

肝细胞癌源性乳酸介导组蛋白 H3K18 乳酸化修饰调控 S100A2 转录促进肝癌细胞的增殖、迁移、侵袭及上皮间质转化

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摘要:目的 探究组蛋白 H3 第 18 位赖氨酸乳酸化(H3K18la)修饰调控 S100 钙结合蛋白 A2(S100A2)转录在肝细胞癌(HCC)发生发展中的作用及机制。方法 生物信息学分析 HCC 单细胞转录组数据, 筛选差异基因及富集通路; 检测 HCC 细胞系及肝正常细胞系的细胞内乳酸含量及组蛋白乳酸化水平; 使用糖酵解抑制剂(2-DG/Oxamate)处理, 通过 Western blotting、CCK-8、克隆形成及 Transwell 评估糖酵解抑制剂对 HCC 细胞增殖、迁移和侵袭的影响。染色质免疫沉淀-实时荧光定量 PCR(ChIP-qPCR)检测 H3K18la 在 *S100A2* 启动子的富集。结合 TCGA 分析 *S100A2* 表达与预后。构建 *S100A2* 过表达/敲低模型, 联合 2-DG 或乳酸进行回复实验, 验证 H3K18la 通过 S100A2 影响 HCC 恶性表型。STRING 在线工具、分子对接及免疫共沉淀(Co-IP)验证 S100A2 与 KRT6A 蛋白的相互作用。Western blotting 检测 HCC 细胞核内 β -catenin 及上皮-间质转化标志物(Vimentin、E-cadherin), 明确 H3K18la 对 Wnt/ β -catenin 通路的调控。结果 HCC 糖酵解及丙酮酸代谢通路上调, 细胞内乳酸含量升高($P<0.001$)。HCC 细胞系 Pan K1a 及 H3K18la 表达水平升高, 缺氧条件下 H3K18la 升高最显著($P<0.001$)。糖酵解抑制剂可浓度依赖性降低 H3K18la, 并抑制 HCC 细胞增殖、克隆形成、迁移和侵袭能力。S100A2 在 HCC 中转录和蛋白水平均上调, 高表达与患者不良预后相关($P<0.05$)。S100A2 促进 HCC 细胞的增殖、迁移及侵袭。经 2-DG 处理后, S100A2 蛋白水平及上述促癌功能均被逆转。S100A2 与 KRT6A 直接相互作用。敲低 KRT6A 降低核内 β -catenin, 过表达则相反; 外源乳酸可逆转敲低 KRT6A 所致的 β -catenin 下调, 该效应被 XAV939 阻断。2-DG 降低 β -catenin 及上皮-间质转化标志物蛋白水平, 过表达 *S100A2* 或 KRT6A 可逆转此效应。结论 HCC 源性乳酸介导组蛋白 H3K18 乳酸化修饰增强并通过激活 *S100A2* 转录正向调控 Wnt/ β -catenin 通路, 最终促进 HCC 细胞的增殖、迁移、侵袭及上皮-间质转化。

关键词:组蛋白乳酸化修饰; S100A2; 肝细胞癌; Wnt/ β -catenin 信号通路; 上皮-间质转化

Hepatocellular carcinoma-derived lactate-mediated histone H3K18 lactylation modifies S100A2 transcription to promote proliferation, migration, invasion and epithelial-mesenchymal transition of hepatocellular carcinoma cells

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Abstract: Objective To investigate the role of histone H3 lysine 18 lactylation (H3K18la) regulating the transcription of S100 calcium-binding protein A2 (S100A2) in the development of hepatocellular carcinoma (HCC) and its molecular mechanism. **Methods** HCC single-cell transcriptome sequencing data were used to screen the differentially expressed genes and pathways. Intracellular lactate and histone lactylation were measured in HCC and normal hepatocyte cell lines. The effects of glycolysis inhibitors (2-DG/Oxamate) on proliferation, migration, and invasion of the cells were evaluated, and ChIP-qPCR was used to detect the enrichment of H3K18la at the *S100A2* promoter. TCGA database was used to analyze the association of S100A2 expression with the prognosis of HCC patients. *S100A2* overexpression and knockdown cell models were established, and rescue experiments using 2-DG or lactate were performed to validate the role of H3K18la in mediating the effects of S100A2. The interaction between S100A2 and KRT6A proteins was validated by STRING, molecular docking, and Co-IP. Western blotting was performed to determine the nuclear expression of β -catenin and the EMT markers (vimentin and E-cadherin) in HCC cells, and to elucidate the regulatory effect of H3K18la on the Wnt/ β -catenin pathway. **Results** Glycolysis and pyruvate metabolism pathways were upregulated in HCC, and lactate levels were elevated in HCC cells. Pan-K1a and H3K18la were significantly overexpressed in HCC cell lines, especially under hypoxia. Glycolysis inhibitors dose-dependently reduced H3K18la and suppressed malignant phenotypes of HCC cells. S100A2 mRNA and protein levels were upregulated in HCC, and its high expression was correlated with poor prognosis. S100A2 promoted HCC cell proliferation, migration, and invasion, which were reversed by 2-DG. S100A2 directly interacted with KRT6A, the expression of nuclear β -catenin was reduced after KRT6A knockdown and increased after KRT6A overexpression; exogenous lactate reversed KRT6A overexpression-induced inhibition of β -catenin, which was blocked by XAV939. Treatment with 2-DG lowered the expressions of β -catenin and EMT markers, which were reversed by *S100A2* or KRT6A overexpression. **Conclusion** HCC-derived lactate enhances H3K18la modification,

which activates *S100A2* transcription to positively regulate the Wnt/ β -catenin pathway, ultimately promoting the proliferation, migration, invasion and EMT of HCC cells.

Keywords: histone lactylation; S100A2; hepatocellular carcinoma; Wnt/ β -catenin signaling pathway; epithelial-mesenchymal transition

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肝细胞癌(HCC)是全球第6大常见癌症,也是癌症相关死亡的第3主要原因。中国是HCC发病人数最多的国家,每年新发和死亡病例均占全球近一半^[1]。手术、化疗和放疗是目前HCC的主要治疗手段,但术后复发率高、早诊率低、可治愈人群比例有限,使得肝癌患者的长期生存率仍然较低^[2]。因此,深入解析HCC的分子机制,对开发精准有效的HCC诊断及治疗方案至关重要。

翻译后修饰(PTM)是蛋白质合成后修饰的重要生化过程,可参与影响蛋白质的功能、稳定性和相互作用^[3]。各种类型的PTMs已被证实能够调节肿瘤微环境中的多种生物学过程,如恶性细胞增殖、免疫细胞激活和代谢重编程,靶向PTM在癌症治疗中具有巨大的潜力。此外,癌细胞中糖酵解激活和乳酸积累是各种类型癌症的常见特征^[4]。研究表明,乳酸可以驱动一种新型的蛋白质PTM——组蛋白赖氨酸乳酸化(Kla)的形成^[5]。这种新型的蛋白质PTM在基因转录调控中至关重要,尤其是组蛋白H3赖氨酸18位点的乳酸化修饰(H3K18la),已被报道参与调控多种生物学过程,如肿瘤发生^[6]、肿瘤进展^[7]、肿瘤免疫逃逸^[8]和癌细胞代谢重编程^[4]等,然而其在HCC的生物学过程中的影响尚不明确。研究发现,在HCC细胞系中组蛋白乳酸化水平高于肝正常细胞系,且H3组蛋白的乳酸化上调可有效促进HCC进展^[9,10]。有研究通过组蛋白乳酸化ChIP-seq鉴定出7个在肿瘤组织中高表达且其启动子区域存在H3K18la富集的候选基因,包括*KLK10*、*LEMD1*、*MMP7*、*RUBCNL*、*S100A2*、*VEGFA*、*VSNL1*^[11]。*S100A2*钙结合蛋白A2(*S100A2*)作为人类*S100*钙结合蛋白家族成员之一,主要位于上皮细胞^[12]并广泛表达于细胞核中,在细胞质中也有适度表达^[13]。研究表明,*S100A2*具有干细胞功能和上皮-间质转化(EMT)调节作用,*S100A2*沉默可通过抑制Wnt/ β -catenin信号通路缓解肺纤维化的EMT^[14],同时,*S100A2*还可通过协调TGF- β /Smad2/3信号通路诱导胰腺癌的EMT和转移^[15],且*S100A2*在乳腺癌中被视为上皮-间质可塑性特征基因之一^[16],其表达上调与乳腺癌的转移及不良预后相关。新近研究亦发现*S100A2*在HCC患者体内的高表达并与其临床晚期特征^[17]和较差的总生存期^[18]相关。

