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·基础研究·

lncRNA HAGLR对骨关节炎软骨细胞焦亡的影响及机制研究

孟晓源[1],马 乐[2],宁 凯[2],安梦宇[1] (1.新疆维吾尔自治区人民医院骨科中心关节运动病区,新疆 乌鲁木齐 830001;2.新疆维吾尔自治区人民医院麻醉手术中心,新疆 乌鲁木齐 830001)

[摘 要] **目的** 探讨lncRNA HAGLR对骨关节炎(OA)软骨细胞焦亡的影响及机制。**方法** 动物实验:将34只SD大鼠随机分为Saline组(左侧膝关节腔注射50 μL生理盐水)和OA模型组(左侧膝关节腔注射50 μL含5 mg碘乙酸单钠的生理盐水),每组17只。造模2周后,采用HE染色、番红O/固绿染色评估软骨组织损伤程度,qRT-PCR检测lncRNA HAGLR、miR-106b-5p表达,Western blot检测硫氧还蛋白互作蛋白(TXNIP)、NOD样受体热蛋白结构域相关蛋白3(NLRP3)、Caspase-1、消皮素D-N端(GSDMD-N)蛋白表达,ELISA测定血清白细胞介素-1β(IL-1β)、白细胞介素-18(IL-18)水平。体外实验:将人软骨细胞系C28/I2分为对照组(常规培养)、OA组(添加IL-1β 20 ng/mL刺激48 h构建体外OA模型)、si-NC组(转染si-NC 24 h后建模)、si-HAGLR组(转染si-HAGLR后建模)、mimic-NC组(转染mimic-NC后建模)、miR-106b-5p-mimic组(转染miR-106b-5p-mimic后建模)、si-HAGLR+miR-106b-5p-mimic组(共转染si-HAGLR和miR-106b-5p-mimic后建模)。采用CCK-8法检测细胞增殖活力,qRT-PCR检测lncRNA HAGLR、miR-106b-5p表达,免疫荧光染色和ELISA检测IL-1β、IL-18表达,Western blot检测TXNIP、NLRP3、Caspase-1、GSDMD-N蛋白表达,双荧光素酶报告基因实验验证lncRNA HAGLR与miR-106b-5p的靶向结合关系。**结果** 动物实验:OA模型组大鼠软骨损伤较Saline组严重;与Saline组相比,OA模型组大鼠Mankin评分升高,lncRNA HAGLR、TXNIP、NLRP3、Caspase-1、GSDMD-N、IL-1β、IL-18表达上调,miR-106b-5p表达下调,差异均有统计学意义($P<0.05$)。体外实验:与对照组相比,OA组细胞中lncRNA HAGLR、TXNIP、NLRP3、Caspase-1、GSDMD-N、IL-1β、IL-18表达上调,细胞增殖活力降低,miR-106b-5p表达下调,差异均有统计学意义($P<0.05$);与si-NC组相比,si-HAGLR组细胞中上述焦亡及炎症相关指标表达下调,细胞增殖活力增加,miR-106b-5p表达上调,差异均有统计学意义($P<0.05$);与mimic-NC组相比,miR-106b-5p-mimic组细胞中lncRNA HAGLR表达无显著变化($P>0.05$),miR-106b-5p表达上调($P<0.05$),细胞增殖活力增加($P<0.05$),TXNIP、焦亡相关蛋白和炎症相关指标下调($P<0.05$);si-HAGLR+miR-106b-5p-mimic组细胞中上述焦亡及炎症相关指标表达进一步下调($P<0.05$),细胞增殖活力进一步增加($P<0.05$)。双荧光素酶报告基因实验证实lncRNA HAGLR与miR-106b-5p存在直接结合位点。**结论** lncRNA HAGLR通过靶向调控miR-106b-5p,促进TXNIP表达,并激活NLRP3/Caspase-1/GSDMD-N通路,从而诱导OA软骨细胞焦亡,加剧软骨细胞炎症和损伤。

[关键词] lncRNA HAGLR;miR-106b-5p;硫氧还蛋白互作蛋白;骨关节炎;软骨细胞;细胞焦亡
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Effect of lncRNA HAGLR on pyroptosis of chondrocytes in osteoarthritis and its mechanism

