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LncRNA NORAD 靶向抑制 miR-363-3p 对多发性骨髓瘤细胞生物学行为的影响及机制

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[摘要] 目的: 探索长链非编码RNA NORAD(lncRNA NORAD)对多发性骨髓瘤细胞增殖和凋亡的影响及其与微小RNA-363-3p(miR-363-3p)的靶向关系。方法: RT-qPCR检测健康人成骨细胞hFOB1.19和多发性骨髓瘤细胞U266, LP-1, H929中NORAD和miR-363-3p表达。在U266细胞中转染si-NORAD, 或共转染si-NORAD和anti-miR-363-3p, 噻唑蓝(MTT)法检测细胞增殖, 流式细胞术检测细胞凋亡, 蛋白质印迹法检测细胞周期蛋白D1(cyclin D1)、细胞周期蛋白依赖性激酶4(CDK4)、活化的含半胱氨酸的天冬氨酸蛋白水解酶3(cleaved caspase-3)、B细胞淋巴瘤/白血病-2(Bcl-2)、Bcl-2相关X蛋白(Bax)蛋白表达。starBase软件预测结合双荧光素酶报告实验分析NORAD与miR-363-3p的靶向结合。结果: 多发性骨髓瘤细胞系U266, LP-1和H929中NORAD表达明显上调($P < 0.05$), miR-363-3p表达显著下调($P < 0.05$)。沉默NORAD表达显著降低48, 72 h的U266细胞活性和cyclin D1, CDK4, Bcl-2蛋白水平($P < 0.05$), 明显提高细胞凋亡率和cleaved caspase-3, Bax蛋白表达量($P < 0.05$)。NORAD靶向调控miR-363-3p表达。抑制miR-363-3p表达逆转沉默NORAD表达对U266细胞增殖、cyclin D1, CDK4和Bcl-2表达的抑制作用, 以及逆转沉默NORAD表达对细胞凋亡、cleaved caspase-3和Bax蛋白表达的促进作用。结论: LncRNA NORAD通过靶向miR-363-3p表达影响多发性骨髓瘤细胞增殖和凋亡。

[关键词] 多发性骨髓瘤; lncRNA NORAD; miR-363-3p; 增殖; 凋亡

Effects of lncRNA NORAD targeted inhibition of miR-363-3p on biological behavior of multiple myeloma cells and its mechanisms

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Abstract **Objective:** To explore the effects of long noncoding RNA NORAD (lncRNA NORAD) on proliferation and apoptosis of multiple myeloma cells and its targeting relationship with microRNA-363-3p (miR-363-3p). **Methods:** The expression of NORAD and miR-363-3p in healthy human osteoblasts hFOB1.19 and multiple

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myeloma cells U266, LP-1 and H929 were detected by RT-qPCR. The U266 cells were transfected with si-NORAD, or co-transfected with si-NORAD and anti-miR-363-3p. MTT assay was used to determine cell proliferation. Flow cytometry was applied to test cell apoptosis. Western blotting was employed to assay the expression of cyclin D1, CDK4, cleaved caspase-3, Bcl-2 and Bax. StarBase software prediction combined with dual luciferase reporting experiment was used to analyze the targeted binding of NORAD to miR-363-3p. **Results:** The expression of NORAD was greatly up-regulated in multiple myeloma cell lines U266, LP-1 and H929 ($P < 0.05$), while the expression of miR-363-3p was obviously down-regulated ($P < 0.05$). Silencing NORAD expression significantly decreased the activity of U266 cells at 48 and 72 h and cyclin D1, CDK4, bcl-2 protein levels ($P < 0.05$), and evidently increased the apoptotic rate and the expression of cleaved caspase-3 and Bax protein ($P < 0.05$). NORAD targeting regulated the expression of miR-363-3p. Inhibiting the expression of miR-363-3p reversed the inhibition of silenced NORAD expression on the proliferation of U266 cells, the expression of cyclin D1, CDK4 and Bcl-2, and the promotion of silenced NORAD expression on cell apoptosis, cleaved caspase-3 and Bax protein expression. **Conclusion:** LncRNA NORAD affects the proliferation and apoptosis of multiple myeloma cells by targeting the expression of miR-363-3p.

