

KHSRP通过激活JAK/STAT信号通路对结直肠癌细胞生物学行为的影响

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[摘要] **目的:** 探讨KH型剪切调节蛋白(KHSRP)激活Janus激酶(JAK)/信号转导与转录激活因子(STAT)信号通路对结直肠癌(CRC)恶性生物学行为的影响, 并阐明其可能的作用机制。**方法:** 选取64例CRC患者的CRC组织和癌旁正常组织, 体外培养人CRC HT29、SW620、SW480、DLD-1、LOVO和RKO细胞及人正常结直肠黏膜FHC细胞, 提取CRC组织和细胞中总RNA, 采用实时荧光定量PCR(RT-qPCR)法检测CRC组织、癌旁正常组织和各种细胞中KHSRP mRNA表达水平。将HT29和SW620细胞分为sh-NC组(无相关性的核苷酸序列插入慢病毒质粒)和sh-KHSRP组(转染敲降KHSRP慢病毒), SW480和DLD-1细胞分为oe-NC组(无相关性的核苷酸序列插入慢病毒质粒)和oe-KHSRP组(转染过表达KHSRP慢病毒)。采用免疫组织化学(IHC)染色法分析CRC组织和癌旁正常组织中KHSRP蛋白表达情况, 细胞计数试剂盒8(CCK-8)法检测各组CRC细胞增殖活性, Transwell小室实验检测各组CRC细胞的迁移细胞数和侵袭细胞数, Western blotting法检测各组CRC细胞中KHSRP、JAK1、磷酸化JAK1(p-JAK1)、JAK2、磷酸化JAK2(p-JAK2)、STAT1、STAT2、STAT3和STAT5蛋白表达水平, 裸鼠皮下移植瘤实验检测各组小鼠肿瘤质量和肿瘤体积。**结果:** 与癌旁正常组织比较, CRC组织中KHSRP mRNA表达水平升高($P<0.05$); 与人正常结直肠黏膜FHC细胞比较, CRC细胞中KHSRP mRNA表达水平平均升高($P<0.05$), 后续实验选择HT29和SW620细胞进行KHSRP敲降, 选择SW480和DLD-1细胞进行KHSRP过表达。Western blotting法检测, CRC组织和细胞中KHSRP蛋白表达量高于癌旁正常组织和FHC细胞。IHC染色法分析, 与癌旁正常组织比较, CRC组织中KHSRP蛋白表达水平升高($P<0.01$)。RT-qPCR法检测, 与sh-NC组比较, sh-KHSRP组HT29和SW620细胞中KHSRP mRNA表达水平降低($P<0.01$); 与oe-NC组比较, oe-KHSRP组SW480和DLD-1细胞中KHSRP mRNA表达水平升高($P<0.01$), 提示细胞转染成功。CCK-8法检测, 与sh-NC组比较, 敲降KHSRP后, sh-KHSRP组HT29和SW620细胞增殖活性降低($P<0.05$ 或 $P<0.01$); 与oe-NC组比较, 过表达KHSRP后, oe-KHSRP组SW480和DLD-1细胞增殖活性升高($P<0.05$ 或 $P<0.01$)。与sh-NC组比较, 敲降KHSRP后, sh-KHSRP组HT29和SW620细胞的迁移细胞数和侵袭细胞数减少($P<0.05$); 与oe-NC组比较, 过表达KHSRP后, oe-KHSRP组SW480和DLD-1细胞的迁移细胞数和侵袭细胞数增多($P<0.05$)。敲降KHSRP后, oe-KHSRP组小鼠肿瘤体积和质量小于sh-NC组($P<0.05$ 或 $P<0.01$); 过表达KHSRP后, oe-KHSRP组小鼠肿瘤体积和质量大于oe-NC组($P<0.05$ 或 $P<0.01$)。与sh-NC组比较, sh-KHSRP组CRC细胞

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中 JAK1、p-JAK1 和 STAT3 蛋白表达水平明显降低 ($P < 0.05$ 或 $P < 0.01$); 与 oe-NC 组比较, oe-KHSRP 组 CRC 细胞中 JAK1、p-JAK1 和 STAT3 蛋白表达水平明显升高 ($P < 0.05$ 或 $P < 0.01$)。

结论: KHSRP 高表达可促进 CRC 细胞增殖、迁移和侵袭, 促进小鼠 CRC 细胞皮下移植瘤的生长, 其机制可能与其激活 JAK1/STAT3 信号通路有关联。

[关键词] 结直肠肿瘤; KH 型剪切调节蛋白; Janus 激酶; 信号转导与转录激活因子; 恶性进展; 细胞增殖; 细胞侵袭; 细胞迁移

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Effect of KHSRP on biological behavior of colorectal cancer cells through activation of JAK/STAT signaling pathway

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ABSTRACT Objective: To discuss the effect of KH-type splicing regulatory protein (KHSRP) on the malignant biological behaviors of colorectal cancer (CRC) by activating the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway, and to clarify its possible mechanism.

Methods: The CRC tissue and adjacent normal tissue from 64 CRC patients were selected. The human CRC cells (HT29, SW620, SW480, DLD-1, LOVO, and RKO) and normal human colorectal mucosal FHC cells were cultured *in vitro*. The total RNA from CRC tissue and cells were extracted, real-time fluorescence quantitative PCR (RT-qPCR) was used to detect the expression levels of KHSRP in the CRC tissue, adjacent normal tissue and all kinds of cells. The HT29 and SW620 cells were divided into sh-NC group (lentiviral plasmid inserted with non-targeting nucleotide sequence) and sh-KHSRP group (transfected with KHSRP knockdown lentivirus). The SW480 and DLD-1 cells were divided into oe-NC group (lentiviral plasmid inserted with non-targeting nucleotide sequence) and oe-KHSRP group (transfected with KHSRP overexpression lentivirus). Immunohistochemistry (IHC) staining method was used to analyze the expressions of KHSRP in CRC tissue and adjacent normal tissue; CCK-8 method was used to detect the proliferation activities of the CRC cells in various groups; Transwell assay was used to detect the numbers of migration and invasion cells in various groups; Western blotting method was used to detect the expression levels of KHSRP, JAK1, phosphorylated JAK1 (p-JAK1), JAK2, phosphorylated JAK2 (p-JAK2), STAT1, STAT2, STAT3, and STAT5 proteins in the CRC cells in various groups. The subcutaneous xenograft tumor models in the nude mice were used to measure the tumor volumes and weights of the mice in various groups. **Results:** Compared with adjacent normal tissue, the expression level of KHSRP in the CRC tissue was increased ($P < 0.05$). Compared with FHC cells, the expression levels of KHSRP in the CRC cells were increased ($P < 0.05$). Therefore, the HT29 and SW620 cells were selected for knockdown of KHSRP, while the SW480 and DLD-1 cells were selected for overexpression of KHSRP. The Western blotting results showed that the expression amounts of KHSRP protein in the CRC tissue and cells were higher than those in adjacent normal tissue and FHC cells. The IHC results showed that compared with adjacent normal tissue, the expression level of KHSRP protein in CRC tissue was increased ($P < 0.01$). The RT-qPCR results showed that compared with sh-NC group, the expression levels of KHSRP mRNA in the HT29 and SW620 cells in sh-KHSRP group were decreased ($P < 0.01$); compared with oe-NC group, the expression levels of KHSRP mRNA in the SW480 and

