



乌头酸脱羧酶1通过衣康酸-自噬轴促进肝细胞癌生长的机制研究*

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【摘要】目的 探讨乌头酸脱羧酶1(aconitate decarboxylase 1, ACOD1)在肝细胞癌中的表达水平及其对肝癌细胞生长的作用与相关机制。**方法** 基于GTEX与TCGA数据库分析ACOD1基因在肝癌中的表达及其与患者生存的关系,采用免疫印迹法检测4对肝癌及癌旁组织中ACOD1的蛋白表达。在肝癌细胞HepG2和PLC/PRF/5中敲低ACOD1基因后,通过MTT与LDH实验分别检测细胞增殖与死亡情况。在敲低ACOD1基因的同时给予4-辛基衣康酸(4-octyl itaconate, 4-OI),观察细胞增殖变化。利用cBioPortal数据库通过KEGG富集分析预测潜在机制,并采用免疫印迹检测自噬相关蛋白LC3-Ⅱ与p62的表达;进一步在敲低ACOD1基因后联合氯喹(chloroquine, CQ)处理或回补4-OI,观察自噬标志蛋白变化及细胞增殖情况。**结果** ACOD1在肝癌组织中的蛋白表达显著高于癌旁组织(约2倍),且高表达患者总生存期较短($P<0.05$)。在ACOD1高表达的HepG2和PLC/PRF/5细胞中,敲低ACOD1基因可抑制HepG2细胞(降低约20%)和PLC/PRF/5细胞(降低约30%)增殖(均 $P<0.05$),并提高细胞死亡率(均上升约30%,均 $P<0.05$)。4-OI以浓度依赖方式促进细胞生长,并能逆转敲低ACOD1基因导致的细胞活力下降,分别恢复至对照组的93%和90%(均 $P<0.05$)。KEGG分析提示ACOD1可能与自噬通路相关;敲低ACOD1基因后, HepG2和PLC/PRF/5细胞中LC3-Ⅱ水平显著上升(分别约7倍和10倍,均 $P<0.05$),而p62水平下降(分别40%和30%,均 $P<0.05$); CQ处理后, LC3-Ⅱ/LC3-Ⅰ比例和p62蛋白表达水平均增加2倍($P<0.05$),而联合敲低ACOD1基因可进一步增加LC3-Ⅱ/LC3-Ⅰ比例(约10%, $P<0.05$),且p62蛋白表达水平未见明显增加($P>0.05$)。回补4-OI可逆转敲低ACOD1基因引起的LC3-Ⅱ和p62蛋白表达水平变化;同时, CQ处理也能部分挽救敲低ACOD1基因所致的HepG2和PLC/PRF/5细胞活力下降(分别恢复至75%和80%,均 $P<0.05$)。**结论** ACOD1在肝癌中高表达且与不良预后相关;其通过合成衣康酸抑制细胞自噬,从而促进肝癌细胞生长。

【关键词】 肝细胞癌 乌头酸脱羧酶1 衣康酸 自噬 预后

Mechanism Study of Aconitase 1 Promoting Hepatocellular Carcinoma Growth via the Itaconic Acid-Autophagy Axis

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【Abstract】 Objective To investigate the expression level of aconitate decarboxylase 1 (ACOD1) in hepatocellular carcinoma (HCC) and its role in HCC cell growth, as well as the underlying mechanisms. **Methods** The expression of the ACOD1 gene in HCC and its correlation with patient survival were analyzed using the GTEX and TCGA databases. ACOD1 protein expression in four paired HCC and adjacent non-tumor tissues was detected by Western blotting. After ACOD1 knockdown in HepG2 and PLC/PRF/5 HCC cells, cell proliferation and death were assessed by MTT and LDH assays, respectively. Following ACOD1 knockdown, 4-octyl itaconate (4-OI) was administered to observe changes in cell proliferation. Potential mechanisms were predicted using the cBioPortal database through KEGG enrichment analysis, and the expression of autophagy-related proteins LC3-Ⅱ and p62 was measured by Western blotting. After ACOD1 knockdown, cells were treated with chloroquine (CQ) or supplemented with 4-OI to observe changes in autophagy marker proteins and cell proliferation. **Results** ACOD1 protein expression was significantly overexpressed in HCC tissues than in adjacent tissues (approximately 2-fold). Patients with ACOD1 overexpression had shorter overall survival