Keywords multiple myeloma; lncRNA NORAD; miR-363-3p; proliferation; apoptosis

多发性骨髓瘤(multiple myeloma)是一种血液恶性肿瘤,其特征在于骨髓中浆细胞的克隆增殖^[1-2]。多发性骨髓瘤占有所有癌症的1%,占有所有血液系统恶性肿瘤的约10%^[3]。目前,多发性骨髓瘤的病因和分子机制尚不清楚。尽管蛋白酶体抑制剂、免疫调节药物、自体干细胞移植等治疗手段取得了进展^[4],但多发性骨髓瘤仍然无法治愈,导致大多数患者复发^[5]。因此,寻找补充或替代现有策略的治疗方法以延长患者生存率尤为重要。长链非编码RNA(long noncoding RNA, lncRNA)长度>200个核苷酸,在细胞增殖、分化、凋亡和迁移等生物学过程中起关键作用^[6]。研究表明:lncRNA NORAD在乳腺癌中表达上调,过表达NORAD促进乳腺癌细胞的增殖、迁移和侵袭^[7],NORAD通过抑制微小RNA-136-5p(miRNA-136-5p, miR-136-5p)功能在非小细胞肺癌中发挥促进生长作用^[8]。然而NORAD对多发性骨髓瘤细胞增殖和凋亡的作用尚不清楚。因此,本研究以多发性骨髓瘤细胞为对象,研究NORAD在多发性骨髓瘤细胞中的表达,并探讨其对细胞增殖和凋亡的影响和作用机制,旨在为多发性骨髓瘤提供新的治疗策略。

1 材料与方法

1.1 材料

健康人成骨细胞(hFOB1.19)、多发性骨髓瘤细胞系(U266, LP-1, H929)购自中国典型培养物

保藏中心细胞库;DMEM, RPMI-1640培养基购自美国Gibco公司;胎牛血清购自美国Hyclone公司;TRIzol, lipofectamine 2000购自美国Invitrogen公司;胰酶、噻唑蓝(Methyl thiazolyl tetrazolium, MTT)、RIPA裂解液购自美国Sigma公司;si-NORAD, pcDNA-NORAD, miR-363-3p, anti-miR-363-3p购自广州锐博生物有限公司;Annexin V-FITC/PI细胞凋亡检测试剂盒购自江苏凯基生物公司;ELC发光液购自美国Millipore公司; β 肌动蛋白(β -actin)、细胞周期蛋白D1(cyclin D1)、细胞周期蛋白依赖性激酶4(cyclin-dependent kinase 4, CDK4)、活化的含半胱氨酸的天冬氨酸蛋白水解酶3(cleaved cysteinyl aspartate specific proteinase 3, cleaved caspase-3)、B细胞淋巴瘤/白血病-2(B cell lymphoma/leukemia 2, Bcl-2)、Bcl-2相关X蛋白(bcl-2 associated X protein, Bax)抗体购自美国Cellular Signaling Technology公司;辣根过氧化物酶标记二抗购自博士德生物公司;荧光素酶报告载体购自上海吉玛生物公司。ABI 7500荧光定量PCR仪购自美国ABI公司,细胞培养瓶购自上海生工科技有限公司,酶标仪购自德国Eppendorf公司。

1.2 细胞培养

健康人成骨细胞hFOB1.19加入DMEM培养基(含10%胎牛血清),多发性骨髓瘤细胞系U266, LP-1, H929加入RPMI-1640培养基(含10%胎牛血清),置于5% CO₂, 37 °C培养箱中培养,每周换液

