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CircADAMTS-6调控AECOPD细胞模型抗病毒固有免疫的机制研究

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[摘要] **目的:** 探讨环状RNA circADAMTS-6通过调控miR-1260b/IFNAR2轴对慢性阻塞性肺疾病急性加重(acute exacerbation of chronic obstructive pulmonary disease, AECOPD)细胞模型抗病毒固有免疫防御反应的影响。**方法:** (1)香烟烟雾提取物(cigarette smoke extract, CSE)干预人支气管上皮细胞(BEAS-2B)建立COPD细胞模型, 实时荧光定量PCR(qRT-PCR)检测circADAMTS-6、miR-1260b、IFNAR2表达, 免疫印迹(Western blot, WB)检测IFNAR2蛋白表达; (2)过表达circADAMTS-6的BEAS-2B经CSE+poly(dA:dT)干预建立AECOPD细胞模型, 检测抗病毒固有免疫通路ISG15和CXCL10 mRNA表达及STAT1、STAT2磷酸化水平; (3)双荧光素酶报告基因检测circADAMTS-6、miR-1260b、IFNAR2之间的关系; (4)BEAS-2B细胞分别转染miR-1260b激活剂+过表达IFNAR2质粒、或转染过表达IFNAR2质粒+小干扰circADAMTS-6, 随后经CSE+poly(dA:dT)干预, WB检测STAT1、STAT2磷酸化水平。**结果:** (1)COPD细胞模型circADAMTS-6、IFNAR2表达降低, miR-1260b表达升高($P<0.05$); (2)过表达circADAMTS-6促进AECOPD细胞模型ISG15和CXCL10 mRNA表达及STAT1、STAT2磷酸化($P<0.05$); (3)miR-1260b分别与circADAMTS-6及IFNAR2结合($P<0.05$); (4)过表达IFNAR2促进STAT1、STAT2磷酸化, 联合过表达miR-1260b或沉默circADAMTS-6逆转IFNAR2过表达的促进作用($P<0.05$)。**结论:** circADAMTS-6可能通过抑制miR-1260b促进IFNAR2表达, 激活STAT1/2介导的抗病毒免疫通路, 增强AECOPD细胞模型的抗病毒免疫防御。

[关键词] 慢性阻塞性肺疾病急性加重; 环状RNA ADAMTS-6; 微小RNA 1260b; I型干扰素受体亚基2; 抗病毒固有免疫

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Research on the mechanism of circADAMTS-6 regulating antiviral innate immunity in AECOPD cell model

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View from specialist: It is creative, and of certain scientific and educational value.

[Abstract] **Objective:** To explore the effect of circADAMTS-6 on antiviral innate immune response in cell model of acute exacerbation of chronic obstructive pulmonary disease (AECOPD) via regulating miR-1260b/IFNAR2 axis. **Methods:** (1) Cigarette smoke extract (CSE) was used to intervene in BEAS-2B cells to conduct a COPD cell model. The expressions of circAD-

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AMTS-6, *miR-1260b* and *IFNAR2* were detected by real-time fluorescence quantitative PCR (qRT-PCR), and the *IFNAR2* protein expression was detected by Western blot (WB). (2) After overexpression of circADAMTS-6, BEAS-2B cells were treated with CSE combined with viral analogue poly (dA:dT) to establish an AECOPD cell model. The antiviral innate immune pathway molecules *ISG15* and *CXCL10* mRNA, as well as STAT1 and STAT2 proteins phosphorylation levels were detected; (3) The relationships among circADAMTS-6, *miR-1260b* and *IFNAR2* were verified *via* dual-luciferase reporter gene; (4) After overexpression of *IFNAR2* combined with overexpression of *miR-1260b* or silencing of circADAMTS-6, BEAS-2B cells were intervened by CSE plus poly (dA:dT). Western blots were used to detected the phosphorylation levels of STAT1 and STAT2 proteins. **Results:** (1) The expressions of circADAMTS-6 and *IFNAR2* in COPD cell models were decreased, while *miR-1260b* expression was increased ($P<0.05$). (2) circADAMTS-6 overexpression promoted *ISG15* and *CXCL10* mRNA expressions and the STAT1 and STAT2 proteins phosphorylation in AECOPD cell models ($P<0.05$). (3) *miR-1260b* interacted with circADAMTS-6 and *IFNAR2* ($P<0.05$). (4) *IFNAR2* overexpression promoted the STAT1 and STAT2 proteins phosphorylation. Combined overexpression of *miR-1260b* or silencing circADAMTS-6 reversed the promoting effect of *IFNAR2* overexpression ($P<0.05$). **Conclusion:** circADAMTS-6 may play a protective role in AECOPD cell model *via* increasing the *IFNAR2* expression by inhibiting *miR-1260b*, promoting the activation of STAT1/2-mediated antiviral immune pathway.