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($P < 0.05$). Knocking down *ACOD1* in HepG2 and PLC/PRF/5 cells, which expressed high levels of *ACOD1*, inhibited cell proliferation (by approximately 20% and 30%, respectively; both $P < 0.05$) and increased cell death (by approximately 30% in both cell lines; both $P < 0.05$). 4-OI promoted cell growth in a concentration-dependent manner and reversed the decrease in cell viability caused by *ACOD1* knockdown, restoring it to 93% and 90% of control levels, respectively (both $P < 0.05$). KEGG analysis suggested a potential association between *ACOD1* and the autophagy pathway. *ACOD1* knockdown significantly increased LC3- II levels (by approximately 7-fold and 10-fold, respectively; both $P < 0.05$) and decreased p62 levels (by 40% and 30%, respectively; both $P < 0.05$) in HepG2 and PLC/PRF/5 cells. CQ treatment increased the LC3- II/LC3- I ratio and p62 protein expression (both by approximately 2-fold; $P < 0.05$). Combined *ACOD1* knockdown further increased the LC3- II/LC3- I ratio (by approximately 10%; $P < 0.05$), while p62 protein expression did not increase significantly ($P > 0.05$). Supplementation with 4-OI reversed the changes in LC3- II and p62 protein expression induced by *ACOD1* knockdown. Furthermore, CQ treatment partially rescued the decrease in viability of HepG2 and PLC/PRF/5 cells caused by *ACOD1* knockdown (restoring viability to 75% and 80%, respectively; both $P < 0.05$). **Conclusion** *ACOD1* is overexpressed in HCC and its' overexpression is associated with poor prognosis. *ACOD1* promotes HCC cell growth by synthesizing itaconate, which inhibits cellular autophagy.

[Key words] Hepatocellular Carcinoma *ACOD1* Itaconic acid Autophagy Prognosis

肝癌是全球高致死性消化道恶性肿瘤。在我国,其发病率与死亡率分别居所有肿瘤的第四和第二位,患者多在晚期确诊,五年总体生存率仅约6.5%^[1]。现有手术、肝移植、放化疗、靶向及免疫治疗等手段虽能改善预后,但对长期生存率的提升仍有限^[2]。因此,阐明肝癌发生机制、寻找新治疗靶点,对开发有效疗法和改善患者结局至关重要。

细胞自噬是高度保守的细胞内降解过程。通过自噬包裹受损组分形成自噬体,进而与溶酶体融合降解,该动态过程称为自噬流^[3-4]。自噬在肿瘤中具有双重作用,可抑制或促进肿瘤发展,其效应取决于自噬流的动态平衡^[4-5]。因此,准确评估自噬流状态是理解自噬在肿瘤中功能的关键。

LEE等最早在小鼠巨噬细胞中鉴定出免疫应答基因1 (immune-responsive gene 1, *Irg1*)^[6]。研究证实,脂多糖可诱导巨噬细胞产生大量衣康酸^[7];而*IRG1*编码的乌头酸脱羧酶1 (aconitate decarboxylase 1, *ACOD1*)正是催化顺式乌头酸生成衣康酸的关键酶^[8]。近年来研究表明,衣康酸在免疫调节、炎症及肿瘤微环境中发挥重要作用,靶向*ACOD1*/衣康酸轴已成为潜在治疗策略^[9]。然而,现有研究多集中于肿瘤微环境中免疫细胞来源的衣康酸在免疫逃逸中的作用,对肿瘤细胞内源性*ACOD1*的表达及其在肿瘤进展中的功能尚缺乏深入探讨^[10-12]。

衣康酸及其衍生物(如4-辛基衣康酸)可通过迈克尔加成反应修饰半胱氨酸残基,调控蛋白功能。例如,衣康酸能促进转录因子EB(transcription factor EB, TFEB)核转位调控溶酶体生成^[13];也可通过修饰Kelch样ECH关联蛋白1(kelch like ECH associated protein 1, KEAP1)稳定核因子红细胞2-相关因子2(nuclear factor erythroid 2-related factor 2, Nrf2)^[14]。TFEB是自噬的关键调控因子,而Nrf2可通过调

控自噬受体蛋白p62影响自噬,研究表明衣康酸可能通过这些途径影响自噬及相关病理过程^[15-17]。此外,4-辛基衣康酸(4-octyl itaconate, 4-OI)也能通过抑制PI3K/AKT/mTOR通路激活软骨细胞自噬^[18]。上述证据提示,衣康酸及其衍生物可通过调控自噬参与多种疾病进展,但其在肿瘤自噬及进展中的作用尚不明确。本研究旨在分析*ACOD1*在肝癌组织中的表达特征,并探究*ACOD1*及其代谢产物衣康酸对肝癌细胞自噬与增殖的调控机制。

1 材料与方法

1.1 细胞系及主要试剂

人肝癌细胞株SK-Hep-1、Huh7、PLC/PRF/5均购自中国科学院上海细胞库;人肝癌细胞株HepG2购自武汉普诺赛生命科技有限公司。胎牛血清、DMEM培养基和胰蛋白酶均购自Gibco公司;青霉素-链霉素购自白鲨生物有限公司;siRNA购自上海吉玛基因,具体序列为si*ACOD1*-1: 5'-CCATCAAGATGAAACACACAA-3', si*ACOD1*-2: 5'-CAGCGTTTCCTGCACACTTTA-3';乳酸脱氢酶(lactate dehydrogenase, LDH)细胞毒性检测试剂盒和lipo6000试剂购自上海碧云天生物;MTT购自APEX BIO; 4-OI购自MedChemexpress;微管相关蛋白1轻链3(microtubule-associated protein 1 light chain 3, LC3)、选择性自噬接头蛋白p62/sequestosome 1(selective autophagy junction protein p62/sequestosome 1, P62)、乌头酸脱羧酶(*ACOD1*/*IRG1*)和内参蛋白Vinculin抗体均购自武汉三鹰生物技术有限公司。