DLD-1 cells in oe-KHSRP group were increased ($P<0.01$), indicating successful transfection. The CCK-8 results showed that compared with sh-NC group, the proliferation activities of the HT29 and SW620 cells in sh-KHSRP group after knockdown of KHSRP were decreased ($P<0.05$ or $P<0.01$); compared with oe-NC group, the proliferation activities of the SW480 and DLD-1 cells in oe-KHSRP group after over-expression of KHSRP were increased ($P<0.05$ or $P<0.01$). Compared with sh-NC group, the numbers of migration and invasion cells in the HT29 and SW620 cells in sh-KHSRP group after knockdown of KHSRP were decreased ($P<0.05$); compared with oe-NC group, the numbers of migration and invasion cells in the CRC cells in oe-KHSRP group after over-expression of KHSRP were increased ($P<0.05$). After knockdown of KHSRP, the tumor volume and weight in sh-KHSRP group were smaller than those in sh-NC group ($P<0.05$ or $P<0.01$), while the tumor volume and weight in oe-KHSRP group were larger than those in oe-NC group after over-expression of KHSRP ($P<0.05$ or $P<0.01$). Compared with sh-NC group, the expression levels of JAK1, p-JAK1, and STAT3 proteins in the CRC cells in sh-KHSRP group were significantly decreased ($P<0.05$ or $P<0.01$); compared with oe-NC group, the expression levels of JAK1, p-JAK1, and STAT3 proteins in the CRC cells in oe-KHSRP group were significantly increased ($P<0.05$ or $P<0.01$). **Conclusion:** High expression of KHSRP promotes the proliferation, migration, and invasion of the CRC cells and enhances the growth of subcutaneous xenograft tumors in the mice; its mechanism may be associated with its activation of the JAK1/STAT3 signaling pathway.

KEYWORDS Colorectal neoplasms; KH-type splicing regulatory protein; Janus kinase; Signal transducer and activator of transcription; Malignant progression; Cell proliferation; Cell invasion; Cell migration

结直肠癌(colorectal cancer, CRC)是全球第三大常见恶性肿瘤和第二大致命癌症,2020年约有190万例CRC患者,死亡约90万例^[1-3]。尽管CRC的治疗取得了进展,如手术切除和系统化疗,但由于复发和远处转移,其死亡率仍然很高^[4-5]。因此,探讨促进肿瘤启动和进展的新生物标志物并阐明其潜在的分子机制,对CRC的靶向治疗至关重要。KH型剪切调节蛋白(KH-type splicing regulatory protein, KHSRP)是一种多功能核酸结合蛋白,在肿瘤侵袭中起重要作用,其表达水平及蛋白结构的改变与肿瘤的发生发展存在密切关联^[6-7]。Janus激酶/信号转导与转录激活因子(Janus kinase/signal transducer and activator of transcription, JAK/STAT)信号通路是一种跨膜信号转导的进化保守机制,在癌症的发生发展、转移和预后过程中起调控作用^[8-9],但KHSRP-JAK/STAT信号轴在CRC发病中的具体作用机制尚未明确。本文作者通过预实验初步验证了KHSRP和JAK/STAT信号通路在CRC细胞中的表达水平,在此基础上,本研究探讨KHSRP对CRC进展的影响,以及KHSRP是否通过调节JAK/STAT信号转导促进或抑制CRC发展,从而为CRC分子靶向治疗提供理论依据。

1 材料与方法

1.1 组织标本、细胞、实验动物、主要试剂和仪器

本研究选取2017年1月—2018年12月在河南大学淮河医院确诊的64例CRC患者的CRC组织及其癌旁正常组织标本。患者纳入标准:①影像和病理确诊的CRC;②进行根治性切除术治疗,并有完整的临床病理记录。排除标准:①曾经接受手术、放化疗或辅助治疗的患者;②存在基础疾病及其他恶性肿瘤病史;③临床病理资料记录不全。本研究经河南大学淮河医院伦理委员会批准(伦理批准号:HUSOM2023-348),所有参与者已签署知情同意书。人正常结肠黏膜FHC细胞(宁波明舟生物公司),人CRC HT29、SW620、SW480、DLD-1、LOVO和RKO细胞(武汉普诺赛公司)。36只SPF级BALB/c雄性裸鼠,4~6周龄,体质量16~22 g,购自北京维通利华公司,饲养于河南大学医学院[动物使用许可证号:SYXK(豫)2016-0006],实验遵循实验动物使用的3R原则。

TRIzol试剂、细胞计数试剂盒8(cell counting kit-8, CCK-8)试剂盒和10%聚偏氟乙烯(polyvinylidene fluoride, PVDF)膜购自美国Sigma Aldrich公司,HiScript® II逆转录酶试剂盒、通用型高特异性染料法定量PCR检测试剂盒、放射

性免疫沉淀分析 (radioimmunoprecipitation assay buffer, RIPA) 缓冲液和胎牛血清 (fatal bovine serun, FBS) 购自北京索莱宝公司, BCA 蛋白质定量试剂盒购自南京诺唯赞公司, Transwell小室购自美国 Corning Incorporated 公司, 慢病毒试剂购自上海吉玛公司, 山羊抗兔一抗和二抗 (1:2 000) 购自翌圣生物科技 (上海) 股份有限公司。