2~3次, 细胞生长至70%~80%融合度时, 加入胰酶消化传代。

1.3 RT-qPCR 检测 NORAD 和 miR-363-3p 表达

细胞中加入 TRIzol 充分提取总 RNA, 反转录成 cDNA, 以 cDNA 为模板, 进行 qPCR 检测。NORAD 正向引物序列为: 5'-TGATAGGATACATCTTGGACATGGA-3', 反向引物序列为: 5'-AACCTAATGAACAAGTCCTGACATACA-3'; miR-363-3p 正向引物序列为: 5'-CGAATTGCACGGTATCCATCT-3', 反向引物序列为: 5'-GTGCAGGGTCCGAGGT-3'。以 U6 为内参, 利用 $2^{-\Delta\Delta Ct}$ 法计算 NORAD 和 miR-363-3p 相对表达量。

1.4 细胞转染

转染前 24 h, 收集处于对数生长期的 U266 细胞, 密度调整成 1×10^5 个/mL, 并接种至 6 孔细胞板, 细胞生长至 70% 融合度时, 严格按照 lipofectamine 2000 说明书操作, 在细胞中转染 si-NORAD, pcDNA-NORAD, miR-363-3p, anti-miR-363-3p 及各自阴性对照, 培养 48 h, 收集细胞进行后续实验。

1.5 MTT 法检测细胞增殖

使用胰酶消化转染后的 U266 细胞, 密度调整成 5×10^4 个/mL, 并接种于 96 孔细胞板, 分别培养 24, 48, 72 h。培养结束后, 每孔细胞中加入 100 μ L MTT 溶液 (5 mg/mL), 37 $^{\circ}$ C 培养 4 h, 弃去上清, 每孔添加 200 μ L 二甲基亚砷 (Dimethyl sulphoxide, DMSO), 37 $^{\circ}$ C 摇床震荡 10 min, 置酶标仪读取 490 nm 波长处的吸光度值 (OD_{490 nm} 值), OD_{490 nm} 值越大的 U266 细胞活性越强。

1.6 流式细胞术检测细胞凋亡

收集转染后的 U266 细胞 1×10^5 个, 将细胞重悬于 500 μ L 缓冲液, 加入 Annexin V-FITC 5 μ L 混匀, 加入 PI 5 μ L 混匀, 室温避光条件下反应 10 min, 置流式细胞仪检测细胞凋亡情况。

1.7 蛋白质印迹法检测目的蛋白表达

使用 RIPA 裂解液充分提取 U266 细胞总蛋白, 蛋白样品经沸水变性, 进行电泳, 转聚偏二氟乙烯 (polyvinylidene fluoride, PVDF) 膜。之后在 5% 脱脂奶粉中封闭 1 h; 加入相应一抗 (稀释度为 1:1 000), 4 $^{\circ}$ C 孵育过夜, 用 Tris-HCl-Tween 缓冲盐

溶液 (Tris buffered saline with Tween, TBST) 充分洗膜, 加入二抗 (稀释度为 1:5 000), 室温孵育 2 h, TBST 充分洗膜, ECL 发光液显影, 以 β -actin 为内参蛋白, 进行目的蛋白相对表达量的分析。

1.8 双荧光素酶报告实验

StarBase 网站 (<http://starbase.sysu.edu.cn/>) 预测出 NORAD 的靶基因 miR-363-3p。分别构建野生型 NORAD (WT-NORAD)、突变型 NORAD (MUT-NORAD) 的 3'-非编码区域 (3'-untranslated region, 3'-UTR) 荧光素酶报告基因质粒, 并与 miR-363-3p 共转染, 培养 48 h, 测定 U266 细胞双荧光素酶活性。

1.9 统计学处理

采用 SPSS 22.0 统计软件进行数据分析。计量资料以均数 \pm 标准差 ($\bar{x} \pm s$) 表示, 两组间数据比较采用 *t* 检验, 多组数据间比较采用单因素方差分析 (one-way analysis of variance), 组间多重比较用 SNK-*q* 检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 多发性骨髓瘤细胞系中 NORAD 和 miR-363-3p 的表达

