

lncRNA ROR1-AS1通过靶向miR-758-3p调控神经母细胞瘤SK-N-SH细胞的增殖、迁移、侵袭和凋亡

王青松 夏鹰* 陈焕雄

(海口市人民医院, 神经外科, 海口 570000)

摘要 该研究旨在探讨ROR1-AS1对神经母细胞瘤细胞SK-N-SH增殖、迁移、侵袭和凋亡的影响及作用机制。收集67例神经母细胞瘤组织和瘤旁组织, 实时荧光定量PCR(RT-qPCR)检测组织中ROR1-AS1和miR-758-3p的表达情况。转染ROR1-AS1小干扰RNA、miR-758-3p模拟物或共转染ROR1-AS1小干扰RNA与miR-758-3p抑制剂至SK-N-SH细胞, 细胞计数试剂盒-8(CCK-8)法检测细胞增殖, Transwell检测细胞迁移和侵袭, 流式细胞术检测细胞凋亡, 蛋白质印迹法检测CyclinD1、p21、Bcl-2、Bax、MMP-2和MMP-9的蛋白表达情况。双荧光素酶报告基因实验验证ROR1-AS1和miR-758-3p的调控关系。结果显示, 神经母细胞瘤组织中ROR1-AS1的表达明显高于瘤旁组织, 而miR-758-3p表达明显低于瘤旁组织。抑制ROR1-AS1表达或过表达miR-758-3p降低了SK-N-SH细胞活性、迁移数和侵袭数及CyclinD1、Bcl-2、MMP-2和MMP-9蛋白表达水平, 而提高了细胞凋亡率及p21和Bax蛋白表达水平。ROR1-AS1在SK-N-SH细胞中靶向负调控miR-758-3p表达, 干扰miR-758-3p可逆转抑制ROR1-AS1对SK-N-SH细胞增殖、迁移、侵袭和凋亡的影响。这提示抑制ROR1-AS1表达可能通过靶向上调miR-758-3p阻碍SK-N-SH细胞增殖、迁移和侵袭, 并促进细胞凋亡, ROR1-AS1有可能成为神经母细胞瘤治疗的分子靶点。

关键词 神经母细胞瘤; ROR1-AS1; miR-758-3p; 细胞增殖; 迁移; 侵袭; 凋亡

lncRNA ROR1-AS1 Regulates the Proliferation, Migration, Invasion and Apoptosis of Neuroblastoma Cells SK-N-SH by Targeting miR-758-3p

WANG Qingsong, XIA Ying*, CHEN Huanxiong

(Department of Neurosurgery, Haikou People's Hospital, Haikou 570000, China)

Abstract The purpose of this study was to explore the effect and mechanism of ROR1-AS1 on the proliferation, migration, invasion and apoptosis of neuroblastoma cells SK-N-SH. Sixty-seven cases of neuroblastoma tissue and adjacent tissues were collected, and RT-qPCR was used to detect the expression levels of ROR1-AS1 and miR-758-3p in the tissues. ROR1-AS1 small interfering RNA or miR-758-3p mimics were transfected into SK-N-SH cells, or ROR1-AS1 small interfering RNA and miR-758-3p inhibitor were co-transfected into SK-N-SH cells. CCK-8 method was used to detect cell proliferation. Transwell was used to detect cell migration and invasion. Flow cytometry was used to detect cell apoptosis, and Western blot was used to detect the protein expression levels of

收稿日期: 2021-04-22 接受日期: 2021-06-30

国家自然科学基金(批准号: 81760234)资助的课题

*通讯作者。Tel: 13687581177, E-mail: xiaying008@163.com

Received: April 22, 2021 Accepted: June 30, 2021

This work was supported by the National Natural Science Foundation of China (Grant No.81760234)

*Corresponding author. Tel: +86-13687581177, E-mail: xiaying008@163.com

CyclinD1, p21, Bcl-2, Bax, MMP-2 and MMP-9. The dual luciferase reporter gene experiment verified the regulatory relationship between ROR1-AS1 and miR-758-3p. The results showed that the expression of ROR1-AS1 in neuroblastoma tissues was higher than that in adjacent tissues, but the expression of miR-758-3p was lower than that in adjacent tissues. Inhibiting ROR1-AS1 or overexpressing miR-758-3p reduced the activity of SK-N-SH cells, the number of migrating cells, the number of invasion cells, as well as the protein expression of CyclinD1, Bcl-2, MMP-2 and MMP-9 in cells. But inhibiting ROR1-AS1 or overexpressing miR-758-3p increased the apoptosis rate of SK-N-SH cells and the protein expression of p21 and Bax in cells. ROR1-AS1 negatively regulated the expression of miR-758-3p in SK-N-SH cells. Interfering with miR-758-3p reversed the effect of inhibiting ROR1-AS1 on proliferation, migration, invasion and apoptosis of SK-N-SH cells. This suggested that inhibiting ROR1-AS1 might block the proliferation, migration and invasion of SK-N-SH cells and promote cell apoptosis by targeting to up-regulate the expression of miR-758-3p. ROR1-AS1 may become a molecular target for neuroblastoma treatment.

Keywords neuroblastoma; ROR1-AS1; miR-758-3p; cell proliferation; migration; invasion; apoptosis

神经母细胞瘤(neuroblastoma, NB)是常见的儿童颅外恶性肿瘤,极大威胁儿童的生命健康。NB的病因尚未明确,其恶性程度高,早期易转移^[1]。NB的治疗方式主要有手术、化疗、放疗和自体干细胞移植等,但治疗效果不佳,患者预后差,尤其是早期转移患者5年生存率较低^[2]。因此,深入探究NB发生发展的分子机制并寻找有效的治疗靶点具有重要意义。受体酪氨酸激酶样孤儿受体1的反义RNA1(receptor tyrosine kinase-like orphan receptor 1 antisense RNA1, ROR1-AS1)是一种长链非编码RNA(long non-coding RNA, lncRNA),其在鼻咽癌^[3]、结直肠癌^[4]等肿瘤中表达水平升高,上调ROR1-AS1表达可促进肿瘤细胞的恶性表型,进而促进肿瘤的发展进程。但目前,ROR1-AS1在NB中的表达和机制及其对NB细胞恶性生物学行为的影响还未见相关报道。