1.2 实验分组及处理

实验分为①敲低*ACOD1*组:分别转染siNC和si*ACOD1* 48 h;②敲低*ACOD1*+CQ组:分别转染siNC和

siACOD1作用24 h后,将细胞消化重悬,待细胞贴壁后,HepG2和PLC/PRF/5细胞再分别加入10 $\mu\text{mol/L}$ 的CQ作用24 h;③敲低ACOD1+4-OI组:转染siACOD1作用24 h后,将细胞消化重悬,待细胞贴壁后,HepG2和PLC/PRF/5细胞再分别加入40 $\mu\text{mol/L}$ 和10 $\mu\text{mol/L}$ 的4-OI作用24 h;④4-OI组:分别用10、20、40、80、160、320 $\mu\text{mol/L}$ 的4-OI处理细胞24 h。

1.3 细胞增殖检测

将细胞接种至96孔板,待细胞贴壁后按照分组处理细胞24 h后,每孔加入20 μL 5 mg/mL的MTT溶液,放入CO₂培养箱,37 $^{\circ}\text{C}$ 孵育6 h后,弃去上清液,每孔加入200 μL DMSO,避光置于振荡器上低速振荡1 min。使用酶标仪在570 nm处测量吸光度。

1.4 细胞毒性检测

将细胞接种至96孔板,待细胞贴壁后按照分组处理细胞24 h后,加入原有培养基体积10%的LDH释放试剂,放入CO₂培养箱,37 $^{\circ}\text{C}$ 孵育1 h后,加入LDH检测工作液,室温避光孵育30 min后,在490 nm处测定吸光度。

1.5 免疫印迹

收集细胞,加入RIPA蛋白裂解液(按照1:100加入蛋白酶抑制剂),4 $^{\circ}\text{C}$ 裂解20 min后离心取上清液进行蛋白定量,随后置于金属浴中100 $^{\circ}\text{C}$ 变性5 min。SDS-PAGE电泳结束后在恒流300 mA、120 min条件下进行转膜。转膜结束后采用5%脱脂奶粉在室温下封闭2 h,4 $^{\circ}\text{C}$ 过夜孵育一抗(ACOD1, 1:1 000; LC3, 1:2 000; P62, 1:1 000; Vinculin, 1:10 000),用1 $\times$ TBST清洗条带3次,室温孵育二抗1 h(用5%脱脂奶粉按照1:5 000稀释),再次用1 $\times$ TBST清洗条带3次后,采用ECL化学发光体系检测条带。使用Image J软件进行灰度扫描,以目的条带与内参条带Vinculin的比值为蛋白的相对表达量。

1.6 生信分析

利用基因型-组织表达(the genotype-tissue expression, GTEx)数据库和肿瘤基因组图谱(the cancer genome atlas, TCGA)数据库分析ACOD1 mRNA在肝癌及正常肝组织中的表达差异。利用cBioPortal数据库查找与ACOD1共表达的基因,随后使用在线数据分析库Sangerbox(<http://www.sangerbox.com/tool>)进行KEGG富集分析与ACOD1基因显著相关的信号通路^[19]。利用cBioPortal数据库分析ACOD1基因表达量与总生存期(overall survival, OS)的相关性。

1.7 统计学方法

使用Graphpad Prism软件进行数据分析,结果以 $\bar{x} \pm s$ 表示。两组比较采用 t 检验,多组比较采用双因素方

差分析,针对同一目的分析,若存在多次假设检验,考虑Bonferroni等方法控制检验水准(图注中已注明采用的具体方法);而针对组学生信分析,进行标志物筛选,采用Bonferroni法校正。生存期分析通过Kaplan-Meier曲线表示并采用对数秩检验进行组间比较。 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 ACOD1在肝癌中高表达且与患者不良预后相关

根据GTEx数据库分析,ACOD1基因mRNA在正常肝组织中呈低表达,其表达水平在42种正常组织中处于整体下半区(图1A)。基于TCGA数据库的比对显示,与正常肝组织相比,ACOD1基因mRNA在肝癌组织中显著上调(图1B, $P < 0.05$)。Kaplan-Meier生存分析进一步表明,ACOD1基因mRNA高表达患者的总体生存期显著缩短(图1C)。同时,对4对肝癌及癌旁组织的蛋白检测结果显示,其中3对肝癌组织中ACOD1蛋白水平是对应癌旁组织的两倍,表明ACOD1在肝癌组织中显著上调(图1D, $P < 0.05$)。以上结果提示,ACOD1在肝癌中高表达,并与患者不良预后相关。

