论著·肝癌诊疗新策专题

INKA2-AS1促进肝细胞癌迁移、侵袭和巨噬细胞相关免疫抑制机制研究

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［摘要］ **目的** 探究INKA2-AS1促进肝细胞癌(hepatocellular carcinoma,HCC)迁移、侵袭和巨噬细胞相关免疫抑制的机制。**方法** 对癌症基因组图谱(the Cancer Genome Atlas Program,TCGA)-HCC数据和健康组织基因表达数据(Genotype-Tissue Expression,GTEx)数据库中374例HCC组织和160例正常肝组织中的基因表达水平进行差异分析。通过生存分析研究INKA2-AS1与HCC患者预后的关系,并对INKA2-AS1进行Cox回归分析。通过肿瘤免疫估计资源数据库分析INKA2-AS1与HCC组织免疫浸润的关系。于2019年1月至2021年1月收集在重庆海吉亚医院肝胆外科接受治疗的90例HCC患者的HCC组织和癌旁正常组织。将HCC细胞Hepa1-6分为NC、INKA2-AS1、siNC和siINKA2-AS1组。通过转染来过表达和沉默INKA2-AS1。分别通过CCK-8、EdU、TUNEL染色、划痕愈合和Transwell实验检测各组细胞的增殖、凋亡、迁移、侵袭情况。将各组Hepa 1-6细胞分别与巨噬细胞共培养分析其对巨噬细胞的影响。将20只4周龄SPF级裸鼠(体质量15~19 g,雌雄各半)通过随机数字表法分为4组(n=5):NC、INKA2-AS1、siNC和siINKA2-AS1组,分别通过皮下注射2×10^6个INKA2-AS1沉默和过表达的Hepa 1-6细胞构建荷瘤裸鼠模型,检测INKA2-AS1对肿瘤体积、质量的影响。通过Western blot检测肿瘤组织中IL-10和FAP蛋白表达水平来分析肿瘤相关巨噬细胞水平。**结果** HCC组织中INKA2-AS1的水平显著高于正常组织(P<0.001)。INKA2-AS1与HCC的组织学分级、病理分期有关(P<0.05)。NKA2-AS1高表达的HCC患者的生存率(P=0.001)、疾病特异性生存期(P=0.036)和无进展间期(P=0.024)显著低于INKA2-AS1低表达患者。Cox回归分析显示INKA2-AS1是HCC患者OS的独立预测因素(P=0.005)。INKA2-AS1的表达与T辅助细胞、巨噬细胞等免疫细胞相关。HCC组织中INKA2-AS1水平显著高于癌旁正常组织(P<0.05)。siINKA2-AS1组的增殖、迁移、侵袭能力低于siNC组(P<0.05),凋亡率显著高于siNC组(P<0.05),抑制与其共培养的巨噬细胞中IL-10和FAP mRNA和蛋白的表达(P<0.05)。INKA2-AS1组的增殖、迁移、侵袭能力高于NC组(P<0.05),凋亡率显著低于NC组(P<0.05),促进与其共培养的巨噬细胞中IL-10和FAP mRNA和蛋白的表达(P<0.01)。siINKA2-AS1组的肿瘤体积和质量显著低于siNC组(P<0.05),而INKA2-AS1组的肿瘤体积(P<0.05)和质量显著高于NC组(P<0.01)。siINKA2-AS1组肿瘤组织中IL-10和FAP蛋白水平显著低于siNC组(P<0.05),INKA2-AS1组肿瘤组织中IL-10和FAP蛋白水平显著高于NC组(P<0.05)。**结论** INKA2-AS1升高与HCC患者的预后不良有关,INKA2-AS1促进HCC细胞的增殖和转移,并诱导肿瘤相关巨噬细胞的转化。

［关键词］ 肝细胞癌;INKA2-AS1;肿瘤免疫;巨噬细胞

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INKA2-AS1 promotes migration, invasion and macrophage-related immunosuppression in hepatocellular carcinoma

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[**Abstract**] Objective To investigate the mechanism by which INKA2-AS1 promotes hepatocellular carcinoma(HCC) migration, invasion, and macrophage-related immunosuppression. Methods Based on the Cancer Genome Atlas Program (TCGA) -HCC data and Genotype-Tissue Expression (GTEx) databases, differentially expressed genes were analyzed in 374 HCC tissues and 160 normal liver tissues. Survival analysis was used to investigate the relationship between INKA2-AS1 and the prognosis of HCC patients. Cox regression analysis was performed on INKA2-AS1. The relationship between INKA2-AS1 and immune infiltration in HCC tissues was analyzed using the Tumor Immune Estimation Resource Database. A total of 90 HCC patients were recruited from the Department of Hepatobiliary Surgery, Chongqing Hygeia Hospital from January 2019 to January 2021. The HCC tissues and adjacent normal tissues were collected. Hepa 1-6 liver cancer cells were grouped into: normal control (NC), INKA2-AS1, siNC and siINKA2-AS1. INKA2-AS1 was overexpressed and silenced by transfection. The proliferation, migration and invasion of cells in each group were detected by CCK-8, EdU, TUNEL, wound healing and Transwell assays. Each group of Hepa 1-6 cells were co-cultured with macrophages to analyze their effects on macrophages. Twenty nude mice (SPF grade, 4 weeks old, 15~19 g, half male and half female) were randomly divided into 4 groups (n=5): NC, INKA2-AS1, siNC and siINKA2-AS1. Tumor-bearing nude mouse models were established by subcutaneous injection of INKA2-AS1 silenced and overexpressed Hepa1-6 cells. The effect of INKA2-AS1 on tumor volume and mass were detected. Macrophage immunity was analyzed by Western blot analysis of IL-10 and FAP protein levels in tumor tissues. Results The level of INKA2-AS1 in HCC tissues was significantly higher than that in normal tissues(P<0. 05). INKA2-AS1 was associated with the histological grade, pathological stage of HCC (P<0. 05). The survival rate (P=0. 05), disease-specific survival (P=0. 036) and progression-free interval (P=0. 024) of HCC patients with high INKA2-AS1 expression were significantly lower than those of patients with low INKA2-AS1 expression. The level of INKA2-AS1 in HCC tissues was significantly higher than that in adjacent normal tissues (P<0. 05). Cox regression analysis showed that INKA2-AS1 was an independent predictor of OS in HCC patients (P=0. 005). INKA2-AS1 expression was associated with immune cells such as T cells and macrophages. The proliferation, migration, and invasion abilities of the siINKA2-AS1 group were lower than those of the siNC group (P<0. 05), the apoptotic rate was higher than that of the siNC group (P<0. 05), and inhibited the expression of IL-10 and FAP at mRNA and protein levels in co-cultured macrophages (P<0. 05). The proliferation, migration and invasion abilities of the INKA2-AS1 group were higher than those of the NC group (P<0. 05), the apoptotic rate was lower than that of the NC group (P<0. 05), and promoted the expression of IL-10 and FAP at mRNA and protein levels in co-cultured macrophages (P<0. 05). The tumor volume and mass of the siINKA2-AS1 group were lower than those of the siNC group (P<0. 05), while the tumor volume and mass of the INKA2-AS1 group were higher than those of the NC group (P<0. 01). The levels of IL-10 and FAP proteins in tumor tissues of the siINKA2-AS1 group were lower than those of the siNC group (P<0. 05), while the levels of IL-10 and FAP proteins were increased (P<0. 05). Conclusion INKA2-AS1 increment is related to poor prognosis of HCC patients, and the molecule promotes the proliferation and metastasis of HCC cells and induces the transformation of tumor-associated macrophages.

[**Key words**] hepatocellular carcinoma; INKA2-AS1; tumor immunity; macrophages

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肝细胞癌(hepatocellular carcinoma,HCC)是常见和致命的癌症,据统计,全世界每年新发HCC约90万例,占所有恶性肿瘤的4.7%,排名第七;而每年死于HCC的患者高达83万例,占所有恶性肿瘤的8.3%,排名第二[1],超过70%接受手术切除或消融的HCC患者在5年内出现肿瘤复发[2]。因此,寻找新的HCC生物标志物、探究HCC发生和进展的分子机制具有重要意义。

免疫抑制是导致HCC发生和发展的关键机制之一,免疫细胞可产生多种炎性分子,如细胞因子、化学物质和氧自由基等,这些分子可刺激肿瘤细胞的生长、扩散和血管生成,进而促进癌症的发展[3]。而肿瘤细胞可以通过多种方式逃避机体免疫系统的监视和清除,如降低肿瘤抗原的表达、改变表面分子以避免免疫识别[4]。肿瘤相关巨噬细胞(tumor-associated macrophages,TAM)在肿瘤微环境中表现出免疫抑制的特性,它们能够高表达成纤维细胞激活蛋白(fibroblast activation protein-α,FAP)和白介素-10(interleukin-10,IL-10)并抑制其他免疫细胞的活性,从而为癌细胞的生长提供有利条件[5],但是相关调控机制仍需要进一步探究。