1.2 细胞培养、转染和分组 人正常结直肠黏膜 FHC 细胞和 CRC HT29、SW620、SW480、DLD-1、LOVO 及 RKO 细胞在含 100 U·mL⁻¹ 青霉素 + 0.1 g·L⁻¹ 链霉素 + 10% FBS 的培养基中培养, 并置于 37℃、5% CO₂ 的湿化培养箱中。根据获取的 KHSRP 全长序列设计特异性敲降和过表达的 RNA 序列, 构建到载体上进行敲降或过表达处理 (HT29 和 SW620 细胞进行 KHSRP 敲降, SW480 和 DLD-1 细胞进行 KHSRP 过表达)。将细胞接种至 6 孔细胞培养板中, 加入无双抗的完全培养基 DMEM 至 2 mL, 置入培养箱中进行培养, 当细胞生长至 70%~80% 密度时开始转染, 从 -80℃ 冰箱取出 sh-KHSRP 和敲降对照 sh-NC 慢病毒及 KHSRP 慢病毒和过表达对照 Vector 慢病毒置于冰上, 待融化后分别吸取 20 μL 慢病毒和 2 μL polybrene 液加入原先铺好的 6 孔细胞培养板中, 混匀后置于培养箱中培养, 48~72 h 后弃去板中带病毒液的培养基, 加入嘌呤霉素 (至其浓度为 4 mL·L⁻¹), 24~48 h 后, 其浓度增加至 8 mL·L⁻¹, 根据细胞状态继续筛选、观察直至无死亡细胞后得到稳定转染的细胞。将 HT29 和 SW620 细胞分为 sh-NC 组 (为无相关性的核苷酸序列插入慢病毒质粒) 及 sh-KHSRP 组 (转染敲降 KHSRP 的慢病毒); 将 SW480 和 DLD-1 细胞分为 oe-NC 组 (为无相关性的核苷酸序列插入慢病毒质粒) 及 oe-KHSRP 组 (转染过表达 KHSRP 的慢病毒)。

1.3 实时荧光定量 PCR (real-time fluorescence quantitative PCR, RT-qPCR) 法检测 CRC 组织和 CRC 细胞中 KHSRP mRNA 表达水平 采用 TRIzol 试剂盒从 CRC 组织和细胞中提取总 RNA, 采用 M-MLV RTase 试剂盒将总 RNA 逆转录至 cDNA 中。然后采用 RT-qPCR 试剂盒对 KHSRP mRNA 表达水平进行定量分析, 以 GAPDH 作为内参基因, 采用 2^{-ΔΔCt} 法计算 KHSRP mRNA 表达水平。PCR 引物序列见表 1。

表1 PCR引物序列
Tab. 1 Primer sequences of PCR

Gene		Primer sequences(5'-3')
KHSRP	R	AATGAGTACGGATCTCGGATTGG
	F	CCGTCATCTTGCTTGAAGTGA
JAK1	R	ACGCTCTGGGAAATCTGCTA
	F	ATGATGGCTCGGAAGAAAGG
STAT3	R	CTGGCCTTTGGTGTTGAAAT
	F	AAGGCACCCACAGAAACAAC
GAPDH	R	GAAGGTGAAGGTCGGAGTC
	F	GAAGATGGTGATGGGATTTC

1.4 免疫组织化学 (immunohistochemistry, IHC) 染色法检测 CRC 组织中 KHSRP 蛋白表达水平

将癌旁正常组织和 CRC 组织石蜡标本进行烤片、二甲苯脱蜡及酒精水化处理。然后进行微波炉抗原修复, 并采用 3% 过氧化氢溶液孵育以阻断内源性过氧化物酶。KHSRP 抗体稀释后 4℃ 孵育过夜。第 2 天, 磷酸盐缓冲液 (phosphate buffer saline, PBS) 清洗后加入二抗室温孵育 30 min, DAB 显色, 苏木素复染细胞核, 氨水返蓝, 最后封片。在光学显微镜下按染色强度 (无染色计 0 分, 浅棕色弱染色计 1 分, 棕色中度染色计 2 分, 深棕色强染色计 3 分) 和阳性范围 (1 分: 0% < 阳性率 < 25%, 2 分: 25% ≤ 阳性率 < 50%, 3 分: 50% ≤ 阳性率 < 75%, 4 分: 75% ≤ 阳性率 ≤ 100%) 评分。上述 2 项评分相加, 0~2 分为低表达, 3~7 分为高表达。以 IHC 染色评分代表 KHSRP 蛋白表达水平。

1.5 CCK-8 法检测各组 CRC 细胞增殖活性 将细胞接种于 96 孔细胞培养板中, 每孔 1×10⁴ 个细胞, 并置于含 10% FBS 的 100 μL 培养基中培养。向含 CCK-8 试剂的每孔中加入 10 μL, 并将混合物在 37℃ 下孵育 2 h。在酶标仪 450 nm 波长处测量吸光度 (A) 值, 计算细胞增殖活性, 评估细胞的生长情况。细胞增殖活性 = (实验孔 A 值 - 空白孔 A 值) / (对照孔 A 值 - 空白孔 A 值) × 100%, 其中对照孔为空白对照组, 空白孔为培养基对照组 (用于调零)。

1.6 Transwell 小室实验检测各组 CRC 细胞的迁移细胞数和侵袭细胞数 选择 8 μm 孔径的 Transwell 小室进行细胞侵袭和迁移能力的评估。细胞迁移实验: 将 5×10⁴ 个细胞加入 200 μL 无血清培养基中, 并接种至无包膜的上腔室孔中, 同时向下腔室中加入 800 μL 含 10% FBS 的培养基。采用 100% 甲醇

固定通过聚碳酸酯膜(孔径 $0.1\sim 12.0\ \mu\text{m}$)迁移的细胞,采用 0.1% 结晶紫染色 $30\ \text{min}$,在光学显微镜下成像和计数。细胞侵袭实验:将细胞接种至预铺基质胶的Transwell小室的上室中,其他实验步骤与细胞迁移实验相同。