2.2 敲低ACOD1可抑制肝癌细胞生长

为明确ACOD1在肝癌生长中的作用,我们首先检测了ACOD1在SK-Hep-1、HepG2、Huh7、PLC/PRF/5肝癌细胞系中的蛋白表达水平。结果显示,ACOD1在HepG2和PLC/PRF/5细胞中表达较高(图2A)。随后,我们在上述两种高表达细胞中分别转染siACOD1以敲低ACOD1表达。蛋白免疫印迹验证显示,转染后,HepG2细胞中ACOD1蛋白水平降低至30%,PLC/PRF/5细胞中ACOD1蛋白水平降低至50%,证明siACOD1能够显著抑制ACOD1蛋白表达(图2B, $P < 0.05$)。进一步功能实验表明,siACOD1能显著抑制HepG2(降低约20%)和PLC/PRF/5细胞(降低约30%)的增殖能力(图2C, $P < 0.05$),并促进两种肝癌细胞死亡,死亡率均上升约30%(图2D, $P < 0.05$)。以上结果提示,敲低ACOD1可抑制肝癌细胞生长。

2.3 ACOD1促进细胞生长依赖其产物衣康酸

为探究代谢产物衣康酸在ACOD1促进肝癌细胞生长中的作用,我们首先使用不同浓度衣康酸衍生物4-OI处理2种肝癌细胞24 h^[20]。结果显示,4-OI以剂量依赖性方式促进细胞增殖(图3A),但两种细胞对4-OI的敏感性存在差异,HepG2细胞在40 $\mu\text{mol/L}$ 时生长显著增强,而PLC/PRF/5细胞在10 $\mu\text{mol/L}$ 时即可见显著促进作用,因此后续实验分别选用相应浓度进行处理。