LncBase Predicted v.2在线软件预测显示,ROR1-AS1可能靶向结合微小RNA-758-3p(miR-758-3p)。研究显示,miR-758-3p在乳腺癌^[5]、甲状腺乳头状癌^[6]和胃癌^[7]等肿瘤中表达下调,过表达miR-758-3p可抑制肿瘤细胞的恶性生物学行为。但目前,miR-758-3p对NB细胞恶性生物学行为的影响也还未知。本研究首先检测了ROR1-AS1和miR-758-3p在NB组织中的表达情况,并以NB细胞SK-N-SH为研究对象,观察抑制ROR1-AS1对SK-N-SH细胞增殖、迁移、侵袭和凋亡的影响及ROR1-AS1能否靶向调控miR-758-3p表达发挥作用。

1 资料与方法

1.1 临床资料

收集2016年2月至2018年12月于海口市人民医院就诊的67例神经母细胞瘤患者的瘤组织及瘤旁组织。男性42例,女性25例,平均年龄(7.16±3.21)岁。所有患者均经病理学检查确诊,术前未进行化疗、放疗等治疗。瘤旁组织距离瘤组织>2 cm,病理证实无肿瘤浸润等病变。本研究经海口市人民医院医学伦理委员会批准,且经患者或家属知情同意。

1.2 细胞和试剂

神经母细胞瘤细胞SK-N-SH购自中国科学院上海细胞库;RPMI 1640培养基、细胞计数试剂盒-8(cell counting kit-8, CCK-8)、二喹啉甲酸(bicinchoninic acid, BCA)蛋白检测试剂盒和膜联蛋白V(Annexin V)-异硫氰酸荧光素(fluorescein isothiocyanate, FITC)/碘化丙啶(propidium iodide, PI)细胞凋亡试剂盒均购自北京索莱宝科技有限公司;逆转录试剂盒和PCR试剂盒购自大连宝生物工程有限公司;胎牛血清(fetal bovine serum, FBS)购自美国Hycolne公司;Trizol试剂和Lipofectamine™ 2000试剂盒购自美国Invitrogen公司;PCR引物、ROR1-AS1小干扰RNA(si-ROR1-AS1)及乱序无意义阴性序列(si-NC)、miR-758-3p模拟物(mimcs)及模拟对照序列(miR-NC)、ROR1-AS1过表达载体(pcDNA-ROR1-AS1)、空载体(pcDNA)、miR-758-3p抑制剂(anti-miR-758-3p)及抑制剂阴性序列(anti-miR-NC)均购自上海吉玛制药技术有限公司;双荧光素酶活性检测试剂盒购自美国Promega公司;细胞周期蛋白D1

(CyclinD1)、p21、基质金属蛋白酶(matrix metalloproteinases, MMP)-2、MMP-9、B淋巴细胞瘤-2(B-cell lymphoma-2, Bcl-2)、B淋巴细胞瘤-2相关蛋白(Bcl-2-associated X protein, Bax)和甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase, GAPDH)抗体均购自美国Santa Cruz公司。

1.3 实验方法

1.3.1 RT-qPCR检测ROR1-AS1和miR-758-3p表达 用Trizol试剂提取组织中RNA, 逆转录为cDNA后, 行PCR扩增。PCR反应体系共25 μ L: 0.2 μ L Taq 酶(5 U/ μ L, TaKaRa)、2.5 μ L 10 $\times$ PCR缓冲液、1.6 μ L脱氧核糖核苷三磷酸(dNTP)、0.5 μ L上游引物(10 μ mol/L)、0.5 μ L下游引物(10 μ mol/L)、1.0 μ L cDNA、18.7 μ L 双蒸水。扩增程序为: 95 $^{\circ}$ C预变性5 min; 95 $^{\circ}$ C变性10 s, 60 $^{\circ}$ C退火30 s, 72 $^{\circ}$ C延伸30 s, 共35个循环。引物序列: ROR1-AS1上游5'-AGC AGG CAT TTT GGA GGA AA-3', 下游5'-TCG TGT TAC CCC AAG AGC TT-3'; miR-758-3p上游5'-GTG CCA ACG CTG GGT GC-3', 下游5'-CGA ACG TGT TCC GAC GGT C-3'; GAPDH上游5'-CTC CTG CAC CAC CAA CTG CT-3', 下游5'-GGG CCA TCC ACA GTC TTC TG-3'; U6上游5'-ATA GGC GCG CGA AAA GCC-3', 下游5'-CGG GCA ACT GAC GCG AGG-3'。2^{- $\Delta\Delta C_t$} 法计算ROR1-AS1相对GAPDH、miR-758-3p相对U6的表达量。

1.3.2 细胞培养 SK-N-SH细胞用含10%胎牛血清(fetal bovine serum, FBS)的RPMI 1640培养基培养, 当细胞融合至80%时, 0.25%胰蛋白酶消化, 以1:3比例传代培养。将对数期SK-N-SH细胞接种于6孔板(1.0×10^5 个/孔)中, 采用LipofectamineTM 2000脂质体法, 分别转染si-ROR1-AS1(si-ROR1-AS1组)、si-NC(si-NC组)、pcDNA-ROR1-AS1(pcDNA-ROR1-AS1组)、pcDNA(pcDNA组)、miR-758-3p mimics(miR-758-3p组)、miR-NC(miR-NC组)、共转染si-ROR1-AS1与anti-miR-758-3p(si-ROR1-AS1+anti-miR-758-3p组)、si-ROR1-AS1与anti-miR-NC(si-ROR1-AS1+anti-miR-NC组)。转染6 h后, 更换培养基。再培养24 h, 收集细胞备用。

1.3.3 CCK-8法检测细胞增殖 si-ROR1-AS1组、si-NC组、miR-758-3p组、miR-NC组、si-ROR1-AS1+anti-miR-758-3p组和si-ROR1-AS1+anti-miR-NC组细胞接种于96孔板(1.0×10^4 个/孔)中, 分别培养24、48和72 h后, 加入10 μ L CCK-8。待其孵育1.5 h后, 酶标仪于450 nm波长处测光密度(D)值, 实验重复3次。

1.3.4 Transwell检测细胞迁移和侵袭 将si-ROR1-AS1组、si-NC组、miR-758-3p组、miR-NC组、si-ROR1-AS1+anti-miR-758-3p组和si-ROR1-AS1+anti-miR-NC组细胞调整密度为 5×10^4 个/mL。迁移实验: Transwell上室加100 μ L细胞悬液, 下室加500 μ L培养基。培养48 h后, 弃培养基, 经多聚甲醛固定、结晶紫染色后, 显微镜观察。随机取5个视野, 计数。侵袭实验: 除了预先在Transwell上室铺Matrigel基质胶外, 其他步骤同迁移实验。