MENG Xiao-yuan[1], MA Le[2], NING Kai[2], AN Meng-yu[1] (1. Joint Movement Disease Ward, Orthopedic Center, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi Xinjiang 830001, China; 2. Anesthesiology and Surgical Center, People's Hospital of Xinjiang Uygur Autonomous Region, Urumqi Xinjiang 830001, China)

Abstract: Objective To investigate the effect of lncRNA HAGLR on pyroptosis of chondrocytes in osteoarthritis (OA) and its mechanism. **Methods** Animal experiment: thirty-four SD rats were randomly divided into the Saline group (injected with 50 μL of normal saline into the left knee joint cavity) and the OA model group (injected with 50 μL of normal saline containing 5 mg monosodium iodoacetate into the left knee joint cavity), with 17 rats in each group. Two weeks after modeling, the degree of cartilage tissue injury was evaluated by HE staining and Safranin O/Fast green staining. The expressions of lncRNA HAGLR and miR-106b-5p were detected by qRT-PCR. The protein expressions of thioredoxin interacting protein (TXNIP), NOD-like receptor family pyrin domain containing 3 (NLRP3), Caspase-1, and gasdermin D N-terminal (GSDMD-N) were detected by Western blot. Serum levels of interleukin-1β (IL-1β) and interleukin-18 (IL-18) were determined by ELISA. In vitro experiments: the human chondrocyte cell line C28/I2 was divided into the following groups: the control group (cultured under normal conditions), OA group (stimulated with 20 ng/mL IL-1β for 48 hours to establish an in vitro OA model), si-NC group (transfected with si-NC for 24 hours followed by modeling), si-HAGLR group (transfected with si-HAGLR followed by modeling), mimic-NC group (transfected with mimic-NC followed by modeling), miR-106b-5p-mimic group (transfected with miR-106b-5p-mimic followed by modeling), and si-HAGLR+miR-106b-5p-mimic group (co-transfected with si-HAGLR and miR-106b-5p-mimic followed by

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[通信作者] 宁 凯,E-mail:13579233099@163.com

modeling). Cell proliferation activity was detected by CCK-8 assay. The expressions of lncRNA HAGLR and miR-106b-5p were detected by qRT-PCR. The expressions of IL-1β and IL-18 were detected by immunofluorescence staining and ELISA. The protein expressions of TXNIP, NLRP3, Caspase-1, and GSDMD-N were detected by Western blot. The targeting relationship between lncRNA HAGLR and miR-106b-5p was verified by dual-luciferase reporter gene assay. **Results** Animal experiments: the cartilage injury in the OA model group was more serious than that in the Saline group. Compared with the Saline group, the OA model group exhibited high Mankin score, upregulated expressions of lncRNA HAGLR, TXNIP, NLRP3, Caspase-1, GSDMD-N, IL-1β, and IL-18, and downregulated expression of miR-106b-5p, with all differences being statistically significant (P<0.05). In vitro experiments: compared with the control group, the OA group exhibited upregulated expressions of lncRNA HAGLR, TXNIP, NLRP3, Caspase-1, GSDMD-N, IL-1β, and IL-18, significantly decreased cell proliferation activity, as well as downregulated expression of miR-106b-5p, with all differences being statistically significant (P<0.05). Compared with the si-NC group, the si-HAGLR group showed significantly downregulated expressions of the aforementioned pyroptosis and inflammation-related indicators, significantly increased cell proliferation activity, along with upregulated expression of miR-106b-5p, with all differences being statistically significant (P<0.05). Compared with the mimic-NC group, the miR-106b-5p-mimic group showed no significant change in lncRNA HAGLR expression (P>0.05), but an upregulation in miR-106b-5p expression (P<0.05), increased in cell proliferation activity (P<0.05), and downregulation in TXNIP, pyroptosis-related proteins and inflammation-related indicators (P<0.05). The si-HAGLR+miR-106b-5p-mimic group showed further downregulation of the above pyroptosis and inflammatory indicators and further increase of cell proliferation activity (P<0.05). Dual-luciferase reporter gene assay confirmed the existence of direct binding sites between lncRNA HAGLR and miR-106b-5p. **Conclusion** LncRNA HAGLR promotes TXNIP expression and activates the NLRP3/Caspase-1/GSDMD-N pathway by targeting miR-106b-5p, thereby inducing pyroptosis of OA chondrocytes and exacerbating chondrocyte inflammation and injury.