长链非编码RNA(long noncoding RNA,LncRNA)是指长度超过200个核苷酸但不能编码蛋白质的RNA,过去LncRNA被认为是"转录噪音",因为它们不参与蛋白质的产生过程[6]。然而,最近的研究表明,LncRNA具有生物学功能,且在HCC中异常表达并参与肿瘤进展和免疫调控,如铁死亡相关的LncRNA NRAV通过miR-375-3P/SLC7A11轴影响肝细胞癌的预后[7]。LI等[8]的研究结果显示,LncRNA CRNDE通过诱导免疫抑制微环境来驱动HCC的进展。INKA2-AS1又名FAM212B-AS1,是一种LncRNA,有研究显示它在子宫内膜癌组织中高表达且可用于早期诊断,但是其在HCC中的作用仍不清楚[9]。本研究拟通过生物信息学分析、临床样本检测、细胞和动物实验分析INKA2-AS1对HCC的调控作用,为HCC的临床诊断和治疗提供理论基础和新思路。

1 材料与方法

1.1 生物信息学分析

通过癌症基因组图谱(the Cancer Genome Atlas Program,TCGA)-HCC数据和健康组织基因表达数据(Genotype-Tissue Expression,GTEx)数据库(https://xenabrowser.net/datapages/)获得374例HCC组织和160例健康肝组织中mRNA表达水平。

1.2 差异分析

通过R语言的"Limma R"包来进行差异分析,差异基因筛选标准:|Log_2倍数变化(fold change,FC)|>2,且$P<0.05$,错误发现率(false discovery rat,FDR)<0.05,制作火山图和热图。

1.3 INKA2-AS1在HCC中的预后和生存分析

通过受试者工作特征(receiver operating characteristic,ROC)曲线分析INKA2-AS1对HCC患者的诊断效用。单独比较HCC组织和正常肝组织中INKA2-AS1的差异,以及INKA2-AS1在其他33种癌症中的表达差异。比较不同年龄、性别和临床分期的HCC患者的INKA2-AS1表达差异。根据INKA2-AS1表达中位数水平将HCC患者分为INKA2-AS1低表达和高表达组,通过Kaplan-Meier分析和对数秩检验比较2组的生存差异,包括总生存期(overall survival,OS)、疾病特异性生存期(disease specific survival,DSS)和无进展间期(progression-free interval,PFI)。通过单变量、多变量Cox回归分析探究影响HCC患者预后的因素。

1.4 富集分析

通过基因本体论(Gene Ontology,GO)和京都基因和基因组百科全书(Kyoto Encyclopedia of Genes and Genomes,KEGG)进行富集分析。使用R语言中的"Cluster Profiler"包分别进行GO和KEGG富集分析,以0.25的FDR作为2次富集分析之间统计差异的阈值,应用基因注释工具Metascape筛选条件,将$P<0.05$、最小计数为3、且富集因子>1定义为差异具有统计学意义。

1.5 免疫浸润分析

为分析HCC中INKA2-AS1与肿瘤免疫之间的关系,应用肿瘤免疫估计资源(https://cistrome.shinyapps.io/timer/)数据库来探究免疫细胞(包括B细胞、中性粒细胞、CD8+ T细胞、巨噬细胞、CD4+ T细胞和树突状细胞等)与INKA2-AS1水平之间的关联。使用R语言的"sparcl"包,根据INKA2-AS1表达中位数水平,将374个HCC患者分为INKA2-AS1低表达和高表达组,比较INKA2-AS1低表达和高表达组中免疫细胞浸润差异。

1.6 HCC组织获取

于2019年1月至2021年1月收集我院肝胆外科的HCC患者90例,收集其HCC组织和癌旁正常组织。纳入标准:①年龄40~70岁;②通过病理学诊断确诊为HCC;③所有患者知情同意。排除标准:①非首次确诊;②入组前3个月接受过抗癌治疗;③信息不全者。本研究已经重庆海吉亚医院临床科研伦理委员会批准(K2024-012-1202)。

1.7 细胞培养、分组和转染

HCC细胞系Hepa1-6和人单核细胞系THP-1来自中国医学科学院。细胞培养使用含有10%胎牛血清(FBS)

的RPMI 1640培养基(美国Life Technologies公司)。所有细胞被保存在37 ℃、含有5%CO_2的培养箱中。

将Hepa 1-6细胞分为4组:NC组、INKA2-AS1组、siNC组、siINKA2-AS1组。根据Lipofectamine 3000 Regent试剂盒(美国Invitrogen公司)说明书转染INKA2-AS1过表达质粒、INKA2-AS1 siRNA以及相应的对照(中国Generay公司)。当达到约70%细胞汇合时,向Hepa1-6细胞中添加50 nmol siRNA或10 μg过表达质粒。转染6 h后,使用新鲜的完全培养基更换细胞培养基。48 h后收集细胞进行后续实验。

通过将INKA2-AS1过表达和沉默Hepa 1-6细胞与巨噬细胞共培养探究其对巨噬细胞的影响。分别将转染NC、INKA2-AS1、siNC和siINKA2-AS1的HCC细胞与巨噬细胞共培养,具体方法如下:将THP-1细胞(1×10^5个/孔)分装到12孔板中,用佛波酯(100 ng/mL,美国MedChemExpress公司)刺激24 h,生成M0巨噬细胞。然后将M0巨噬细胞与转染NC、INKA2-AS1、siNC和siINKA2-AS1的Hepa1-6细胞在共培养系统(0.4 μm孔径,中国LABSELECT公司)中共培养,以模拟肿瘤相关巨噬细胞的生成,72 h后收集巨噬细胞。

1.8 RT-qPCR实验

总RNA提取采用TRIzol试剂盒(中国Maokang公司)。PrimeScript Ⅱ first-strand cDNA合成试剂盒(中国TaKaRa公司)用于cDNA合成。然后,使用SYBR Green PCR Master Mix试剂盒(中国SolarBio公司)通过Applied Biosystems 7500实时PCR系统进行定量RT-qPCR测定。反应条件如下:95 ℃变形15 s,60 ℃持续30 s,进行40个循环的扩增,72 ℃下进行30 s的延伸。SLC38A1的相对表达标准化为GAPDH,通过$2^{-\Delta\Delta Ct}$方法计算。引物(中国Songjiang公司)序列见表1。

表1 引物序列

Table 1 Primer sequences

Primer name	Primer sequences(5'→3')
β-actin	forward:CCTGTTCCTCCCTGGAGAAGAGCTA
	reverse:CGATCCACACAGAGTACTTGCG
INKA2-AS1	forward:GAAGAGGACCCTGGGAAGGT
	reverse:AATGCCTCACCCAGGATGTC

1.9 Western blot实验

采用RIPA裂解缓冲液试剂盒(中国SolarBio公司)来获取各实验组的总细胞裂解物。接下来,将蛋白质样品加载到4%~12%的梯度凝胶上,进行SDS-PAGE电泳。电泳结束后,对膜进行封闭处理(使用5%BSA),分别与并与以下一抗在4 ℃条件下孵育12 h:FAP(1∶800,美国Abcam公司)、IL-10(1∶800,美国ProteinTech公司)。次日,使用相应的二抗与膜共同孵育,随后利用ECL试剂盒(中国ZOMAN bio公司)来观察和分析蛋白条带。

1.10 CCK-8检测细胞活力

将各组细胞放入96孔板(每孔5×10^3个细胞)中,再培养12 h以贴壁。然后每孔加入CCK-8试剂,37 ℃孵育2 h后,使用酶标仪(美国BioTek公司)读取450 nm处的光密度[D(450)]值。

1.11 EdU检测增殖

取2 μL的10 mmol/L的EdU试剂(中国碧云天公司)稀释于1 mL细胞培养基。将预热后的EdU溶液与细胞混合,在适宜条件下孵育3 h,让细胞充分摄取EdU。之后用PBS清洗细胞2次,去除未结合的EdU和杂质。再用4%多聚甲醛固定细胞15 min,保持细胞形态。接着用0.3%Triton X-100溶液透化细胞15 min,增加通透性。充分洗涤后,加点击反应液,在避光条件下孵育30 min。然后将Hoechst 33342与PBS按1∶500稀释,对细胞核染色5 min。洗涤后收集细胞,荧光显微镜下观察拍照。

1.12 TUNEL染色

使用4%多聚甲醛固定细胞爬片,需要15~30 min,用PBS缓冲液洗涤细胞或固定后的玻片,至少洗涤3次(5 min/次)。使用蛋白酶K进行处理以增强TUNEL试剂的进入,然后洗涤。准备TUNEL反应混合物,在微量离心管中,混合TUNEL试剂盒中的TdT酶和标记的dUTP,按照试剂盒的说明进行,将TUNEL反应混合物添加到样本上,覆盖所有细胞,在37 ℃下孵育30 min。用试剂盒中提供的停止溶液停止TUNEL反应,使用DAPI染色细胞核。洗涤、封片并显微镜观察。

1.13 划痕愈合实验

各组细胞在8孔板中培养以构成单层细胞形态,采用1 mL移液器组合200 μL的吸头,利用其自重在尺子的辅助下精确地在细胞层划出1条直线伤痕。之后,使用PBS缓冲液洗涤3次,清除漂浮的细胞,并添加无血清培养基。在0 h时,记录下各组细胞的初始状态,然后在37 ℃、5%CO_2的培养箱中继续培养。24 h后,再次拍摄并记录细胞划痕状态。

1.14 Transwell实验

使用Matrigel基质胶稀释后包被Transwell小室底部膜的上室,然后放置在37 ℃环境中使其聚合成凝胶,加入含BSA的无血清培养液进行水化。细胞撤血清饥饿12 h,消化细胞后离心弃去培养液,用PBS洗涤后用无血清培养基重悬细胞并加入到Transwell小室