然而,现有研究主要集中于H3K18la通过调控代谢相关基因或信号通路影响HCC细胞增殖和代谢重编程,对于H3K18la是否通过调控特定靶基因进而影响HCC EMT过程尚未见报道。*S100A2*在EMT中的具体作用分子机制尚未完全阐明。因此,本研究旨在阐明糖酵解-H3K18la-*S100A2*-KRT6A-Wnt/ β -catenin信号轴在HCC EMT中的调控机制。在HCC中糖酵解代谢激活会导致乳酸积累,驱动组蛋白H3K18la修饰上调,该修饰直接促进*S100A2*转录激活;进一步证实*S100A2*与Wnt/ β -catenin信号通路核心因子KRT6A存在蛋白

相互作用,正向调控Wnt/ β -catenin信号通路,最终驱动HCC细胞EMT过程。

1 材料和方法

1.1 生物信息学分析

利用GEO公共数据库(<https://www.ncbi.nlm.nih.gov/geo/>)中的公开数据集(数据集编号GSE242889),从该数据集中选取并分析中国HCC患者的肿瘤与配对正常肝组织(各3例)的单细胞转录组测序(scRNA-seq)数据。根据唯一分子标识符、基因数量和线粒体基因百分比等标准筛选高质量细胞。经质量控制后,将筛选后的全细胞降维聚类,对聚类后的细胞簇标记已知的标记基因以识别细胞类型。标记基因注释完成后提取肝上皮细胞,比较HCC与正常肝组织样本间的基因表达差异,以筛查差异表达基因(DEGs)供进一步的功能富集分析,包括基因集富集分析(GSEA)和基因本体论(GO)分析。使用GSEA软件,基于HCC组和正常肝组织组的基因表达变化排序列表,对MSigDB中的Hallmark基因集行富集分析。使用clusterProfiler包,对筛选出的HCC上皮细胞中上调的差异基因行GO富集分析,从生物过程(BP)、细胞组分(CC)和分子功能(MF)3个维度,确定DEGs富集的生物学功能条目,以具体阐释在HCC中发生改变的细胞活动、定位和分子机制。上述数据使用R(版本4.3.2)进行分析。

通过UALCAN平台(<https://ualcan.path.uab.edu/analysis.html>)对TCGA数据库进行分析,检测了*S100A2*在肝细胞癌(Liver hepatocellular carcinoma, LIHC)中的表达水平。随后,使用UALCAN平台对TCGA数据库进行分析,检测了*S100A2*表达水平与肝细胞癌患者总生存期的关系,该工具直接提供了基于TCGA等数据库的Kaplan-Meier生存曲线及P值统计结果。所有图表与分析结果均直接从上述网站获取并导出。

1.2 细胞培养

人源肝癌细胞系(HepG2、Hep3B、Huh-7、SMMC-7721、Bel-7402)和人源肝正常细胞系(L-O2)购自中国科学院细胞库,细胞株均用含10%胎牛血清(Vazyme, F101-01)的DMEM高糖(普诺赛, PM150210)培养液培养。常氧条件下,细胞于37℃、5% CO₂气体环境培养箱中培养。为模拟缺氧条件,细胞于37℃、1% O₂、5% CO₂、94% N₂气体环境培养箱中培养。为检测乳酸及糖酵解抑制剂的作用,分别将不同浓度乳酸(0.5、10、15 mmol/L)或2-DG、oxamate(0.1、2、4 mmol/L)加入培养液,随后置于低氧条件(1% O₂、5% CO₂、94% N₂)下培养24 h,以0 mmol/L作为相应对照。

1.3 细胞转染

*S100A2*及*KRT6A*的siRNA及过表达质粒由Gene Create(中国武汉)设计(表1)。分别筛选HCC细胞系中*S100A2*蛋白表达最高与最低的细胞系进行siRNA或过

表达质粒的转染,使用Lipofectamine 3000(Invitrogen)转染 siRNA 或质粒,具体操作步骤参照说明书,转染后分别通过 Western blotting 和 RT- qPCR 方法验证转染效果,转染株构建成功后用于后续实验。

表1 siRNA靶向序列

Tab.1 Targeted sequences of siRNA

siRNA	Sequence
siRNA- <i>Ctrl</i>	5'-CCTAAGGTTAAGTCGCCCTCGCTC-3'
siRNA- <i>S100A2</i>	5'-GATGAGAACAGTGACCAGCAG-3'
siRNA- <i>KRT6A</i>	5'-CCAGCAGGAAGAGCUAUA-3'

1.4 逆转录实时定量聚合酶链式反应(RT-qPCR)

将不同处理组的细胞分别接种于6孔培养板内培养,收集细胞样品,Trizol法提取总RNA,参照试剂盒说明书逆转录为cDNA。使用SYBR Green PCR预混液(Vazyme)和实时荧光定量PCR系统7500(Thermo Scientific,)进行RT-qPCR。以 β -actin为内参,采用 $2^{-\Delta\Delta Ct}$ 法计算*S100A2*的mRNA相对表达量。引物序列来自美国国立生物技术信息中心NCBI数据库,由擎科生物(中国北京)合成,引物序列见表2。

表2 RT-qPCR引物序列

Tab.2 Primers for RT-qPCR

Gene		Sequence
<i>S100A2</i>	Forward	GCCAAGAGGGCGACAAGTT
	Reverse	AGGAAAACAGCATACTCCTGGA
β -actin	Forward	GACCTGACTGACTACCTCATGGAAGAT
	Reverse	GTCACCTCATGATGGAGTTGAAGG

1.5 Western blotting检测

用PBS清洗HCC细胞3次后,于冰上用RIPA强裂解液(含1%蛋白酶抑制剂、磷酸酶抑制剂及PMSF)裂解细胞,超声处理后4℃、12 000 g离心30 min取上清。BCA法测定蛋白浓度。取蛋白与5×上样缓冲液按4:1混合,100℃变性10 min。经SDS-PAGE分离后转至PVDF膜,5%脱脂牛奶封闭1 h,一抗4℃孵育过夜,PBST洗涤后与HRP标记的山羊抗小鼠IgG二抗(1:10 000,BA1050)或HRP标记的山羊抗兔IgG二抗(1:10 000,BA1054,BOSTER)室温孵育1 h,最后采用ECL化学发光法检测。使用的一抗为:anti-L-Lactyl Lysine (PTM-1425RM)、anti- L-Lactyl-Histone H3 (Lys18) (PTM-1427RM)、anti-L-Lactyl-Histone H3 (Lys9) (PTM-1419RM)、anti-L-Lactyl-Histone H4 (Lys8) (PTM-1415RM, PTM BIO), anti-Histone H3 (Abmart, P30266), anti-S100A2 (15454-1-AP)、anti-KRT6A (10590-1-AP)、anti- β -catenin (51067-2-AP)、

anti-Vimentin (10366-1-AP)、anti-E-cadherin (20874-1-AP)、anti- β -actin(66009-1-Ig, Proteintech)。以上一抗稀释比例均为1:1000。