Keywords: lncRNA HAGLR; miR-106b-5p; thioredoxin interacting protein; osteoarthritis; chondrocyte; pyroptosis

骨关节炎(osteoarthritis, OA)是常见的退行性关节病,软骨细胞异常死亡及炎症是其核心病理特征[1]。WHO的数据显示,60岁以上人群中OA患病率已超过20%,目前其治疗侧重于症状缓解,但难以修复关节结构损伤,因此阐明OA的分子机制和寻找新型治疗靶点具有重要意义[2]。研究显示,细胞焦亡在OA进展中起关键作用[3-4]。lncRNA作为表观遗传调控的重要参与者,其在OA发病机制中的作用逐渐受到关注[5]。lncRNA HAGLR已被证实与软骨细胞炎症相关[6-7],但其对OA软骨细胞焦亡的调控机制尚未明确。本研究旨在探讨lncRNA HAGLR对OA软骨细胞焦亡的影响及机制,为OA治疗提供靶点。

1 材料与方法

1.1 试剂与耗材

lncRNA HAGLR siRNA(序列:5′-GCAUGUACUUCAGGACAAATT-3′)及其阴性对照(si-NC序列:5′-UUCUCCGAACGUGUCACGUTT-3′)、miRNA-106b-5p-mimic(序列:5′-UAAAGUGCUGACAGUGCAGAU-3′)及其阴性对照(mimic-NC序列:5′-UUCUCCGAACGUGUCACGUTT-3′)购自上海吉玛公司;碘乙酸单钠购自美国Sigma-Aldrich公司;白细胞介素(interleukin,IL)-1β购自美国PeproTech公司;IL-1β、IL-18的ELISA试剂盒购自美国R&DSystems公司;DMEM/F12培养基、胎牛血清购自美国Gibco公司;Lipofectamine 3000购自美国ThermoFisherScientific公司;硫氧还蛋白互作蛋白(thioredoxin interacting protein,TXNIP)、NOD样受体热蛋白结构域相关蛋白3(NOD-like receptor family pyrin domain containing 3,NLRP3)、Caspase-1、消皮素D-N端(gasdermin D N-terminal,GSDMD-N)抗体及免疫荧光二抗均购自英国Abcam公司;CCK-8试剂盒购自日本Dojindo公司;双荧光素酶报告基因系统购自美国Promega公司。

1.2 方法

1.2.1 动物分组 34只雄性SD大鼠[8周龄,体质量(220±20)g,生产许可证号:SCXK(新)2023-0001]适应性饲养1周后,随机分为生理盐水组(Saline组)和OA模型组,每组17只。OA模型组大鼠左侧膝关节腔注射50 μL含5 mg碘乙酸单钠的生理盐水,Saline组大鼠注射等量生理盐水。造模2周后采集血清,对大鼠进行安乐死并分离左侧膝关节软骨组织,部分固定后用于免疫荧光化学检测,其余冻存用于Western blot和qRT-PCR检测。

1.2.2 细胞实验 人软骨细胞系C28/I2(购自美国ATCC)常规培养。将细胞分为对照组(常规培养)、OA组(添加20 ng/mL的IL-1β刺激48 h构建体外OA模型)、si-NC组(转染si-NC 24 h后建模)、si-HAGLR组(转染si-HAGLR后建模)、mimic-NC组(转染mimic-NC后建模)、miR-106b-5p-mimic组(转染miR-106b-5p-mimic后建模)、si-HAGLR+miR-106b-5p-mimic组(共

转染si-HAGLR和miR-106b-5p-mimic后建模)。转染浓度均为50 nmol/L,按Lipofectamine 3000说明书操作。

1.2.3 检测方法 组织学检测:软骨组织脱钙、包埋切片后,行HE染色及番红O/固绿染色。依据Mankin系统[8-9]综合评估软骨损伤情况,包括软骨表面完整性(0~3分)、细胞形态(0~3分)、潮线完整性(0~3分)及基质染色(0~3分),总分12分,5分及以上为OA。