1.7 Western blotting法检测CRC组织和细胞中KHSRP及其相关蛋白表达水平 采用T-PER[®]蛋白提取试剂提取细胞和组织蛋白,并采用Pierce[™] BCA蛋白质检测试剂盒测定蛋白质浓度。采用十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SDS-PAGE)分离蛋白质,并转移到PVDF膜中。在室温条件下,采用 5% 脱脂牛奶溶液封闭膜 $1\ \text{h}$,以减少非特异性结合。将膜与GAPDH抗体和针对KHSRP的一抗 $4\ ^\circ\text{C}$ 过夜孵育。孵育完成后,采用TBST缓冲液清洗膜3次,以去除未结合的抗体。将膜与二抗一起在室温下孵育 $1.5\ \text{h}$ 后进行信号检测,以可视化蛋白条带。采用ECL化学发光试剂盒曝光显影,采用Image J软件分析蛋白质条带的灰度值,计算目的蛋白表达水平。目的蛋白表达水平=目的蛋白条带灰度值/GAPDH蛋白条带灰度值。

1.8 裸鼠皮下移植瘤实验检测各组小鼠肿瘤生长情况 取sh-NC组和sh-KHSRP组HT29细胞以及oe-NC组和oe-KHSRP组SW480细胞,密度为 $5\times 10^6\ \text{mL}^{-1}$,分别皮下接种至36只裸鼠的右背侧(每

组8只)。每3 d测量1次小鼠肿瘤长和宽及质量,并计算肿瘤体积,肿瘤体积=(长 $\times$ 宽²)/2。3~4周后,处死小鼠,取出肿瘤并固定于甲醛溶液中。

1.9 统计学分析 采用GraphPad Prism 8统计软件进行统计学分析和绘图。各组细胞中KHSRP mRNA和蛋白表达水平、JAK/STAT蛋白表达水平、细胞增殖活性、迁移细胞数、侵袭细胞数、肿瘤质量和肿瘤体积均符合正态分布,以 $\bar{x}\pm s$ 表示,多组间样本均数比较采用单因素方差分析,组间样本均数两两比较采用LSD- t 检验,2组间样本均数比较采用两独立样本 t 检验。以 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 CRC组织和细胞中KHSRP mRNA及蛋白表达水平 RT-qPCR检测结果显示:与癌旁正常组织比较,CRC组织中KHSRP mRNA表达水平升高($P<0.05$);与人正常结直肠黏膜FHC细胞比较,CRC细胞中KHSRP mRNA表达水平平均升高($P<0.05$),后续实验选择HT29和SW620细胞进行KHSRP敲降,选择SW480和DLD-1细胞进行KHSRP过表达。Western blotting法检测结果显示: CRC组织和细胞中KHSRP蛋白表达水平高于癌旁组织及FHC细胞。IHC染色法分析结果显示:与癌旁正常组织比较,在CRC组织中KHSRP蛋白表达水平升高($P<0.01$)。见图1~4。

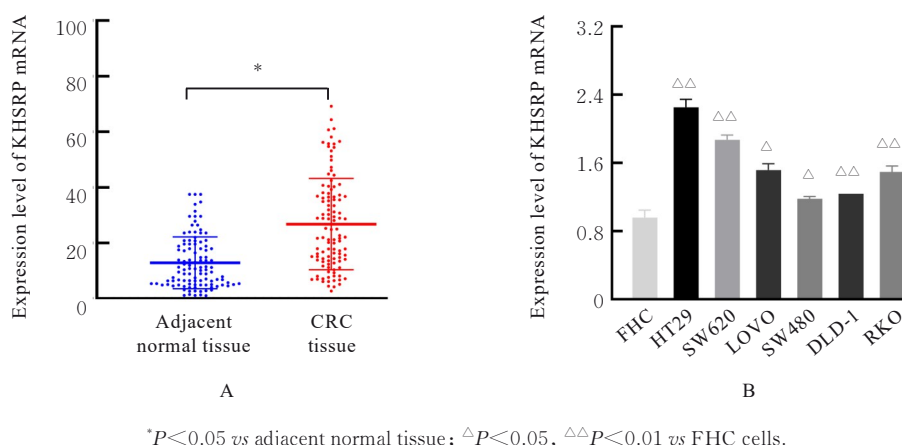
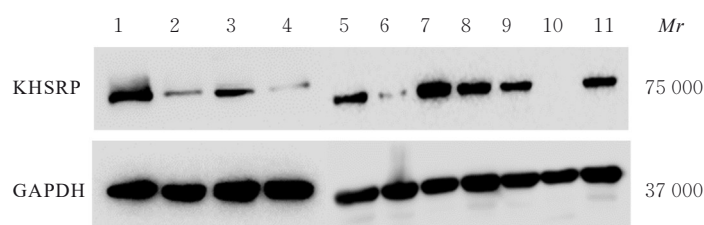


图1 RT-qPCR法检测CRC组织(A)和细胞(B)中KHSRP mRNA表达水平

Fig.1 Expression levels of KHSRP mRNA in CRC tissue(A) and cells(B) detected by RT-qPCR method

2.2 敲降或过表达KHSRP后CRC细胞中KHSRP mRNA表达水平 RT-qPCR法检测结果显示:与sh-NC组比较,sh-KHSRP组HT29和SW620细胞

中KHSRP mRNA表达水平降低($P<0.01$);与oe-NC组比较,oe-KHSRP组SW480和DLD-1细胞中KHSRP mRNA表达水平升高($P<0.01$),



Lane 1, 3: CRC tissue; Lane 2, 4: Adjacent normal tissue; Lane 5: FHC cells; Lane 6: SW480 cells; Lane 7: HT29 cells; Lane 8: SW620 cells; Lane 9: LOVO cells; Lane 10: DLD-1 cells; Lane 11: RKO cells.

图2 Western blotting法检测CRC组织和细胞中KHSRP蛋白表达电泳图

Fig. 2 Electrophoregrams of expression of KHSRP protein in CRC tissue and cells detected by Western blotting method

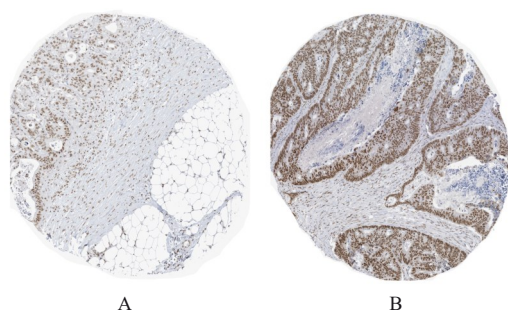
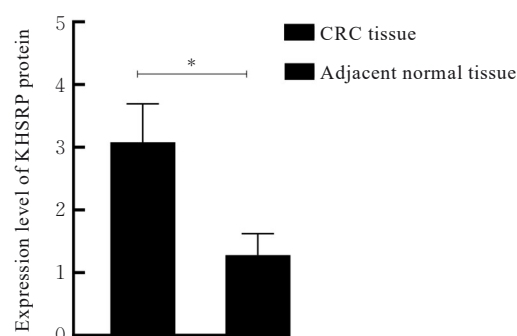


图3 IHC染色法检测CRC组织(A)和癌旁正常组织(B)中KHSRP蛋白表达情况($\times 40$)

Fig. 3 Expressions of KHSRP protein in CRC tissue (A) and adjacent normal tissue (B) detected by IHC staining method ($\times 40$)



* $P < 0.01$ compared with adjacent normal tissue.

