

S100A2在结直肠癌进展中的作用机制研究

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摘要 目的 探究钙结合蛋白S100A2在结直肠癌(CRC)进展中的作用及其与CRC细胞果糖代谢的关系。方法 通过肿瘤数据库GEPIA2网站分析S100A2在CRC患者和健康人群中的表达差异。通过Western blot和qRT-PCR分析S100A2在CRC细胞系HCT116、SW480、Caco-2以及人正常结肠上皮细胞系NCM460中的表达差异。通过免疫组织化学染色分析S100A2在CRC组织和癌旁组织中的表达差异。通过慢病毒转染的方法构建敲低S100A2的HCT116和Caco-2 CRC稳转细胞系及其阴性对照细胞系。通过CCK-8实验、伤口愈合实验和Transwell实验检测S100A2的下调对CRC细胞增殖、迁移和侵袭能力的影响。通过Western blot和细胞免疫荧光染色检测S100A2的下调对CRC细胞内果糖转运蛋白5(GLUT5)、酮己糖激酶(KHK)表达水平的影响。通过果糖检测试剂盒检测敲低S100A2后CRC细胞的果糖浓度。利用敲低S100A2的结直肠癌HCT116细胞系构建裸鼠CRC荷瘤模型,明确S100A2对CRC体内生长的影响,通过Western blot和免疫荧光检测成瘤小鼠的肿瘤组织中GLUT5、KHK的表达。结果 S100A2在CRC患者中的表达水平显著高于健康人群。S100A2在3种CRC细胞中的表达水平均显著高于正常结肠上皮细胞系。S100A2敲低后,CRC细胞的增殖、迁移和侵袭能力受到抑制。S100A2敲低后,CRC细胞中果糖代谢相关蛋白GLUT5和KHK的表达水平下调,细胞对果糖的摄取量减少。小鼠体内实验显示S100A2的敲低抑制CRC肿瘤增殖及肿瘤组织中GLUT5和KHK表达水平。结论 下调S100A2表达通过影响CRC细胞果糖代谢抑制CRC进展。

关键词 S100A2; 结直肠癌; 果糖代谢; 葡萄糖转运蛋白5; 酮己糖激酶

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结直肠癌(colorectal cancer, CRC)是主要的恶性肿瘤之一,其发生和进展涉及一系列病理生理机制^[1]。钙结合蛋白S100A2(S100 calcium-binding protein A2, S100A2)是S100蛋白家族的重要成员,其在癌症中过表达,被认为主要起到促癌作用^[2]。例如,靶向S100A2被证明可能是子宫内膜癌的一种治疗方法^[3]。此外,S100A2的表达与脑源性肿瘤^[4]的不良预后及肝细胞癌^[5]的不良体内总生存期相关。S100A2也被认为与胶质母细胞瘤肿瘤细胞体内增殖、凋亡、侵袭和迁移有关^[6]。

果糖是葡萄糖的一种可互换的单糖底物,它主要通过果糖转运蛋白5(glucose transporter 5, GLUT5)输入细胞,文献^[7]报道升高的GLUT5促进CRC进展。酮己糖激酶(ketohexokinase, KHK)是果

糖代谢中的第一个限速酶,研究^[7]表明GLUT5-KHK轴在介导CRC细胞的果糖驱动的糖酵解和三羧酸循环中发挥关键作用。然而,关于S100A2与CRC细胞果糖代谢的关系尚无深入研究。该研究通过在CRC细胞中下调S100A2的表达,初步探索了CRC中S100A2与果糖代谢的关系。

1 材料与方法

1.1 细胞系、动物和病理组织切片

人CRC细胞系HCT116、SW480、Caco-2以及人正常结肠上皮细胞系NCM460均购自美国ATCC公司。HCT116、SW480以及NCM460细胞使用含10%胎牛血清和1%青-链霉素混合液的RPMI1640培养基培养,Caco-2细胞使用含20%胎牛血清、1%青-链霉素混合液和1%非必需氨基酸的DMEM培养基培养。18只4周龄雄性BALB/C裸鼠(体质量16~18 g)购自北京斯贝福生物公司。所有小鼠在新疆医科大学附属肿瘤医院SPF级动物实验室内适应性饲养1周后,随机分为3组(对照组与两个敲低组),分组时3组间体质量无显著差异。正式实验开始时,小鼠年龄为5周龄,体质量约为18~20 g。本研究动物实验

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方案获得新疆医科大学附属肿瘤医院动物保护和伦理委员会审批(批号:IACUC-20250305-44)。10例CRC患者病理组织及其癌旁组织切片来自新疆医科大学附属肿瘤医院,样本的采集获得医院伦理委员会审批(批号:KY2021052635),患者均签署知情同意书。