1.6 免疫共沉淀(Co-IP)实验

使用含有1% PMSF、1%蛋白酶抑制剂及磷酸酶抑制剂的IP级裂解液(KeyGEN BioTECH, KGB5202-100)于冰上裂解样品30 min。裂解后,将样品于4℃、12 000 r/min离心30 min并收集上清液。上清液分为Input、IP和IgG组,Input组作为对照,IP组中加入3 μ g S100A2抗体,IgG组中加入3 μ g IgG抗体,4℃翻转孵育过夜。第2天,加入30 μ L/管Protein A/G免疫沉淀磁珠,继续4℃翻转孵育过夜。第3天,用预冷的PBST与PBS缓冲液交替洗涤磁珠复合物各3次。最后,向磁珠中加入1×IP级上样缓冲液,煮沸变性,样品保存于-80℃。第4天,取样品进行Western blotting检测。

1.7 细胞增殖、迁移和侵袭实验

采用CCK-8和克隆形成实验检测HCC细胞增殖能力。采用Transwell小室迁移和侵袭实验分别检测HCC细胞的迁移和侵袭能力。

1.7.1 CCK-8法 将处于对数生长期、经不同处理的HCC细胞以 1×10^3 /孔的密度接种于96孔板(5复孔)。培养24 h后,每天同一时间加入CCK-8溶液(10 μ L/孔),孵育2 h,酶标仪测定吸光度 A_{450nm} ,连续检测5 d。

1.7.2 克隆形成实验 将处于对数生长期、经不同处理的HCC细胞以 1×10^3 /孔的密度接种于6孔板中,培养7 d(定期更换培养液)。PBS洗涤后,苏木素染色过夜,流水漂洗、干燥,拍照并计数克隆。

1.7.3 Transwell迁移实验 将处于对数生长期、经不同处理的Huh-7和HepG2细胞,用无血清培养液重悬,调整细胞密度。取 2×10^5 细胞(无血清培养基,300 μ L)接种于上室(孔径8 μ m),下室加600 μ L含20% FBS的培养基。培养24 h后,擦去上室面细胞,下室面细胞用4%多聚甲醛固定、结晶紫染色,显微镜下随机选5个视野拍照计数。

1.7.4 Transwell侵袭实验 实验步骤同1.6.3迁移实验,但上室预包被Matrigel胶(康宁,354277),细胞密度为 2×10^5 /300 μ L,其余条件一致。孵育48 h后擦去未侵袭细胞及Matrigel,固定、染色、拍照并计数。

1.8 染色质免疫沉淀-实时荧光定量PCR(ChIP-qPCR)实验

利用EZ-Magna ChIP™试剂盒(Merck Millipore, 56383)进行染色质免疫沉淀(ChIP)实验。细胞经1%甲醛室温交联10 min,甘氨酸终止后裂解,超声剪切染色质。取上清与抗H3K18la抗体(10 μ L/IP)于4℃孵育过夜,再与Protein G磁珠结合2 h。磁珠洗涤后,洗脱免疫复合物,并用NaCl/蛋白酶K于65℃过夜解交联。纯化DNA进行RT-qPCR分析,引物序列见表3。

表3 ChIP-qPCR引物序列
Tab 3. Primers for ChIP-qPCR

Gene	Sequence
S100A2 promoter	Forward 5'-GAAGTGGGAGGTGTCGTGG-3'
	Reverse 5'-CGAGCACTGGACCATGAAGT-3'

1.9 分子对接分析

利用 GRAMM-X (<http://gramm.compbio.ku.edu/>) 软件对 S100A2 和 KRT6A 进行蛋白-蛋白刚性对接, 分析 S100A2 和 KRT6A 之间的关系。S100A2 和 KRT6A 的蛋白结构域从 Protein Data Bank PDB 数据库 (<http://www.rcsb.org/>) 中获得。Pymol (版本 3.0.3) 和 PDBePISA (<https://www.ebi.ac.uk/pdbe/pisa/>) 用于研究蛋白质之间的相互作用, 并进一步进行可视化分析。

1.10 统计学分析

采用 GraphPadPrism8.0 版和 SPSS26.0 进行统计分析。计量资料以均数±标准差表示, 使用单因素方差分析, 两组比较采用 Student's test 检验多组比较。 $P<0.05$ 为差异具有统计学意义。

2 结果

2.1 HCC 中糖酵解代谢激活且乳酸水平升高

GEO 数据库分析显示, 对该数据集进行降维聚类分析, 基于 UMAP 的细胞分群鉴定出 T/NK 细胞、肝上皮细胞、髓系细胞、B 细胞等 5 类细胞亚群 (图 1A~C)。进一步筛选了正常组与肿瘤组上皮细胞之间的 DEGs, 发现糖酵解关键基因 *LDHA*、*ALDOB* 在肿瘤组中表达上调 (图 1D)。*ALDOB* 是糖酵解途径中催化果糖-1,6-二磷酸转化为 3-磷酸甘油醛的催化酶, 而 *LDHA* 则是催化丙酮酸转变为乳酸的关键酶 (图 1E)。GSEA 和 GO 富集分析结果显示 HCC 中丙酮酸代谢活性通路富集 (图 1F、G)。HCC 细胞系中的乳酸水平高于肝正常细胞系 (图 1H)。

2.2 糖酵解驱动的组蛋白 H3K18la 修饰促进 HCC 进展

检测 HCC 细胞系及肝正常细胞系 Pan K1a、H3K18la 蛋白水平发现, 在 HCC 细胞系中 Pan K1a 及 H3K18la 表达水平升高 ($P<0.01$, 图 2A、B)。缺氧条件下, HepG2 细胞中 H3K9la、H3K18la 及 H4K8la 水平均升高 ($P<0.05$, 图 2B、C), H3K18la 水平升高最为显著 ($P<0.001$)。2-DG 与 Oxamate 均可浓度依赖性地降低 HepG2 细胞中 H3K18la 水平 ($P<0.05$, 图 2E、F)。CCK-8 实验结果显示, 糖酵解抑制剂组 (2-DG/Oxamate) 细胞的增殖速率减慢 ($P<0.001$, 图 2G)。克隆形成实验显示, 糖酵解抑制剂组形成的细胞克隆数量明显少于对照组, 且克隆体积更小 (图 2H、I)。Transwell 迁移和侵袭实验结果显示, 2-DG 或 Oxamate 处理后, 迁移和侵袭的细胞数量较对照组减少 ($P<0.05$, 图 2J、K)。

2.3 H3K18la 介导 *S100A2* 转录激活并与 HCC 不良预后相关

ChIP-qPCR 实验结果显示, H3K18la 在 *S100A2* 启动子区域的富集水平高于 IgG 对照组 ($P<0.001$, 图 3A)。缺氧条件下 HepG2 细胞中 S100A2 蛋白表达水平随时间延长而逐渐升高 ($P<0.05$, 图 3B、C)。乳酸处理可浓度依赖性上调 S100A2 蛋白表达 ($P<0.05$, 图 3D、E)。Western blotting 检测结果显示, 2-DG 处理可降低 H3K18la 与 S100A2 蛋白水平; 而乳酸处理可逆转 2-DG 对 S100A2 的下调作用 ($P<0.05$, 图 3F~H)。TCGA 数据库分析结果显示, *S100A2* 在 LIHC 中表达上调 ($P<0.001$, 图 3I)。Kaplan-Meier 生存分析显示, 高表达 *S100A2* 的患者组总生存期低于中/低表达组 ($P<0.05$, 图 3J)。Western blotting 检测结果显示, HCC 细胞系中 S100A2 蛋白水平高于肝正常细胞系 ($P<0.01$, 图 3K、L)。