ELISA:按ELISA试剂盒说明书检测大鼠血清及细胞上清中IL-1β、IL-18水平。

qRT-PCR:TRIzol法提取RNA,逆转录后行qPCR,以GAPDH和U6为内参,采用$2^{-\Delta\Delta Ct}$法计算lncRNA HAGLR和miR-106b-5p的相对表达量。

Western blot:提取蛋白并定量,经电泳、转膜后孵育TXNIP(1∶800)、NLRP3(1∶1 000)、Caspase-1(1∶1 000)、GSDMD-N(1∶1 000)一抗及二抗(1∶5 000),ECL显影,Image J软件分析蛋白条带灰度值。

CCK-8:细胞接种于96孔板,加入CCK-8试剂后,酶标仪检测450 nm波长处吸光度值。

免疫荧光染色:细胞经固定、通透、封闭后,孵育IL-1β、IL-18一抗(1∶100)及荧光二抗(1∶500),DAPI染色,荧光显微镜下观察。

双荧光素酶报告基因实验:构建lncRNA HAGLR野生型(WT)和突变型(MUT)载体,与miR-106b-5p mimic或mimic-NC共转染细胞,检测细胞荧光素酶活性。

1.3 统计学分析

使用GraphPad Prism 9.0软件进行数据录入和统计学分析,经*Levene*法行方差齐性检验,数据以均数±标准差($\bar{x}\pm s$)表示,2组间差异采用独立样本*t*检验,多组差异采用单因素方差分析(*ANOVA*),进一步两两比较采用*LSD-t*检验。以$P<0.05$为差异有统计学意义。

2 结果

2.1 大鼠Mankin评分和血清炎症因子水平

HE染色显示,OA模型组大鼠软骨表面粗糙,出现明显缺损,软骨细胞排列紊乱,可见细胞簇集现象,软骨基质染色不均,部分区域可见血管翳侵入软骨深层;番红O/固绿染色结果显示,软骨染色显著变淡,蛋白多糖大量丢失,软骨全层结构破坏,软骨层变薄。上述结果提示,OA模型组大鼠软骨损伤严重,见图1。与Saline组比较,OA模型组大鼠软骨组织Mankin评分增加,血清IL-1β和IL-18水平升高,差异均有统计学意义($P<0.001$),见表1。

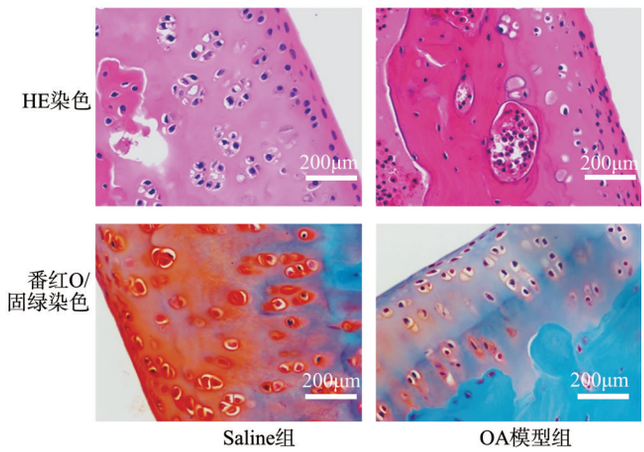


图1 HE染色和番红O/固绿染色观察OA大鼠软骨组织的病理变化

表1 2组大鼠Mankin评分和血清炎症因子水平比较($\bar{x}\pm s$)

组别	Mankin评分(分)	IL-1β(pg/mL)	IL-18(pg/mL)
Saline组	2.15±0.31	35.81±4.73	28.59±3.12
OA模型组	7.83±0.95	89.72±7.21	75.34±6.58
t	24.368	21.895	20.986
P	<0.001	<0.001	<0.001

2.2 大鼠lncRNA HAGLR、miR-106b-5p、TXNIP及焦亡相关蛋白表达

与Saline组比较,OA模型组大鼠软骨组织中lncRNA HAGLR表达上调,而miR-106b-5p表达下调,TXNIP、NLRP3、Caspase-1、GSDMD-N蛋白表达均上调,差异有统计学意义($P<0.001$),见图2、表2。