[Key words] Acute exacerbation of chronic obstructive pulmonary disease; CircADAMTS-6; MiR-1260b; IFNAR2; Antiviral innate immune response

慢性阻塞性肺疾病(chronic obstructive pulmonary disease, COPD)是全球人口死亡的主要原因之一。慢阻肺急性加重(acute exacerbation of COPD, AECOPD)使患者肺功能及生活质量下降,增加住院率和病死率,造成巨大经济和社会负担^[1-3]。呼吸道病毒感染是AECOPD的主要原因。气道上皮细胞是抵御呼吸道感染的第一道防线,识别病毒、产生I型干扰素(type I interferons, IFN-I), IFN-I与受体(IFN-I receptor, IFNAR, 由IFNAR1和IFNAR2组成)结合并促进信号转导及转录激活因子1/2(signal transducer and activator of transcription 1/2, STAT1/2)磷酸化及IFN刺激基因(IFN-stimulated genes, ISGs)表达以抵御病毒^[4,5]。香烟烟雾(cigarette smoke, CS)是COPD的主要危险因素,抑制抗病毒固有免疫反应使其对病毒易感性增加^[6],具体机制尚不明确。

环状RNA(Circular RNAs, circRNAs)由前体mRNA反向剪接形成,无5'帽结构和3'poly(A)结构,据报道circRNA与COPD发病机制相关^[7],研究发现AECOPD患者血清circRNA0001859丰度降低,可能作为其预后标志物,但未涉及机制研究^[8]。circRNA也调节抗病毒防御反应,参与新型冠状病毒及甲型流感病毒等的免疫调控^[9,10]。研究报道具有凝血反应蛋白基序-6的崩解素和金属蛋白酶基因(a disintegrin and metalloprotease with thrombospondin motif-6 ADAMTS-6)产生的环状RNA circADAMTS-6(hsa_circ_0008667)通过miR-324-5p/PIK3R3对IL-1 β 刺激的软骨细胞发挥保护作用^[11],但hsa_circ_0008667(即circADAMTS-6)在AECOPD中作用尚不明确。本研究通过香烟烟雾提取物

(cigarette smoke extract, CSE)联合poly(dA:dT)干预BEAS-2B细胞构建AECOPD细胞模型,探索circADAMTS-6、miR-1260b、IFNAR2在AECOPD抗病毒固有免疫中的作用,为AECOPD防治提供潜在生物靶点。

1 材料与方法

1.1 材料

1.1.1 细胞 人支气管上皮细胞(BEAS-2B, AWCNH004)和人胚肾细胞(HEK-293T, AWCNH086)购买于长沙艾碧维科技有限公司。

1.1.2 主要试剂 DMEM培养基、胎牛血清、胰酶(Gibco, 美国), 链青霉素、RIPA裂解液(索莱宝, 北京), 香烟(白沙牌, 湖南长沙)。BCA蛋白浓度测定试剂盒、抗体稀释液(碧云天, 中国)。5 $\times$ 蛋白上样缓冲液、蛋白分子量marker、HRP发光试剂、poly(dA:dT)、Lipofectamine2000脂质体、TransZol、circRNA及mRNA逆转录和PCR试剂盒(赛默飞, 美国), PCR引物(生工, 上海)。microRNA逆转录及PCR试剂盒(康维世纪, 江苏)。小干扰RNA和过表达质粒、双荧光素酶报告质粒circADAMTS-6-3'UTR-WT、circADAMTS-6-3'UTR-MUT及检测试剂盒、miR-1260b mimic、miR-NC(吉玛基因, 上海)。IFNAR2-3'UTR-WT、IFNAR2-3'UTR-MUT双荧光素酶报告质粒(汉恒生物, 上海)。circRNA原位杂交探针及试剂盒(lnc1100621, 锐博, 广州)。抗体 GAPDH、STAT1、p-STAT1、STAT2、IFNAR2(abcam, 英国), 抗体p-STAT2(CST, 美国)及羊抗兔IgG(H+L)二抗(三鹰, 武汉)。

1.1.3 主要仪器 分光光度仪、超净工作台、多功能酶标仪(赛默飞, 美国), PVDF膜(Minipore, 美

国),制胶架、电泳槽、转膜仪(天能,上海),激光共聚焦显微镜系统(徕卡,德国),PCR仪及凝胶成像分析系统(赛默飞,美国)。

1.2 方法

1.2.1 制备CSE 充分燃烧一支去除过滤嘴的香烟(湖南中烟工业有限责任公司,白沙牌香烟,成分:焦油量10 mg,烟气烟碱量0.8 mg,烟气一氧化碳含量12 mg)产生的烟雾通过负压装置(LeyNow,广州)溶解于5 mL PBS。溶液经0.22 μm过滤嘴(Lida,美国)过滤,制备成无菌CSE经分光光度仪检测320 nm和540 nm波长的吸光值在0.9~1.2,定为100%CSE。提前30 min制备新鲜的100%CSE溶液,使用前稀释至所需的浓度。