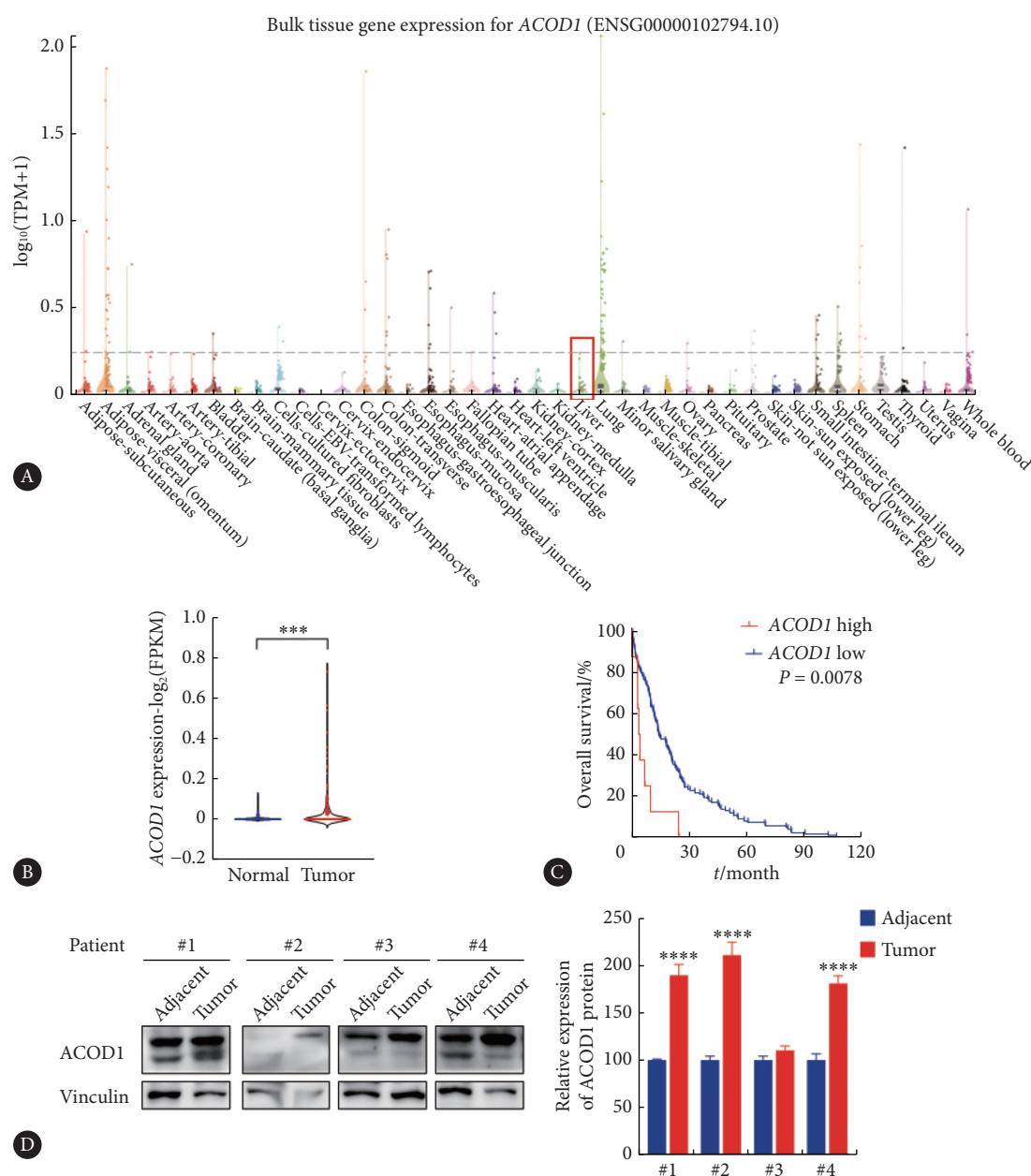


图1 ACOD1在肝癌中的表达水平及与患者预后的相关性

Fig 1 The expression of *ACOD1* and its correlation with patient prognosis

A, The expression of *ACOD1* mRNA in different human tissues; B, the expression of *ACOD1* mRNA in normal tissues ($n = 102$) and HCC tissues ($n = 373$), a two-tailed Student's *t*-test was performed; C, the correlation between *ACOD1* and overall survival ($n = 376$), Kaplan-Meier curves were generated and compared with the log-rank test; D, analysis of *ACOD1* expression in HCC tissues and paired normal tissues, a two-way ANOVA with Šidák correction was used (compared with normal). *** $P < 0.001$, **** $P < 0.0001$.

为验证ACOD1对肝癌细胞生长的促进作用是否依赖于衣康酸,我们在敲低ACOD1后外源性回补4-OI。结果显示,4-OI能够显著逆转敲低ACOD1所引起的两种肝癌细胞生长抑制,分别恢复至对照组的93%和90%(图3B, $P < 0.05$)。以上结果表明,ACOD1通过其代谢产物衣康酸促进肝癌细胞的生长。

2.4 敲低ACOD1基因诱导细胞自噬

通过cBioPortal数据库进行Spearman相关性分析,筛

选出与ACOD1共表达且显著相关的基因($P < 0.05$)。KEGG通路富集分析显示,细胞自噬是其中显著富集的细胞进程之一(图4A, $P < 0.05$ 且FDR < 0.05),提示ACOD1可能与细胞自噬密切相关。为验证这一关系,我们检测了敲低ACOD1基因后细胞自噬标志物的表达。结果显示,siACOD1可显著促进HepG2和PLC/PRF/5细胞中LC3-II的积累(分别上调约7倍和10倍)并降低p62蛋白水平,分别降低约40%和30%(图4B),表明自噬水平升高。