1.2 生物信息学分析 在肿瘤数据库GEPIA2网站(<http://gepia2.cancer-pku.cn/#index>)中检索S100A2在CRC患者与健康人群中的表达情况,下载分析结果图。

1.3 CCK-8实验 将转染后的细胞按 5×10^3 个/孔接种于96孔中,分别在培养的第12、24、48、72 h向每孔加入10 μ L的CCK-8试剂(上海碧云天公司)^[8],继续避光孵育2 h。然后,用酶标仪于450 nm波长处读取各孔的吸光度(optical density, OD)。

1.4 Western blot实验 对于细胞来源蛋白,首先收集细胞,用RIPA裂解液(上海碧云天公司)裂解细胞获得总蛋白。蛋白在金属浴中煮沸变性,经过SDS-PAGE凝胶电泳分离,再转移到PVDF膜(美国Millipore公司)上。常温下,用5%脱脂牛奶封闭PVDF膜2 h。膜与稀释好的一抗在4 °C下孵育过夜。然后膜与二抗在常温下孵育1 h。最后使用ECL化学发光液(美国赛默飞公司)进行显影。对于动物组织来源蛋白,首先将动物组织小心切取,加入裂解液,再放在冷冻研磨机上充分研磨,获得总蛋白之后蛋白处理步骤与细胞来源蛋白处理步骤相同。本研究所使用抗 β -actin抗体(1:5 000)、抗S100A2抗体(1:2 000)均购自美国abcam公司,抗KHK抗体(1:2 000)、抗GLUT5抗体(1:2 000)均购自美国Proteintech公司。羊抗兔二抗(1:5 000)购自武汉赛维尔公司。

1.5 细胞划痕实验 将转染后的细胞接种于6孔板内,待汇合度达60%,弃去培养基,用无菌10 μ L移液枪头在细胞平面十字划痕,制造“缺口”。然后以无血清培养基继续培养细胞,分别于第0 h和第48 h在显微镜下观察划痕“缺口”面积。

1.6 Transwell侵袭实验 将转染后的细胞消化并离心,用无血清培养基进行重悬。然后,将细胞按 1×10^3 个/孔加入至铺好Matrigel胶的24孔Transwell板(美国康宁公司)上室内,下室加入700 μ L含10%胎牛血清的完全培养基。48 h后撤去培养基,用4%多聚甲醛固定细胞,0.1%结晶紫染色细胞,PBS轻轻洗涤3次,在显微镜下观察并统计敲低S100A2

的HCT116和Caco-2 CRC稳转细胞系发生侵袭的细胞数量。

1.7 qRT-PCR 根据制造商的说明,使用TRIzol试剂(南京Vazyme公司)提取细胞总RNA。然后,使用SuperScript IV试剂盒(美国赛默飞公司)对RNA进行逆转录反应,获得cDNA。在7500型PCR仪(美国ABI公司)上,使用ChamQ SYBR qPCR Master Mix试剂盒(南京Vazyme公司)对cDNA进行qPCR,获得产物。使用GAPDH转录水平归一化处理各基因转录水平。各基因引物序列见表1。

表1 qRT-PCR引物序列

Tab. 1 Sequences of qRT-PCR Primers

Name	Primer sequences (5' - 3')
GAPDH	Forward: TGTGGGCATCAATGGATTGG Reverse: ACACCATGTATTCCGGGTCAAT
S100A2	Forward: CAGCGGATAGACCCACACAA Reverse: GCCACATCTTGCTGACAAAC
GLUT5	Forward: GAGGCTGACGCTTGCTT Reverse: CCACGTTGTACCCATACTGG
KHK	Forward: CTAAGGAGGACTCGGAGATAAGG Reverse: CATTGAGCCCATGAAGGCAC

1.8 免疫组织化学染色 使用石蜡包埋和福尔马林固定的CRC组织及癌旁组织样本进行免疫组化检测。样品用4%多聚甲醛固定20 min,0.1%TritonX-100通透10 min,S100A2抗体4 °C下孵育过夜,二抗37 °C下孵育2 h。阳性判定标准为:超过50%的细胞显示明确的阳性染色。

1.9 慢病毒转染细胞 包含S100A2基因表达干扰质粒(shS100A2-#1、shS100A2-#2、shS100A2-#3)及阴性对照质粒(shS100A2-NC)的慢病毒载体购自上海吉玛基因公司。干扰质粒序列见表2。按照制造商的说明,将慢病毒与培养基混合,加入至HCT116细胞和Caco-2细胞培养体系。经过0.01 mg/L嘌呤霉素(上海碧云天公司)筛选获得转染成功的细胞株。