1.2.2 细胞培养 BEAS-2B和293T细胞系培养于

含10%胎牛血清和1%青霉素-链霉素的DMEM培养基中,培养条件为37℃、5%CO₂。按照实验目的将BEAS-2B、293T细胞接种于培养皿或培养板中,细胞生长至70%~80%,接受不同分组处理。后续使用3%CSE及2 μg/mL poly(dA:dT)干预24 h进行AECOPD细胞体外实验。

1.2.3 细胞转染 用Lipofectamine 2000将对照小干扰RNA si-NC、si-circADAMTS-6、对照质粒Vector、过表达质粒pcDNA3.1IFNAR2(OE-IFNAR2)、pcDNA3.1circADAMTS-6(OE-circADAMTS-6)、miR-NC、miR-1260b mimic按转染说明书转染至BEAS-2B细胞,24 h后检测转染效率,再按照不同实验目的予以不同干预,24 h后收集细胞进行检测。寡核苷酸序列见表1。

表1 RNA寡核苷酸序列
Tab 1 Sequences of oligo RNA

基因名称	核酸序列
<i>si-circADAMTS-6</i>	sense:5'-UUCUAAUACUGAAGGUGUCTT-3'
	antisense:5'-GACACCUUCAGUAUUAGAATT-3'
<i>si-NC</i>	sense:5'-UUCUCCGAACGUGUCACGUTT-3'
	antisense:5'-ACGUGACACGUUCGGAGAATT-3'
<i>miR-1260b mimic</i>	sense:5'-AUCCCACCACUGCCACCAU-3'
	antisense:5'-AUGGUGGCAGUGGUGGGAU-3'
<i>miR-NC</i>	sense:5'-UCACAACCUCCUAGAAAGAGUAGA-3'
	antisense:5'-UCUACUCUUUCU AGGAGGUUGUGA-3'

1.2.4 荧光原位杂交实验 BEAS-2B接种至12孔板爬片,经4%多聚甲醛固定、0.1%TritonX-100渗透细胞膜、PBS洗涤。按照说明书进行预杂交,避光条件下分别加入Cy3标记的circADAMTS-6探针和18S探针杂交,37℃避光孵育过夜。次日予DAPI行核染色,使用激光共聚焦显微镜系统进行图像采集。

1.2.5 双荧光素酶报告基因检测 用Lipofectamine 2000将双荧光素酶报告基因质粒野生型或突变型分别与miR-1260bmimic或miR-NC共转染至293T细胞培养48 h,分组如下:miR-NC+circADAMTS-6-3'UTR-WT,miR-1260bmimic+circADAMTS-6-3'UTR-WT,miR-NC+circADAMTS-6-3'UTR-MUT,miR-1260bmimic+circADAMTS-6-3'UTR-MUT,miR-NC+IFNAR2-3'UTR-WT,miR-1260bmimic+IFNAR2-3'UTR-WT,miR-NC+IFNAR2-3'UTR-MUT,miR-1260bmimic+IFNAR2-3'UTR-MUT。48 h后冰上裂解细胞、离心收集上清,双荧光素酶报告基因试剂盒检测各组萤火虫荧光Firefly和海肾荧光Renilla活性,并

计算比值。

1.2.6 定量实时聚合酶链反应(qRT-PCR) BEAS-2B按照不同实验分组干预,TransZol提取总RNA并测浓度,按照说明书逆转录circRNA、miRNA及mRNA合成cDNA,circRNA、mRNA用GAPDH作参照,miRNA用U6作参照,按照qPCR试剂盒对上述cDNA进行荧光定量扩增,表2为引物序列。以2^{-ΔΔCt}值统计分析相关分子相对表达水平。

1.2.7 Western Blot BEAS-2B接种于6孔板,按照不同分组干预,冰上裂解细胞30 min,收集于4℃,12 000×g离心20 min取上清,BCA检测蛋白浓度。蛋白与上样缓冲液按照4:1混合充分,95℃金属浴10 min使蛋白充分变性。通过SDS-PAGE进行电泳分离蛋白,于转膜槽将蛋白转至PVDF膜,蛋白快速封闭液封闭30 min,与相应抗体IFNAR2(1:1 000)、STAT1(1:1 000)、p-STAT1(1:1 000)、STAT2(1:1 000)、p-STAT2(1:1 000)、GAPDH(1:1 000)在4℃孵育过夜。次日,TBST溶液充分漂洗3次,二抗(1:5 000)室温孵育2 h,