1.3.5 流式细胞仪检测细胞凋亡 si-ROR1-AS1组、si-NC组、miR-758-3p组、miR-NC组、si-ROR1-AS1+anti-miR-758-3p组和si-ROR1-AS1+anti-miR-NC组细胞接种于24孔板(2.5×10^4 个/孔)中, 培养48 h后, 收集细胞。参照Annexin V-FITC/PI试剂盒说明书, 用流式细胞仪检测细胞凋亡。

1.3.6 蛋白质印迹法检测蛋白表达 细胞接种及处理时间同1.3.5。RIPA试剂提取总蛋白, BCA法测蛋白浓度, 行10% SDS-PAGE电泳。将分离蛋白湿转至PVDF膜, 于5%脱脂奶粉中封闭室温2 h。分别用CyclinD1(1:500)、p21(1:500)、Bcl-2(1:1 000)、Bax(1:1 000)、MMP-2(1:500)、MMP-9(1:500)和GAPDH(1:1 000)一抗4 $^{\circ}$ C孵育过夜。再用山羊抗兔二抗(1:2 000)37 $^{\circ}$ C孵育1 h。加化学发光试剂避光显影, 曝光拍照, ImageJ软件分析目的蛋白相对GAPDH的表达量。

1.3.7 双荧光素酶报告基因实验 利用PCR技术扩增含miR-758-3p结合位点的ROR1-AS1核苷酸序列, 并将其克隆至pmirGLO载体, 构建ROR1-AS1野生型荧光素酶载体(WT-ROR1-AS1)。同时, 利用基因突变技术将结合位点突变后, 克隆至pmirGLO载体, 构建ROR1-AS1突变型荧光素酶载体(MUT-ROR1-AS1), 该过程由上海吉玛制药技术有限公司完成。将处于对数生长期的SK-N-SH细胞以 1×10^5 个/孔的密度接种于6孔板, 采用LipofectamineTM 2000脂质体法, 分别共转染WT-ROR1-AS1与miR-758-3p mimics或miR-NC、MUT-ROR1-AS1与miR-758-3p mimics或miR-NC。转染6 h后, 更换培养基。再培养24 h, 收集细胞并裂解。3 500 r/min离心5 min后, 取上清, 利用双荧光素酶活性检测试剂盒检测荧光素酶活性, 结果以荧光强度比值表示。

1.4 统计学分析

SPSS 22.0软件进行统计学分析。计量资料以

均数±标准差($\bar{x}\pm s$)表示。两组间比较用独立样本 t 检验;多组间比较用单因素方差分析,进一步两两比较用SNK- q 检验。以 $P<0.05$ 表示差异有统计学意义。

2 结果

2.1 ROR1-AS1和miR-758-3p在神经母细胞瘤组织中的表达

神经母细胞瘤组织中ROR1-AS1的表达高于瘤旁组织($P<0.05$),而miR-758-3p的表达水平低于瘤旁组织($P<0.05$)(表1)。

2.2 抑制ROR1-AS1对SK-N-SH细胞增殖的影响

转染si-ROR1-AS1、si-NC的SK-N-SH细胞中ROR1-AS1表达量分别为 0.47 ± 0.04 、 1.00 ± 0.07 ,两者比较差异具有统计学意义($t=19.722$, $P<0.05$),表明SK-N-SH细胞中ROR1-AS1的表达受到抑制。si-ROR1-AS1组SK-N-SH细胞活性及CyclinD1蛋白水平低于si-NC组($P<0.05$),而p21蛋白水平高于si-NC组($P<0.05$)(图1和表2)。

2.3 抑制ROR1-AS1对SK-N-SH细胞迁移和侵袭的影响

si-ROR1-AS1组SK-N-SH细胞迁移数和侵袭数

表1 ROR1-AS1和miR-758-3p在神经母细胞瘤组织中的表达

Table 1 The expression of ROR1-AS1 and miR-758-3p in neuroblastoma tissues

组别 Group	ROR1-AS1	miR-758-3p
Adjacent tissue	1.00 ± 0.25	1.00 ± 0.22
Neuroblastoma tissue	$2.40\pm 0.32^*$	$0.55\pm 0.12^*$
t	28.220	14.698
P	0.000	0.000

$\bar{x}\pm s$; $n=67$; $*P<0.05$, 与瘤旁组织比较。

$\bar{x}\pm s$; $n=67$; $*P<0.05$ compared with adjacent tissues.

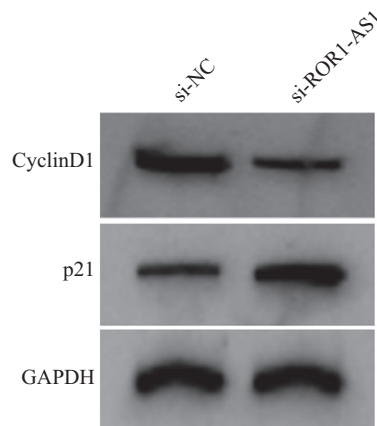


图1 抑制ROR1-AS1对SK-N-SH细胞中CyclinD1和p21蛋白表达的影响

Fig.1 The effect of inhibiting ROR1-AS1 on the protein expression of CyclinD1 and p21 in SK-N-SH cells

表2 抑制ROR1-AS1对SK-N-SH细胞增殖的影响

Table 2 The effect of inhibiting ROR1-AS1 on the proliferation of SK-N-SH cells

组别 Group	D_{450}			CyclinD1	p21
	24 h	48 h	72 h		
si-NC	0.38 ± 0.03	0.73 ± 0.06	1.14 ± 0.11	0.49 ± 0.04	0.23 ± 0.02
si-ROR1-AS1	$0.21\pm 0.02^*$	$0.37\pm 0.03^*$	$0.55\pm 0.05^*$	$0.14\pm 0.02^*$	$0.59\pm 0.04^*$
t	14.145	16.100	14.649	23.479	24.150
P	0.000	0.000	0.000	0.000	0.000

$\bar{x}\pm s$; $n=9$; $*P<0.05$, 与si-NC组比较。

$\bar{x}\pm s$; $n=9$; $*P<0.05$ compared with si-NC group.