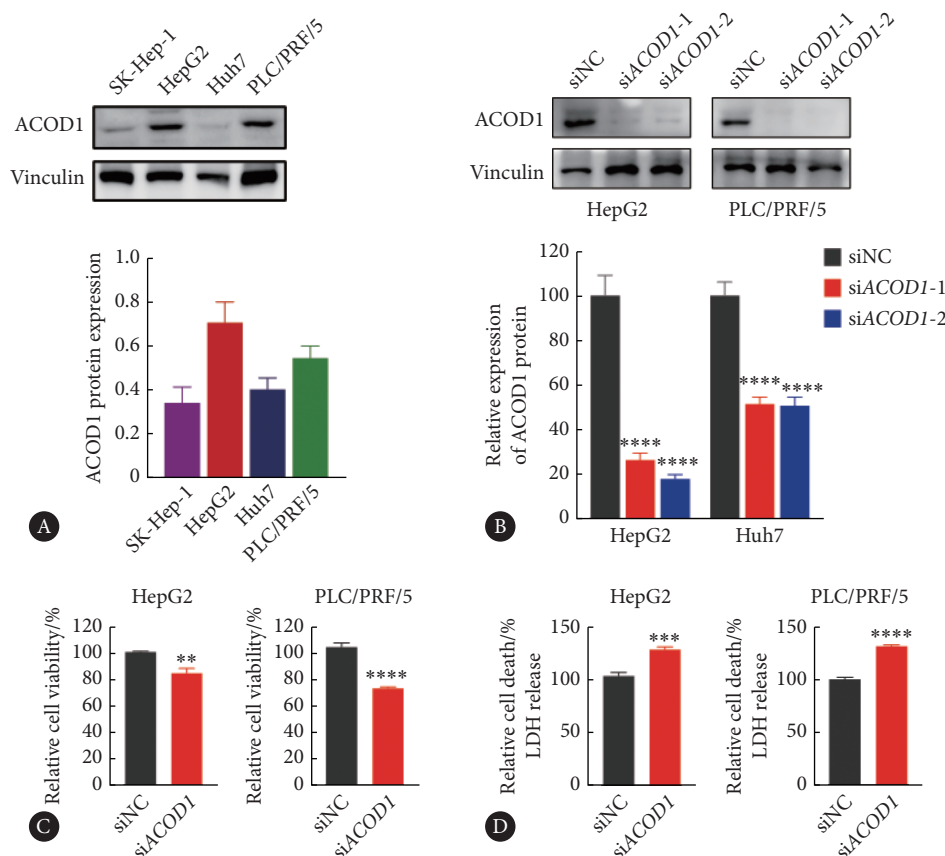


图 2 敲低ACOD1对肝癌细胞生长的影响

Fig 2 The role of ACOD1 knockdown in cell growth

A, Analysis of ACOD1 expression in different HCC cells; B, Western blot analysis of ACOD1 knockdown efficiency, a two-way ANOVA with Dunnett correction was used (compared with siNC); C, cell viability was measured by MTT assays, a two-tailed Student's *t*-test was performed; D, cell death was determined by measuring LDH release in the culture supernatants, a two-tailed Student's *t*-test was performed. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, $n = 3$.

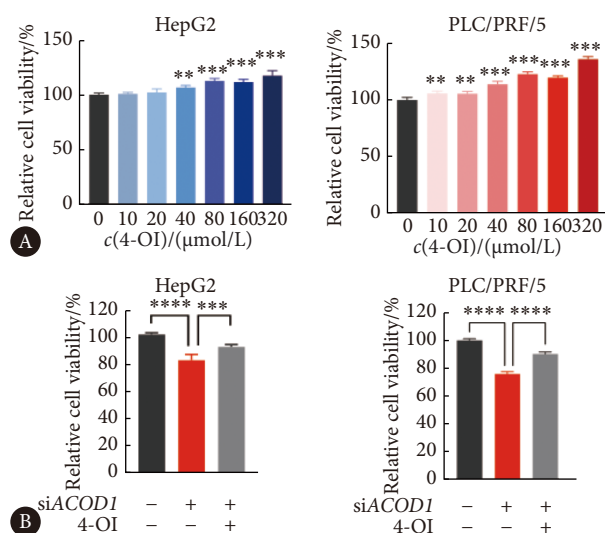


图 3 衣康酸在ACOD1促进肝癌生长中的作用

Fig 3 The role of 4-OI in ACOD1-mediated carcinogenesis

A, Cell viability was measured by MTT assays, a two-way ANOVA with Dunnett correction was used (vs. 0 $\mu\text{mol/L}$), $n = 5$; B, cell viability was measured by MTT assays, $n = 3$, a two-way ANOVA with Dunnett correction was used (compared with siACOD1). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

为明确这一变化是由自噬体生成增加还是自噬流受阻引起,我们在敲低ACOD1基因的基础上联合使用自噬流阻断剂CQ进行处理。结果显示,CQ单独处理显著增加了LC3- II 和p62的蛋白表达水平(均上调2倍),证明其有效抑制了自噬流;而在CQ存在下,siACOD1进一步增加两种肝癌细胞中LC3- II 的蛋白水平,LC3- II /LC3- I 比值约升高10%,且p62在HepG2细胞中降低、在PLC/PRF/5细胞中无明显变化(图4C)。这些结果表明,敲低ACOD1促进了自噬体生成,增强了自噬流。

2.5 衣康酸可逆转敲低ACOD1基因诱导的自噬

为探究衣康酸在ACOD1调控自噬中的作用,我们检测了敲低ACOD1基因后回补4-OI对自噬标志蛋白的影响。结果显示,与siACOD1组相比,siACOD1+4-OI组的HepG2和PLC/PRF/5细胞中LC3- II 表达显著降低,分别降低约40%和30%;而p62表达则显著升高,分别升高约1.7倍和1.3倍;ACOD1表达则无明显变化(图5)。该结果表明,衣康酸能够部分逆转敲低ACOD1基因所诱导的自噬激活。