表2 基因PCR引物序列
Tab 2 Primers sequences of gene for PCR

基因名称	引物序列
<i>circ_0008667(circADAMTS-6)</i>	正:5'-CCTGAGTGGTTCATTGGGGA-3' 反:5'-GTCACTAGATGCCTGTCCGG-3'
<i>miR-1260b</i>	正:5'-AACACGTGATCCCACCACTGC-3'反:5'-ATCCAGTGCAGGGTCCGAGG-3'
<i>GAPDH</i>	正:5'-ATTTGGTCGTATTGGGCGC-3' 反:5'-GGGTCATTATGGCAACAATATCCA-3'
<i>IFNAR2</i>	正:5'-GGTCTCGCTAAGGGCTGGAATG-3' 反:5'-CACAGGGATGCACGCTTGTAATC-3'
<i>CXCL10</i>	正:5'-ACTGTACGCTGTACCTGCATCAG-3'反:5'-ACGTGGACAAAATTGGCTTGACAG-3'
<i>ISG15</i>	正:5'-CTGCTGGTGGTGGACAAATGC-3'反:5'-CCGCTCACTTGCTGCTTCAG-3'

TBST 漂洗 3 次。通过凝胶成像系统进行化学发光。

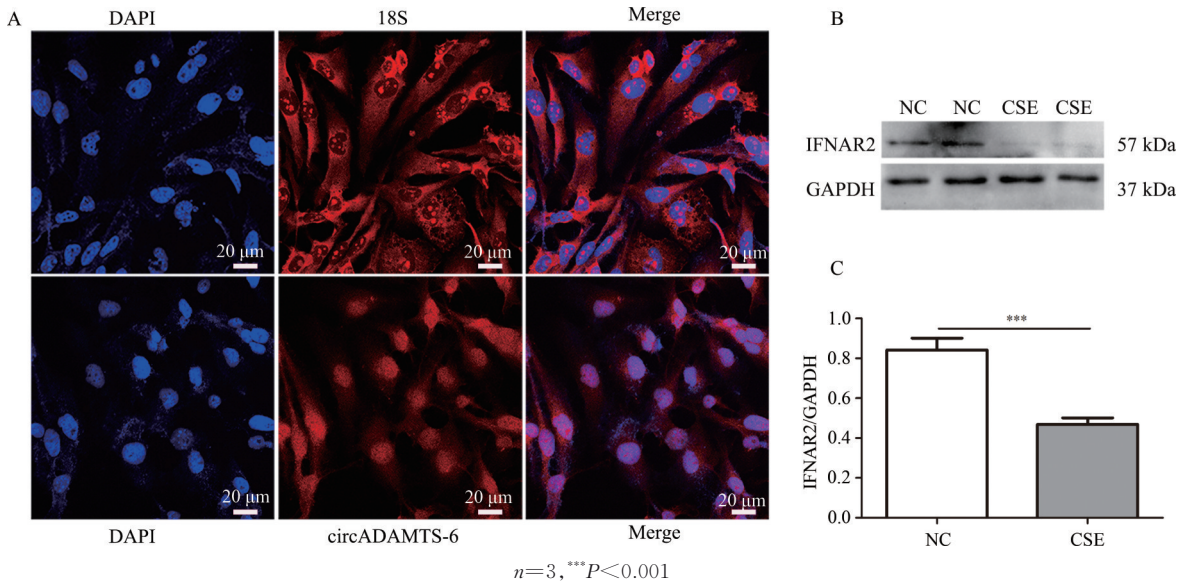
1.3 统计学处理

使用 ImageJ 检测 WB 条带灰度值、GraphPad Prism 9 软件进行数据统计分析。本研究计量资料符合正态分布,以 $\bar{x} \pm s$ 表示,两组间比较采用独立样本 *t* 检验,多组间比较采用单因素方差分析,组间两两比较采用 Bonferroni's 多重比较检验。*P* < 0.05 为差异具有统计学意义。

2 结果

2.1 circADAMTS-6、miR-1260b、IFNAR2 在 CSE 干预的人支气管上皮细胞内表达情况

荧光原位杂交实验 FISH 显示 circADAMTS-6 定位于细胞质和细胞核(图 1A)。PCR 表明与空白组(NC)相比,CSE 组 *circADAMTS-6*、*IFNAR2* 表达明显降低,而 *miR-1260b* 表达增加(*P* < 0.05),见表 3。WB 结果表明 CSE 抑制 IFNAR2 蛋白表达(图 1B、C)。



A: FISH 检测 circADAMTS-6 细胞内定位; B, C: CSE 抑制 IFNAR2 蛋白表达

图 1 circADAMTS-6 的定位及 CSE 对 IFNAR2 表达的影响

Fig 1 The location of circADAMTS-6 and the effect of CSE on the expression of IFNAR2