表2 S100A2基因表达干扰质粒序列

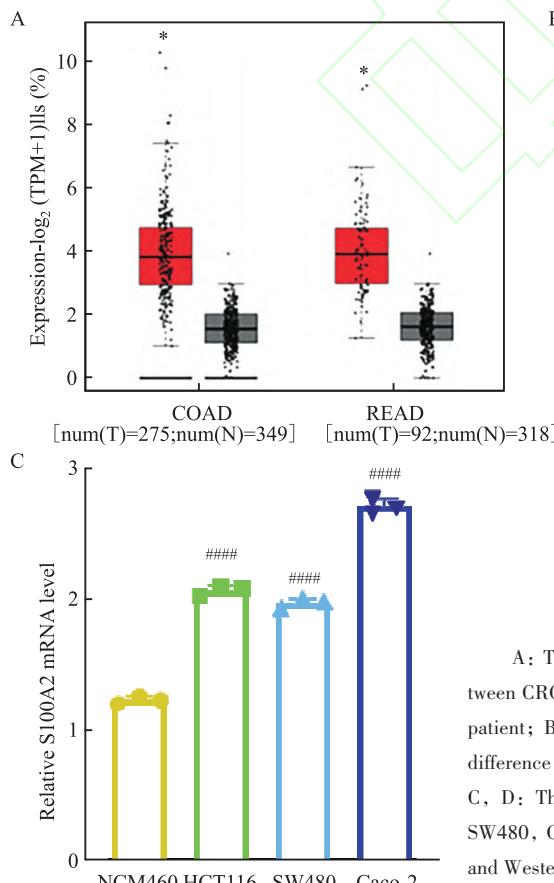
Tab. 2 Sequences of S100A2 gene expression interference plasmid

Name	Primer sequences (5' - 3')
shS100A2-#1	CCGGCAAGTCTGGAGGAGATGAAACTCGAGTT CATCTCCTCCAGAACATTGTTTTT
shS100A2-#2	CCCGCAG-ACAAGTCTGGAGGAGATCTCGAGA TCTCCTCCAGAACATTGCTCTTT
shS100A2-#3	CCGGACCACTGTTCTCTGTTAAGAACCTCGAG-TTC TTAACAGAACAGTGTCTTTTT

1.10 免疫荧光染色 将细胞接种在培养皿中并培养36 h。使用4%多聚甲醛固定细胞20 min,PBS冲洗3次,并用含有0.1%Triton-X 100的PBS溶液

在室温下通透细胞15 min, PBS冲洗3次。将细胞与山羊血清在37 °C下避光孵育30 min。滴加GLUT5一抗,并将细胞放在湿盒中4 °C下孵育过夜。去除一抗后,将细胞在PBS中漂洗3次。滴加荧光二抗,并将细胞在37 °C下孵育1.5 h。细胞与KHK一抗孵育的步骤与前文GLUT5一抗孵育相同。然后细胞用PBS洗涤3次。将DAPI染色液(武汉赛维尔公司)加入细胞中,孵育5 min。最后,将细胞用PBS冲洗3次,并用抗荧光猝灭剂(美国赛默飞公司)进行封闭,在共聚焦荧光显微镜(德国蔡司公司)下拍摄细胞。对于动物组织切片进行的免疫荧光染色,按上述操作将切片与抗体进行孵育后,在共聚焦荧光显微镜下观察拍照。

1.11 裸鼠荷瘤模型构建 18只4周龄BALB/C雄性裸鼠饲养在医院洁净动物房内,适应性饲喂1周。将转染后的HCT116细胞按1×10⁶个/只接种于裸鼠背部皮下,根据接种细胞转染质粒的不同将裸鼠分为:sh-#2-nude组、sh-#3-nude组和shNC-nude组,每组各6只。接种第5天,所有裸鼠背部皮下均观察到有明显凸起组织形成,表明荷瘤模型构建成功。接种3周后,处死动物并完整取下肿瘤,冻存于-80 °C冰箱中备用。



1.12 果糖含量检测 根据制造商的说明,使用含相同浓度果糖(5 mmol/L)不含葡萄糖的培养体系培养细胞,在培养的第0 h和第48 h使用果糖含量检测试剂盒(北京索莱宝公司)定量检测细胞培养体系上清液中果糖浓度。

1.13 统计学处理 所有实验重复3次,数据以 $\bar{x} \pm s$ 表示,使用SPSS 22.0软件分析,通过Graphpad Prism 8.0软件绘制统计图形。通过t检验分析两组数据,One-way ANOVA比较多组数据。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 S100A2在CRC中高表达 通过检索GEPIA2数据库,获得S100A2在CRC患者和健康人群中的表达情况,S100A2在CRC患者中表达水平高于健康人群($P < 0.05$)(图1A)。对10例CRC患者病理组织和癌旁组织切片进行免疫组化,结果表明S100A2在CRC组织中高表达(图1B)。提取CRC细胞系和NCM460细胞RNA和蛋白,分别进行qRT-PCR和Western blot分析(图1C、1D),显示S100A2基因在CRC细胞中的转录和翻译表达水平均上调($P < 0.01$)。