2.4 *S100A2* 促进 HCC 细胞的增殖、迁移和侵袭能力

RT-qPCR 及 Western blotting 检测结果显示, *S100A2* 在 5 种 HCC 细胞系中存在 mRNA 及蛋白表达差异 (图 4A~C)。Huh-7 细胞中敲低 *S100A2* 后, *S100A2* 蛋白及 mRNA 水平降低 ($P<0.05$, $P<0.01$), HepG2 细胞中过表达 *S100A2* 后, *S100A2* 蛋白及 mRNA 水平升高 ($P<0.001$, 图 4D~G)。CCK-8 实验结果显示, 敲低 *S100A2* 可抑制 Huh-7 细胞增殖, 过表达 *S100A2* 促进 HepG2 细胞增殖 ($P<0.05$, 图 4H、I)。克隆形成实验结果显示, *S100A2* 敲低组细胞克隆数量少于对照组, 克隆体积更小 ($P<0.01$, 图 4J)。*S100A2* 过表达组细胞克隆形成能力增强 ($P<0.001$, 图 4K)。Transwell 迁移及侵袭实验结果显示, *S100A2* 敲低组迁移和侵袭的细胞相对数量较对照组降低 ($P<0.01$, $P<0.05$), *S100A2* 过表达组迁移和侵袭的细胞相对数量较对照组增加 ($P<0.01$, 图 4L、M)。

2.5 H3K18la 修饰通过调控 *S100A2* 表达促进 HCC 细胞的增殖、迁移和侵袭能力

Western blotting 检测结果显示, 在 *S100A2* 敲低细胞中补充乳酸可升高 H3K18la 及 S100A2 蛋白水平 ($P<0.01$, 图 5A)。功能实验表明, *S100A2* 敲低组细胞克隆形成数量、迁移及侵袭细胞相对数量均减少 ($P<0.05$), 而乳酸处理可促进上述指标 ($P<0.001$), 部分逆转了 *S100A2* 敲低的作用 (图 5B~D)。反之, 在 *S100A2* 过表达的 HepG2 细胞中, 糖酵解抑制剂 2-DG 处理可下调 H3K18la 及 S100A2 蛋白水平 ($P<0.001$, 图 5E)。功能实验显示, *S100A2* 过表达组细胞克隆形成数量、迁移及侵袭细胞相对数量均增加 ($P<0.01$), 而 2-DG 处理可降低上述指标 ($P<0.01$), 部分逆转了 *S100A2* 过表达的作用 (图 5F~H)。

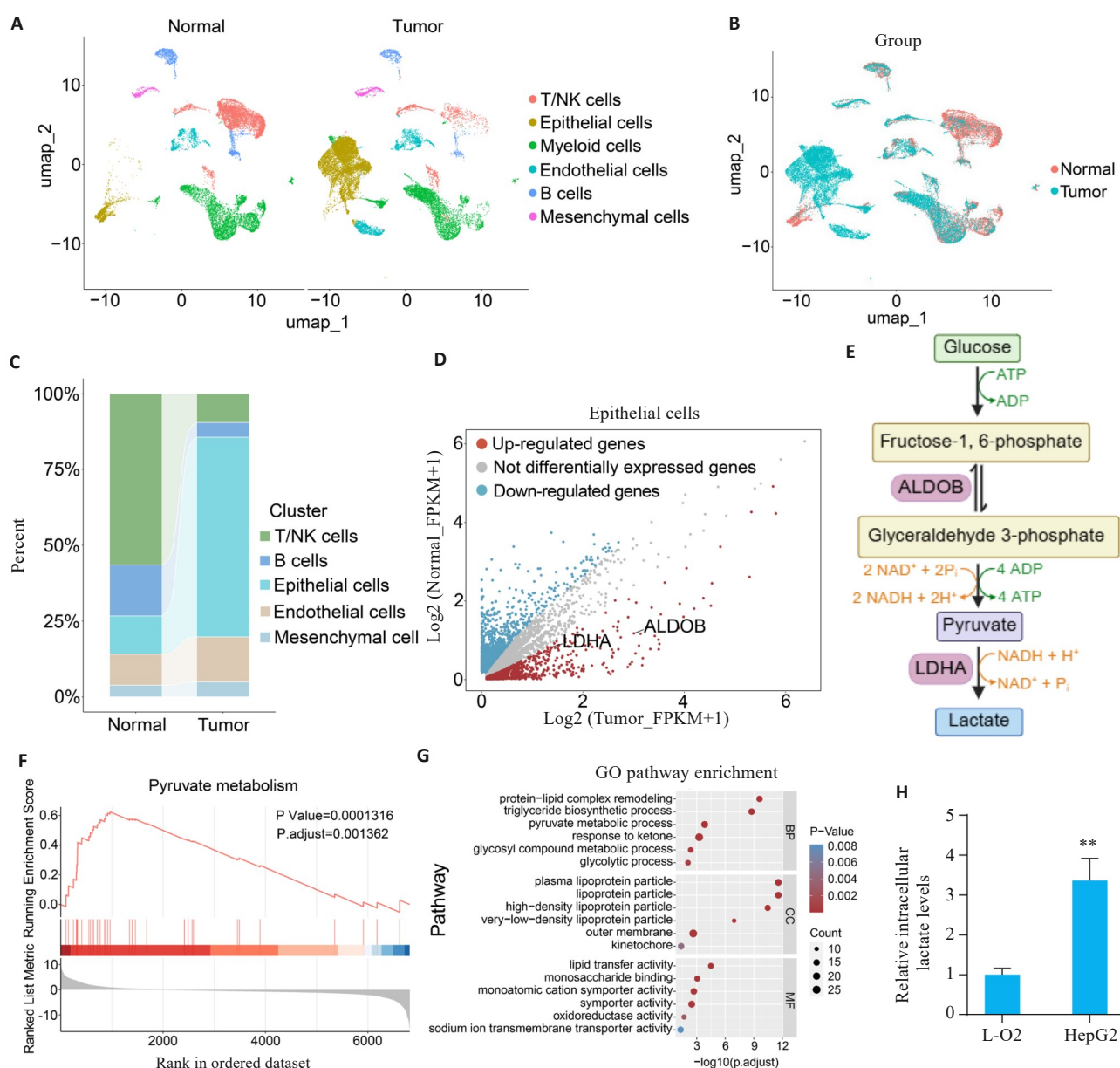


图1 HCC中糖酵解代谢途径及乳酸水平变化情况

Fig. 1 Changes in pyruvate metabolic process and lactate levels in HCC. **A-C**: ScRNA-seq analysis of HCC tissues and paired adjacent normal liver tissues from 3 patients with primary HCC. **D**: Scatter plot of DEGs between normal and tumor samples. Red dots, upregulated genes in tumors; blue dots, downregulated genes; gray dots, genes without significant changes. Selection criteria: adjusted *P* value (*P*_{adj}) < 0.05 and |log₂(fold change)| ≥ 1. **E**: Schematic illustration of the glycolytic pathway. **F**: GSEA showing enrichment of pyruvate metabolism in HCC. **G**: GO enrichment analysis of upregulated DEGs in epithelial cells from tumor samples. **H**: Lactate levels in HCC and normal hepatic cell lines. Data are presented as Mean ± SD (*n* = 3). ***P* < 0.01 vs L-O2 group.