低于si-NC组($P<0.05$), 细胞中MMP-2和MMP-9蛋白水平低于si-NC组($P<0.05$)(图2和表3)。

2.4 抑制ROR1-AS1对SK-N-SH细胞凋亡的影响

si-ROR1-AS1组SK-N-SH细胞凋亡率、Bax蛋白水平高于si-NC组($P<0.05$), 而Bcl-2蛋白水平低于si-NC组($P<0.05$)(图3和表4)。

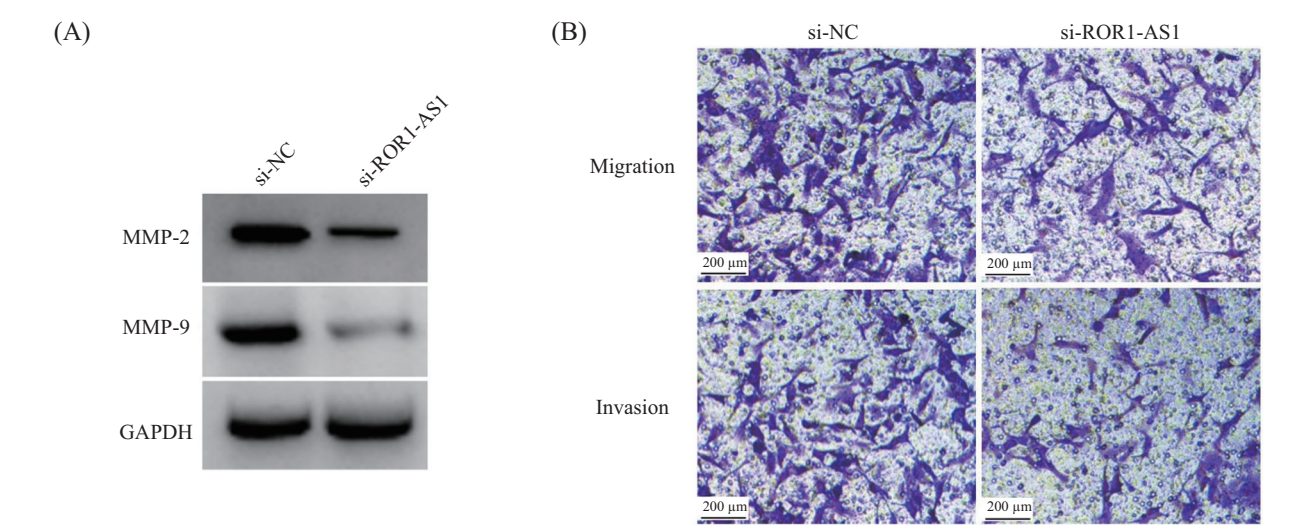
2.5 ROR1-AS1靶向负调控miR-758-3p的表达

LncBase Predicted v.2在线软件预测显示的ROR1-AS1与miR-758-3p的结合位点见图4。与共转染WT-ROR1-AS1与miR-NC的细胞比较, 共转染WT-ROR1-AS1与miR-758-3p mimics的细胞荧光素酶活性降低(0.63 ± 0.05 比 1.05 ± 0.04 , $t=19.678$, $P<0.05$)。与共转染MUT-ROR1-AS1与miR-NC的细胞比较, 共转染MUT-ROR1-AS1与miR-758-3p mimics的细胞荧光素酶活性无显著变化(1.03 ± 0.07

比 1.06 ± 0.09 , $t=0.789$, $P=0.441$)。pcDNA-ROR1-AS1组miR-758-3p表达水平低于pcDNA组(0.58 ± 0.06 比 1.00 ± 0.06 , $t=14.849$, $P<0.05$), si-ROR1-AS1组miR-758-3p表达水平显著高于si-NC组(2.77 ± 0.23 比 0.99 ± 0.05 , $t=22.687$, $P<0.05$)。

2.6 过表达miR-758-3p对SK-N-SH细胞增殖、迁移、侵袭和凋亡的影响

转染miR-758-3p mimics、miR-NC的SK-N-SH细胞中miR-758-3p表达量分别为 2.54 ± 0.22 、 1.00 ± 0.08 , 两者比较差异具有统计学意义($t=19.736$, $P<0.05$), 表明miR-758-3pSK-N-SH细胞中miR-758-3p过表达。miR-758-3p组SK-N-SH细胞活性、迁移数和侵袭数及细胞中CyclinD1、MMP-2、MMP-9和Bcl-2蛋白水平均低于miR-NC组($P<0.05$), 而细胞凋亡率和细胞中p21、Bax的蛋白水平均高于miR-NC组($P<0.05$)(图



A: 抑制ROR1-AS1对SK-N-SH细胞中MMP-2和MMP-9蛋白表达的影响; B: 抑制ROR1-AS1对SK-N-SH细胞迁移和侵袭的影响。
A: the effect of inhibiting ROR1-AS1 on the protein expression of MMP-2 and MMP-9 in SK-N-SH cells; B: the effect of inhibiting ROR1-AS1 on the migration and invasion of SK-N-SH cells.

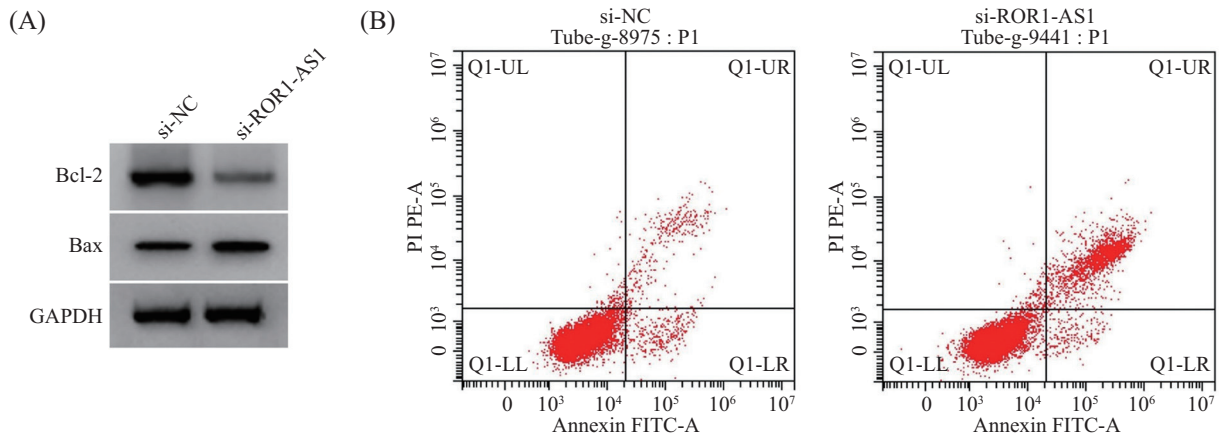
图2 抑制ROR1-AS1对SK-N-SH细胞中MMP-2和MMP-9蛋白表达及细胞迁移和侵袭的影响
Fig.2 The effects of inhibiting ROR1-AS1 on the protein expression of MMP-2 and MMP-9 and migration and invasion of SK-N-SH cells