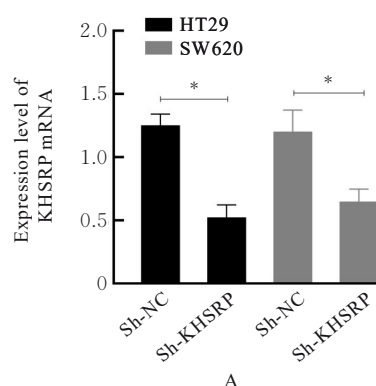
图4 CRC和癌旁正常组织中KHSRP蛋白表达水平

Fig. 4 Expression levels of KHSRP protein in CRC tissue and adjacent normal tissue

提示细胞转染成功。见图5。

2.3 各组CRC细胞增殖活性 CCK-8法检测结果显示:与sh-NC组比较,敲降KHSRP后,sh-KHSRP组HT29和SW620细胞增殖活性降低

($P < 0.05$ 或 $P < 0.01$);与oe-NC组比较,过表达KHSRP后,sh-KHSRP组SW480和DLD-1细胞的增殖活性升高($P < 0.05$ 或 $P < 0.01$)。见图6。



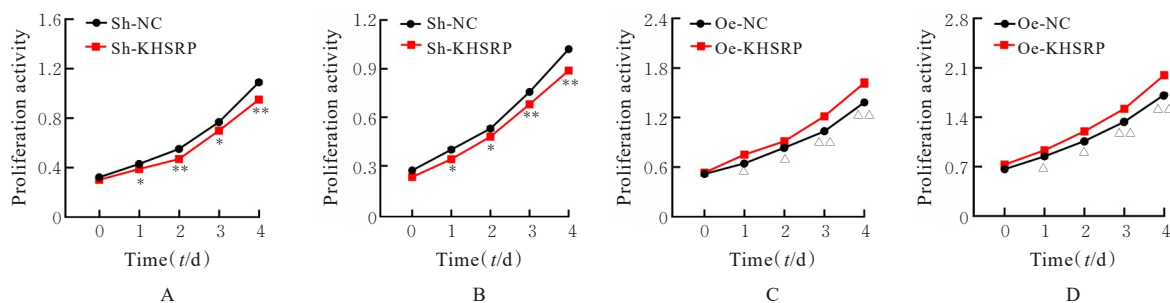
* $P < 0.01$ compared with sh-NC group; $\Delta P < 0.01$ compared with oe-NC group.

图5 敲降和过表达KHSRP后CRC细胞中KHSRP mRNA表达水平

Fig. 5 Expression levels of KHSRP mRNA in CRC cells after knockdown or over-expression of KHSRP

2.4 各组CRC细胞的迁移细胞数和侵袭细胞数与sh-NC组比较,sh-KHSRP组HT29和SW620细胞的迁移细胞数和侵袭细胞数减少($P < 0.05$);

与oe-NC组比较,oe-KHSRP组SW480和DLD-1细胞的迁移细胞数和侵袭细胞数增多($P < 0.05$)。见图7~14。



A,B: Knockdown of KHSRP; C,D: Over-expression of KHSRP; A:HT29 cells; B:SW620 cells; C:SW480 cells; D:SK-GT-4 cells. * $P<0.05$, ** $P<0.01$ compared with sh-NC group; $\triangle P<0.05$, $\triangle\triangle P<0.01$ compared with oe-NC group.

图6 敲降和过表达KHSRP后各组CRC细胞增殖活性

Fig. 6 Proliferation activities of CRC cells in various groups after knockdown and over-expression of KHSRP

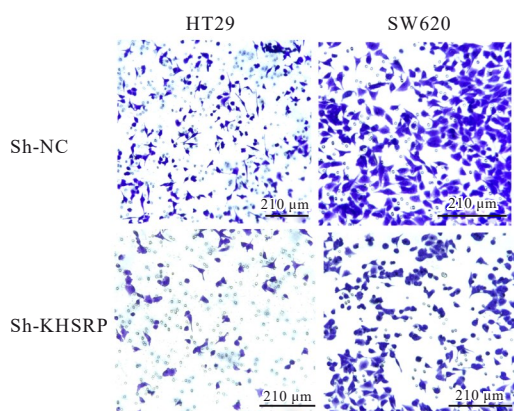


图7 敲降KHSRP后2组HT29和SW620细胞迁移情况(结晶紫)

Fig. 7 Morphology of migration of HT29 and SW620 cells in two groups after knockdown of KHSRP (Crystal violet)

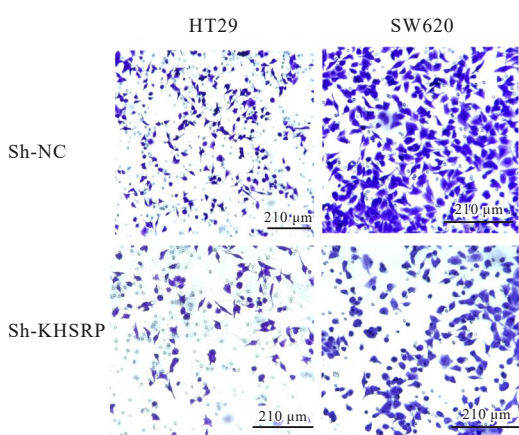
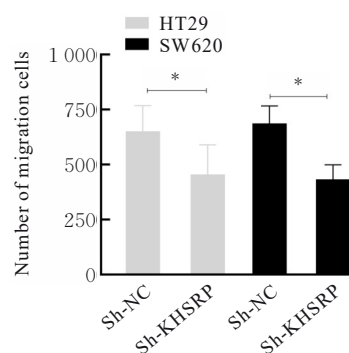


图9 敲降KHSRP后2组HT29和SW620细胞侵袭情况(结晶紫)

Fig. 9 Morphology of invasion of HT29 and SW620 cells in two groups after knockdown of KHSRP (Crystal violet)

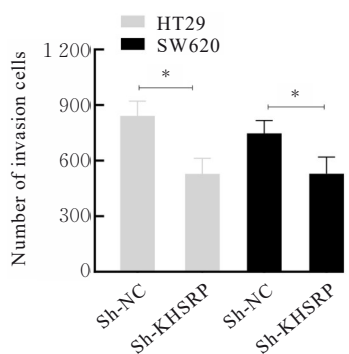
2.5 各组裸鼠皮下移植瘤的体积和质量 敲降KHSRP后, sh-KHSRP组小鼠肿瘤体积和质量小于



* $P<0.05$ compared with sh-NC group.