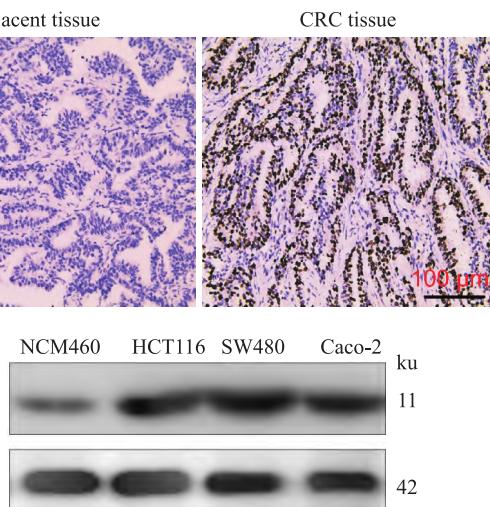


图1 S100A2在CRC中高表达

Fig. 1 S100A2 was highly expressed in CRC

A: The GEPIA2 database was used to analyze the difference in S100A2 expression between CRC patients and healthy people; COAD: Colon cancer; READ: rectal cancer; Red: patient; Black: healthy people; **B**: Immunohistochemical staining was used to analyze the difference of S100A2 expression in CRC tissues and corresponding paracancer tissues $\times 40$; **C, D**: The transcripts and protein levels of S100A2 in three CRC cell lines (HCT116, SW480, Caco-2) and normal colon epithelial cell line NCM460 were analyzed by qRT-PCR and Western blot; $^*P < 0.05$ vs healthy people; $^{****}P < 0.0001$ vs NCM460 cells.

2.2 S100A2敲低抑制了CRC细胞增殖、迁移和侵袭活性 为了探究S100A2的表达对CRC进展的作用,本研究构建了稳定敲低S100A2的HCT116和Caco-2细胞株及其阴性对照细胞株(图2A)。CCK-8

实验结果表明,S100A2敲低后,HCT116和Caco-2细胞增殖活性受到抑制(图2B)。细胞划痕实验和Transwell实验结果显示(图2C、2D),在HCT116细胞和Caco-2细胞中,sh组的迁移和侵袭能力弱于