的上室中。将Transwell系统放置在37 ℃、5%CO_2的培养箱中进行培养。24 h后取出Transwell小室,去除上室中未侵袭的细胞。对下室中已侵袭的细胞进行固定、染色和计数,以评估细胞的侵袭能力。

1.15 荷瘤裸鼠实验

20只4周龄SPF级裸鼠(15~19 g,雌雄各半)购自中国贝恩特生物科技有限公司,并通过随机数字表法分为4组:NC、INKA2-AS1、siNC、siINKA2-AS1组,按照组别,通过皮下注射2×10^6个INKA2-AS1沉默和过表达的Hepa 1-6细胞构建荷瘤裸鼠模型,转染细胞按照1.7的方法获得。将各组转染后的Hepa 1-6细胞从培养皿中分离、洗涤、消化并制备成单细胞悬浮液。使用血细胞计数板计算细胞数和浓度,将肿瘤细胞的浓度调整为1×10^7个/mL。在无菌条件下,使用无菌注射器注射到裸鼠的腋下。注射1周后可观察并触摸到肿瘤,每3天测量1次肿瘤体积,第21天安乐死小鼠,取出瘤体测量质量。

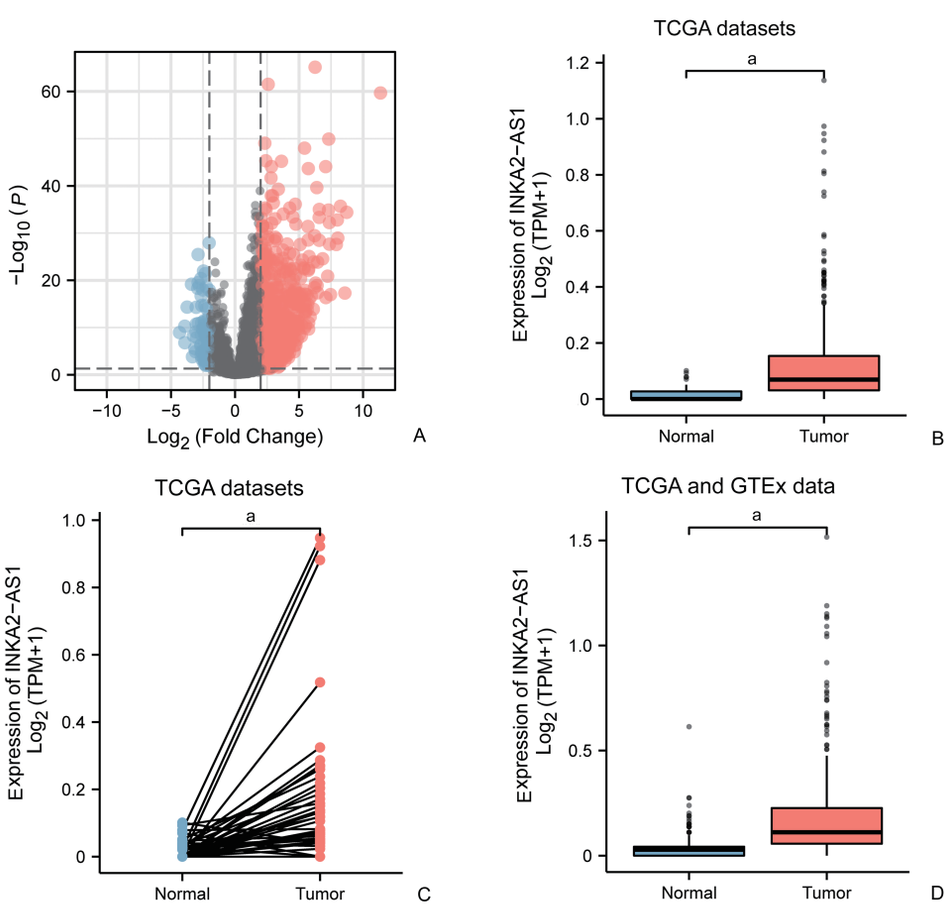
1.16 统计学分析

所有统计计算采用SPSS 16.0软件进行。计数指标以例(%)表示,2组比较通过χ^2检验。计量指标以$\bar{x}\pm s$表示,两组比较采用独立t检验,多组比较采用ANOVA和Tukey事后检验。$P<0.05$表示差异具有统计学意义。

2 结果

2.1 INKA2-AS1在HCC组织中上调

通过差异分析对TCGA和GTEx数据库中374例HCC和160例非肿瘤标本的信息进行分析。共发现938个差异基因,包括859个显著上调的基因和79个显著下调的基因(图1A)。其中,INKA2-AS1在HCC组织中的表达显著高于正常组织($P<0.05$,图1B~D)。提示INKA2-AS1在HCC组织中上调。



a: $P<0.05$

A: Volcano plot of differentially expressed genes between HCC and non-tumor specimens; B~D: Comparison of INKA2-AS1 expression levels in HCC specimens and non-tumor specimens from the TCGA and GTEx databases

图1 INKA2-AS1在HCC中的表达特点

Figure 1 Expression profiles of INKA2-AS1 in HCC

2.2　INKA2-AS1对HCC具有诊断价值

通过ROC分析INKA2-AS1对HCC的诊断价值。结果显示：INKA2-AS1对TCGA数据库中的HCC患者的诊断价值，AUC值为0.810（95%*CI*：0.762~0.859，图2A）。在GTEx数据库中，HCC中INKA2-AS1高表达的AUC值为0.817（95%*CI*：0.779~0.855，图2B）。INKA2-AS1在HCC的组织学分级、病理分期和T分期的AUG分别为0.583、0.600和0.568（图2C~E）。提示INKA2-AS1对HCC具有诊断价值。

2.3　INKA2-AS1与HCC临床病理特征有关

根据INKA2-AS1表达水平中位数将374例HCC患者分为2组，即高表达组（n=187）和低表达组（n=187），分析INKA2-AS1与HCC临床病理特征的关系。结果显示，INKA2-AS1表达与年龄和性别无关（图3A、B），但与组织学分级和病理分期相关（图3C、D）。此外，χ^2检验的结果也显示INKA2-AS1表达与组织学分级和病理分期有关（表2），提示INKA2-AS1水平与HCC的组织学分级和病理分期相关。

2.4　高水平INKA2-AS1与HCC患者预后不良有关

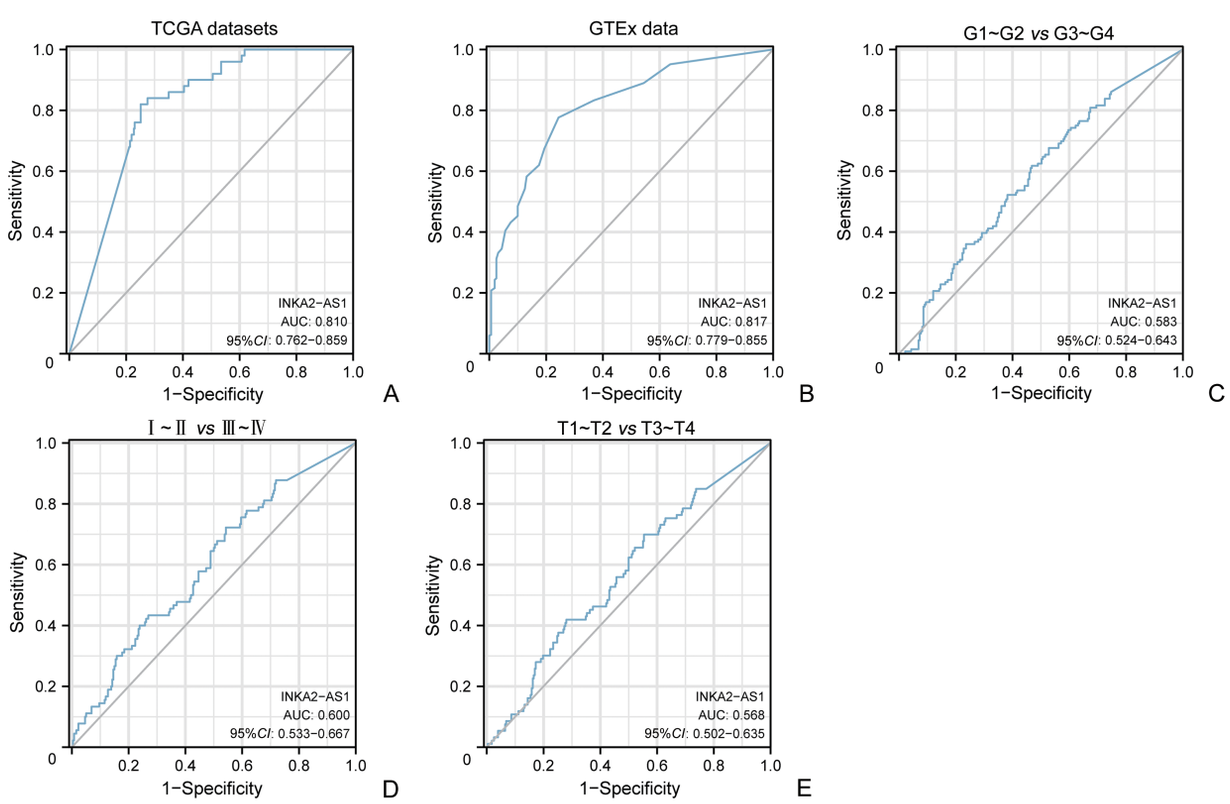
通过Kaplan-Meier分析INKA2-AS1与HCC患者预后的关系。研究结果显示，INKA2-AS1高表达的HCC患者的OS（P=0.001，图4A）、DSS（P=0.036，图4B）和PFI（P=0.024，图4C）显著低于INKA2-AS1低表达的HCC患者。并且Cox回归分析显示，高表达INKA2-AS1是HCC患者OS的独立预测因素（P=0.005，表3）。提示高表达INKA2-AS1与HCC患者预后不良有关。