2.6 S100A2与KRT6A存在相互作用

STRING在线工具预测结果显示, S100A2蛋白与KRT6A蛋白可能存在相互作用(图6A)。分子对接结果显示, S100A2与KRT6A可通过氨基酸残基位点成键(图6B)。Co-IP实验结果显示, S100A2与KRT6A存在蛋白相互作用(图6C、D)。

2.7 KRT6A调控Wnt/β-catenin信号通路与EMT促进HCC进展

Western blotting实验结果显示, 敲低KRT6A降低了细胞核内β-catenin蛋白水平(*P* < 0.001, 图7A)。过表

达KRT6A提高了细胞核内β-catenin蛋白水平(*P* < 0.001, 图7B)。外源乳酸可促进核内β-catenin蛋白表达, 该效应可被XAV939阻断; 此外, 外源乳酸能够部分逆转敲低KRT6A所引起的β-catenin下调(图7C)。糖酵解抑制剂2-DG处理可下调核内β-catenin及Vimentin、上调E-cadherin的蛋白表达水平, 而在2-DG处理基础上过表达S100A2或KRT6A, 可部分逆转上述分子变化(*P* < 0.01, 图7D、E)。敲低KRT6A下调Vimentin、上调E-cadherin表达, 过表达KRT6A上调Vimentin、下调E-cadherin表达(*P* < 0.05, 图7F、G, 图8)。

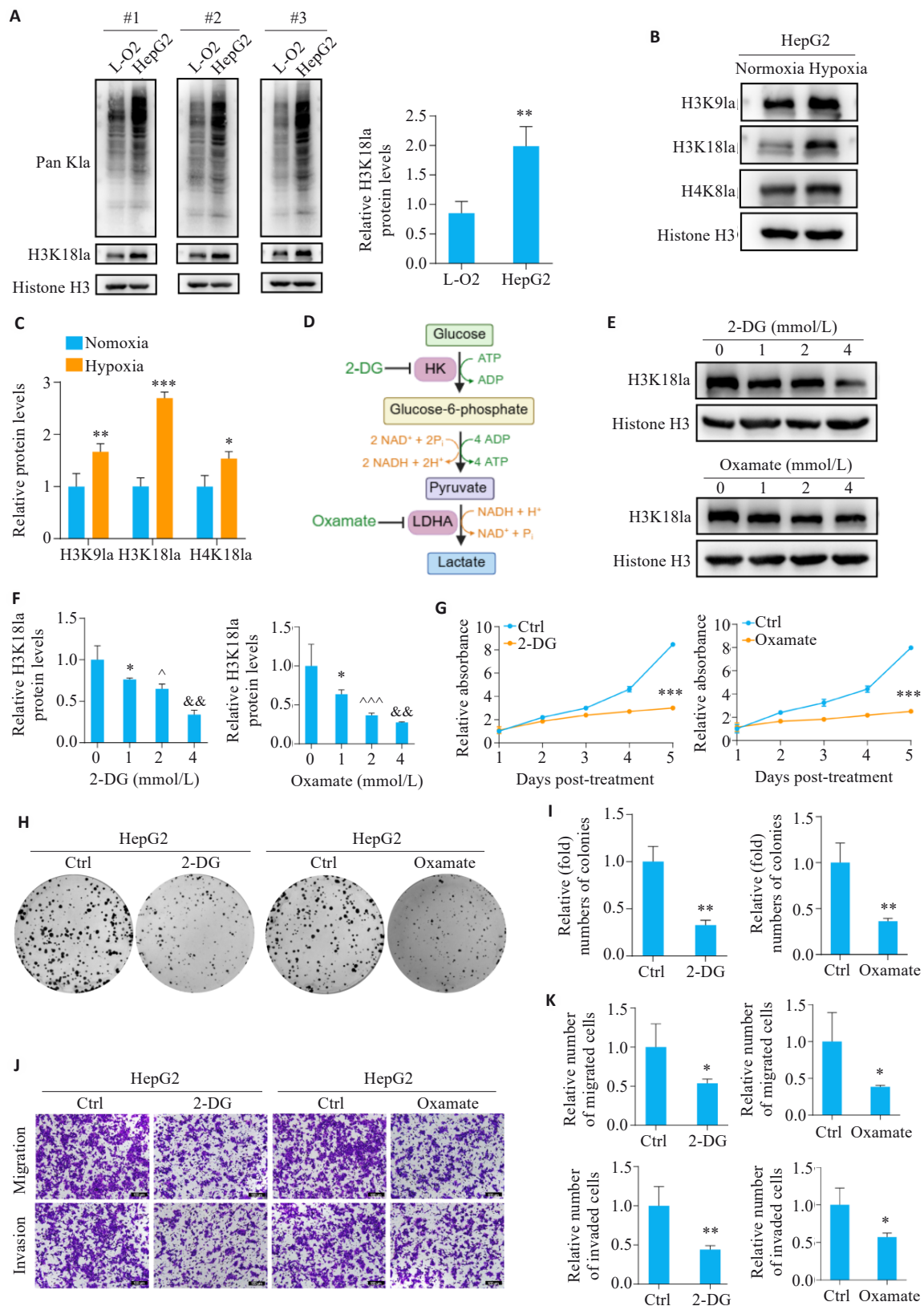


图2 糖酵解驱动的组蛋白H3K18la修饰促进HCC进展

Fig.2 Glycolysis-driven H3K18la promotes HCC progression. **A**: Western blotting of Pan K1a and H3K18la levels in HCC and normal hepatic cell lines. **B**, **C**: Western blotting of H3K9la, H3K18la, and H4K8la levels in HepG2 cells under normoxia and hypoxia. **D**: Schematic of glycolytic pathway and its inhibitors. **E**, **F**: Western blotting and of H3K18la levels following treatment with graded concentrations of 2-DG and oxamate for 24 h in hypoxia (1% oxygen). **G**: CCK-8 assay for the effect of 2-DG and oxamate for 24 h in hypoxia (1% oxygen) on the proliferation of HepG2 cells. **H-I**: Colony formation assay of HepG2 cells in different treatment groups with hematoxylin staining. **J-K**: Transwell migration and invasion assays of HepG2 cells in different treatment groups with crystal violet staining (Scale bar=100 μ m). Data are presented as Mean $\pm$ SD (n=3). * P <0.05, ** P <0.01, *** P <0.001 vs Normal, Normoxia or Ctrl group; ^ P <0.05, ^^ P <0.001 vs 2-DG (1 mmol/L) or oxamate (1 mmol/L) group; && P <0.01 vs 2-DG (2 mmol/L) or oxamate (2 mmol/L) group.