图8 敲降KHSRP后2组HT29和SW620细胞迁移细胞数

Fig. 8 Number of migration cells of HT29 and SW620 cells in two groups after knockdown of KHSRP



* $P<0.05$ compared with sh-NC group.

图10 敲降KHSRP后2组HT29和SW620细胞侵袭细胞数

Fig. 10 Numbers of invasion HT29 and SW620 cells in two groups after knockdown of KHSRP

sh-NC组 ($P<0.05$ 或 $P<0.01$); 过表达KHSRP后, oe-KHSRP组小鼠肿瘤体积和质量大于oe-NC组 ($P<0.05$ 或 $P<0.01$)。见图15。

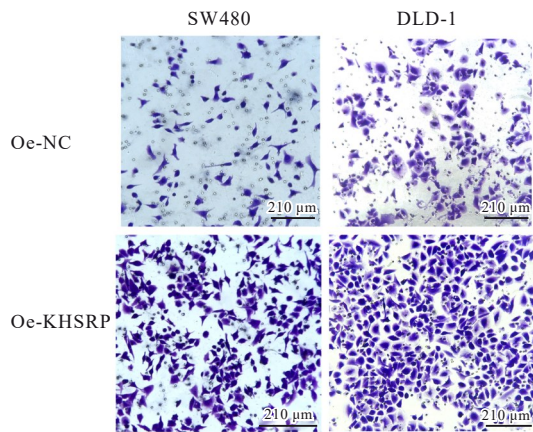


图 11 过表达 KHSRP 后 2 组 SW480 和 DLD-1 细胞迁移情况(结晶紫)

Fig. 11 Morphology of migration of SW480 and DLD-1 cells in two groups after over-expression of KHSRP (Crystal violet)

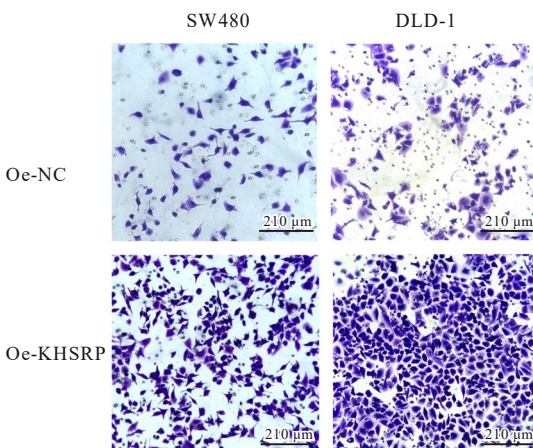


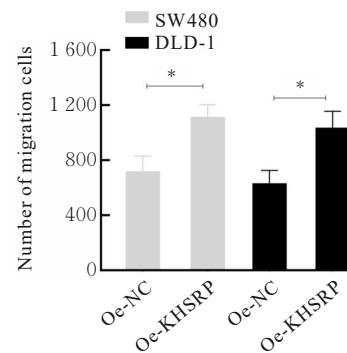
图 13 过表达 KHSRP 后 2 组 SW480 和 DLD-1 细胞侵袭情况(结晶紫)

Fig. 13 Morphology of invasion of SW480 and DLD-1 cells in two groups after over-expression of KHSRP (Crystal violet)

2.6 敲降或过表达 KHSRP 后 CRC 细胞中 JAK/STAT 信号通路相关蛋白表达水平 与 sh-NC 组比较, sh-KHSRP 组 CRC 细胞中 JAK1、p-JAK1 和 STAT3 蛋白表达水平明显降低 ($P < 0.05$ 或 $P < 0.01$); 与 oe-NC 组比较, oe-KHSRP 组 CRC 细胞中 JAK1、p-JAK1 和 STAT3 蛋白表达水平明显升高 ($P < 0.05$ 或 $P < 0.01$)。见图 16 和 17。

3 讨论

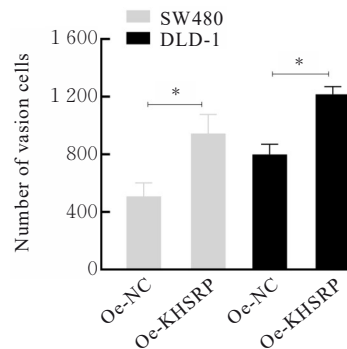
癌症的发病机制是一个复杂的多因素过程, 癌细胞具有生存优势和增殖、侵袭及迁移能力^[10]。



* $P < 0.05$ compared with oe-NC group.

图 12 过表达 KHSRP 后 2 组 SW480 和 DLD-1 细胞迁移细胞数

Fig. 12 Numbers of migration SW480 and DLD-1 cells in two groups after over-expression of KHSRP