表3 抑制ROR1-AS1对SK-N-SH细胞迁移和侵袭的影响

Table 3 The effects of inhibiting ROR1-AS1 on the migration and invasion of SK-N-SH cells

组别 Group	迁移细胞数 Number of migration cells	侵袭细胞数 Number of invasion cells	MMP-2	MMP-9
si-NC	102.13±8.89	86.09±7.98	0.79±0.07	0.63±0.05
si-ROR1-AS1	57.59±5.08*	44.69±3.75*	0.32±0.03*	0.24±0.03*
t	13.050	14.086	18.514	20.065
P	0.000	0.000	0.000	0.000

$\bar{x}\pm s$; $n=9$; * $P<0.05$, 与si-NC组比较。
 $\bar{x}\pm s$; $n=9$; * $P<0.05$ compared with si-NC group.



A: 抑制ROR1-AS1对SK-N-SH细胞中Bcl-2和Bax蛋白表达的影响; B: 抑制ROR1-AS1对SK-N-SH细胞凋亡的影响。

A: the effect of inhibiting ROR1-AS1 on the protein expression of Bcl-2 and Bax in SK-N-SH cells; B: the effect of inhibiting ROR1-AS1 on apoptosis of SK-N-SH cells.

图3 抑制ROR1-AS1对SK-N-SH细胞中Bcl-2和Bax蛋白表达及细胞凋亡的影响

Fig.3 The effects of inhibiting ROR1-AS1 on the protein expression of Bcl-2 and Bax and apoptosis of SK-N-SH cells

表4 抑制ROR1-AS1对SK-N-SH细胞凋亡及Bcl-2、Bax蛋白表达的影响

Table 4 The effects of inhibiting ROR1-AS1 on the apoptosis and the protein expression of Bcl-2 and Bax in SK-N-SH cells

组别 Group	凋亡率/% Apoptosis rate /%	Bcl-2	Bax
si-NC	8.36±0.69	0.84±0.07	0.25±0.02
si-ROR1-AS1	23.34±2.33*	0.38±0.04*	0.62±0.05*
<i>t</i>	18.494	17.117	20.612
<i>P</i>	0.000	0.000	0.000

$\bar{x} \pm s$; $n=9$; * $P<0.05$, 与si-NC组比较。

$\bar{x} \pm s$; $n=9$; * $P<0.05$ compared with si-NC group.

WT-ROR1-AS1 5' ccggagaUGUAAGUUGUCACAAg 3'
 miR-758-3p 3' aucACCUGGUCCAGUGUUu 5'
 MUT-ROR1-AS1 5' ccggagaCUUGAGUUACUGACGg 3'

红色表示突变位点。

Red indicates the site of mutation.

图4 ROR1-AS1与miR-758-3p互补的核苷酸序列

Fig.4 The complementary nucleotide sequence of ROR1-AS1 and miR-758-3p

5、表5、图6、表6)。

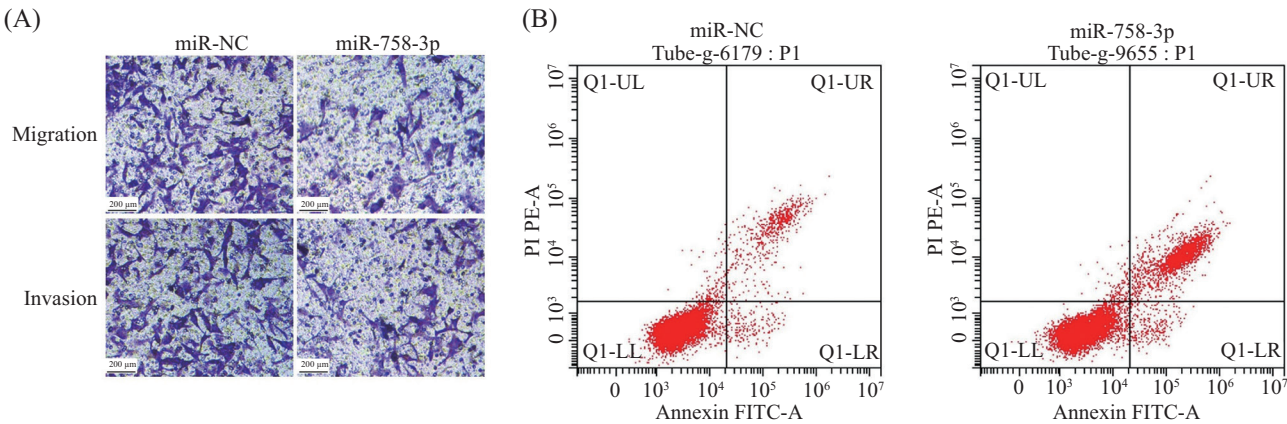
2.7 干扰miR-758-3p逆转抑制ROR1-AS1对SK-N-SH细胞增殖、迁移、侵袭和凋亡的作用

共转染 si-ROR1-AS1与 anti-miR-758-3p、si-ROR1-AS1与 anti-miR-NC的SK-N-SH细胞中miR-758-3p表达量分别为 0.49 ± 0.04 、 1.00 ± 0.06 , 两者比较差异具有统计学意义($t=21.217$, $P<0.05$), 表明SK-N-SH细胞中miR-758-3p表达受到抑制。si-ROR1-AS1+anti-miR-758-3p细胞活性、迁移数和侵袭数及细胞中CyclinD1、MMP-2、MMP-9和Bcl-2蛋白

水平均高于 si-ROR1-AS1+anti-miR-NC组 ($P<0.05$), 而细胞凋亡率降低、p21和Bax蛋白水平均低于 si-ROR1-AS1+anti-miR-NC组 ($P<0.05$) (图7、表7、图8、表8)。

3 讨论

ROR1是ROR家族受体酪氨酸激酶成员之一, 在乳腺癌^[8]、非小细胞肺癌^[9]和结直肠癌^[10]等肿瘤中广泛表达, ROR1表达的上调促进肿瘤的发生发展。ROR1-AS1是ROR1的反义RNA, 属于lncRNA。



A: 过表达miR-758-3p对SK-N-SH细胞迁移和侵袭的影响; B: 过表达miR-758-3p对SK-N-SH细胞凋亡的影响。
A: the effects of overexpression of miR-758-3p on the migration and invasion of SK-N-SH cells; B: the effect of overexpression of miR-758-3p on apoptosis of SK-N-SH cells.