2.2 过表达 circADAMTS-6 削弱 CSE 对 BEAS-2B 细胞抗病毒免疫应答的抑制作用

使用 poly(dA:dT) 模拟病毒感染,与 Vector 组相比,poly(dA:dT)能明显诱导 *ISG15* 和 *CXCL10* mRNA 表达及 STAT1、STAT2 磷酸化,CSE 明显抑制 poly(dA:dT)的诱导效应;而过表达 circAD-

AMTS-6 明显增强 poly(dA:dT)诱导的 *ISG15* 和 *CXCL10* mRNA 表达及 STAT1、STAT2 磷酸化,且明显削弱 CSE 对 poly(dA:dT)的抑制作用(*P* < 0.05),见表 4、5 及图 2。

2.3 circADAMTS-6、IFNAR2 和 miR-1260b 三者

表3 CSE对 *circADAMTS-6*、*miR-1260b*、*IFNAR2*表达的影响
($n=3, \bar{x} \pm s$)

Tab 3 The effect of CSE on the expression of *circADAMTS-6*, *miR-1260b*, *IFNAR2*($n=3, \bar{x} \pm s$)

分组	<i>circADAMTS-6</i>	<i>miR-1260b</i>	<i>IFNAR2</i>
NC组	1.036±0.074	0.968±0.026	1.038±0.121
CSE组	0.623±0.029*	1.391±0.105*	0.682±0.031*
<i>t</i>	10.430	6.771	4.949
<i>P</i>	<0.05	<0.05	<0.05

注:与NC组比较,* $P<0.05$ 。

表4 过表达 *circADAMTS-6*对CSE干预的BEAS-2B细胞内
CXCL10、*ISG15*mRNA的影响($n=3, \bar{x} \pm s$)

Tab 4 *circADAMTS-6* overexpression affected the levels of *CXCL10*, *ISG15*mRNA in CSE-treated BEAS-2B cells($n=3, \bar{x} \pm s$)

分组	<i>CXCL10</i>	<i>ISG15</i>
Vector	0.965±0.067	0.929±0.030
Vector+DAT	2.783±0.124*	4.523±0.034*
Vector+DAT+CSE	1.955±0.084 [#]	2.517±0.336 [#]
OE- <i>circADAMTS-6</i> +DAT	4.594±0.353 [#]	5.636±0.157 [#]
OE- <i>circADAMTS-6</i> + DAT+CSE	2.702±0.134 [△]	3.595±0.275 [△]
<i>F</i>	157.1	229.6
<i>P</i>	<0.05	<0.05

注:与Vector比较,* $P<0.05$;与Vector+DAT比较,[#] $P<0.05$;与Vector+DAT+CSE比较,[△] $P<0.05$;DAT代表poly(dA:dT)。

表5 过表达 *circADAMTS-6*对AECOPD细胞模型内STAT1/2
磷酸化的影响($n=3, \bar{x} \pm s$)

Tab 5 Overexpression of *circADAMTS-6* affected phosphorylation levels of STAT1/2 in AECOPD cell model($n=3, \bar{x} \pm s$)

分组	p-STAT1/ STAT1	p-STAT2/ STAT2
Vector	0.993±0.007	0.094±0.048
Vector+CSE	0.938±0.084	1.096±0.043
Vector+DAT+CSE	0.942±0.067 [△]	1.321±0.045 [△]
Vector+DAT	1.951±0.057*	1.460±0.036*
OE- <i>circADAMTS-6</i>	0.915±0.058	1.041±0.039
OE- <i>circADAMTS-6</i> +CSE	0.853±0.100	1.126±0.052
OE- <i>circADAMTS-6</i> +DAT	2.186±0.011 [△]	1.653±0.041 [△]
OE- <i>circADAMTS-6</i> +DAT+ CSE	2.069±0.075 [#]	1.588±0.016 [#]
<i>F</i>	249.000	121.100
<i>P</i>	<0.05	<0.05

注:与Vector比较,* $P<0.05$;与Vector+DAT+CSE比较,[#] $P<0.05$;与Vector+DAT比较,[△] $P<0.05$;DAT代表poly(dA:dT)。