2.5　INKA2-AS1的富集分析

通过GO和KEGG对INKA2-AS1进行富集分析探究其生物学功能。GO分析结果显示，INKA2-AS1功能主要与表皮、皮肤、消化、突触、跨膜转运蛋白、通道和激素功能的生长有关（图5A）。KEGG分析结果显示，INKA2-AS1的主要富集区域是尼古丁成瘾和神经活性配体-受体相互作用（图5B）。

2.6　INKA2-AS1促进HCC中的免疫浸润

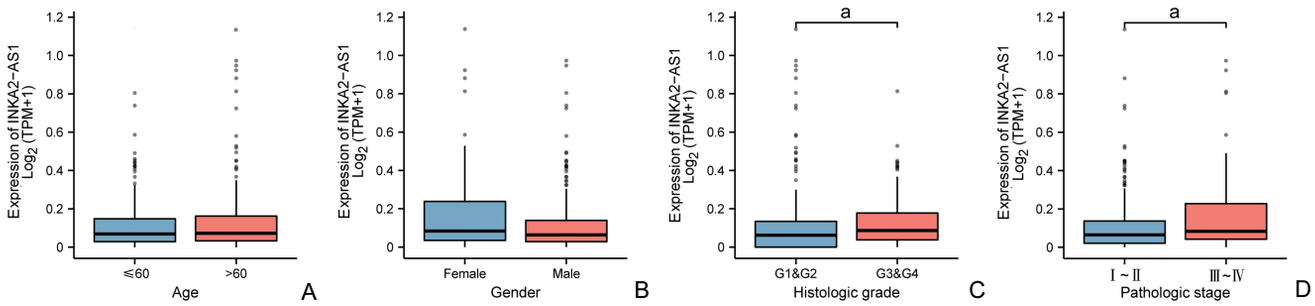
通过肿瘤免疫估计资源数据库进行免疫浸润分析。INKA2-AS1的表达与T辅助细胞、Th2细胞、巨噬细胞、TFH和NK CD56bright细胞呈正相关，与Th17细



A and B：Diagnostic value of INKA2-AS1 in HCC in TCGA and GTEx databases；C：Relationship between INKA2-AS1 and HCC patients' G1~G2 *vs* G3~G4 grades；D：relationship between INKA2-AS1 and HCC patients' stages Ⅰ~Ⅱ *vs* Ⅲ~Ⅳ；E：Relationship between INKA2-AS1 and HCC patients' T1~T2 *vs* T3 ~T4 stages

图2　INKA2-AS1对HCC的诊断价值

Figure 2　Diagnostic value of INKA2-AS1 in HCC



a: $P<0.05$

A: Relationship between INKA2-AS1 expression level and age; B: Relationship between INKA2-AS1 expression level and gender; C: Relationship between INKA2-AS1 expression level and histological grade; D: Relationship between INKA2-AS1 expression level and pathological stage

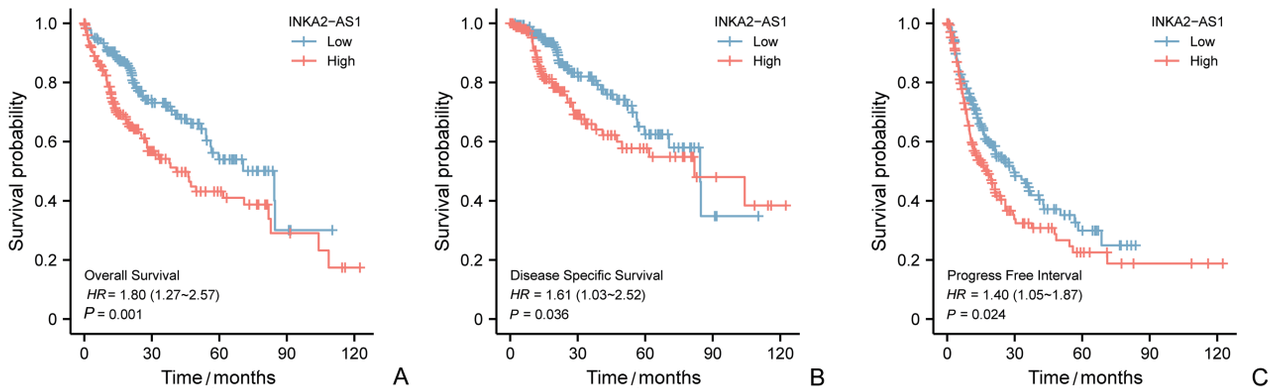
图3 INKA2-AS1与HCC临床病理特征的关系

Figure 3 Relationship between INKA2-AS1 and clinicopathological characteristics of HCC patients

表2 INKA2-AS1表达与HCC临床病理特征的相关性[n=187,例(%)]

Table 2 Correlation of INKA2-AS1 expression with clinicopathological characteristics of HCC patients[n=187, n(%)]

Characteristics	INKA2-AS1 low expression	INKA2-AS1 high expression	P
Age/years			0.874
≤60	90(24.1)	88(23.5)	
>60	97(26.0)	99(26.5)	
Gender			0.062
Female	49(13.1)	72(19.3)	
Male	138(36.9)	115(30.7)	
Histologic grade			0.036
G1	38(10.2)	19(5.1)	
G2	91(24.3)	87(23.3)	
G3	52(13.9)	72(19.2)	
G4	6(1.6)	9(2.4)	
Vascular invasion			0.217
No	136(36.4)	95(50.8)	
Yes	51(13.6)	92(49.2)	
Residual tumor			0.220
R0	180(48.1)	159(42.5)	
R1	6(1.6)	17(4.5)	
R2	1(0.3)	11(3.0)	
Pathologic stage			0.023
Ⅰ	113(30.2)	73(19.5)	
Ⅱ	37(9.9)	50(13.4)	
Ⅲ	35(9.4)	50(13.4)	
Ⅵ	2(0.5)	14(3.7)	
T stage			0.030
T1	105(28.3)	78(21.0)	
T2	39(10.5)	56(15.1)	
T3	35(9.4)	45(12.1)	
T4	5(1.3)	8(2.2)	
M stage			1.000
M0	133(35.6)	135(36.1)	
M1	54(14.4)	52(13.9)	
N stage			0.122
N0	129(34.5)	125(33.4)	
N1	58(15.5)	62(16.6)	



A: Kaplan-Meier survival curve analysis of the relationship between INKA2-AS1 expression level and OS in HCC patients;
B: Kaplan-Meier survival curve analysis of the relationship between INKA2-AS1 expression level and DSS in HCC patients;
C: Kaplan-Meier survival curve analysis of the relationship between INKA2-AS1 expression level and PFI in HCC patients

图4 INKA2-AS1表达水平与HCC患者预后的关系

Figure 4 Relationship between INKA2-AS1 expression level and prognosis of HCC patients

表3 OS的单变量和多变量Cox回归分析

Table 3 Univariate and multivariate Cox regression analyses for OS characteristics

Characteristics	*n*	Univariate analysis		Multivariate analysis	
		Hazard ratio(95%*CI*)	*P*	Hazard ratio(95%*CI*)	*P*
Gender	373				
Female	121	Reference			
Male	252	0.793(0.557~1.130)	0.200		
Age/years	373				
≤60	177	Reference			
>60	196	1.205(0.850~1.708)	0.295		
Histologic grade	368				
G1~G2	233	Reference			
G3~G4	135	1.091(0.761~1.564)	0.636		
Pathologic stage	349				
Ⅰ~Ⅱ	259	Reference			
Ⅲ~Ⅳ	90	2.504(1.727~3.631)	<0.05	2.352(1.618~3.419)	<0.05
INKA2-AS1	373				
Low expression	187	Reference			
High expression	186	1.804(1.268~2.568)	<0.05	1.722(1.180~2.514)	0.005