RT-qPCR 检测结果显示 (表 1): 与健康人成骨细胞 hFOB1.19 比较, 多发性骨髓瘤细胞系 U266, LP-1 和 H929 中 NORAD 表达量明显增加 ($P < 0.05$), miR-363-3p 表达量显著降低 ($P < 0.05$)。选择 NORAD 表达量差异最显著的 U266 细胞进行后续研究。

表 1 多发性骨髓瘤细胞系和健康人成骨细胞中 NORAD 和 miR-363-3p 的表达 ($n=9$, $\bar{x} \pm s$)

Table 1 Expression of NORAD and miR-363-3p in multiple myeloma cell lines and healthy human osteoblasts ($n=9$, $\bar{x} \pm s$)

组别	NORAD	miR-363-3p
hFOB1.19	1.00 \pm 0.07	1.00 \pm 0.08
U266	4.48 \pm 0.35*	0.45 \pm 0.04*
LP-1	3.33 \pm 0.38*	0.58 \pm 0.06*
H929	4.33 \pm 0.53*	0.38 \pm 0.05*
F	168.119	196.830
P	<0.001	<0.001

与 hFOB1.19 组比较, * $P < 0.05$ 。

Compared with hFOB1.19 group, * $P < 0.05$.

2.2 干扰 NORAD 对多发性骨髓瘤细胞 U266 增殖的影响

在U266细胞中转染si-NORAD, NORAD表达量明显低于si-con组($P<0.05$, 表2)。MTT检测结果显示(表2): 与si-con组比较, 沉默NORAD

表达对24 h的细胞活性无明显影响, 显著降低48, 72 h的细胞活性($P<0.05$)。蛋白质印迹法检测结果显示(图1, 表2): 与si-con组比较, 沉默NORAD表达显著降低cyclin D1和CDK4蛋白水平($P<0.05$)。

表2 沉默NORAD表达对多发性骨髓瘤细胞U266增殖和cyclin D1, CDK4蛋白表达的影响($n=9, \bar{x} \pm s$)

Table 2 Effect of silencing NORAD expression on proliferation of multiple myeloma cells U266 and expression of cyclin D1 and CDK4 proteins ($n=9, \bar{x} \pm s$)

组别	NORAD	细胞活性 (OD _{490 nm})			Cyclin D1	CDK4
		24 h	48 h	72 h		
NC	1.00 ± 0.07	0.38 ± 0.02	0.71 ± 0.05	0.99 ± 0.05	0.87 ± 0.06	0.37 ± 0.03
si-con	0.96 ± 0.09	0.36 ± 0.03	0.65 ± 0.07	0.93 ± 0.07	0.78 ± 0.08	0.41 ± 0.05
si-NORAD	0.23 ± 0.04*	0.35 ± 0.04	0.47 ± 0.06*	0.68 ± 0.06*	0.25 ± 0.04*	0.18 ± 0.03*
<i>F</i>	347.486	2.172	38.291	66.355	261.233	94.814
<i>P</i>	<0.001	0.136	<0.001	<0.001	<0.001	<0.001

与 si-con 组比较, * $P<0.05$ 。

Compared with the si-con group, * $P<0.05$ 。

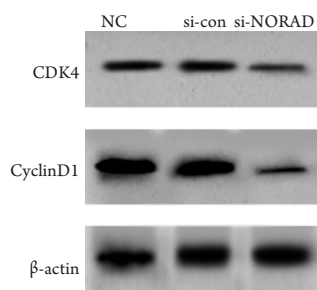


图1 蛋白质印迹法检测多发性骨髓瘤细胞U266增殖相关蛋白表达

Figure 1 U266 proliferation-related protein expression in multiple myeloma cells detected by Western blotting