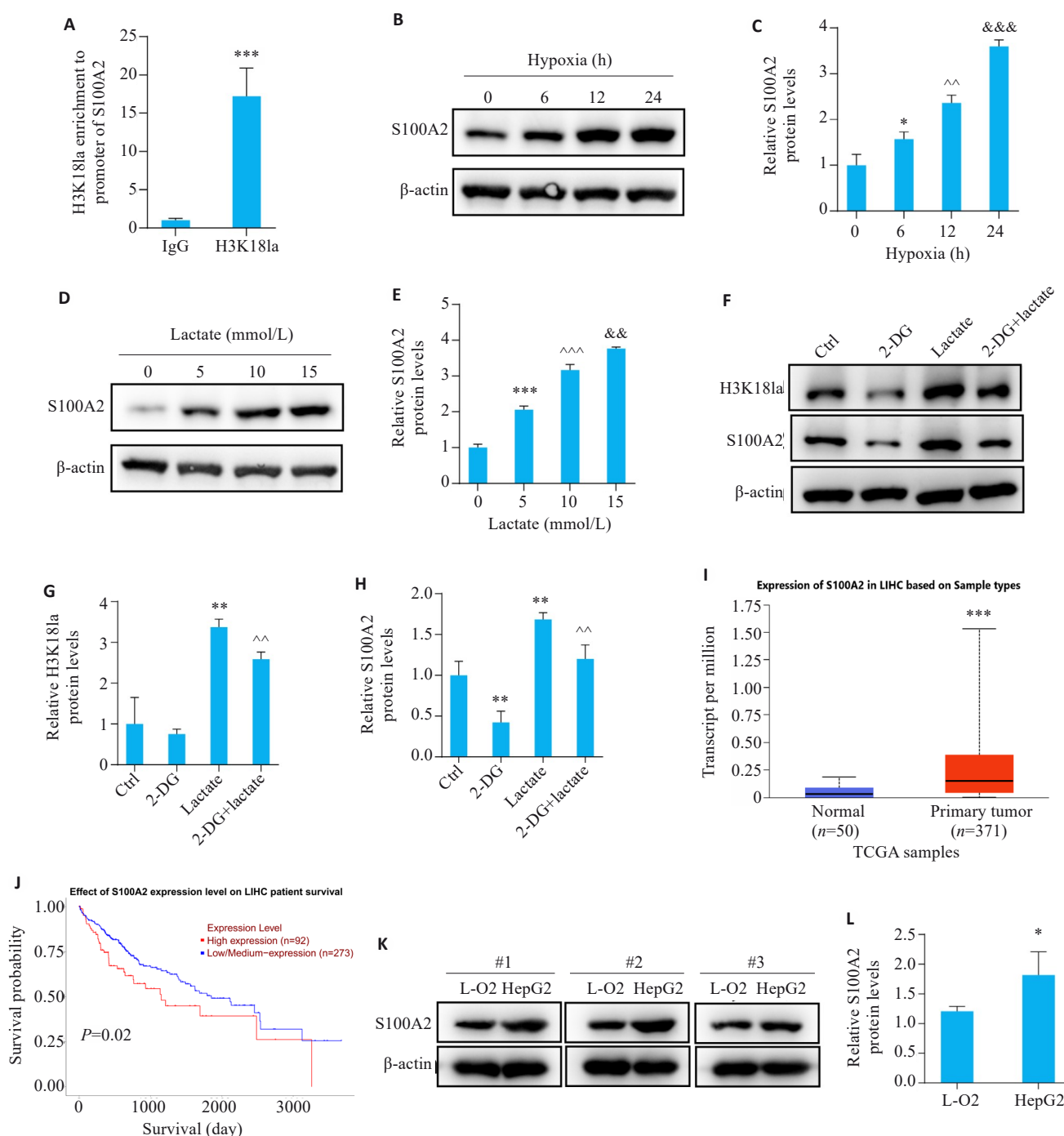


图3 H3K18la介导S100A2转录激活并与HCC不良预后相关

Fig.3 H3K18la-mediated transcriptional activation of *S100A2* is associated with poor prognosis in HCC. **A:** ChIP-qPCR analysis of H3K18la enrichment at the *S100A2* promoter. **B, C:** Western blotting of *S100A2* expression in HepG2 cells under hypoxia at indicated time points. **D, E:** Western blotting of *S100A2* expression in HepG2 cells treated with lactate at indicated time points. **F-H:** Western blotting of H3K18la and *S100A2* expression in HepG2 cells treated with 2-DG, lactate or 2-DG with lactate. **I:** Analysis of *S100A2* expression in LIHC using TCGA database. **J:** Kaplan-Meier analysis of the association between *S100A2* expression and prognosis in LIHC patients. **K, L:** Western blotting of *S100A2* expression in HCC and normal hepatic cell lines. Data are presented as Mean $\pm$ SD (n=3). *P<0.05, **P<0.01, ***P<0.001 vs IgG, Hypoxia (0 h), Lactate (0 mmol/L), Ctrl or L-O₂ group; ^^P<0.01, ^^^P<0.001 vs Hypoxia (6 h), Lactate (5 mmol/L) group or Lactate group; &&P<0.01, &&&P<0.001 vs Hypoxia (12 h) or Lactate (10 mmol/L) group.

3 讨论

代谢重编程已成为恶性肿瘤的核心标志之一^[19],癌细胞会通过重塑自身代谢过程来维持其快速增殖、迁移及侵袭等恶性生物学行为。癌细胞中糖酵解代谢激活和代谢产物乳酸积累是各种类型癌症的常见特征^[4]。研究

发现,乳酸可作为一种新型的表观遗传修饰直接刺激基因转录^[20],促进组蛋白赖氨酸的乳酸化修饰,这一新型修饰过程已被证明与肿瘤细胞的糖酵解代谢激活密切相关,尤其是H3K18la修饰,被报道参与调控多种生物学过程^[11]。然而,既往研究多聚焦H3K18la在肿瘤中的广谱

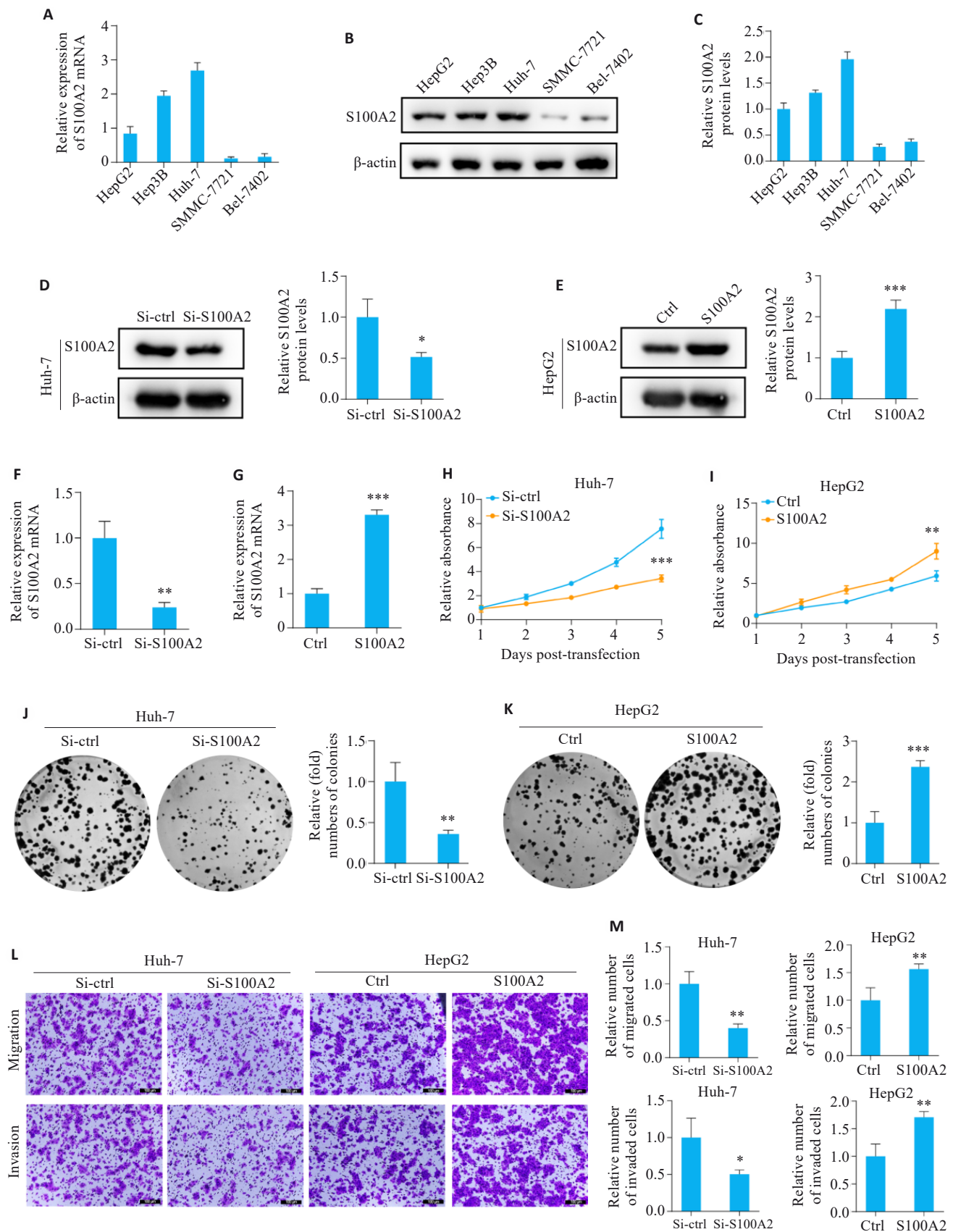


图4 S100A2促进HCC细胞的增殖、迁移和侵袭能力

Fig.4 S100A2 promotes proliferation, migration, and invasion of HCC cells. A-C: RT-qPCR and Western blotting of S100A2 mRNA and protein expression in 5 HCC cell lines. D-G: Western blotting and RT-qPCR analysis of S100A2 expression in HCC cells following overexpression or knockdown. H-I: CCK-8 assay for cell proliferation in different treatment groups. J, K: Colony formation assay of the cells with hematoxylin staining. L, M: Transwell migration and invasion assays with crystal violet staining (Scale bar=100 μ m). Data are presented as *Mean* $\pm$ *SD* (*n*=3). **P*<0.05, ***P*<0.01, ****P*<0.001 vs si-ctrl or Ctrl group.