图5 过表达miR-758-3p对SK-N-SH细胞迁移、侵袭和凋亡的影响
Fig.5 The effects of overexpression of miR-758-3p on the migration, invasion and apoptosis of SK-N-SH cells

表5 过表达miR-758-3p对SK-N-SH细胞增殖、迁移、侵袭和凋亡的影响
Table 5 The effects of overexpression of miR-758-3p on the proliferation, migration, invasion and apoptosis of SK-N-SH cells

组别 Group	D_{450}			迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invasion cells	凋亡率/% Apoptosis rate /%
	24 h	48 h	72 h			
miR-NC	0.39±0.03	0.75±0.06	1.12±0.08	105.12±9.41	84.96±7.31	7.93±0.64
miR-758-3p	0.29±0.03*	0.46±0.04*	0.64±0.05*	64.37±4.98*	53.90±4.56*	20.22±1.99*
<i>t</i>	7.071	12.065	15.264	11.483	10.815	17.638
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000

$\bar{x} \pm s$; $n=9$; * $P<0.05$, 与miR-NC组比较。
 $\bar{x} \pm s$; $n=9$; * $P<0.05$ compared with miR-NC group.

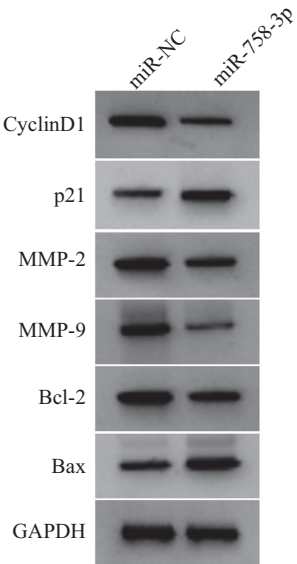


图6 过表达miR-758-3p对SK-N-SH细胞中增殖、迁移、侵袭和凋亡相关蛋白表达的影响
Fig.6 The effects of overexpression of miR-758-3p on the expression of proteins related to proliferation, migration, invasion and apoptosis in SK-N-SH cells

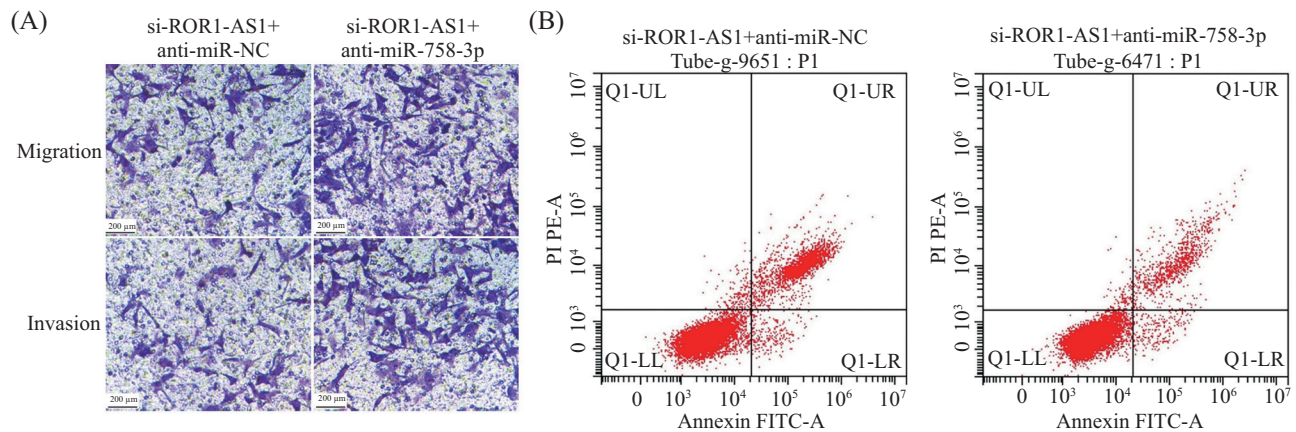
表6 过表达miR-758-3p对SK-N-SH细胞中增殖、迁移、侵袭和凋亡相关蛋白表达的影响

Table 6 The effects of overexpression of miR-758-3p on the expression of proteins related to proliferation, migration, invasion and apoptosis in SK-N-SH cells

组别 Group	CyclinD1	p21	MMP-2	MMP-9	Bcl-2	Bax
miR-NC	0.48±0.03	0.24±0.03	0.77±0.06	0.62±0.06	0.85±0.06	0.22±0.03
miR-758-3p	0.20±0.02*	0.51±0.05*	0.37±0.03*	0.29±0.03*	0.41±0.04*	0.57±0.04*
<i>t</i>	23.297	13.891	17.889	14.758	18.305	21.000
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000

$\bar{x} \pm s$; $n=9$; * $P<0.05$, 与miR-NC组比较。

$\bar{x} \pm s$; $n=9$; * $P<0.05$ compared with miR-NC group.



A: 同时抑制miR-758-3p和ROR1-AS1对SK-N-SH细胞迁移和侵袭的影响; B: 同时抑制miR-758-3p和ROR1-AS1对SK-N-SH细胞凋亡的影响。

A: the effect of inhibiting miR-758-3p and ROR1-AS1 on the migration and invasion of SK-N-SH cells; B: the effect of inhibiting miR-758-3p and ROR1-AS1 on apoptosis of SK-N-SH cells.