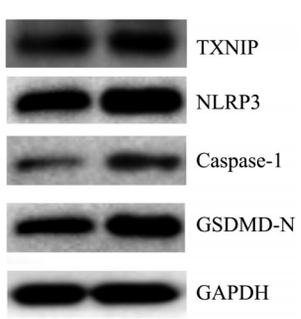


图2 大鼠软骨组织中TXNIP及焦亡相关蛋白表达

表2 大鼠软骨组织中lncRNA HAGLR、miR-106b-5p、TXNIP及焦亡相关蛋白的表达水平($\bar{x}\pm s$)

组别	lncRNA HAGLR	miR-106b-5p	TXNIP	NLRP3	Caspase-1	GSDMD-N
Saline组	1.02±0.15	0.98±0.11	0.95±0.12	0.93±0.10	0.94±0.13	0.96±0.14
OA模型组	3.57±0.42	0.31±0.06	3.85±0.38	2.36±0.35	2.79±0.41	2.88±0.37
t	23.816	25.142	22.953	21.768	22.342	22.679
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

2.3 沉默lncRNA HAGLR对miR-106b-5p表达和软骨细胞增殖的影响

与对照组比较，OA组细胞中lncRNA HAGLR表达上调，miR-106b-5p表达下调，细胞增殖活力降低，差异均有统计学意义（$P<0.05$）。与si-NC组比较，si-HAGLR组细胞中lncRNA HAGLR表达下调，miR-106b-5p表达上调，细胞增殖活力增加，差异均有统计学意义（$P<0.05$）。与mimic-NC组比较，miR-106b-5p-mimic组细胞中lncRNA HAGLR表达无显著变化（$P>0.05$），miR-106b-5p表达上调（$P<0.05$），细胞增殖活力增加（$P<0.05$）。与miR-106b-5p-mimic组比较，si-HAGLR+miR-106b-5p-mimic组细胞中lncRNA HAGLR表达下调，miR-106b-5p表达上调，细胞增殖活力增加，差异均有统计学意义（$P<0.05$）；与si-HAGLR组比较，si-HAGLR+miR-106b-5p-mimic组细胞中miR-106b-5p表达上调，细胞增殖活力增加，差异均有统计学意义（$P<0.05$），见表3。

表3 各组细胞中lncRNA HAGLR、miR-106b-5p表达和细胞增殖活力比较（$\bar{x}\pm s$）

组别	lncRNA HAGLR	miR-106b-5p	细胞增殖活力（%）
对照组	1.00±0.11	1.02±0.12	97.23±8.96
OA组	3.25±0.38*	0.31±0.05*	51.42±7.12*
si-NC组	3.18±0.36*	0.33±0.04*	50.96±8.35*
si-HAGLR组	1.42±0.17*#	0.87±0.10*#	75.73±6.07*#
mimic-NC组	3.22±0.37*	0.29±0.05*	52.05±5.36*
miR-106b-5p-mimic组	3.27±0.39*	1.98±0.18*△	70.12±7.33*△
si-HAGLR+miR-106b-5p-mimic组	1.38±0.16*□	2.62±0.19*▲□	86.89±9.32*▲□
F	151.833	143.670	69.225
P	<0.001	<0.001	<0.001

*：与对照组比较，$P<0.05$；#：与si-NC组比较，$P<0.05$；△：与mimic-NC组比较，$P<0.05$；▲：与si-HAGLR组比较，$P<0.05$；□：与miR-106b-5p-mimic组比较，$P<0.05$

2.4 沉默lncRNA HAGLR对TXNIP及焦亡相关蛋白的影响

与对照组比较，OA组细胞中TXNIP、NLRP3、Caspase-1、GSDMD-N蛋白表达均上调，差异有统计学意义（$P<0.05$）。与si-NC组比较，si-HAGLR组细胞中TXNIP、NLRP3、Caspase-1、GSDMD-N蛋白表达均下调，差异有统计学意义（$P<0.05$）。与mimic-NC组比较，miR-106b-5p-mimic组细胞中TXNIP、NLRP3、Caspase-1、GSDMD-N蛋白表达均下调，差异有统计学意义（$P<0.05$）。与si-HAGLR组和miR-106b-5p-mimic组比较，si-HAGLR+miR-106b-5p-mimic组细胞中TXNIP、NLRP3、Caspase-1、GSDMD-N蛋白表达均下调，差异有统计学意义（$P<0.05$），见图3、表4。