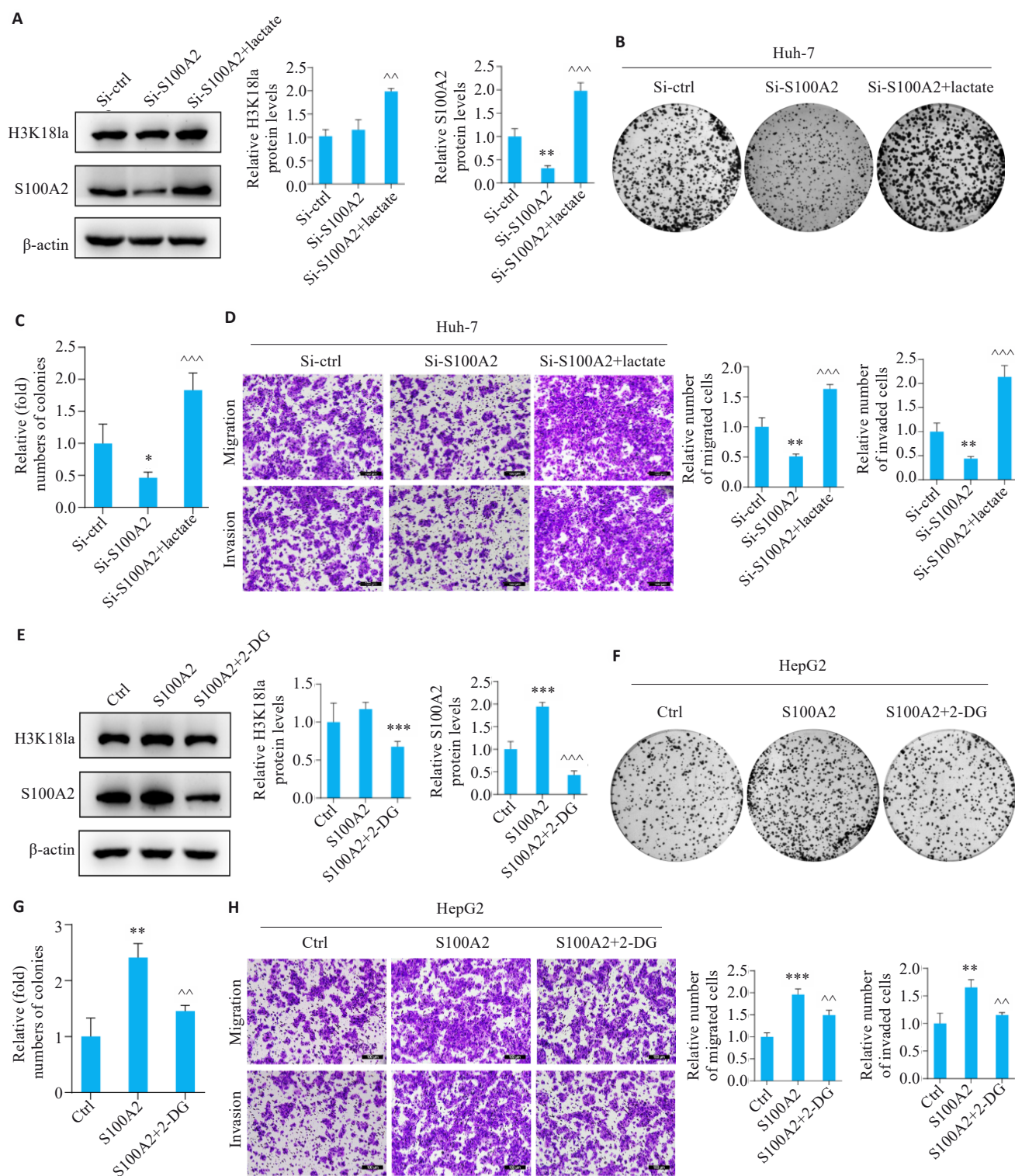


图5 H3K18la修饰通过调控S100A2表达促进HCC细胞的增殖、迁移和侵袭能力

Fig. 5 H3K18la modification promotes proliferation, migration, and invasion of HCC cells by regulating S100A2 expression. **A**: Western blotting of H3K18la and S100A2 expression in HepG2 cells in different treatment groups. **B**, **C**: Colony formation assay with hematoxylin staining. **D**: Transwell migration and invasion assays with crystal violet staining (Scale bar=100 μ m). **E**: Western blotting of H3K18la and S100A2 expression in Huh-7 cells in different treatment groups. **F**, **G**: Colony formation assay with hematoxylin staining. **H**: Transwell migration and invasion assays with crystal violet staining (Scale bar=100 μ m). Data are presented as $Mean \pm SD$ ($n=3$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs si-Ctrl or Ctrl group; ^^ $P<0.01$, ^^ $P<0.001$ vs si-S100A2 or S100A2 group.

调控作用,较少系统阐明其在HCC中特异性调控的靶基因及完整信号轴。本研究通过HCC单细胞转录组数据分析发现,糖酵解关键基因显著上调,丙酮酸代谢通路明显富集;细胞实验亦证实,HCC细胞中乳酸水平显著高

于正常肝细胞,且Pan K1a及H3K18la修饰水平均明显上调,缺氧可通过增强糖酵解进一步加剧该修饰。使用糖酵解抑制剂处理后,H3K18la水平降低,HCC细胞增殖、迁移及侵袭能力均被抑制。上述结果提示,H3K18la

