制。

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