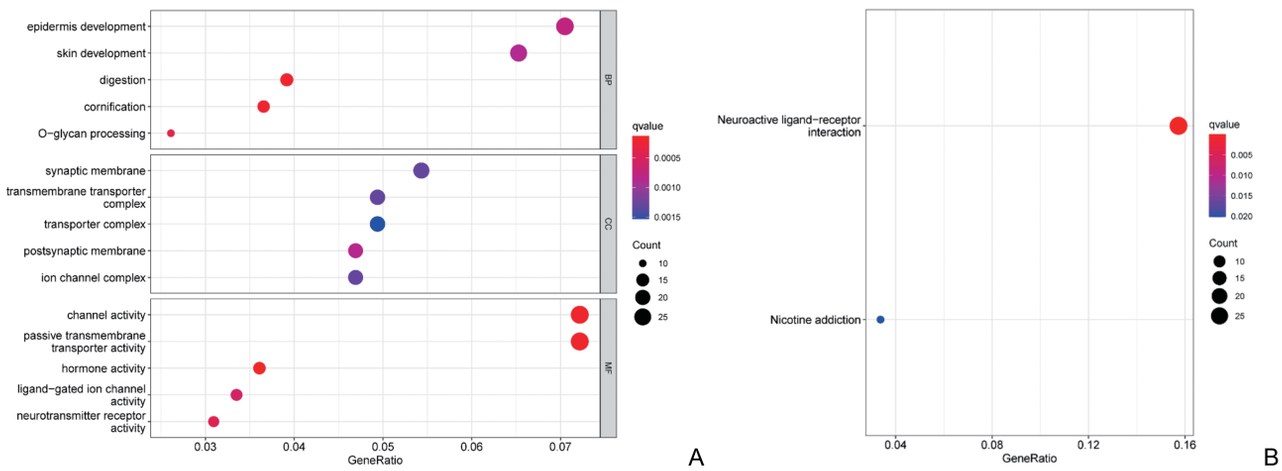
胞、pDC、细胞毒性细胞、DC、Treg、Tgd和Tcm呈负相关(图6)。提示INKA2-AS1促进HCC中的免疫浸润。

2.7 INKA2-AS1在HCC组织中高表达且与不良预后有关

收集HCC组织分析INKA2-AS1在HCC组织中的表达特点和与预后的关系。HCC组织中INKA2-AS1水平显著高于癌旁正常组织(P<0.05,图7A)。INKA2-AS1高表达HCC患者的生存率显著低于INKA2-AS1低表达者(P=0.012,图7B)。提示INKA2-AS1在HCC组织中高表达且与不良预后有关。

2.8 沉默INKA2-AS1抑制HCC细胞的增殖、迁移和侵袭

通过转染沉默INKA2-AS1分析INKA2-AS1对HCC细胞增殖、迁移和侵袭的影响。INKA2-AS1沉默的HCC细胞被成功构建(图8A);siINKA2-AS1组的细胞活力和增殖能力显著低于siNC组(P<0.05,图8B~D),siINKA2-AS1组的凋亡率显著高于siNC组(P<0.05,图8E、F),且siINKA2-AS1组的迁移和侵袭能力显著低于siNC组(P<0.05,图8G~J),提示沉默INKA2-AS1抑制HCC细胞的增殖、迁移和侵袭。



A: GO enrichment analysis of INKA2-AS1; B: KEGG enrichment analysis of INKA2-AS1

图5 INKA2-AS1的富集分析

Figure 5 Enrichment analysis of INKA2-AS1

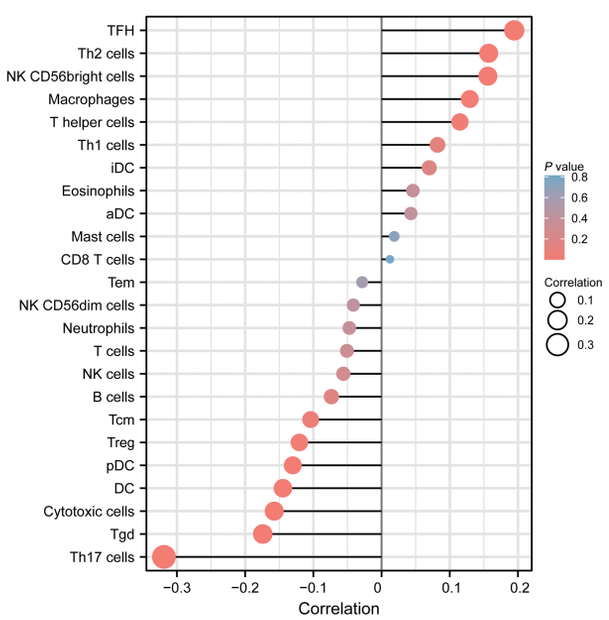


图6 HCC中INKA2-AS1表达与免疫细胞浸润的关系

Figure 6 Relation of immune cell infiltration with INKA2-AS1 expression in HCC

2.9 过表达INKA2-AS1促进HCC细胞的增殖、迁移和侵袭

通过转染过表达INKA2-AS1分析INKA2-AS1对HCC细胞增殖、迁移和侵袭的影响。INKA2-AS1过表达的HCC细胞被构建(图9A)。INKA2-AS1组的细胞活力和增殖能力显著高于NC组(P<0.05,图9B~D),INKA2-AS1组的凋亡率显著低于NC组(P<0.05,图9E、F),并且INKA2-AS1组的迁移和侵袭能力显著高于NC组(P<0.05,图9G~J),提示过表达INKA2-AS1促进HCC细胞的增殖、迁移和侵袭。

2.10 INKA2-AS1促进巨噬细胞相关免疫抑制

通过将转染NC、INKA2-AS1、siNC和siINKA2-AS1的HCC细胞与巨噬细胞共培养分析INKA2-AS1对巨噬细胞免疫的影响。结果显示,INKA2-AS1组巨噬细胞中FAP、IL-10 mRNA和蛋白水平显著高于NC组(P<0.01);siINKA2-AS1组巨噬细胞中FAP、IL-10 mRNA和蛋白水平显著低于siNC组(P<0.05,图10),提示INKA2-AS1促进巨噬细胞相关免疫抑制。

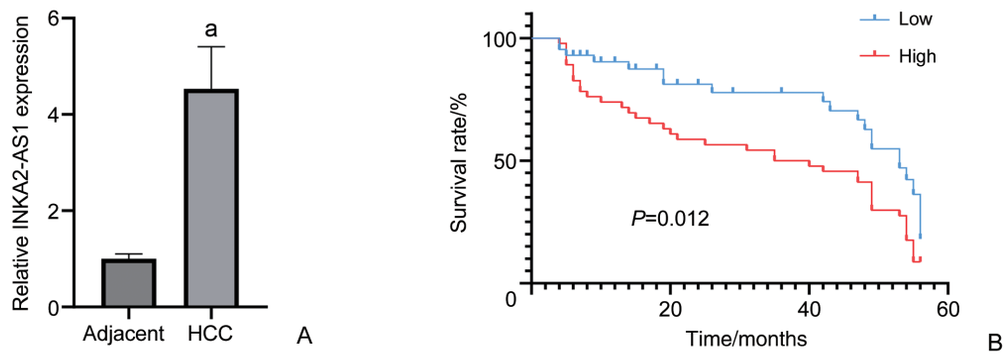
2.11 INKA2-AS1促进HCC荷瘤裸鼠模型的肿瘤生长和免疫抑制

通过荷瘤裸鼠模型在体内水平上分析INKA2-AS1对HCC荷瘤裸鼠模型的肿瘤生长和免疫抑制的影响。siINKA2-AS1组的肿瘤体积和质量显著低于siNC组(P<0.05),而INKA2-AS1组的肿瘤体积和质量显著高于NC组(P<0.01,图11A、B)。siINKA2-AS1组肿瘤组织中IL-10和FAP蛋白水平显著低于siNC组(P<0.05),INKA2-AS1组肿瘤组织中IL-10和FAP蛋白水平显著高于NC组(P<0.05,图11C、D)。提示INKA2-AS1促进HCC荷瘤裸鼠模型的肿瘤生长和免疫抑制。