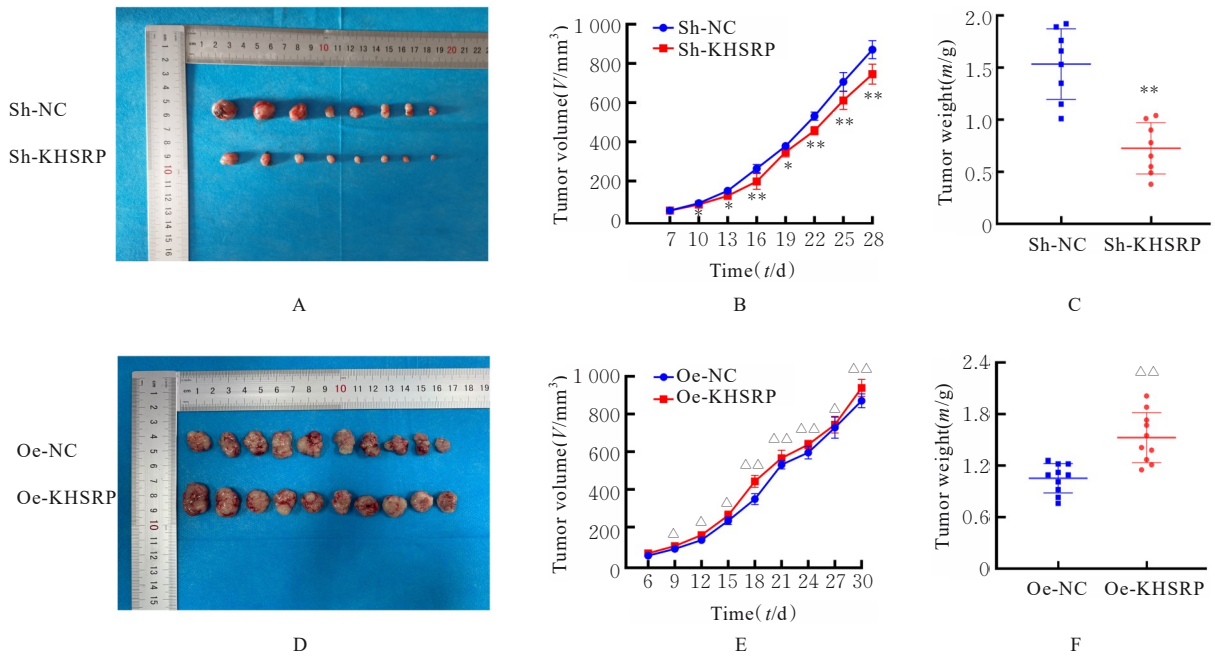
* $P < 0.05$ compared with oe-NC group.

图 14 过表达 KHSRP 后 2 组 SW480 和 DLD-1 细胞侵袭细胞数

Fig. 14 Number of invasion SW480 and DLD-1 cells in two groups after over-expression of KHSRP

近年来, 已有多项研究^[11-13]聚焦于 KHSRP 在肿瘤的形成和发展过程中所扮演的角色。YAN 等^[14]发现: KHSRP 可促进非小细胞肺癌的肿瘤生长和转移。TANIUCHI 等^[6]发现: KHSRP 位于胰腺癌细胞系的细胞核和细胞突起中, 通过小干扰 RNA 抑制 KHSRP, 可减少细胞突起的数量, 抑制胰腺癌细胞的侵袭性和转移性。因此, 本研究从细胞、动物和人体组织 3 个水平上进行分析, 同时在细胞功能实验中采取双细胞系双向验证, 分析 KHSRP 在调节 CRC 细胞侵袭和转移中发挥的生物学功能。

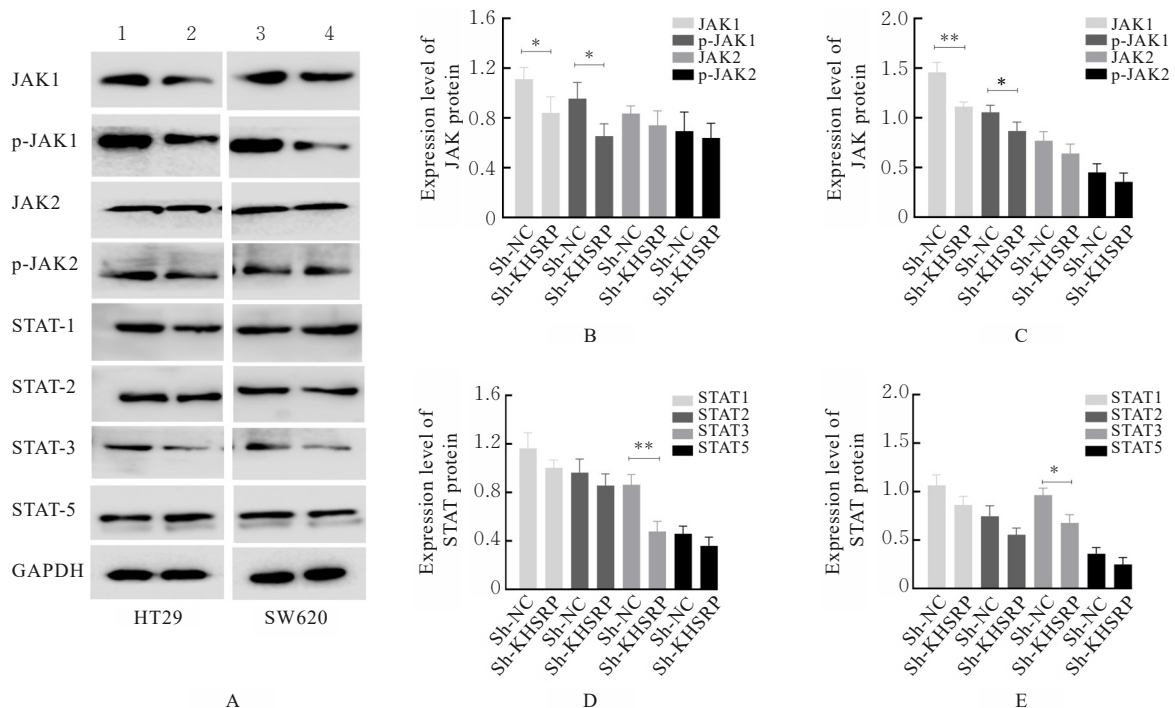
本研究强调了 KHSRP-JAK1/STAT3 在 CRC 发展中的重要作用, 表明其上调与肿瘤细胞增殖、侵袭和迁移的增加存在密切关联。通过体外和体内实验探讨 KHSRP 在调控 CRC 细胞侵袭及转移中的



A: Gross morphology of transplanted tumors in sh-NC group and sh-KHSRP group; B, C: HT29 cells; D: Gross morphology of transplanted tumors in oe-NC group and oe-KHSRP group; E, F: SW480 cells. * $P < 0.05$, ** $P < 0.01$ vs sh-NC group; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ vs oe-NC group.