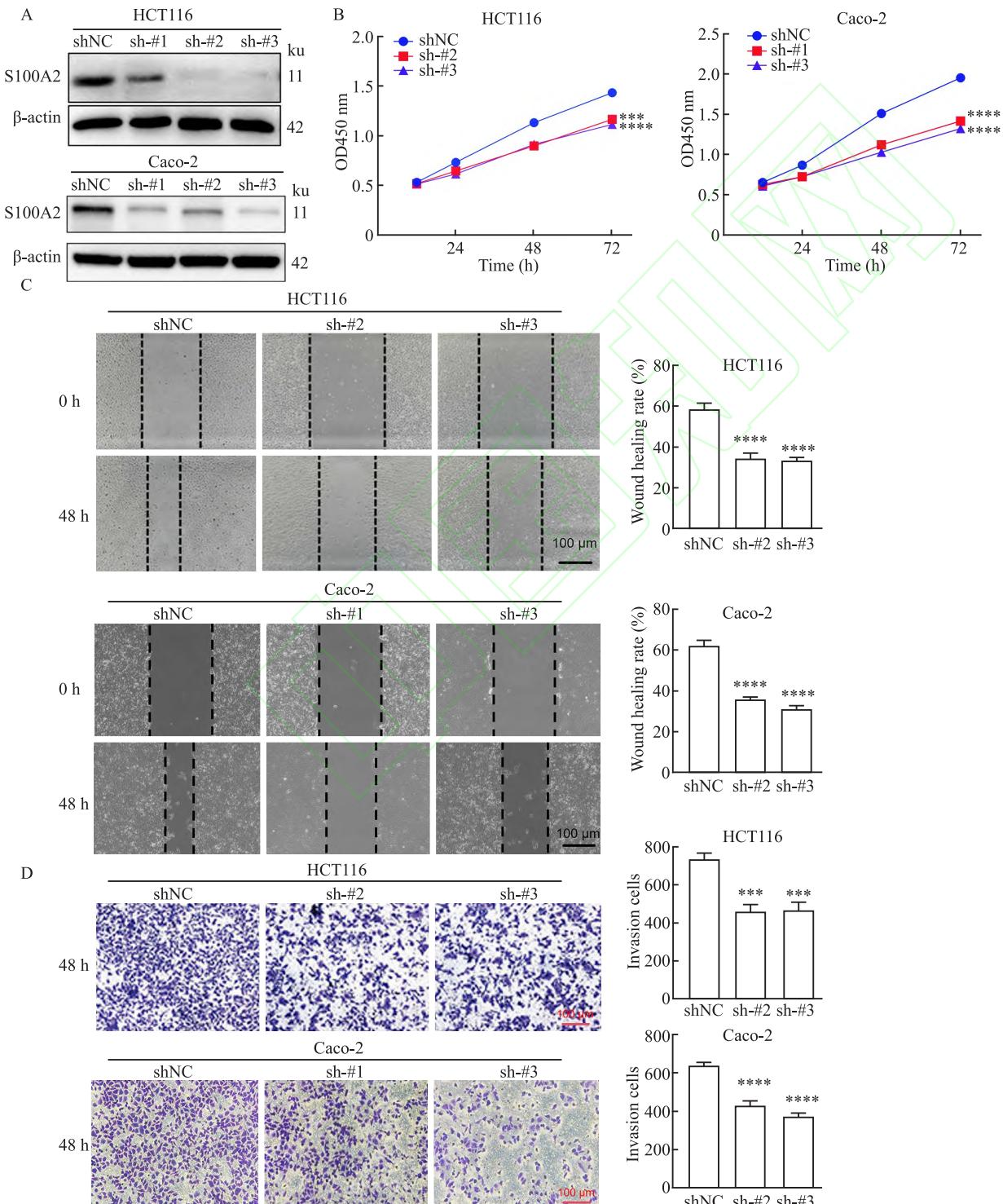


图2 S100A2敲低抑制了CRC细胞增殖、迁移和侵袭活性

Fig. 2 S100A2 knockdown inhibited the proliferation, migration and invasion of CRC cells

A: Western blot verified the low efficiency of S100A2 expression in HCT116 cells and Caco-2 cells; B: Cell proliferation activity was detected by CCK-8 proliferation assay; C: Wound healing test was used to detect cell migration ability $\times 10$; D: Transwell assay was used to detect cell invasion ability $\times 20$; *** $P < 0.001$, **** $P < 0.0001$ vs shNC.

shNC组($P<0.001$)。

2.3 S100A2敲低抑制CRC细胞果糖代谢 与shNC组相比,HCT116和Caco-2细胞中sh组的果糖代谢相关蛋白GLUT5和KHK表达水平显著降低(图3A)。细胞免疫荧光分析得到了相同的结果(图3B)。S100A2敲低可能抑制了CRC细胞果糖代谢。随后,对两组细胞进行相同初始浓度果糖培养,并检测培养48 h后上清中果糖浓度。如图3C结果显示,与shNC组相比,HCT116细胞($P=0.0008$)和Caco-2细胞($P=0.0004$)中sh组的细胞对果糖的摄取量减少。

2.4 S100A2敲低抑制CRC肿瘤体内生长和果糖代谢 通过接种肿瘤细胞的方法构建裸鼠CRC荷

瘤模型。建模3周后完整取下裸鼠肿瘤,测量肿瘤重量,显示sh-#2-nude组和sh-#3-nude组裸鼠肿瘤的重量均低于shNC-nude组($P<0.0001$)(图4A、4B)。从sh-#2-nude组和shNC-nude组裸鼠肿瘤组织中提取蛋白,进行Western blot实验。结果显示,GLUT5和KHK在sh-#2-nude组肿瘤中的表达水平低于shNC-nude组(图4C)。对裸鼠肿瘤组织切片进行免疫荧光染色,显示GLUT5和KHK在sh-#2-nude组的荧光染色强度弱于shNC-nude组,验证了体外实验的结果(图4D)。