之间相互作用

生物信息学预测 miR-1260b 分别与 *circADAMTS-6*、*IFNAR2*-3'UTR 存在靶向关系(图3)。双

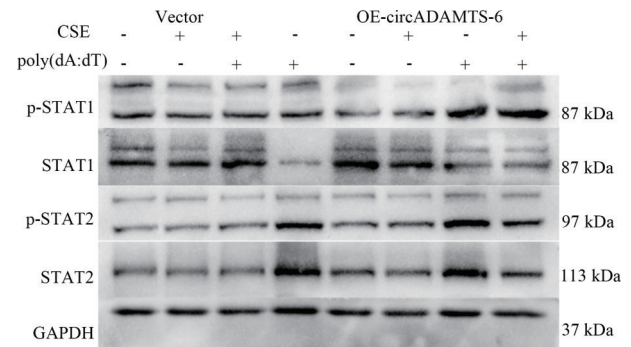
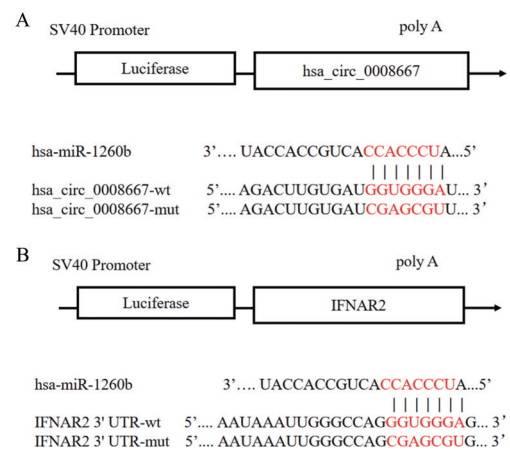


图2 *circADAMTS-6*对AECOPD细胞模型内STAT1/2
蛋白磷酸化的影响

Fig 2 *circADAMTS-6* affected the phosphorylation levels
of STAT1/2 in AECOPD cell models

荧光素酶实验结果显示,与miR-NC比较,miR-1260b mimic 明显减弱 *circADAMTS-6*-WT、*IFNAR2*-3'UTR-WT 组荧光素酶活性,而对 *circADAMTS-6*-MUT、*IFNAR2*-3'UTR-MUT 组荧光素酶活性无明显影响(表6、7)。进一步说明 *circADAMTS-6* 与 miR-1260b 相互作用,miR-1260b 与 *IFNAR2* 相互作用。



A: *circADAMTS-6*、miR-1260b 互作位点; B: miR-1260b、*IFNAR2*
互作位点

图3 *circADAMTS-6*、*IFNAR2*、miR-1260b 互作位点

Fig 3 Interaction sites of *circADAMTS-6*, *IFNAR2*, miR-1260b

2.4 *circADAMTS-6*/miR-1260b 靶向 *IFNAR2* 调控CSE对BEAS-2B细胞的抗病毒免疫应答

过表达 *IFNAR2* 明显减轻 CSE 对 poly(dA:dT) 诱导的 STAT1/STAT2 磷酸化的抑制(图4,表8、9);共转染 miR-1260b mimic 及 OE-*IFNAR2*, 明显削弱 OE-*IFNAR2* 对 STAT1/STAT2 磷酸

表6 双荧光素酶报告基因检测miR-1260b与circADAMTS-6的关系(n=3, $\bar{x} \pm s$)

Tab 6 Dual-luciferase reporter gene assays of the relationship between miR-1260b and circADAMTS-6(n=3, $\bar{x} \pm s$)

分组	荧光素酶活性
miR-NC+circADAMTS-6-WT	1.013±0.045
mimic+circADAMTS-6-WT	0.570±0.061 [*]
miR-NC+circADAMTS-6-MUT	1.097±0.063
mimic+circADAMTS-6-MUT	0.999±0.084 [#]
F	66.900
P	<0.05

注:与miR-NC+circADAMTS-6-WT比较,^{*}P<0.05;与miR-NC+circADAMTS-6-MUT比较,[#]P>0.05。

化的促进作用(图4A,表8)。同样,共同转染si-circADAMTS-6及OE-IFNAR2,结果显示si-circAD-

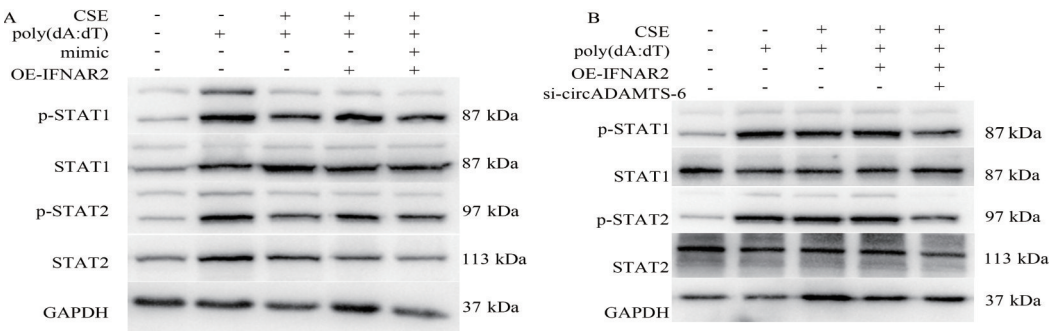
表7 双荧光素酶报告基因检测miR-1260b与IFNAR2的关系(n=3, $\bar{x} \pm s$)