2.3 干扰 NORAD 对多发性骨髓瘤细胞 U266 凋亡的影响

流式细胞术检测结果显示(图2A, 表3), 沉默NORAD表达的U266细胞凋亡率显著高于si-con组($P<0.05$)。蛋白质印迹法检测结果表明(图2B, 表3): 与si-con组比较, 沉默NORAD表达明显增加U266细胞中cleaved caspase-3和Bax蛋白表达量($P<0.05$), 显著降低Bcl-2蛋白水平($P<0.05$)。

2.4 NORAD 靶向调控 miR-363-3p 的表达

StarBase网站(<http://starbase.sysu.edu.cn/>)预测结果显示: miR-363-3p与NORAD的3'-UTR区域

部分核苷酸可形成互补配对(图3)。双荧光素酶报告实验结果表明: miR-363-3p对WT-NORAD荧光素酶相对活性具有明显抑制作用($P<0.05$, 表4), 对MUT-NORAD荧光素酶相对活性无明显影响。转染si-NORAD较si-con组明显增加miR-363-3p表达量, 转染pcDNA-NORAD较pcDNA组显著降低miR-363-3p表达量($P<0.05$, 表5)。

2.5 干扰 NORAD 表达和干扰 miR-363-3p 表达对多发性骨髓瘤细胞 U266 增殖和凋亡的作用

与si-con+anti-miR-con组比较, si-con+anti-miR-363-3p组U266细胞中miR-363-3p表达量明显减少($P<0.05$), 对24 h的细胞活性无显著影响($P>0.05$), 但48和72 h的细胞活性提高($P<0.05$), 细胞凋亡率降低($P<0.05$, 表6); cyclin D1, CDK4, Bcl-2蛋白水平提高, cleaved caspase-3和Bax蛋白表达量减少($P<0.05$, 表7, 图4)。与si-NORAD+anti-miR-con组比较, 共转染si-NORAD和anti-miR-363-3p对24 h的U266细胞活性无明显影响, 显著提高48, 72 h的细胞活性($P<0.05$); 同时U266细胞凋亡率明显降低, miR-363-3p, cleaved caspase-3蛋白, Bax蛋白表达量减少($P<0.05$), 而cyclin D1, CDK4和Bcl-2蛋白水平显著提高($P<0.05$, 表6, 表7, 图4)。

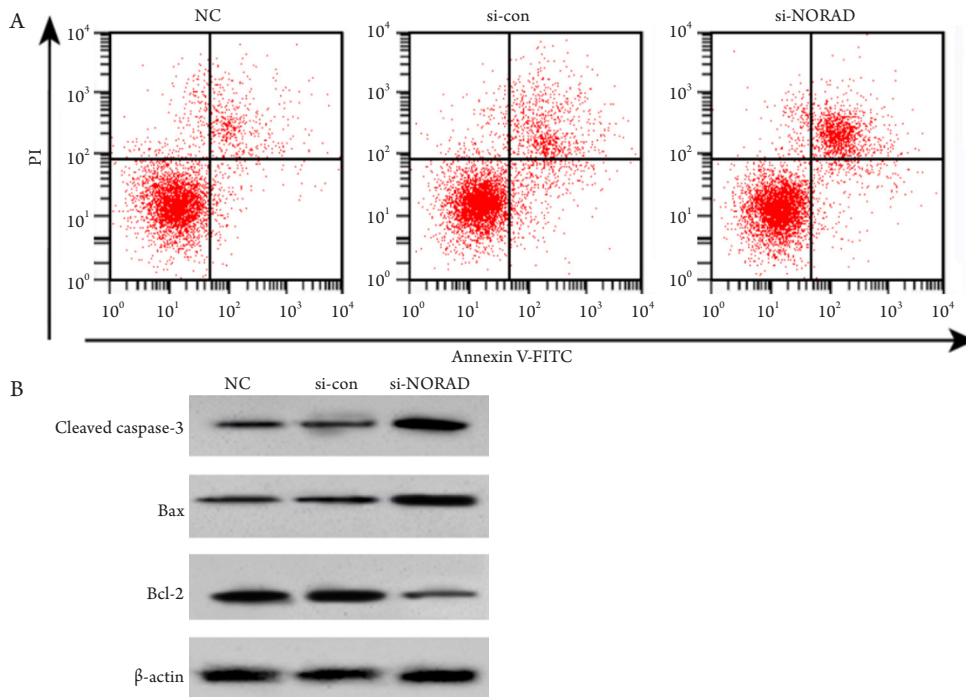


图2 沉默NORAD表达对多发性骨髓瘤细胞凋亡和凋亡相关蛋白表达的影响

Figure 2 Effect of silencing NORAD expression on apoptosis and expression of apoptosis-related proteins in multiple myeloma cells