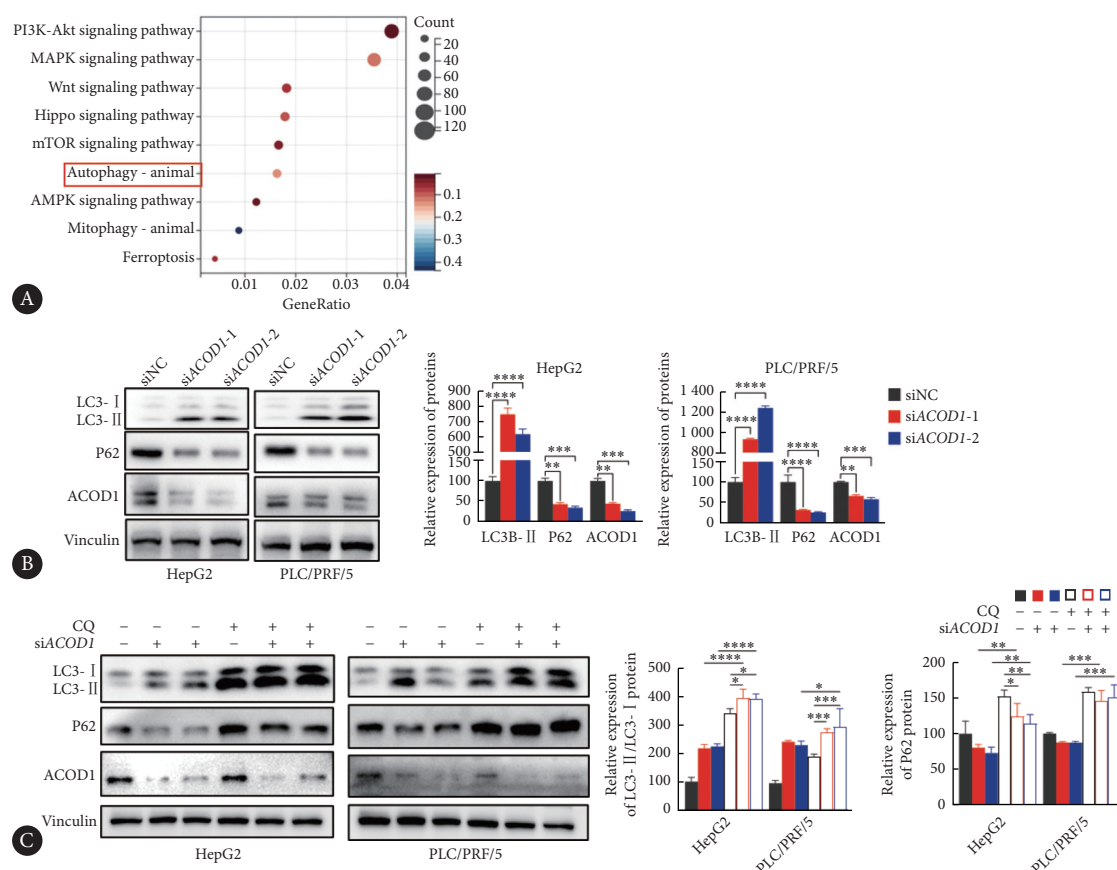


图4 敲低ACOD1对自噬流的调控

Fig 4 *ACOD1* knockdown regulates autophagic flux

A, KEGG enrichment analysis of *ACOD1*-coexpression genes; B, Western blot of LC3-II and P62 expression upon siACOD1, a two-way ANOVA with Dunnett correction was used (vs. siNC); C, Western blot of LC3-II and P62 expression upon siACOD1 in combination with CQ, a two-way ANOVA with Dunnett correction was used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, $n = 3$.

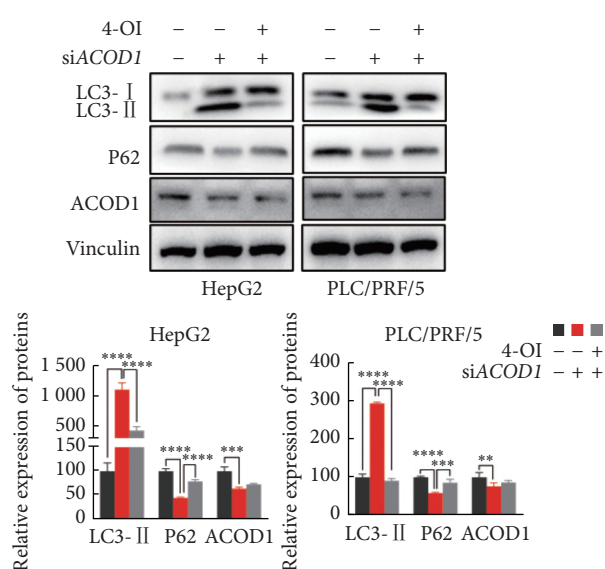


图5 衣康酸在ACOD1调控自噬中的作用

Fig 5 The role of 4-OI in *ACOD1*-mediated autophagy

A two-way ANOVA with Dunnett correction was used (vs. siACOD1). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, $n = 3$.

2.6 ACOD1通过抑制自噬促进肝癌细胞生长

为验证“敲低ACOD1通过诱导自噬抑制肝癌细胞生长”这一假设,我们进一步进行了实验验证。结果显示(图6),相较于单独敲低ACOD1组(siACOD1),联合使用自噬抑制剂氯喹(CQ)后(siACOD1+CQ组)细胞活力显著回升,HepG2细胞恢复至75%,PLC/PRF/5细胞恢复至

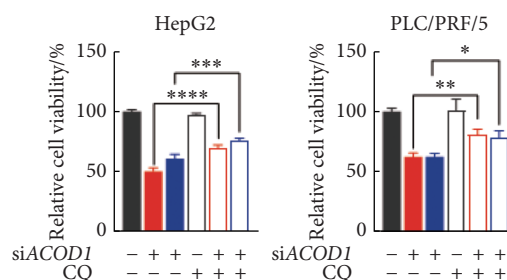


图6 自噬在ACOD1促进肝癌生长中的作用

Fig 6 The role of autophagy in *ACOD1*-mediated carcinogenesis

A two-way ANOVA with Tukey correction was used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, $n = 3$.