Tab 7 Dual-luciferase reporter gene assay of the relationship between miR-1260b and IFNAR2(n=3, $\bar{x} \pm s$)

分组	相对荧光素酶活性
miR-NC+IFNAR2-WT	1.022±0.017
mimic+IFNAR2-WT	0.310±0.038 [*]
miR-NC+IFNAR2-MUT	1.048±0.118
mimic+IFNAR2-MUT	1.158±0.025 [#]
F	167.000
P	<0.05

注:与miR-NC+IFNAR2-WT比较,^{*}P<0.05;与miR-NC+IFNAR2-MUT比较,[#]P>0.05。

AMTS-6明显降低OE-IFNAR2对STAT1、STAT2磷酸化的促进作用(图4B,表9)。



A:过表达IFNAR2和miR-1260b mimic;B:过表达IFNAR2和沉默circADAMTS-6对STAT1/2磷酸化的影响

图4 circADAMTS-6/miR-1260b靶向IFNAR2调控CSE对BEAS-2B细胞抗病毒免疫反应

Fig 4 circADAMTS-6/miR-1260b regulated antiviral innate immunity in CSE-treated BEAS-2B cell via targeting IFNAR2

表8 联合过表达IFNAR2和miR-1260b mimic对STAT1、STAT1磷酸化的影响(n=3, $\bar{x} \pm s$)

Tab 8 The effect of combined overexpression of IFNAR2 and miR-1260b mimic on the phosphorylation of STAT1,STAT1(n=3, $\bar{x} \pm s$)

分组	p-STAT1/STAT1	p-STAT2/STAT2
Vector+miR-NC	0.996±0.013	0.947±0.048
Vector+miR-NC+DAT	2.044±0.020 [*]	2.330±0.068 [*]
Vector+miR-NC+DAT+CSE	1.176±0.046 [#]	1.927±0.037 [#]
OE-IFNAR2+miR-NC+DAT+CSE	1.752±0.064 [△]	3.329±0.079 [△]
OE-IFNAR2+mimic+DAT+CSE	1.352±0.069 [▲]	2.982±0.153 [▲]
F	238.500	341.700
P	<0.05	<0.05

注:与Vector+miR-NC比较,^{*}P<0.05;与Vector+miR-NC+DAT比较,[#]P<0.05;与Vector+miR-NC+DAT+CSE比较,[△]P<0.05;与OE-IFNAR2+miR-NC+DAT+CSE比较,[▲]P<0.05。

表9 联合过表达IFNAR2和沉默circADAMTS-6对STAT1、STAT1磷酸化的影响(n=3, $\bar{x} \pm s$)

Tab 9 The effect of combined overexpression of IFNAR2 and silencing of circADAMTS-6 on STAT1,STAT1 phosphorylation(n=3, $\bar{x} \pm s$)

分组	p-STAT1/STAT1	p-STAT2/STAT2
Vector+siNC	1.098±0.101	1.026±0.108
Vector+siNC+DAT	5.419±0.126 [*]	6.374±0.083 [*]
Vector+siNC+DAT+CSE	4.839±0.117 [#]	6.036±0.067 [#]
DAT+CSE+OE-IFNAR2+siNC	5.599±0.167 [△]	6.550±0.115 [△]
DAT+CSE+OE-IFNAR2+si-circADAMTS-6	3.199±0.155 [▲]	5.483±0.147 [▲]
F	585.800	1381.000
P	<0.05	<0.05

注:与Vector+siNC比较,^{*}P<0.05;与Vector+siNC+DAT比较,[#]P<0.05;与Vector+siNC+DAT+CSE比较,[△]P<0.05;与DAT+CSE+OE-IFNAR2+siNC比较,[▲]P<0.05。

3 讨论

AECOPD是慢阻肺的严重并发症,减少和预防AECOPD发生是慢阻肺治疗的重要目标之一^[12]。慢阻肺是异质性疾病,患者常不能识别急性加重的临床表现而耽误治疗,故预防、及时精准诊断AECOPD将使患者获益^[13]。呼吸道病毒感染是慢阻肺急性加重的主要诱因,COPD患者易合并病毒感染,而后者会加重气道炎症反应^[14]。研究发现香烟损害COPD患者气道抗病毒免疫反应而对病毒易感导致AECOPD发生,确切机制尚不明确^[15]。