3 讨论

CRC是最主要的恶性肿瘤之一,在世界范围内

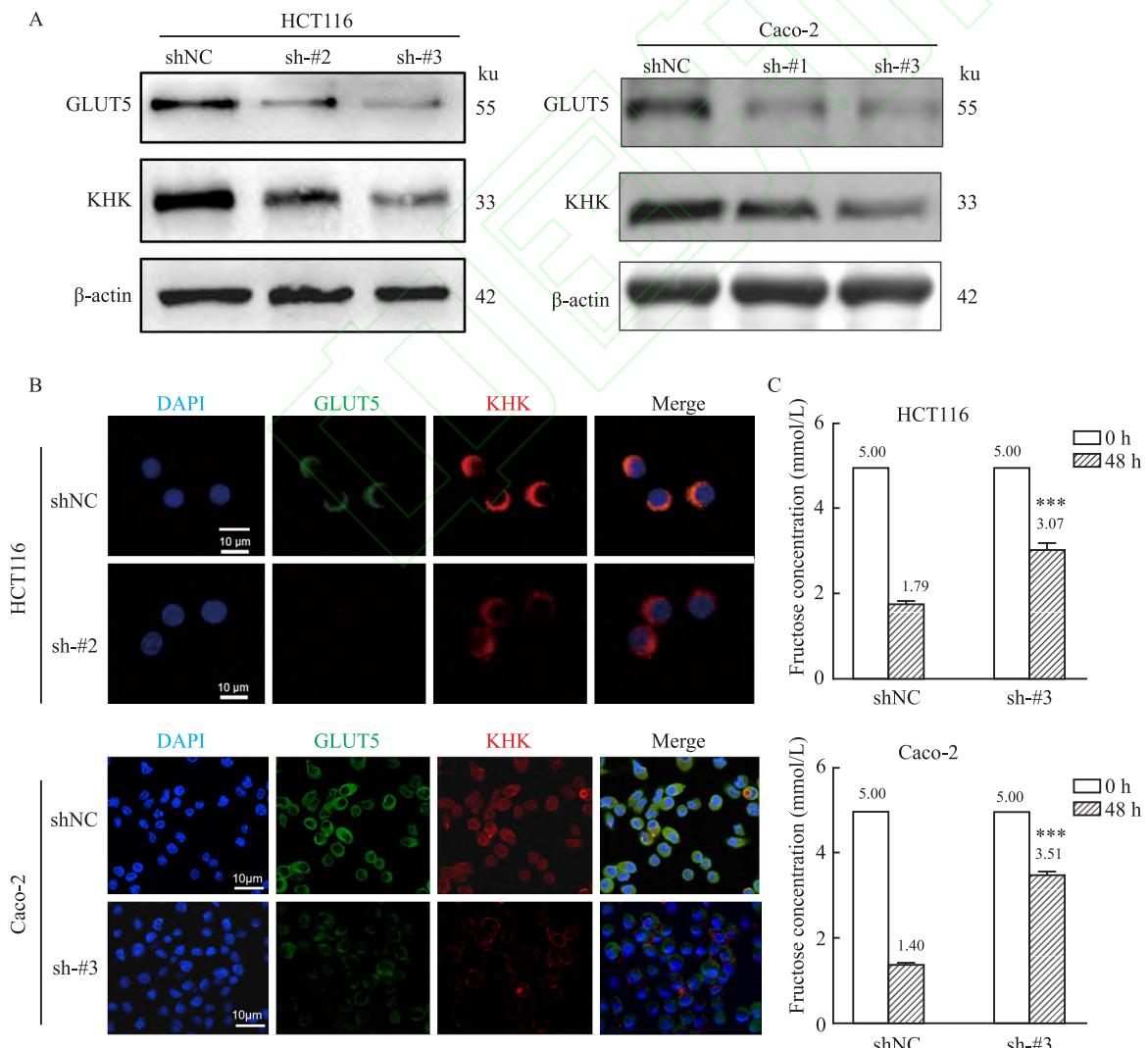


图3 S100A2敲低抑制CRC细胞果糖代谢

Fig. 3 S100A2 knockdown inhibited fructose metabolism in CRC cells

A: The expression levels of GLUT5 and KHK, which are related to fructose metabolism, were detected by Western blot; B: The expression of GLUT5 and KHK in each group was detected by cellular immunofluorescence $\times 40$; C: The concentration of fructose in the supernatant of fructose-cultured cells at 0 h and 48 h was determined; *** $P < 0.001$ vs shNC group.