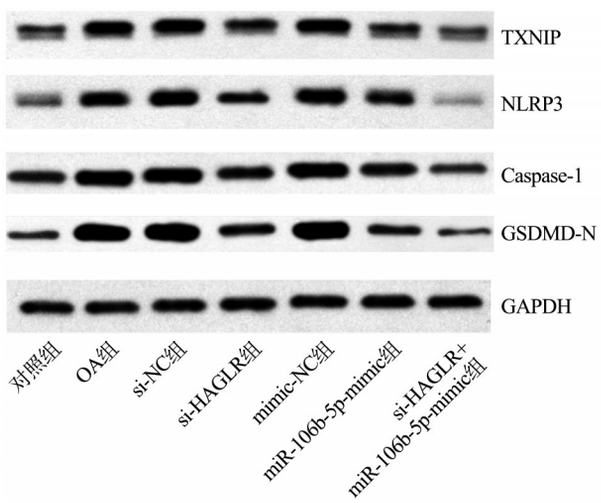


图3 各组细胞中TXNIP和焦亡相关蛋白条带

表4 各组细胞中TXNIP和细胞焦亡蛋白表达水平比较（$\bar{x}\pm s$）

组别	TXNIP	NLRP3	Caspase-1	GSDMD-N
对照组	0.96±0.12	0.93±0.11	0.97±0.13	0.95±0.12
OA组	3.92±0.35*	6.87±0.34*	2.34±0.37*	7.29±0.36*
si-NC组	3.88±0.33*	6.85±0.32*	2.30±0.35*	7.24±0.33*
si-HAGLR组	1.45±0.18*#	3.32±0.17*#	1.38±0.19*#	1.63±0.18*#
mimic-NC组	3.95±0.36*	6.91±0.35*	2.37±0.38*	7.22±0.37*
miR-106b-5p-mimic组	1.48±0.16*△	3.25±0.15*△	1.30±0.17*△	1.67±0.16*△
si-HAGLR+miR-106b-5p-mimic组	0.73±0.10*▲□	0.62±0.09*▲□	0.78±0.11*▲□	0.57±0.10*▲□
F	142.575	138.918	145.311	140.863
P	<0.001	<0.001	<0.001	<0.001

*：与对照组比较，$P<0.05$；#：与si-NC组比较，$P<0.05$；△：与mimic-NC组比较，$P<0.05$；▲：与si-HAGLR组比较，$P<0.05$；□：与miR-106b-5p-mimic组比较，$P<0.05$

2.5 沉默lncRNA HAGLR对炎症因子表达的影响

与对照组比较,OA组细胞中IL-1β和IL-18水平升高,差异均有统计学意义(P<0.05)。与si-NC组比较,si-HAGLR组细胞中IL-1β和IL-18水平降低,差异均有统计学意义(P<0.05)。与mimic-NC组比较,miR-106b-5p-mimic组细胞中IL-1β和IL-18水平降低,差异均有统计学意义(P<0.05)。与si-HAGLR组和miR-106b-5p-mimic组比较,si-HAGLR+miR-106b-5p-mimic组细胞中IL-1β和IL-18水平降低,差异均有统计学意义(P<0.05),见图4、表5。

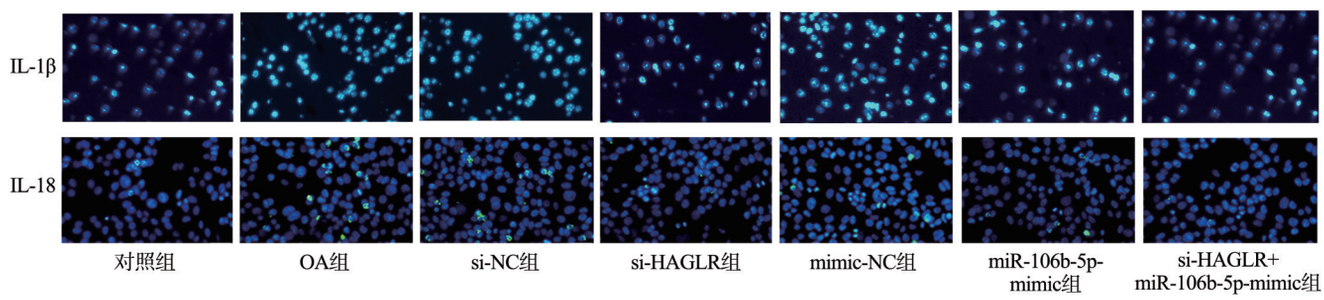


图4 免疫荧光染色检测细胞中炎症因子IL-1β和IL-18的表达(×200)