circRNAs通过调控细胞凋亡、炎症、氧化应激等参与COPD发病^[16]。Zhou等^[17]报道了circFOXO3在CS暴露的肺组织和CSE处理的小鼠肺泡上皮细胞中过表达,敲减circFOXO3后CXCL1和IL-6表达减少,减轻炎症反应。研究发现AECOPD患者血清中circRNA表达失调,提示circRNA可能参与调控慢阻肺急性加重^[18]。本研究利用CSE干预支气管上皮细胞建立COPD细胞模型,发现CSE组circADAMTS-6表达降低。研究发现同样来源于ADAMTS-6亲本基因的hsa_circ_0072688参与COPD肺气肿发生^[19]。然而,尚无对circADAMTS-6在AECOPD中的报道。以往研究已证实circRNA是重要的抗病毒固有免疫防御调节因子,抑制或促进病毒感染^[20]。如hsa_circ_0004445通过与hnRNP-PC的结合明显抑制MERS-CoV的复制^[21]。本研究显示,过表达circADAMTS-6促进支气管上皮细胞对poly(dA:dT)诱导的细胞因子CXCL10、ISG15 mRNA表达及STAT1/STAT2蛋白磷酸化水平,表明circADAMTS-6参与STAT1/2-ISGs抗病毒固有免疫通路调控,缓解CSE对固有免疫反应的抑制。

circRNAs作为miRNA分子海绵调节基因表达,如circBBS9通过靶向miR-30e-5p/ADAR加剧COPD的肺部炎症^[22]。circXPO1通过靶向miR-23b-3p抑制TGF- β 活化激酶1/MAP3K7结合蛋白3(TAB3)的表达,而抑制circXPO1可缓解CS暴露的肺和CSE处理的肺泡上皮细胞的炎症和衰老^[23]。本研究FISH结果显示circADAMTS-6位于细胞质和细胞核中。生物信息学发现、双荧光素酶报告基因检测显示circADAMTS-6与miR-1260b结合,说明circADAMTS-6作为ceRNA结合miR-1260b。既往研究显示miR-1260b在COPD大鼠和NR8383细胞中高表达,并靶向抑制I κ B α 表达促进炎症细胞因子产生^[24]。本研究也表明miR-1260b在CSE干预的BEAS-2B细胞中表达升高,miR-1260b mimic

加剧CSE对poly(dA:dT)诱导的抗病毒相关分子的抑制,说明miR-1260b在AECOPD抗病毒免疫防御受损中发挥作用。

I型IFN受体由IFNAR1和IFNAR2亚基组成,IFNAR2与重症新冠肺炎患者临床预后相关^[25]。香烟通过下调IFNAR抑制IFN-I依赖性抗病毒免疫反应^[26]。与之前研究一致,本研究发现CSE抑制BEAS-2B细胞内IFNAR2 mRNA和蛋白表达,OE-IFNAR2促进STAT1/STAT2磷酸化,增强抗病毒固有免疫。Liu等^[27]发现miR-30c靶向结合并抑制IFNAR2表达从而促进猪繁殖与呼吸综合征病毒复制,提示IFNAR2表达及下游的抗病毒免疫受miRNA靶向调控。本研究通过生物信息学分析及荧光素酶报告基因检测发现IFNAR2与miR-1260b靶向结合,miR-1260 mimic减弱OE-IFNAR2对STAT1/STAT2蛋白磷酸化的促进作用。敲低circADAMTS-6也可削弱OE-IFNAR2的作用。表明circADAMTS-6通过调节miR-1260b靶向IFNAR2参与AECOPD细胞模型的抗病毒固有免疫调控。

综上所述,本研究在AECOPD细胞模型中阐述circADAMTS-6通过miRNA-ceRNA机制对抗病毒固有免疫发挥调控作用。机制研究发现circADAMTS-6在AECOPD细胞模型中表达减少,使miR-1260b丰度增加,对IFNAR2表达抑制作用增强而损害抗病毒固有免疫信号通路。以上结果为AECOPD抗病毒先天免疫异常及病毒易感性增加提供新的机制解释,同时也为预防及治疗AECOPD提供潜在生物靶点。circRNAs由于其稳定性及安全性高,现在逐渐成为疫苗、抗肿瘤生物疗法研究热点^[28]。未来circADAMTS-6能否作为一种预测或预防COPD急性加重的候选靶标、向临床应用转化等,仍需更多体内动物实验甚至临床试验验证。本研究也存在不足之处:除了气道上皮细胞外,多种免疫细胞也参与先天抗病毒免疫反应,探索参与AECOPD发生、发展的其他细胞中circADAMTS-6的作用也十分必要。circRNAs通过多种机制发挥生物功能,除外miRNA-ceRNA,circADAMTS-6是否通过其他机制产生作用,仍待进一步研究。同时,关于circADAMTS-6的免疫调节作用也需在AECOPD动物模型中验证。

作者贡献度说明:

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