3 讨论

3.1 HCC的临床现状和挑战

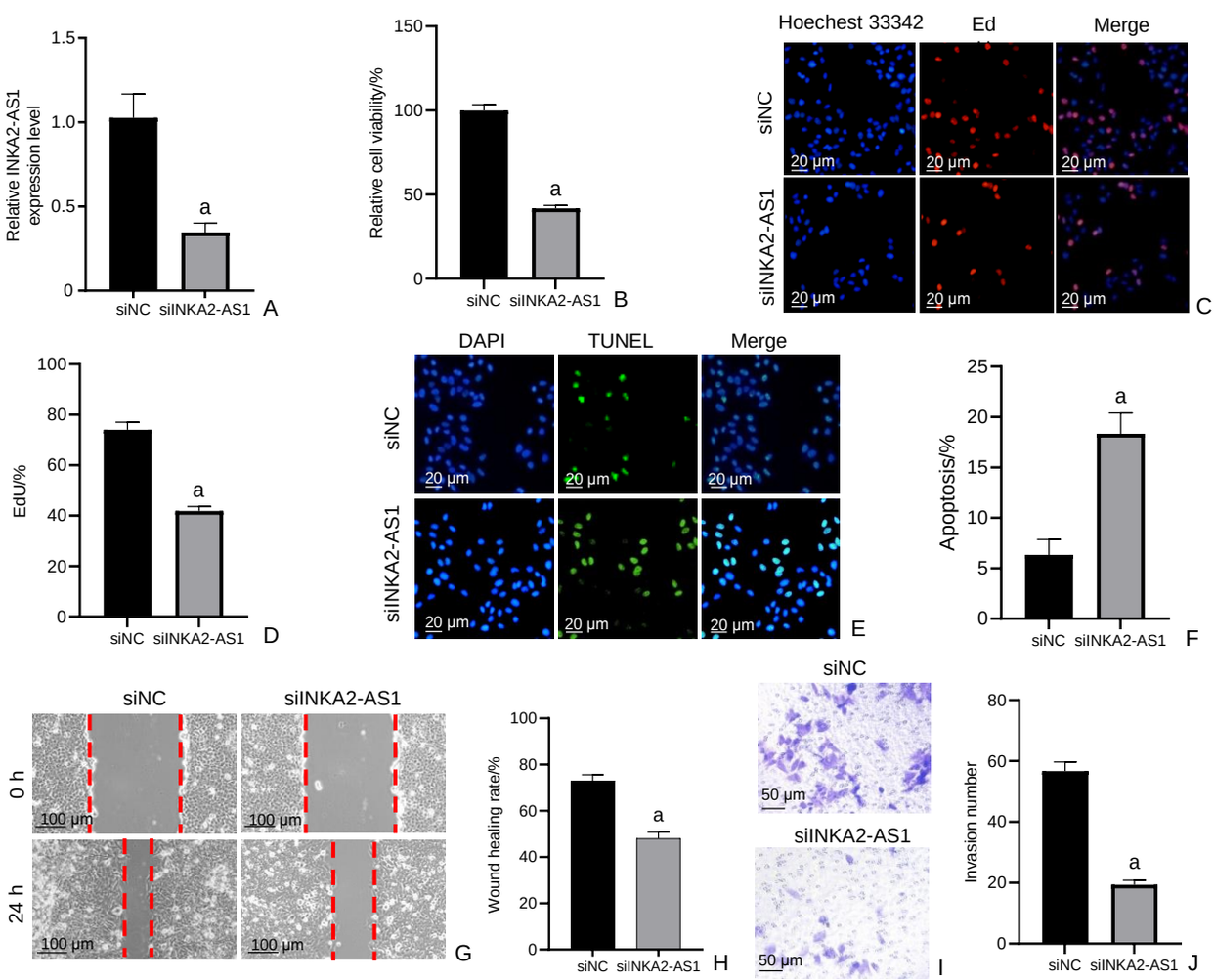
我国HCC发病率约为0.02%,是发达国家的4~5倍[10]。长期饮酒、乙肝病毒感染是HCC的主要致病因素[11]。据统计,我国每年新发HCC患者约40万,其中超过80%的HCC患者在确诊时已经处于中晚期[12]。并且近年来我国HCC的发病呈现年轻化趋势,严重威胁患者的健康和生命,也给医疗系统带来极大负担[13]。



A: Comparison of INKA2-AS1 levels in HCC tissues and adjacent tissues a: $P<0.05$, in comparison with adjacent tissues; B: Relationship between INKA2-AS1 and prognosis of HCC patients

图7 INKA2-AS1在HCC组织的表达特点以及与预后的关系

Figure 7 Expression characteristics of INKA2-AS1 in HCC tissues and its relationship with prognosis

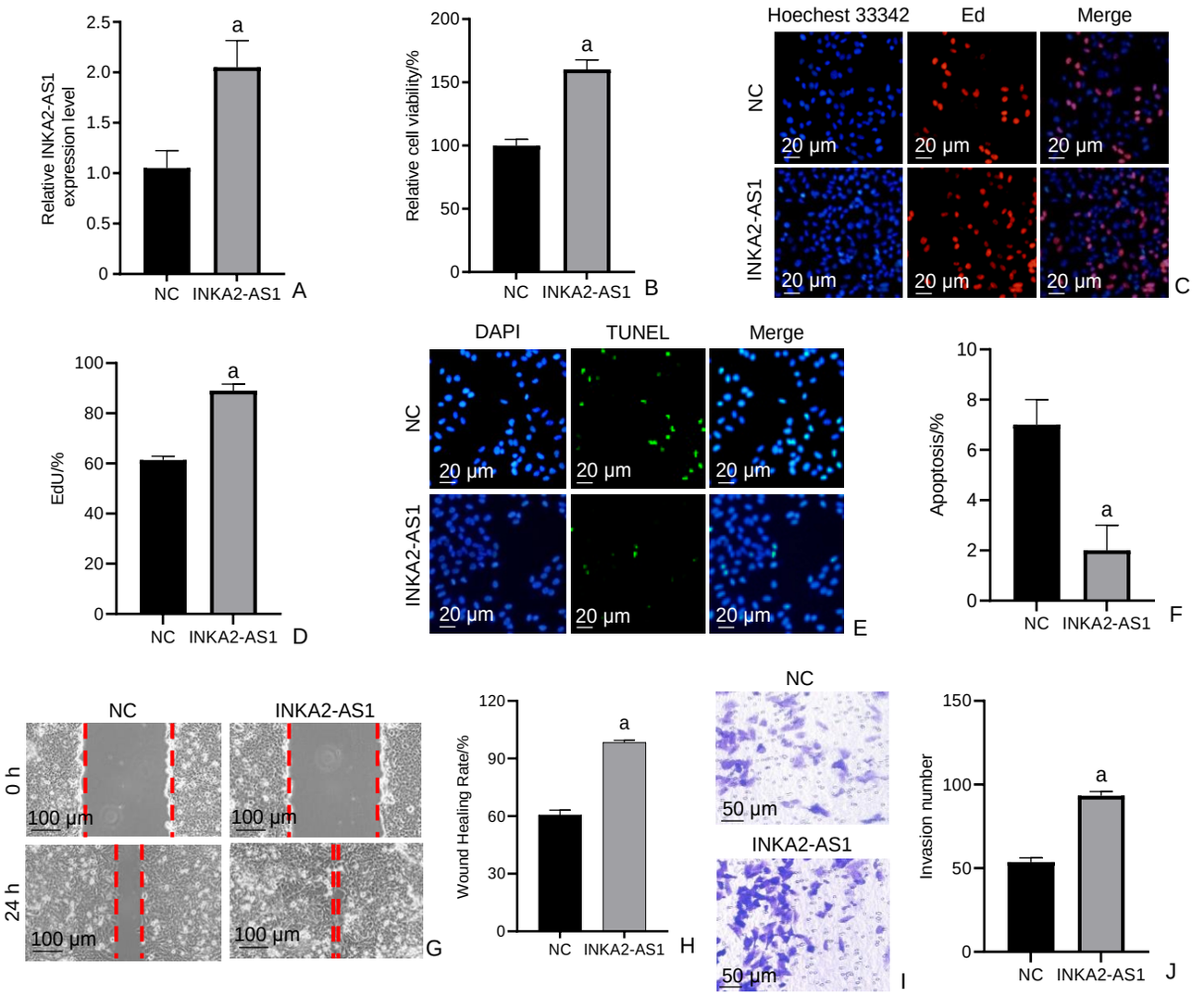


a: $P<0.05$, in comparison with the siNC group

A: RT-qPCR for the transfection results of HCC cells with INKA2-AS1 silencing; B: CCK-8 assay showing the effect of silencing INKA2-AS1 on HCC cell viability; C and D: EdU indicating the effect of silencing INKA2-AS1 on HCC cell proliferation; E and F: TUNEL staining for the effect of silencing INKA2-AS1 on HCC cell apoptosis; G~J: Scratch and Transwell assays revealing the effect of silencing INKA2-AS1 on HCC cell migration and invasion

图8 沉默INKA2-AS1对HCC细胞的影响

Figure 8 Effects of silencing INKA2-AS1 on HCC cells



a: $P<0.05$, in comparison with NC group

A: RT-qPCR showing the transfection results of HCC cells with INKA2-AS1 overexpression; B: CCK-8 assay indicating the effect of overexpression INKA2-AS1 on HCC cell viability; C and D: EdU assay displaying the effect of overexpression INKA2-AS1 on HCC cell proliferation; E and F: TUNEL staining revealing the effect of overexpression INKA2-AS1 on HCC cell apoptosis; G~J: Scratch and Transwell assays demonstrating the effect of overexpression INKA2-AS1 on HCC cell migration and invasion

图9 过表达INKA2-AS1对HCC细胞的影响

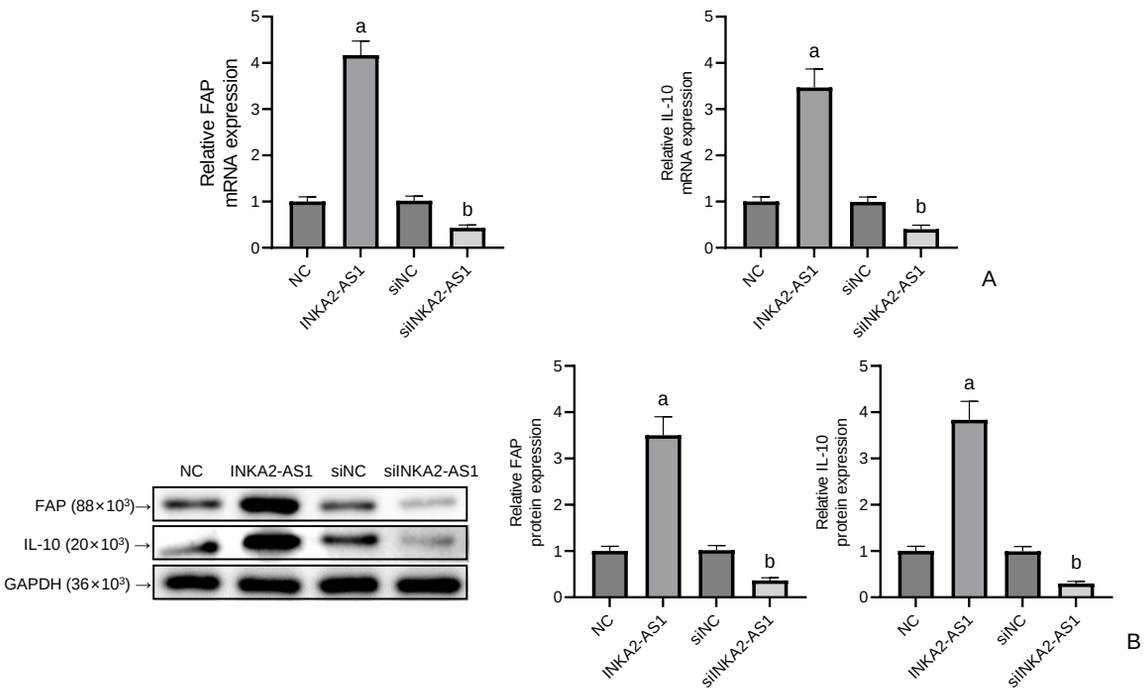
Figure 9 Effects of overexpression of INKA2-AS1 on HCC cells