图 15 各组小鼠体内肿瘤生长情况

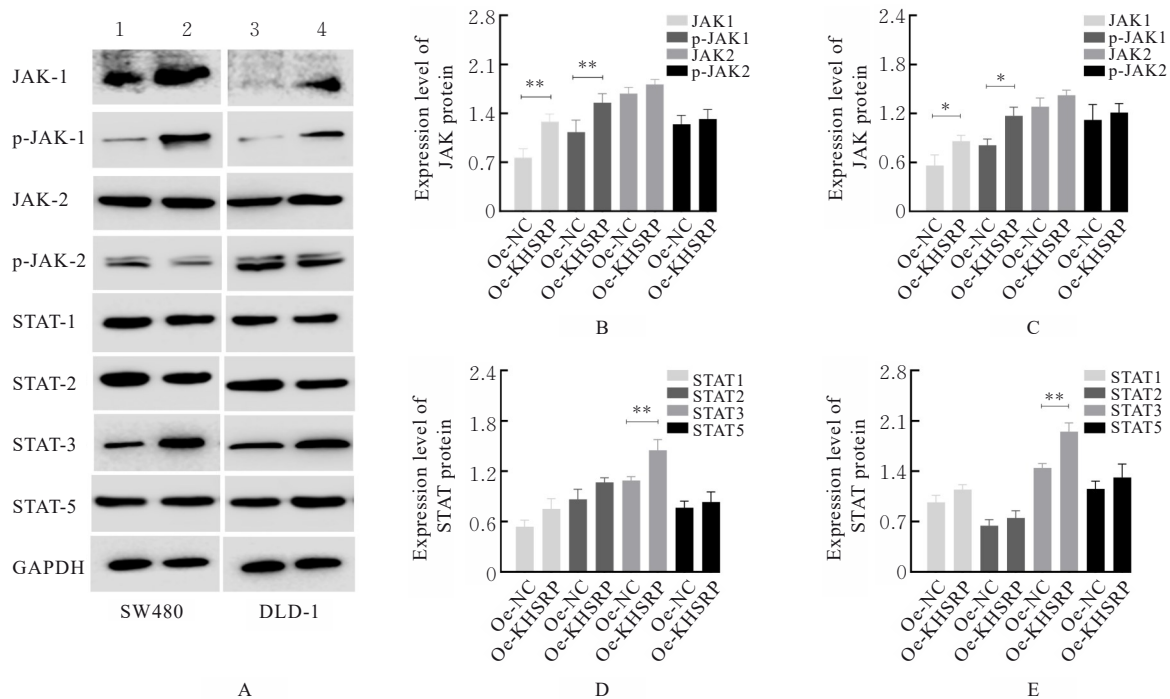
Fig. 15 *In vivo* tumor growth of mice in various groups



A: Electrophoresis (Lane 1: Sh-NC group; Lane 2: Sh-KHSRP group; Lane 3: Sh-NC group; Lane 4: Sh-KHSRP group); B-E: Histograms; B, C: JAK protein; D, E: STAT protein; B, D: HT29 cells; C, E: SW620 cells. * $P < 0.05$, ** $P < 0.01$ compared with sh-NC group.

图 16 Sh-NC 和 sh-KHSRP 组 CRC 细胞中 JAK/STAT 信号通路相关蛋白表达电泳图(A)及直条图(B-E)

Fig. 16 Electrophoresis (A) and histograms (B-E) of expressions of JAK/STAT signaling pathway-related proteins in CRC cells in sh-NC and sh-KHSRP groups



A: Electrophoregram (Lane 1: Sh-NC group; Lane 2: Sh-KHSRP group; Lane 3: Sh-NC group; Lane 4: Sh-KHSRP group); B-E: Histograms; B, C: JAK protein; D, E: STAT protein; B, D: SW480 cells; C, E: DLD-1 cells. * $P < 0.05$, ** $P < 0.01$ compared with oe-NC group.

图 17 Oe-NC 和 oe-KHSRP 组 CRC 细胞中 JAK/STAT 信号通路相关蛋白表达电泳图(A)及直条图(B-E)

Fig. 17 Electrophoregram (A) and histograms (B-E) of expressions of JAK/STAT signaling pathway-related proteins in CRC cells in oe-NC and oe-KHSRP groups

生物学功能。首先验证了在人正常结直肠黏膜细胞和 CRC 细胞中 KHSRP 的表达差异情况, 结果显示: KHSRP 在 CRC 细胞中的表达上调, 并从组织水平上得到进一步验证; 体外细胞功能实验显示: 敲降 KHSRP 在体外可以抑制 CRC 细胞增殖、迁移和侵袭, 过表达 KHSRP 则会促进 CRC 细胞增殖、迁移和侵袭。本研究在裸鼠体内构建皮下移植瘤, 结果显示: 裸鼠体内肿瘤的质量和体积受体内 KHSRP 表达水平的影响, 过表达 KHSRP 会促进裸鼠体内肿瘤质量和体积的增大。

研究^[15-18]显示: JAK/STAT 信号通路参与癌症细胞恶性进展。JAK 是细胞因子和生长激素信号传导的介质。激活的 STAT 蛋白转位到细胞核, 调节参与细胞增殖、分化和凋亡的基因转录^[19]。JAK/STAT 信号转导是癌症进展的基石, 既可以作为癌症生长/转移的肿瘤内在驱动因素, 也可以作为免疫监视的调节剂^[20-22]。WANG 等^[22]发现: CircNOL10 通过调节 miR-767-5p/SOCS2 轴使 JAK2/STAT5 信号失活来抑制乳腺癌细胞的增殖。研究^[23]显示: JAK/STAT 信号通路在 RCC 肿瘤

免疫中的重要调控功能。本研究结果表明: JAK1/STAT3 信号通路可能是 KHSRP 在 CRC 中的发挥致癌作用的关键因素之一, KHSRP 介导的肿瘤生长和转移部分归因于 JAK1/STAT3 信号通路的激活, 在 CRC 的发生和转移过程中发挥重要作用。

综上所述, KHSRP 可以通过 JAK1/STAT3 信号通路促进 CRC 细胞的增殖、侵袭和迁移, 未来 KHSRP 有可能成为 CRC 治疗潜在的分子标志物 and 新的治疗靶点。本研究的局限性包括样本量有限和来源单一, 无法全面分析肿瘤异质性对 KHSRP-JAK1/STAT3 信号通路功能的影响。KHSRP-JAK1/STAT3 信号通路作为治疗靶点的具体作用机制仍需进一步阐明。

利益冲突声明:

所有作者声明不存在利益冲突。

作者贡献声明:

李宏丽参与样本收集和论文撰写, 王梦瑶参与实验操作和数据处理, 刘洋洋参与临床样本病理诊断及处理, 李丽和张卉参与论文审阅。

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