(A) 流式细胞术检测多发性骨髓瘤细胞凋亡率; (B) 蛋白质印迹法检测多发性骨髓瘤细胞凋亡相关蛋白表达。

(A) Flow cytometry detects apoptosis rate of multiple myeloma cells; (B) Detection of apoptosis-related protein expression in multiple myeloma cells by Western blotting.

表3 沉默NORAD表达对多发性骨髓瘤细胞U266凋亡及其相关蛋白表达的影响(n=9, $\bar{x} \pm s$)

Table 3 Effect of silencing NORAD expression on apoptosis and related protein expression in multiple myeloma cells U266 (n=9, $\bar{x} \pm s$)

组别	凋亡率/%	Cleaved caspase-3	Bcl-2	Bax
NC	6.09 ± 0.51	0.43 ± 0.04	0.86 ± 0.07	0.57 ± 0.05
si-con	8.31 ± 0.71	0.48 ± 0.05	0.84 ± 0.08	0.53 ± 0.06
si-NORAD	22.38 ± 1.87*	0.68 ± 0.08*	0.52 ± 0.05*	0.75 ± 0.07*
F	494.510	45.000	71.217	33.709
P	<0.001	<0.001	<0.001	<0.001

与si-con组比较, *P<0.05。

Compared with the si-con group, *P<0.05.

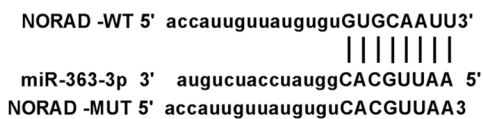


图3 NORAD的3'-UTR中含有与miR-363-3p互补的核苷酸序列

Figure 3 3'-UTR of NORAD contains a nucleotide sequence complementary to miR-363-3p

表4 双荧光素酶报告实验(n=9, $\bar{x} \pm s$)

Table 4 Dual luciferase reporter experiment (n=9, $\bar{x} \pm s$)

组别	WT-NORAD	MUT-NORAD
miR-con	1.00 ± 0.05	0.96 ± 0.09
miR-363-3p	0.43 ± 0.07*	1.03 ± 0.12
t	19.878	1.400
P	<0.001	0.181

与miR-con组比较, *P<0.05。

Compared with the miR-con group, *P<0.05.

表5 NORAD调控miR-363-3p的表达(n=9, $\bar{x} \pm s$)

Table 5 NORAD regulates the expression of miR-363-3p (n=9, $\bar{x} \pm s$)

组别	miR-363-3p
si-con	1.00 ± 0.08
si-NORAD	6.31 ± 0.55*
pcDNA	0.89 ± 0.06
pcDNA-NORAD	0.32 ± 0.04 [#]
F	900.210
P	<0.001

与 si-con 组比较, *P<0.05; 与 pcDNA 组比较, [#]P<0.05。

Compared with the si-con group, *P<0.05; compared with the pcDNA group, [#]P<0.05.