3.2 LncRNA在HCC进展和免疫抑制中具有关键作用

LncRNA是一类长度超过200个核苷酸的RNA分子,不编码蛋白质[14]。LncRNA可以作为转录调控因子,与DNA、蛋白质和其他RNA相互作用,调控癌症相关基因的表达[15]。LncRNA能够通过改变染色体的表观遗传状态来调控癌症相关基因,并通过招募组蛋白修饰酶来改变染色体的乙酰化、甲基化等修饰[16]。LncRNA也可作为miRNA的内源性抑制剂调控癌细胞中的miRNA靶标基因的表达[17]。LncRNA还可以通过调控剪切过程来改变基因的表达,从而影响蛋白质编码[18]。更重要的是,LncRNA在也可在免疫抑制反应中发挥作用,从而导致HCC细胞能够逃脱免疫系统的识别和攻击,例如,LINC00638通过靶向miR-4732-3p/ULBP1促进PD-L1轴介导HCC免疫逃逸反应[19]。

3.3 LncRNA INKA2-AS1在HCC高表达且与不良预后有关

由于LncRNA数目众多,因此,从中寻找能够有效预测HCC预后的LncRNA至关重要。本研究首先通过生物信息学的分析思路和方法,发现HCC组织中

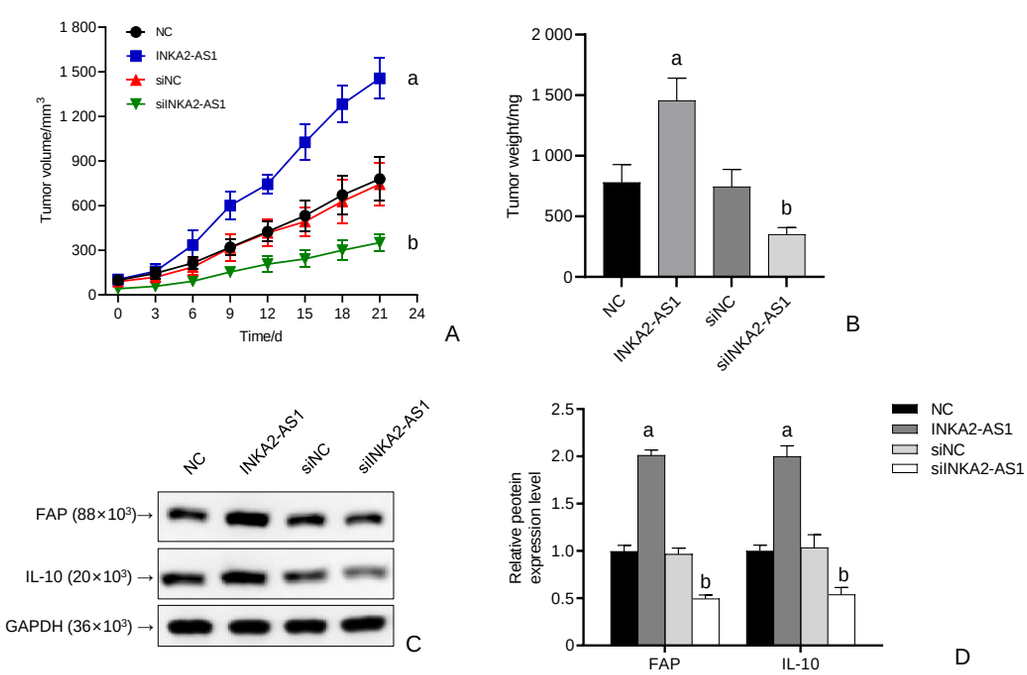


a:P<0.05, in comparison with the NC group; b: P<0.05, in comparison with the siNC group

A: Effects of overexpression and silencing of INKA2-AS1 in HCC cells on the mRNA levels of FAP and IL-10 in co-cultured macrophages; B: Effects of overexpression and silencing of INKA2-AS1 in HCC cells on the protein levels of FAP and IL-10 in co-cultured macrophages

图10 INKA2-AS1对巨噬细胞相关免疫的影响

Figure 10 Effects of INKA2-AS1 on macrophage-related immunity



a: P<0.05, in comparison with the NC group; b: P<0.05, in comparison with the siNC group

A and B: Effects of INKA2-AS1 on HCC nude mouse model; C and D: Effects of INKA2-AS1 on the expression of tumor-associated macrophage marker proteins IL-10 and FAP in HCC nude mice

图11 INKA2-AS1对HCC荷瘤裸鼠模型以及巨噬细胞相关免疫的影响

Figure 11 Effects of INKA2-AS1 on HCC nude mouse model and macrophage-related immunity

INKA2-AS1的水平显著高于正常组织,并且INKA2-AS1对HCC具有诊断价值。INKA2-AS1的表达水平与患者的年龄和性别无关,但是与组织学分级和病理分期相关。此外,INKA2-AS1高表达的HCC患者的OS和PFI显著低于INKA2-AS1低表达的HCC患者。并且Cox回归分析显示INKA2-AS1是HCC患者OS的独立预测因素。一项临床研究显示INKA2-AS1可能是子宫内膜癌潜在核心LncRNA[9]。LncRNA INKA2-AS1在HCC诊断和预后判断中有意义,但是在细胞和动物水平上INKA2-AS1对HCC细胞的影响仍需要进一步研究。

3.4 LncRNA INKA2-AS1在体内外促进HCC

本研究通过转染分别构建INKA2-AS1过表达和沉默的Hepa 1-6细胞,结果显示INKA2-AS1升高后,Hepa 1-6细胞的增殖、迁移和侵袭能力得到了显著的提高,凋亡被明显地抑制;而当INKA2-AS1降低后,Hepa 1-6细胞的增殖、迁移和侵袭能力也被抑制,凋亡率明显的升高。并且荷瘤裸鼠模型的实验结果也在体内水平上证实了INKA2-AS1对HCC的促进作用。

3.5 LncRNA INKA2-AS1在HCC中调控巨噬细胞相关免疫

免疫抑制是HCC发生和进展的重要原因,因此,本研究也分析了LncRNA INKA2-AS1对HCC免疫浸润的影响,结果显示INKA2-AS1与HCC组织中浸润的巨噬细胞有关。肝脏巨噬细胞的功能异常与代谢紊乱、慢性炎症和癌症进展等病理生理状况有关[20]。肿瘤微环境的特点是低pH、缺氧、缺糖,以及富含乳酸、脂质和细胞因子的环境,这都有利于巨噬细胞重新编程为促进肿瘤的TAM表型[21]。TAM具有M1和M2巨噬细胞的混合特征[22-23]。TAM中高表达IL-10和FAP蛋白,诱导免疫抑制并促进新生血管形成和癌细胞转移等[24-25]。

为此,本研究也分析了INKA2-AS1对巨噬细胞的影响。分别将转染NC、INKA2-AS1、siNC和siINKA2-AS1的HCC细胞与巨噬细胞共培养,结果显示INKA2-AS1组巨噬细胞中FAP、IL-10 mRNA和蛋白水平显著高于NC组,而siINKA2-AS1组巨噬细胞中FAP IL-10 mRNA和蛋白水平显著低于siNC组。这提示INKA2-AS1不但会影响HCC细胞的增殖和转移,还会通过巨噬细胞促进HCC的进展。FAP是一种Ⅱ型跨膜丝氨酸蛋白酶,具有促进细胞增殖、上皮-间充质转化和血管生成的作用[26]。最新一项报道显示在HCC中,FAP的升高促进TAM激活并降低了HCC细胞对索拉菲尼的敏感性[27]。此外,TAM也是免疫抑制因子IL-10的主要来源[28]。TAM表达的IL-10可通过调控HCC组织中浸润的T细胞等其他免疫细胞来诱导免疫抑制微环境[29]。本次研究也构建的荷瘤裸鼠模型在体内水平上分析INKA2-AS1对TAM的影响,结果也显示了INKA2-AS1对TAM的促进作用,INKA2-AS1过表达会促进TAM标志FAP和IL-10的蛋白的表达水平,沉默INKA2-AS1会抑制FAP和IL-10的表达。结合文献报道和本次体内外实验结果,说明INKA2-AS1也会通过促进TAM的浸润诱导HCC免疫抑制,进而促进HCC进展。

然而,本研究也存在一些局限性,首先,对于巨噬细胞,INKA2-AS1诱导其向TAM转化的分子机制仍需要进一步分析;其次,INKA2-AS1在体内对HCC中巨噬细胞的影响仍需要组织学和细胞学水平上的观察和验证;最后,INKA2-AS1在HCC组织中浸润的TAM的临床意义仍需要进一步分析。

综上所述,INKA2-AS1升高与HCC患者的预后不良有关,INKA2-AS1促进HCC细胞的增殖和转移,并诱导肿瘤相关巨噬细胞的转化。

利益冲突 本文所有作者声明不存在利益冲突

参考文献:

[1] BRAY F, LAVERSANNE M, SUNG H, *et al*. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA Cancer J Clin, 2024, 74(3): 229-263. DOI: 10.3322/caac.21834.