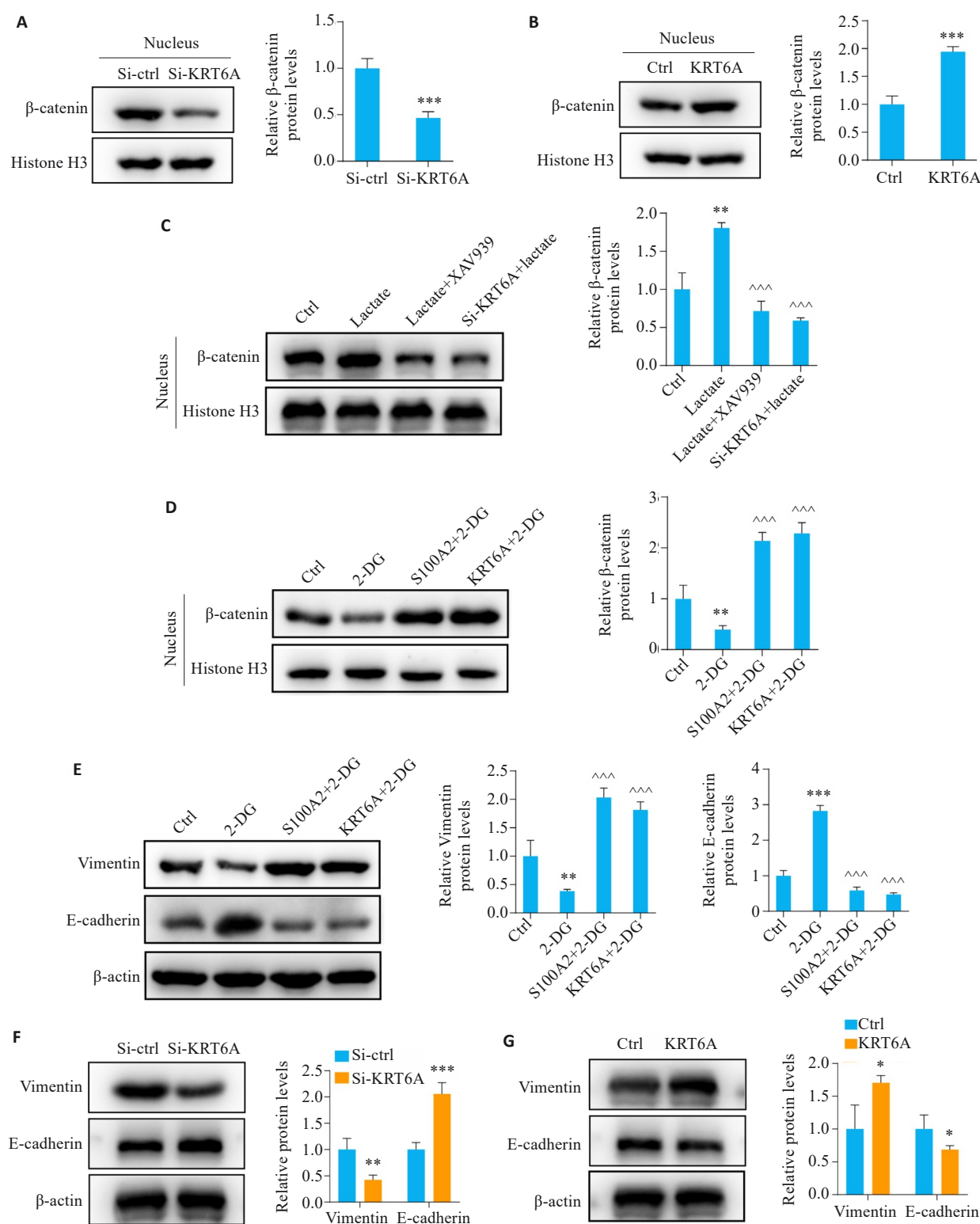


图7 KRT6A调控Wnt/β-catenin信号通路与EMT促进HCC进展

Fig.7 KRT6A promotes HCC progression by regulating the Wnt/β-catenin signaling pathway and EMT. **A, B**: Western blotting of nuclear β-catenin protein levels following KRT6A knockdown and overexpression; **C**: Western blotting of nuclear β-catenin in different treatment groups. **D**: Western blotting analysis of nuclear β-catenin in different treatment groups. **E**: Western blotting of vimentin and E-cadherin expression in different treatment groups. **F-G**: Western blotting analysis of Vimentin and E-cadherin expression following KRT6A knockdown and overexpression. Data are presented as Mean±SD (n=3). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs si-Ctrl or Ctrl group; ^^^ $P<0.001$ vs Lactate or 2-DG group.

调控 Wnt/β-catenin通路活性,这是对既往 KRT6A 仅作为结构蛋白认知的重要补充。

EMT 是一种使肿瘤细胞获得间质细胞特征从而增

强其扩散到远处器官的能力的一种复杂表型改变,在肿瘤的侵袭转移中起着关键作用^[31]。钙粘蛋白是 EMT 的重要标志物, E-cadherin/β-catenin 复合物在维持正常上

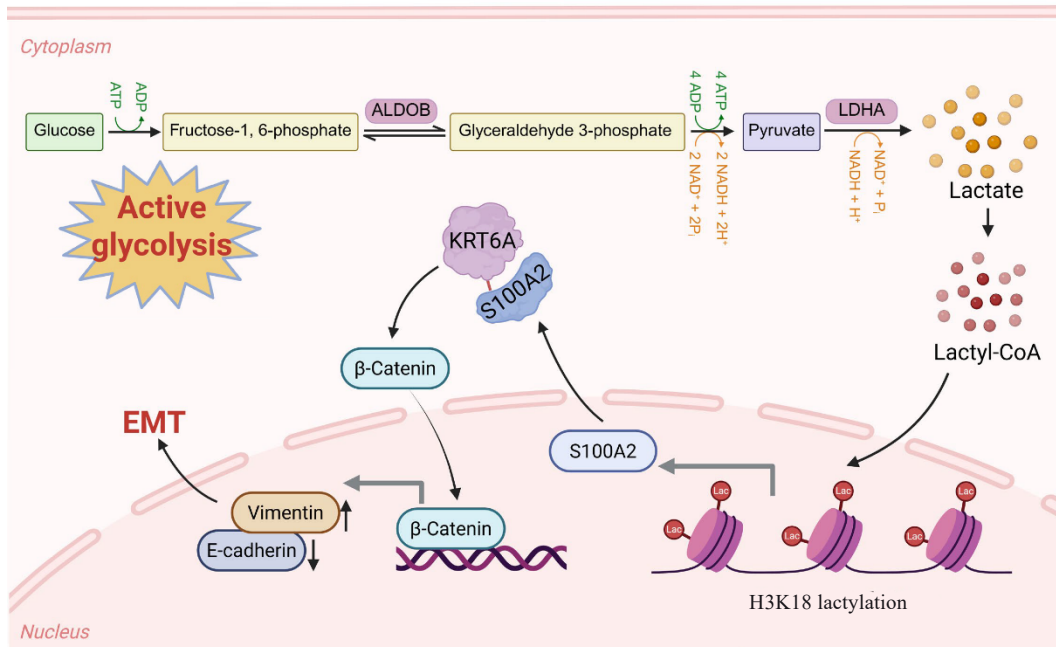


图8 H3K18la激活 S100A2/KRT6A/β-catenin信号轴促进肝细胞癌上皮间质转化过程的机制示意图

Fig.8 Mechanism of H3K18la for activating the S100A2/KRT6A/β-catenin signaling axis to promote the EMT process in HCC.

皮形态和细胞间粘附方面发挥着重要作用。多项研究表明 Wnt/β-catenin 信号通路的激活可促进各类癌症的 EMT 进程^[28, 32-34],与肿瘤细胞的侵袭转移密切相关。但尚未有研究将代谢重编程、组蛋白乳酸化、S100A2/KRT6A 复合物与 HCC 的 EMT 串联为完整机制。因此,本研究进一步检测了敲低与过表达 *KRT6A* 对 HCC 细胞 EMT 过程相关蛋白表达的影响,发现敲低 *KRT6A* 降低 Vimentin 蛋白表达并增加了 E-cadherin 蛋白表达,过表达 *KRT6A* 则逆转了这一效应,提示 *KRT6A* 参与促进了 HCC 细胞的 EMT 进程。本研究主要基于体外细胞实验及生物信息学分析,揭示了 H3K18la-S100A2-KRT6A 轴在 HCC 中的促癌机制。尽管 TCGA 数据提供了初步的临床相关性支持,但仍需在更大规模临床队列及体内动物模型中进一步验证该通路的临床意义与治疗靶向价值,这将是后续研究的重点方向。

综上所述,本研究揭示了 HCC 中糖酵解代谢激活、缺氧及乳酸堆积促进 H3K18la 增加,激活 *S100A2* 基因转录,蛋白表达上调,与 *KRT6A* 蛋白相互作用增强,进一步通过调控 Wnt/β-catenin 信号通路促进 β-catenin 核转位及 EMT 进程,驱动 HCC 细胞向上皮特性减弱、间质特性增强的侵袭性表型转变,最终促进 HCC 细胞增殖、迁移、侵袭及 EMT 的作用机制,证实了组蛋白乳酸化修饰可驱动 HCC 进展。与既往研究多单独关注代谢重编程、表观遗传修饰或单一信号分子不同,本研究将代谢紊乱、乳酸化修饰、S100A2/KRT6A 复合物及 Wnt/β-catenin 通路整合为统一的调控网络,系统阐明了 HCC 恶性进展的新机制,为深入理解肿瘤代谢重编程与表观

遗传调控的调控网络提供了新见解,同时为临床靶向组蛋白乳酸化治疗 HCC 提供了潜在靶点与理论依据。

Declaration of interests: The authors declare no competing interests.

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