表6 抑制miR-363-3p表达逆转沉默NORAD表达对多发性骨髓瘤细胞U266增殖和凋亡的作用($n=9, \bar{x} \pm s$)Table 6 Inhibition of miR-363-3p expression reversed the effect of silencing NORAD expression on proliferation and apoptosis of multiple myeloma cell line U266 ($n=9, \bar{x} \pm s$)

组别	miR-363-3p	细胞活性 (OD _{490 nm})			凋亡率 /%
		24 h	48 h	72 h	
si-con + anti-miR-con	1.00 ± 0.07	0.35 ± 0.04	0.72 ± 0.06	1.03 ± 0.07	7.53 ± 0.63
si-con + anti-miR-363-3p	0.67 ± 0.06*	0.36 ± 0.03	0.99 ± 0.09*	1.37 ± 0.15*	4.55 ± 0.42*
si-NORAD + anti-miR-con	5.84 ± 0.55	0.32 ± 0.05	0.44 ± 0.05	0.64 ± 0.05	21.78 ± 1.88
si-NORAD + anti-miR-363-3p	1.94 ± 0.12 [#]	0.34 ± 0.04	0.63 ± 0.06 [#]	0.89 ± 0.07 [#]	15.33 ± 1.05 [#]
F	626.643	1.591	105.775	95.957	418.871
P	<0.001	0.211	<0.001	<0.001	<0.001

与 si-con + anti-miR-con 组比较, * $P<0.05$; 与 si-NORAD + anti-miR-con 组比较, [#] $P<0.05$ 。

Compared with the si-con + anti-miR-con group, * $P<0.05$; compared with the si-NORAD + anti-miR-con group, [#] $P<0.05$.

表7 抑制miR-363-3p表达和沉默NORAD表达对多发性骨髓瘤细胞U266增殖和凋亡相关蛋白表达的作用($n=9, \bar{x} \pm s$)Table 7 Effect of inhibition of miR-363-3p expression and silencing of NORAD expression on proliferation and apoptosis-related protein expression in multiple myeloma cells U266 ($n=9, \bar{x} \pm s$)

组别	Cyclin D1	CDK4	Cleaved caspase-3	Bax	Bcl-2
si-con + anti-miR-con	0.78 ± 0.08	0.88 ± 0.08	0.43 ± 0.04	0.48 ± 0.05	0.52 ± 0.05
si-con + anti-miR-363-3p	0.93 ± 0.09*	1.17 ± 0.12*	0.27 ± 0.03*	0.36 ± 0.04*	0.79 ± 0.09*
si-NORAD + anti-miR-con	0.36 ± 0.06	0.31 ± 0.05	0.81 ± 0.08	0.88 ± 0.09	0.42 ± 0.05
si-NORAD + anti-miR-363-3p	0.56 ± 0.07 [#]	0.48 ± 0.05 [#]	0.61 ± 0.07 [#]	0.57 ± 0.05 [#]	0.67 ± 0.08 [#]
F	97.709	210.884	141.217	121.041	49.108
P	<0.001	<0.001	<0.001	<0.001	<0.001

与 si-con + anti-miR-con 组比较, * $P<0.05$; 与 si-NORAD + anti-miR-con 组比较, [#] $P<0.05$ 。

Compared with the si-con + anti-miR-con group, * $P<0.05$; compared with the si-NORAD + anti-miR-con group, [#] $P<0.05$.

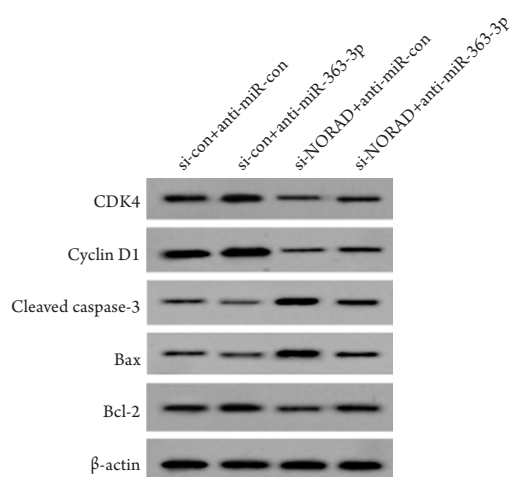


图4 蛋白质印迹法检测多发性骨髓瘤细胞中蛋白表达

Figure 4 Protein expression in multiple myeloma cells detected by Western blotting