图7 同时抑制miR-758-3p和ROR1-AS1对SK-N-SH细胞迁移、侵袭和凋亡的影响

Fig.7 The effects of inhibiting miR-758-3p and ROR1-AS1 on the migration, invasion and apoptosis of SK-N-SH cells

表7 同时抑制miR-758-3p和ROR1-AS1对SK-N-SH细胞增殖、迁移、侵袭和凋亡的影响

Table 7 The effects of inhibiting miR-758-3p and ROR1-AS1 on the proliferation, migration, invasion and apoptosis of SK-N-SH cells

组别 Group	miR-758-3p	D_{450}			迁移细胞数 Number of migrating cells	侵袭细胞数 Number of invasion cells	凋亡率/% Apoptosis rate /%
		24 h	48 h	72 h			
si-ROR1-AS1+anti-miR-NC	1.00±0.06	0.20±0.02	0.36±0.03	0.52±0.05	53.26±4.89	46.17±4.89	24.23±2.53
si-ROR1-AS1+anti-miR-758-3p	0.49±0.04*	0.31±0.03*	0.62±0.06*	1.03±0.07*	89.96±6.26*	78.43±5.08*	12.60±1.16*
<i>t</i>	21.217	9.153	11.628	17.786	13.860	13.725	12.536
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000

$\bar{x} \pm s$; $n=9$; * $P<0.05$, 与si-ROR1-AS1+anti-miR-NC组比较。

$\bar{x} \pm s$; $n=9$; * $P<0.05$ compared with si-ROR1-AS1+anti-miR-NC group.

ROR1-AS1在多种肿瘤中也异常表达, 与肿瘤的发生发展密切相关。CHEN等^[11]研究显示, ROR1-AS1在膀胱癌组织表达升高, 且ROR1-AS1表达与高组织学分级、晚期肿瘤分期、淋巴结转移和患者预

后密切相关, 敲低ROR1-AS1可显著抑制膀胱癌T24和5637细胞生长和迁移, ROR1-AS1可作为膀胱癌的预后生物标志物和治疗靶标。WANG等^[12]研究显示, 结肠癌患者癌组织中ROR1-AS1表达升高, 抑制

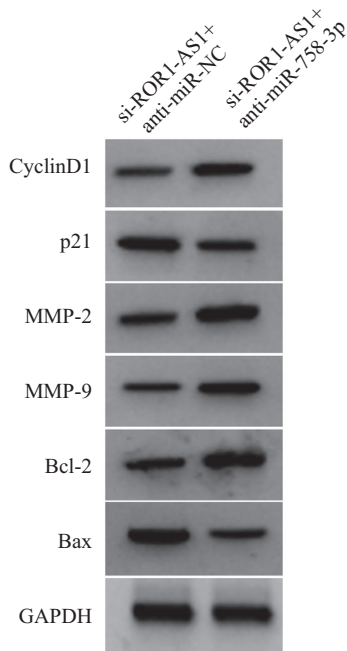


图8 同时抑制miR-758-3p和ROR1-AS1对SK-N-SH细胞中增殖、迁移侵袭和凋亡相关蛋白表达的影响
Fig.8 The effects of inhibiting miR-758-3p and ROR1-AS1 on the expression of proteins related to proliferation, migration, invasion and apoptosis in SK-N-SH cells

表8 同时抑制miR-758-3p和ROR1-AS1后SK-N-SH细胞中增殖、迁移、侵袭和凋亡相关蛋白表达水平
Table 8 The effects of inhibiting miR-758-3p and ROR1-AS1 on the expression of protein related to proliferation, migration, invasion and apoptosis in SK-N-SH cells

组别 Group	CyclinD1	p21	MMP-2	MMP-9	Bcl-2	Bax
si-ROR1-AS1+anti-miR-NC	0.13±0.02	0.60±0.05	0.31±0.02	0.23±0.02	0.37±0.03	0.64±0.04
si-ROR1-AS1+anti-miR-758-3p	0.38±0.03*	0.29±0.02*	0.68±0.06*	0.52±0.04*	0.73±0.07*	0.32±0.03*
<i>t</i>	20.801	17.270	17.551	19.454	14.181	19.200
<i>P</i>	0.000	0.000	0.000	0.000	0.000	0.000

$\bar{x}\pm s$; $n=9$; * $P<0.05$, 与si-ROR1-AS1+anti-miR-NC组比较。
 $\bar{x}\pm s$; $n=9$; * $P<0.05$ compared with si-ROR1-AS1+anti-miR-NC group.

ROR1-AS1表达可降低结肠癌细胞的迁移和侵袭能力, 为结肠癌提供了潜在的治疗靶点。但是, ROR1-AS1对NB发生发展的影响和作用机制还未知。

本研究显示, NB组织中ROR1-AS1呈高表达, 抑制ROR1-AS1可有效削弱SK-N-SH细胞活性及迁移和侵袭能力, 诱导细胞凋亡, 提示ROR1-AS1可作为促癌基因参与NB的发生发展, 其也有可能成为NB治疗的分子靶点。CyclinD1可促进细胞周期由G₁期向S期转变, 促进细胞增殖^[13]。p21基因是一种抑癌基因, 其通过调控细胞周期进程参与细胞的生长和凋亡^[14]。Bax和Bcl-2参与调控细胞凋亡, Bax表达量升高诱导细胞凋亡, 而Bcl-2表达量升高则抑制细胞凋

亡^[15]。MMP-2和MMP-9属于基质金属蛋白酶家族成员, 可降解细胞外基质, 对肿瘤细胞迁移和侵袭起促进作用^[16-17]。本研究显示, 抑制ROR1-AS1表达促进了SK-N-SH细胞中p21和Bax的蛋白表达, 而降低了CyclinD1、Bcl-2、MMP-2和MMP-9的蛋白表达, 提示ROR1-AS1可能通过直接或间接的方式调控与细胞增殖、迁移、侵袭及凋亡相关的蛋白表达来影响抑制SK-N-SH细胞的恶性表型。

为了进一步探究ROR1-AS1影响SK-N-SH细胞恶性表型的分子机制, 本研究证实了ROR1-AS1可结合miR-758-3p, 且抑制ROR1-AS1促进了SK-N-SH细胞中miR-758-3p表达, 而过表达ROR1-AS1则抑制了miR-

758-3p表达,进一步说明ROR1-AS1负调控miR-758-3p,这与NB组织中ROR1-AS1表达升高而miR-758-3p表达降低的结果一致。研究显示,miR-758-3p可通过靶向抑制MDM2和mTOR的表达降低肝癌细胞增殖、迁移和侵袭,是肝癌治疗的潜在分子靶点^[18]; lncRNA DANCER可靶向结合并负调控miR-758-3p表达促进非小细胞肺癌细胞增殖、迁移和侵袭, DANCER/miR-758-3p轴可能是治疗非小细胞肺癌的潜在靶标^[19]。本研究显示,过表达miR-758-3p减弱了SK-N-SH细胞的增殖、迁移和侵袭能力,并加剧了SK-N-SH细胞凋亡,提示miR-758-3p在NB中发挥抑癌基因作用,过表达miR-758-3p可抑制NB的发展进程;此外,干扰miR-758-3p会减弱抑制ROR1-AS1对SK-N-SH细胞恶性表型的影响,与相关报道结果一致^[20],提示抑制ROR1-AS1通过靶向上调miR-758-3p来降低SK-N-SH细胞的恶性表型。