[2] SANKAR K, GONG J, OSIPOV A, *et al*. Recent advances in the management of hepatocellular carcinoma[J]. Clin Mol Hepatol, 2023, 30(1): 1-15. DOI: 10.3350/cmh.2023.0125.

[3] DE MARTINO M, RATHMELL J C, GALLUZZI L, *et al*. Cancer cell metabolism and antitumour immunity[J]. Nat Rev Immunol, 2024, 24(9): 654-669. DOI: 10.1038/s41577-024-01026-4.

[4] CAI N, CHENG K, MA Y, *et al*. Targeting MMP9 in CTNNB1 mutant hepatocellular carcinoma restores $CD8^+$T cell-mediated antitumour immunity and improves anti-PD-1 efficacy[J]. Gut, 2024, 73(6): 985-999. DOI: 10.1136/gutjnl-2023-331342.

[5] XIAO J, SONG Y X, GAO R, *et al*. Changes of immune microenvironment in head and neck squamous cell carcinoma in 3D-4-culture compared to 2D-4-culture[J]. J Transl Med, 2023, 21(1): 771. DOI: 10.1186/s12967-023-04650-1.

[6] ZHANG Y G. LncRNA-encoded peptides in cancer[J]. J Hematol Oncol, 2024, 17(1): 66. DOI: 10.1186/s13045-024-01591-0.

[7] ZONG K, LIN C F, LUO K, *et al*. Ferroptosis-related lncRNA NRAV affects the prognosis of hepatocellular carcinoma *via* the miR-375-3P/SLC7A11 axis[J]. BMC Cancer, 2024, 24(1): 496. DOI: 10.1186/s12885-024-12265-y.

[8] LI X Y, LIANG S H, FEI M M, *et al*. LncRNA *CRNDE* drives the progression of hepatocellular carcinoma by inducing the immunosuppressive niche[J]. Int J Biol Sci, 2024, 20(2): 718-732. DOI: 10.7150/ijbs.85471.

[9] XU J, QIAN Y J, YE M, *et al*. Distinct expression profile of lncRNA in endometrial carcinoma[J]. Oncol Rep, 2016, 36(6): 3405-3412. DOI: 10.3892/or.2016.5173.

[10] CAO W, CHEN H D, YU Y W, *et al*. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020[J]. Chin Med J, 2021, 134(7): 783-791. DOI: 10.1097/cm9.0000000000001474.

[11] ANWANWAN D, SINGH S K, SINGH S, *et al*. Challenges in liver cancer and possible treatment approaches[J]. Biochim Biophys Acta BBA Rev Cancer, 2020, 1873(1): 188314. DOI: 10.1016/j.bbcan.2019.188314.

[12] 何凤蝶, 王秋童, 胡亚男, 等. 中国人群肝癌发病危险因素病例对照研究的meta分析[J]. 现代预防医学, 2022, 49(23): 4230-4240. DOI: 10.20043/j.cnki.MPM.202109297.
HE F D, WANG Q T, HU Y N, et al. Meta-analysis of case-control studies on risk factors for liver cancer in the Chinese population[J]. Mod Prev Med, 2022, 49(23): 4230-4240. DOI: 10.20043/j.cnki.MPM.202109297.

[13] 赵海潮, 高强. 2022年度肝癌研究及诊疗新进展[J]. 中国癌症杂志, 2023, 33(4): 315-326. DOI: 10.19401/j.cnki.1007-3639.2023.04.002.
ZHAO H C, GAO Q. Progress in research, diagnosis, and treatment of hepatocellular carcinoma in 2022[J]. Chin J Oncol, 2023, 33(4): 315-326. DOI: 10.19401/j.cnki.1007-3639.2023.04.002.

[14] WANG F, HU Y H, WANG H D, *et al*. LncRNA FTO-IT1 promotes glycolysis and progression of hepatocellular carcinoma through modulating FTO-mediated N6-methyladenosine modification on GLUT1 and PKM2[J]. J Exp Clin Cancer Res, 2023, 42(1): 267. DOI: 10.1186/s13046-023-02847-2.

[15] ZHAO J J, CHEN P J, TAN C, *et al*. LncRNA LINC00667 gets involved in clear cell renal cell carcinoma development and chemoresistance by regulating the miR-143-3p/ZEB1 axis[J]. Aging, 2023, 15(19): 10057-10071. DOI: 10.18632/aging.205029.

[16] FERREIRA R S, ASSIS R I F, RACCA F, *et al*. Analyzes in silico indicate the lncRNAs MIR31HG and LINC00939 as possible epigenetic inhibitors of the osteogenic differentiation in PDLCs[J]. Genes, 2023, 14(8): 1649. DOI: 10.3390/genes14081649.

[17] 邓钰梅, 陈俊霞. lncRNA ENST 933在乳腺癌中的作用及其机制研究[J]. 陆军军医大学学报, 2024, 46(14): 1676-1694. DOI: 10.16016/j.2097-0927.202401022.
DENG Y M, CHEN J X. Role of long non-coding RNA ENST 933 in breast cancer and its underlying mechanism[J]. J Army Med Univ, 2024, 46(14): 1676-1694. DOI: 10.16016/j.2097-0927.202401022.

[18] ZHANG Q L, ZHENG L M, BAI Y K, *et al*. ITPR1-AS1 promotes small cell lung cancer metastasis by facilitating P21 splicing and stabilizing DDX3X to activate the cRaf-MEK-ERK cascade[J]. Cancer Lett, 2023, 577: 216426. DOI: 10.1016/j.canlet.2023.216426.

[19] QI F, DU X J, ZHAO Z Y, *et al*. Tumor mutation burden-associated LINC00638/miR-4732-3p/ULBP1 axis promotes immune escape *via* PD-L1 in hepatocellular carcinoma[J]. Front Oncol, 2021, 11: 729340. DOI: 10.3389/fonc.2021.729340.

[20] SINGH A, MAYENGBAM S S, YADUVANSHI H, *et al*. Obesity programs macrophages to support cancer progression[J]. Cancer Res, 2022, 82(23): 4303-4312. DOI: 10.1158/0008-5472.can-22-1257.

[21] VITALE I, MANIC G, COUSSENS L M, *et al*. Macrophages and metabolism in the tumor microenvironment[J]. Cell Metab, 2019, 30(1): 36-50. DOI: 10.1016/j.cmet.2019.06.001.

[22] HELM O, HELD-FEINDT J, GRAGE-GRIEBENOW E, *et al*. Tumor-associated macrophages exhibit pro- and anti-inflammatory properties by which they impact on pancreatic tumorigenesis[J]. Int J Cancer, 2014, 135(4): 843-861. DOI: 10.1002/ijc.28736.

[23] OSHI M, TOKUMARU Y, ASAOKA M, *et al*. M1 macrophage and M1/M2 ratio defined by transcriptomic signatures resemble only part of their conventional clinical characteristics in breast cancer[J]. Sci Rep, 2020, 10: 16554. DOI: 10.1038/s41598-020-73624-w.

[24] WU K Y, LIN K J, LI X Y, *et al*. Redefining tumor-associated macrophage subpopulations and functions in the tumor microenvironment[J]. Front Immunol, 2020, 11: 1731. DOI: 10.3389/fimmu.2020.01731.

[25] WENES M, SHANG M, DI MATTEO M, *et al*. Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis[J]. Cell Metab, 2016, 24(5): 701-715. DOI: 10.1016/j.cmet.2016.09.008.

[26] WU Y Y, WU C, SHI T C, *et al*. FAP expression in adipose tissue macrophages promotes obesity and metabolic inflammation[J]. Proc Natl Acad Sci U S A, 2023, 120(51): 5120-5132. DOI: 10.1073/pnas.2303075120.

[27] ZHAO J L, LIN E, BAI Z R, *et al*. Cancer-associated fibroblasts induce sorafenib resistance of hepatocellular carcinoma cells through CXCL12/FOLR1[J]. BMC Cancer, 2023, 23(1): 1198. DOI: 10.1186/s12885-023-11613-8.

[28] CHONG L J, JIANG Y W, WANG D X, *et al*. Targeting and repolarizing M2-like tumor-associated macrophage-mediated MR imaging and tumor immunotherapy by biomimetic nanoparticles[J]. J Nanobiotechnol, 2023, 21(1): 401. DOI: 10.1186/s12951-023-02122-8.

[29] KERZEL T, GIACCA G, BERETTA S, *et al*. *In vivo* macrophage engineering reshapes the tumor microenvironment leading to eradication of liver metastases[J]. Cancer Cell, 2023, 41(11): 1892-1910.e10. DOI: 10.1016/j.ccell.2023.09.014.

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