3 讨论

LncRNA 是一类非编码 RNA, 不能编码蛋白质^[9]。越来越多的证据^[10]表明: lncRNA 参与调控多发性骨髓瘤发生和进展的不同阶段, 如 HOTAIR, MALAT1 及 MEG3。NORAD 作为肿瘤促进因子^[11], 有可能成为癌症诊断和预测的标志物。Yu 等^[12]研究发现: NORAD 在胃癌细胞中表达上调; 下调 NORAD 表达明显抑制胃癌细胞 AGS 和 BGC-823 存活率、迁移和侵袭, 提高细胞凋亡、cleaved caspase-3 和 Bax 水平, 并降低 Bcl-2 蛋白表达, 证明了 NORAD 在胃癌进展中的致癌作用。Zhang 等^[13]报道指出: NORAD 表达在结直肠癌 (colorectal cancer, CRC) 组织中显著上调, 其表达与 CRC 转移和患者预后不良呈正相关; 敲除

NORAD明显抑制CRC细胞增殖、迁移和侵袭,在体外诱导细胞凋亡。He等^[14]和Yang等^[15]学者的研究表明NORAD在乳头状甲状腺癌和肝细胞癌组织与细胞系中表达上调,NORAD过表达显著促进癌细胞的增殖、侵袭和迁移。本研究中NORAD在多发骨髓瘤细胞系U266, LP-1和H929中NORAD表达量明显增加($P<0.05$),沉默NORAD表达显著降低48, 72 h的U266细胞活性($P<0.05$),降低cyclin D1, CDK4和bcl-2蛋白水平,增加U266细胞凋亡率、cleaved caspase-3和Bax蛋白表达量,说明NORAD在多发骨髓瘤进展中充当致癌基因,沉默NORAD表达抑制多发骨髓瘤细胞增殖,并诱导其凋亡,与上述研究结果一致。

大量资料显示:微RNA(microRNA, miRNA)在多发骨髓瘤细胞增殖、凋亡、转移等生物学过程中扮演重要角色^[16-18],如miR-340-5p抑制多发骨髓瘤^[19],miR-15a和miR-16的下调促进多发骨髓瘤增殖^[20]。研究^[21-23]发现:miR-363-3p抑制非小细胞肺癌、胶质瘤及肝癌等肿瘤细胞增殖。MiR-363在多发骨髓瘤患者中表达水平低于健康对照样本,miR-363过表达使多发骨髓瘤细胞生长明显被抑制,破骨细胞减少,成骨细胞增加,骨髓抗肿瘤免疫增强^[24]。MiR-363-3p在骨肉瘤组织中表达显著下调,miR-363-3p过表达抑制骨肉瘤U2OS和MG63细胞的增殖、迁移和侵袭^[25]。本实验中miR-363-3p在多发骨髓瘤细胞中表达下调,这与前述研究结果相符,提示miR-363-3p可能影响多发骨髓瘤进程。

LncRNA和miRNA之间的表观遗传相互作用在调控肿瘤发生、发展、转移或凋亡发挥着重要作用^[26-27],CCAT1调控miR-181a-5p^[28],PVT1调控miR-203a^[29]参与多发骨髓瘤发生发展过程,在本实验中,利用生物学信息预测和双荧光素酶报告实验证实NORAD靶向调控miR-363-3p的表达,另外,si-NORAD明显增加miR-363-3p表达量,pcDNA-NORAD则反之,抑制miR-363-3p表达逆转了沉默NORAD表达抑制U266细胞增殖并促进细胞凋亡的作用,由此得出NORAD在多发骨髓瘤细胞增殖和凋亡过程中是通过调控miR-363-3p表达发挥作用的。

综上所述,LncRNA NORAD在多发骨髓瘤细胞中表达上调,NORAD通过靶向抑制miR-363-3p影响多发骨髓瘤细胞增殖和凋亡,为多发骨髓瘤提供新的生物标志物和潜在治疗靶点。

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