综上,NB组织中ROR1-AS1表达量升高,而miR-758-3p表达量降低;抑制ROR1-AS1可有效降低NB细胞的增殖、迁移和侵袭,同时抑制ROR1-AS1促进了NB细胞凋亡。ROR1-AS1可能通过靶向负调控miR-758-3p影响NB细胞的恶性表型,ROR1-AS1/miR-758-3p轴可能为NB的治疗提供了潜在的分子靶点。

参考文献 (References)

- [1] 张恒瑜, 张虹, 姜忠, 等. 长链非编码RNA牛磺酸上调基因1对人神经母细胞瘤侵袭和转移能力的影响[J]. 中华实验外科杂志(ZHANG H Y, ZHANG H, JIANG Z, et al. Effects of taurine upregulated gene 1 on migration and invasion of human neuroblastoma cells [J]. Chin J Exp Surg, 2018, 35(12): 2284-5.
- [2] 彭妍, 邢思宁, 唐瑚应, 等. 抑制葡萄糖转运蛋白1表达可降低神经母细胞瘤细胞的增殖、侵袭和迁移能力[J]. 细胞与分子免疫学杂志(PENG Y, XING S N, TANG H Y, et al. Inhibiting the expression of glucose transporter 1 reduces the proliferation, invasion and migration of neuroblastoma cells [J]. Chinese Journal of Cellular and Molecular Immunology, 2018, 34(11): 38-43.
- [3] HOU J, YAN J, REN X Y, et al. Long noncoding RNA ROR1-AS1 induces tumor metastasis and epithelial-mesenchymal transition by sponging miR-375 in nasopharyngeal carcinoma [J]. Eur Rev Med Pharmacol Sci, 2020, 24(1): 174-80.
- [4] WANG W, ZHENG W H, ZHANG L, et al. Long non-coding RNA ROR1-AS1 induces tumorigenesis of colorectal cancer by affecting Wnt/ β -catenin signaling pathway [J]. Biosci Rep, 2019, 39(11): 1-9.
- [5] WU X F, CHEN B, SHI H B, et al. miR-758-3p suppresses human bladder cancer cell proliferation, migration and invasion by targeting NOTCH2 [J]. Exp Ther Med, 2019, 17(5): 4273-8.
- [6] CHEN J P, XU Z Y, YU C S, et al. MiR-758-3p regulates papillary thyroid cancer cell proliferation and migration by targeting TAB1 [J]. Pharmazie, 2019, 74(4): 235-8.
- [7] GUO J X, ZHANG Z C, PAN L J, et al. Identification of miR-758-3p as potential modulator of CBX5 expression in gastric cancer [J]. Technol Cancer Res Treat, 2018, 17(5): 1-7.
- [8] FULTANG N, ILLENDULA A, LIN J H, et al. ROR1 regulates chemoresistance in breast cancer via modulation of drug efflux pump ABCB1 [J]. Sci Rep, 2020, 10(1): 1821-32.
- [9] WANG H L, LIU Y C, LONG M P, et al. Blocking ROR1 enhances the roles of erlotinib in lung adenocarcinoma cell lines [J]. Oncol Lett, 2019, 18(3): 2977-84.
- [10] ZHANG R J, WANG Z J, YU Q Y, et al. Atractylenolide II reverses the influence of lncRNA XIST/miR-30a-5p/ROR1 axis on chemo-resistance of colorectal cancer cells [J]. J Cell Mol Med, 2019, 23(5): 3151-65.
- [11] CHEN Q K, FU L M. Upregulation of long non-coding RNA ROR1-AS1 promotes cell growth and migration in bladder cancer by regulation of miR-504 [J]. PLoS One, 2020, 15(1): e0227568-79.
- [12] WANG F Z, ZHANG M Q, ZHANG L, et al. Long non-coding RNA ROR1-AS1 enhances colorectal cancer metastasis by targeting miR-375 [J]. Eur Rev Med Pharmacol Sci, 2019, 23(16): 6899-905.
- [13] CHEN B, HU Z M, CHEN B, et al. Effects and mechanism of Lanthanum Citrate on the proliferation and apoptosis of hepatocellular carcinoma cell line SMMC-7721 [J]. Turk J Gastroenterol, 2020, 31(3): 264-71.
- [14] BAE J H, JEONG H J, KIM H, et al. ZNF746/PARIS overexpression induces cellular senescence through FoxO1/p21 axis activation in myoblasts [J]. Cell Death Dis, 2020, 11(5): 359-71.
- [15] PARK C, LEE H, HWANGBO H, et al. Ethanol extract of hizikia fusiforme induces apoptosis in B16F10 mouse melanoma cells through ROS-dependent inhibition of the PI3K/Akt signaling pathway [J]. Asian Pac J Cancer Prev, 2020, 21(5): 1275-82.
- [16] CAI F Z, GUO S X, HUANG S H, et al. Rubimallin suppresses proliferation, migration and invasion of prostate cancer cells via the Notch-1/MMP signaling pathway [J]. Cell Mol Biol, 2020, 66(2): 130-4.
- [17] LI H Q, ZHAO J, JIA X M, et al. miR-21 promotes growth, invasion and migration of lung cancer cells by AKT/P-AKT/cleaved-caspase 3/MMP-2/MMP-9 signaling pathway [J]. Int J Clin Exp Pathol, 2020, 13(4): 692-700.
- [18] JIANG D, CHO W, LI Z H, et al. MiR-758-3p suppresses proliferation, migration and invasion of hepatocellular carcinoma cells via targeting MDM2 and mTOR [J]. Biomed Pharmacother, 2017, 96: 535-44.
- [19] WANG S, JIANG M. The long non-coding RNA-DANCER exerts oncogenic functions in non-small cell lung cancer via miR-758-3p [J]. Biomed Pharmacother, 2018, 103(1): 94-100.
- [20] HU X W, LI Y, KONG D, et al. Long noncoding RNA CASC9 promotes LIN7A expression via miR-758-3p to facilitate the malignancy of ovarian cancer [J]. J Cell Physiol, 2019, 234(7): 10800-8.