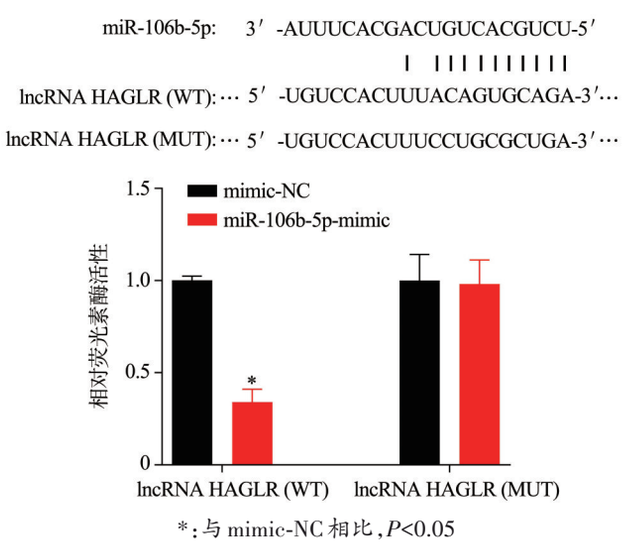
表5 免疫荧光染色法和ELISA检测细胞中炎症因子IL-1β和IL-18的表达($\bar{x}\pm s$,pg/mL)

组别	免疫荧光染色		ELISA	
	IL-1β	IL-18	IL-1β	IL-18
对照组	1.00±0.12	1.00±0.07	36.75±4.82	29.83±3.21
OA组	7.22±0.49*	5.29±0.52*	92.46±8.37*	78.25±7.43*
si-NC组	7.17±0.81*	5.40±0.71*	90.53±8.25*	76.84±7.35*
si-HAGLR组	3.03±0.24*#	2.87±0.15*#	48.63±5.87*#	42.17±4.95*#
mimic-NC组	7.20±0.76*	5.36±0.44*	93.21±8.46*	79.06±7.52*
miR-106b-5p-mimic组	3.52±0.19*△	2.41±0.22*△	45.82±5.64*△	39.75±4.72*△
si-HAGLR+miR-106b-5p-mimic组	1.48±0.16*▲□	1.24±0.05*▲□	30.59±4.12*▲□	20.14±3.05*▲□
F	205.115	178.523	139.243	134.870
P	<0.001	<0.001	<0.001	<0.001

*:与对照组比较,P<0.05;#:与si-NC组比较,P<0.05;△:与mimic-NC组比较,P<0.05;▲:与si-HAGLR组比较,P<0.05;□:与miR-106b-5p-mimic组比较,P<0.05

2.6 lncRNA HAGLR对miR-106b-5p的靶向调控

生物信息学网站预测结果显示,lncRNA HAGLR与miR-106b-5p存在潜在的靶向结合位点。双荧光素酶报告基因实验结果显示,转染miR-106b-5p-mimic降低了lncRNA HAGLR(WT)载体的荧光素酶活性(P<0.05),而转染miR-106b-5p-mimic对lncRNA HAGLR(MUT)载体的荧光素酶活性无显著影响(P>0.05),见图5。