80% ($P < 0.05$)。这表明,抑制自噬能够部分恢复敲低 *ACOD1* 所引起的细胞生长抑制,支持 *ACOD1* 通过调控自噬影响肝癌细胞生长的结论。

3 讨论

越来越多的研究证实, *IRG1/ACOD1* 在肿瘤发生发展中发挥促癌作用。例如,有研究发现,肿瘤浸润性中性粒细胞在粒细胞-巨噬细胞集落刺激因子与其受体结合后,可通过激活 JAK-STAT5-C/EBP β 通路上调 *ACOD1* 表达,进而合成衣康酸,并通过抑制 Nrf2 介导的铁死亡促进乳腺癌转移^[21]。此外,有研究报道,巨噬细胞来源的衣康酸能够上调程序性死亡蛋白 1 (programmed cell death protein 1, PD-1) 和 T 细胞免疫球蛋白黏蛋白结构域蛋白 3 (T-cell immunoglobulin and mucin domain-containing protein 3, TIM-3) 表达,诱导 CD8⁺ T 细胞耗竭,从而促进肝癌免疫逃逸^[22]。这些发现提示, *ACOD1* 可能在肝癌进展中扮演重要角色。

本研究通过公共数据库分析发现, *ACOD1* 在肝癌组织中表达显著上调,且其高表达与患者预后不良相关。进一步检测 4 对肝癌及癌旁组织样本显示, *ACOD1* 蛋白水平在肝癌组织中明显升高。体外实验证实,敲低肝癌细胞中 *ACOD1* 可显著抑制细胞生长,而外源性回补衣康酸可逆转这一抑制效应,提示内源性 *ACOD1* 能促进肝癌细胞增殖,且可能依赖于其酶活功能。

为探究 *ACOD1*-衣康酸轴促进肝癌生长的机制,本研究基于 KEGG 富集分析发现细胞自噬与 *ACOD1* 密切相关。后续实验表明,敲低 *ACOD1* 可诱导自噬并促进自噬流,而外源性回补衣康酸则可逆转该自噬激活现象。自噬在肿瘤进展中具有双重调控作用,其具体效应取决于肿瘤类型、阶段及遗传背景等因素^[23]。一方面,自噬可通过清除受损线粒体增强肿瘤细胞应激适应能力、降解 MHC-I 类分子以促进免疫逃逸、协助抵抗失巢凋亡等途径支持肿瘤存活与转移^[24-25]。另一方面,自噬作为细胞“质量控制”系统,可通过维持基因组稳定性及细胞内稳态抑制肿瘤恶性转化^[26]。多项研究支持自噬具有抑制肿瘤进展的作用,例如:敲低 *MLKL* 可通过 AMPK α 1 磷酸化诱导自噬从而抑制肝癌发展^[27]。降低 PI5P4K α 表达可通过促进自噬流抑制胰腺癌细胞活力^[28]。DAPK3 通过促进 ULK1 磷酸化诱导自噬体形成进而抑制胃癌进展^[29]。

研究表明,衣康酸在多种疾病中可通过调控自噬影响病理进程,如通过促进自噬通量缓解坏死性结肠炎及高氧所致支气管肺发育不良^[15-16]。 *IRG1/ACOD1*-衣康酸轴还可通过增强线粒体自噬抑制神经元铁死亡,减轻蛛

网膜下腔出血后的神经炎症^[30]。然而, *ACOD1*-衣康酸-自噬轴在肿瘤发生发展中的作用尚未明确。本研究发现,使用自噬抑制剂可部分恢复敲低 *ACOD1* 基因引起的肝癌细胞生长抑制,提示敲低 *ACOD1* 基因通过诱导自噬进而抑制肝癌生长,从而揭示了该通路在肝癌中的潜在抑癌作用

本研究尚存在一定局限性。尽管实验证实 *ACOD1* 通过其代谢产物衣康酸促进肝癌细胞生长,但 *ACOD1* 是否完全依赖其酶活性发挥促癌作用仍需进一步验证。同时,敲低 *ACOD1* 基因激活自噬的具体分子机制尚未完全阐明。

综上所述,本研究表明 *ACOD1* 在肝癌中表达上调并与患者不良预后相关; *ACOD1* 可通过衣康酸抑制细胞自噬,进而促进肝癌细胞生长。这些结果提示 *ACOD1* 具有作为肝癌治疗靶点及预后评估标志物的潜力,为今后靶向 *ACOD1*-衣康酸-自噬轴开发肝癌治疗策略提供了理论依据。

* * *

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