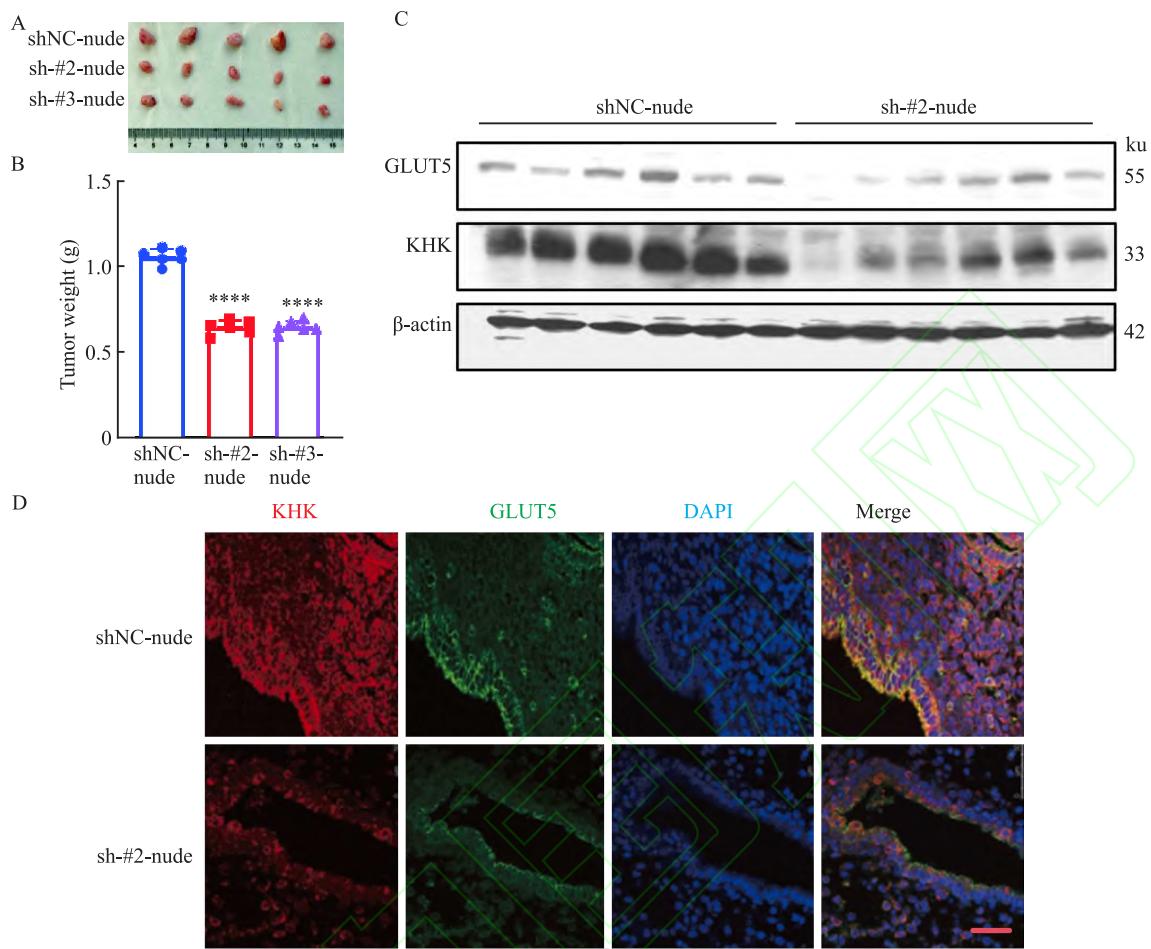


图4 S100A2敲低抑制CRC肿瘤体内生长和果糖代谢

Fig. 4 S100A2 knockdown inhibited CRC tumor growth and fructose metabolism *in vivo*

A: The subcutaneous CRC tumor was removed from the back of nude mice three weeks after the inoculation of tumor cells, then weighed and photographed; B: The weight change of the tumor; C: Western blot was performed on CRC tumor tissues of nude mice to analyze the expression of GLUT5 and KHK; D: The expression of GLUT5 and KHK in CRC tumor tissues of nude mice in control group and S100A2 knockdown group were analyzed by immunohistochemistry $\times 40$; *** $P < 0.0001$ vs shNC-nude group.

有着较高的发病率和病死率。近年来,随着对肿瘤代谢重编程的深入研究,代谢异常在肿瘤发生发展中的关键作用逐渐受到关注^[9]。果糖代谢作为细胞能量代谢的重要途径之一,在肿瘤细胞的增殖、侵袭和转移等过程中发挥着重要作用^[10]。

代谢异常是肿瘤细胞的标志之一。实体肿瘤细胞往往处于缺氧环境,强大的能量需求迫使肿瘤细胞过度摄取环境中的能源物质并增强多种能量代谢途径。肿瘤细胞通过主动调节各种代谢途径通量,以满足增加的生物能量和生物合成需求,并减轻癌细胞增殖和存活所产生的氧化应激^[11]。果糖代谢与肿瘤的发生和发展紧密相关。增加膳食中果糖的摄入量会导致肥胖和代谢综合征的风险增加,肥胖是导致多种癌症发生的重要风险因素。有临床和实验证据^[12]表明,增加果糖摄入量会促进

肿瘤进展。然而,果糖代谢促进肿瘤进展以及肿瘤细胞如何调控果糖代谢的确切机制仍未完全了解。果糖代谢在CRC肝转移过程中发挥了重要作用,转移性CRC细胞可以在新的微环境中重新编程果糖代谢,尤其是在肝脏等代谢活跃的器官中,以利于肿瘤细胞定植。此外,临床研究^[13]表明,总果糖摄入量的增加与近端CRC发病率和病死率增加有关,并且容易诱发女性早发性CRC。