*:与mimic-NC相比,P<0.05

图5 双荧光素酶报告基因实验验证lncRNA HAGLR对miR-106b-5p的靶向作用

3 讨论

miR-106b-5p作为miR-17家族成员,近年被证实参与多种疾病的炎症调控[10-11]。研究表明,lncRNA H19通过靶向调控外泌体中的miR-106b-5p表达从而对OA软骨细胞的增殖、迁移以及基质降解发挥了重要作用[12]。然而,在OA的炎症反应调控领域,关于miR-106b-5p的功能和上下游机制尚未完全阐明。本研究发现,OA模型大鼠中miR-106b-5p和lncRNA HAGLR的表达存在负向调控关系,敲降lncRNA HAGLR可以促进miR-106b-5p的表达,并抑制TXNIP蛋白、细胞焦亡蛋白和相关炎症因子的表达。这说明lncRNA HAGLR/miR-106b-5p/TXNIP轴可能调控了软骨细胞焦亡,此机制很可能在OA病理进程中扮演重要角色。

lncRNA往往通过“分子海绵”的作用抑制靶miRNA的表达,并通过调节下游的细胞焦亡相关靶基因改善或加重细胞焦亡[13-14]。本研究表明,OA软骨组织中lncRNA HAGLR表达显著上调,同时miR-106b-5p表达显著下调,而沉默lncRNA HAGLR不仅显著抑制了NLRP3、Caspase-1、GSDMD-N等细胞焦亡指标的表达,还促进了miR-106b-5p的表达,推测lncRNA HAGLR可能通过“分子海绵”机制吸附miR-106b-5p。本研究双荧光素酶报告基因实验也证实了在软骨细胞中lncRNA HAGLR可直接吸附miR-106b-5p,与Luo等[15]在糖尿病视网膜病变中的研究结果类似。上述研究结果表明,在不同类型病理状态以及细胞类型中,lncRNA HAGLR均可靶向调控miR-106b-5p。

在OA的病理进程中,软骨细胞的焦亡是程序性细胞死亡的重要形式,近年来被证实与炎症微环境和基质降解密切相关[16-17]。lncRNA HAGLR通过靶向调控miR-106b-5p促进人视网膜色素上皮细胞焦亡,从而加速糖尿病视网膜病变的进程[15]。本研究在OA中也揭示了沉默lncRNA HAGLR可靶向激活miR-106b-5p,从而抑制TXNIP、NLRP3、Caspase-1、IL-1β和IL-18的表达,进而抑制OA模型软骨细胞焦亡,为OA的分子靶向治疗提供了新的理论依据。研究还表明,TXNIP可通过直接结合并激活NLRP3,促进Caspase-1前体切割为活性形式,参与焦亡进程[18];同时其还可增加线粒体活性氧生成,触发细胞膜孔道形成,从而调节IL-1β和IL-18前体的成熟与释放[19]。本研究表明,沉默lncRNA HAGLR并联合过表达miR-106b-5p处理协同抑制TXNIP蛋白表达,并伴随NLRP3、Caspase-1、IL-1β和IL-18的同步抑制,这为开发联合靶向OA的治疗策略提供了理论基础。

在细胞焦亡与炎症的级联放大效应过程中,活化的Caspase-1不仅可直接导致细胞膜穿孔,还可切割GSDMD形成GSDMD-N,这是形成孔道结构的关键条件,同时介导IL-1β和IL-18的释放[20-21]。本研究观察到,OA软骨细胞中IL-1β和IL-18的蛋白表达和血清水平均上调,并伴随着GSDMD-N、NLRP3、Caspase-1等焦亡指标的表达上调,这验证了OA中存在细胞焦亡-炎症的正反馈循环[16]。此外,OA的滑膜中IL-1β可通过激活NF-κB通路进一步上调促焦亡基因表达,形成持续炎症微环境[22]。本研究显示,在体外OA软骨细胞模型中过表达miR-106b-5p可导致TXNIP蛋白显著低表达,炎症因子IL-1β和IL-18的表达下调;而沉默lncRNA HAGLR联合过表达miR-106b-5p干预对炎症具有显著抑制作用,这提示lncRNA HAGLR/miR-106b-5p/TXNIP轴可同时作用于焦亡触发和炎症扩散的关键环节。

本研究仍存在一定局限性,后续还需扩大样本,并进一步研究lncRNA HAGLR对OA进展过程中其他信号通路的调控。

综上所述,本研究初步阐明在OA中lncRNA HAGLR通过吸附miR-106b-5p调控TXNIP蛋白的表达,促进NLRP3、Caspase-1、IL-1β和IL-18的表达,从而激活OA软骨细胞焦亡。

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