GLUT5是特异性转运果糖的膜蛋白,其对果糖的亲和力远高于葡萄糖。研究^[14]表明,GLUT5的过度表达可以促进肿瘤细胞果糖代谢通量,增加细胞能量来源,以促进肿瘤进展。在CRC中,GLUT5显示出高水平表达,GLUT5-KHK轴介导的果糖代谢能够增强CRC细胞增殖和化疗耐药性,使用GLUT5抑制剂能够抑制CRC细胞增殖活性^[15]。也有研

究^[16]报道, GLUT5介导的果糖代谢驱动肿瘤细胞增殖可以不依赖 KHK 的表达, 这提示 GLUT5 对肿瘤果糖代谢的促进作用可能涉及多种方式。

有研究^[17]表明, S100A2 对 CRC 具有预后价值, 且与 CRC 肿瘤组织中的免疫浸润具有相关性。更具体地说, S100A2 过度表达可以作为 II 期和 III 期 CRC 患者的预后标志物。S100A2 作为钙结合蛋白, 可能通过调节细胞内钙离子水平进而影响某些基因的转录、翻译过程, 这其中可能就涉及了 GLUT5 基因或者其他参与 GLUT5 基因表达的相关基因。最近的一项研究^[18]结果可以支持本研究的结论, S100A2 可以通过调节葡萄糖转运蛋白 GLUT1 的表达增加 CRC 细胞糖酵解通量, 进而促进肿瘤进展。本研究结果表明, S100A2 可以通过调控 GLUT5/KHK 轴调节 CRC 细胞果糖代谢通量, 促进肿瘤进展。然而, 有关 S100A2 如何影响 GLUT5 的表达, 还需要进一步探讨。综上所述, 本实验结果为 S100A2 与 CRC 细胞果糖代谢之间的联系提供了一定依据, 并且这是首次报道二者之间存在的功能联系, 将为临床 CRC 治疗和早期诊断提供新的思路。

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The role of S100A2 in the progression of colorectal cancer

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Abstract **Objective** To investigate the role of calcium-binding protein S100A2 in colorectal cancer (CRC) progression and its association with fructose metabolism in CRC cells. **Methods** Differential expression of S100A2 between CRC patients and healthy individuals was analyzed using the GEPIA2 tumor database. Western blot and qRT-PCR were performed to compare S100A2 expression levels in CRC cell lines (HCT116, SW480, Caco-2) and normal human colonic epithelial cells (NCM460). Immunohistochemical staining was conducted to assess S100A2 expression in CRC tissues and adjacent non-tumor tissues. S100A2-knockdown stable CRC cell lines and negative control cell lines were established via lentiviral transduction. Functional assays, including CCK-8, wound healing and Transwell experiments were utilized to evaluate the effects of S100A2 downregulation on CRC cell proliferation, migration, and invasion. Western blot and immunofluorescence staining were employed to analyze the impact of S100A2 knockdown on the expression levels of fructose transporter 5 (GLUT5) and ketohexokinase (KHK). Intracellular fructose concentration was measured using a fructose assay kit. A nude mouse CRC xenograft model was established using S100A2-knockdown HCT116 cell lines to investigate the role of S100A2 in tumor proliferation *in vivo*. Tumor tissues from the xenografted mice were analyzed by Western blot and immunofluorescence staining to evaluate the expression levels of GLUT5 and KHK. **Results** S100A2 expression was significantly elevated in CRC patients compared with healthy individuals. All three CRC cell lines exhibited markedly higher S100A2 expression than normal colonic epithelial cells. S100A2 knockdown significantly inhibited CRC cell proliferation, migration, and invasion capacities. Downregulation of S100A2 suppressed the expression of fructose metabolism-related proteins GLUT5 and KHK, accompanied by reduced cellular fructose uptake. *In vivo* experiments demonstrated that S100A2 knockdown effectively inhibited tumor growth and decreased GLUT5/KHK expression in xenograft tissues. **Conclusion** Downregulation of S100A2 inhibits CRC progression by modulating fructose metabolism in CRC cells.

Key words S100A2; colorectal cancer; fructose metabolism; facilitated glucose transporter member 5; ketohexokinase

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of glomerular tubular structure were improved. The number of apoptotic cells in the Cu+TgCtwh6 group (88.36±19) was lower than that in the Cu group (119.0±20). Compared with the Cu+TgCtwh6 group, the expression of SOD1 protein was down-regulated, and the difference was statistically significant ($P<0.05$). TgCtwh6 infection could restore the down-regulation of renal glutaminase (GLS) expression and the up-regulation of ATPase copper transporting beta gene (ATP7B) expression caused by copper overload. **Conclusion** *Toxoplasma gondii* infection can interfere with the copper metabolism pathway in the kidney of mice, improve the kidney damage caused by copper overload, and provide new clues for the treatment of copper overload disease.

Key words *Toxoplasma gondii*; kidney; cuproptosis; Western blot; apoptosis; ICP-MS

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