miR-382-5p通过靶基因*PTEN*阻滞NB4细胞分化

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摘要 该文主要研究在急性早幼粒细胞白血病NB4细胞中, miR-382-5p通过调控靶基因*PTEN*(phosphatase and tensin homologue)抑制了全反式维甲酸(all-trans retinoic acid, ATRA)诱导的急性早幼粒细胞分化。我们运用ATRA(1 μmol/L)诱导细胞分化; Western blot检测PTEN及髓系分化标志物CD11b的蛋白质水平; 实时荧光定量PCR检测miR-382-5p的表达水平; 过表达*PTEN*的慢病毒载体分别感染NB4细胞和HL-60、THP-1细胞; 脂质体转染miR-382-5p的模拟剂(mimics)和特异性抑制剂(inhibitors)NB4细胞。结果显示, PTEN促进ATRA诱导的NB4细胞分化, 而在HL-60和THP-1细胞中并无明显促分化效应。NB4细胞中, 脂质体转染miR-382-5p mimics在mRNA和蛋白水平均抑制了PTEN的表达, 并且抑制了ATRA诱导的分化; 转染miR-382-5p inhibitors则恢复了PTEN表达, 同时促进了ATRA诱导的急性早幼粒细胞NB4细胞的分化。该文结果提示, miR-382-5p靶向抑制了*PTEN*的表达从而抑制ATRA诱导的NB4细胞分化。

关键词 *PTEN*; microR-382-5p; 急性早幼粒细胞白血病; 全反式维甲酸; 分化

miR-382-5p Blocks the Differentiation of NB4 Cells Through Targeting *PTEN*

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Abstract In order to investigate the regulatory effects and mechanism of *PTEN* (phosphatase and tensin homologue) and miR-382-5p on ATRA (all-trans retinoic acid)-induced differentiation of acute promyelocytic leukemia cell line NB4. Enforced expression of PTEN in APL cells (NB4) and no-APL cells (HL-60, THP-1) was achieved through a lentivirus vector. The miR-382-5p mimic, inhibitor and negative controls were infected into NB4 cells using Lipofectamine 2000. ATRA, a typical regent was to induced granulocytic differentiation. The protein levels of CD11b, PTEN were detected by Western blot. The mRNA expression of miRNA-382-5p and *PTEN* was measured by quantitative Real-time polymerase chain reaction (qRT-PCR). Data showed that overexpression of PTEN promoted ATRA-induced differentiation in APL cell line NB4 compared to no-APL line HL-60 and THP-1. Enforced

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expression of miR-382-5p attenuated expression of PTEN and cell differentiation marker CD11b. Conversely, down-regulation of miR-382-5p, increased the expression of PTEN and CD11b. It was concluded that miR-382-5p modulated ATRA-induced differentiation of APL cells by regulating its potential target *PTEN*.

Keywords phosphatase and tensin homologue; microRNA-382-5p; acute promyelocytic leukemia; all-trans retinoic acid; differentiation

急性早幼粒细胞白血病(acute promyelocytic leukemia, APL)是急性髓系白血病(acute myeloid leukemia, AML)的一种特殊亚型, 主要致病机制是15号染色体上的急性早幼粒细胞基因(promyelocytic leukemia, *PML*)与17号染色体上的维甲酸受体α(retinoic acid receptor α, *RARα*)易位形成*PML/RARα*融合基因[1-2]。*PML/RARα*融合基因的形成扰乱了PML核体的组装并使RARα对生理浓度的维甲酸不敏感[3-5], 导致细胞大量阻滞在早幼粒细胞阶段。体内外实验均证实, 药理浓度的全反式维甲酸(all-trans retinoic acid, ATRA)诱导早幼粒白血病细胞迅速分化成为成熟粒细胞[6]。临床使用ATRA联合三氧化二砷使APL患者缓解率可达到90%[7]。但ATRA诱导分化治疗的机制尚不完全, 这使APL的治疗仍存在一些问题, 如对ATRA的治疗抵抗, 复发耐药等[8-10]。因此, 进一步探究APL的发病机理以及ATRA的治疗机制十分必要。

*PTEN*是一种具有蛋白磷酸酶和脂质磷酸酶双重磷酸酶活性的抑癌基因, 通过抑制PI3K/AKT/mTOR通路调控多种细胞进程, 如生存、增殖、细胞的代谢和构建等[11]。研究显示, PTEN在许多肿瘤中存在缺失或者突变[12], 随着研究的深入, 我们发现PTEN在造血祖/干细胞的增殖和分化中也起着重要的作用[13-14]。

微小RNA(microRNA)是一种由19~25个核苷酸组成的小非编码RNA, 在细胞凋亡、增殖和分化等过程中发挥重要调控作用[15-17]。临床APL患者和APL细胞株NB4在全反式维甲酸处理之后, miR-15a、miR-15b、miR-223等水平明显上调, 而miR-181b水平下调[18]。已有报道指出, miR-181a和miR-181b作为APL中PML/RARa相关的致癌miRNA, 证明了*RASSF1A*作为APL诱导的粒细胞分化中的关键因子[19]。对52例具有常见易位的急性骨髓性白血病患者样本进行全基因组miRNA表达分析发现, 相比其他染色体易位组[inv(16)、t(8;21)、t(9;11)]以及CD34+的正常造血干/祖细胞, t(15;17)/PML/RARa患者骨髓中miR-382-5p表达明显上调[20-21]。miR-382-5p在APL中的报道较少, 这促使我们进一步探究miR-382-5p在APL中的作用及其相关机制。

1 材料与方法

1.1 材料

全反式维甲酸(ATRA)购自美国Sigma公司, 用助溶剂二甲基亚砜(DMSO)溶解成0.01 mol/L的母液, 于–80 ℃保存待用。人早幼粒白血病细胞株NB4细胞、人髓系白血病细胞株HL-60和THP-1细胞均由重庆医科大学临床检验诊断学教育部重点实验室提供。

1.2 试剂及仪器

胎牛血清购自美国Gemini公司。RPMI-1640购自美国Gibco公司。PTEN慢病毒(载体带荧光蛋白GFP及Flag标 签)、miR-382-5p mimics与inhibitor购自上海吉玛制药技术有限公司。Lipofectamine购自美国Invitrogen公司。兔抗人PTEN、CD11b单克隆抗体和嘌呤霉素均购自英国Abcam公司。流式抗体CD11b购自美国Biolegend公司。鼠抗人β-actin单克隆抗体购自上海博士德生物技术有限责任公司。RNA提取试剂、逆转录试剂盒、SYBR Green PCR试剂盒购于日本TaKaRa公司。miR-382-5p引物购自上海吉玛制药技术有限公司。其他引物均由生工生物工程(上海)股份有限公司合成。

仪器包括: 细胞培养皿(美国Thermo Fisher Scifentific公司)、倒置荧光显微镜(日本Nikon公司)、凝胶成像系统和荧光定量PCR仪(美国Bio-Rad公司)。

1.3 细胞培养

NB4细胞培养于RPMI-1640培养基中, 内含有10%新鲜胎牛血清、100 μg/mL青霉素和100 μg/mL链霉素, 置于37 ℃、5.0% CO_2的饱和湿度培养箱中培养, 待细胞汇合达到70%时进行传代培养。

1.4 过表达*PTEN*慢病毒感染细胞

取对数生长期细胞, 以5×10^{4}个/孔接种于24孔

板中, 以最适感染复数(MOI=50)加入病毒溶液, 同时加入1 mg/mL polybrence, 培养24 h后加入新鲜培养基继续培养。病毒感染7天后荧光显微镜观察感染效率, 用含5 μg/mL嘌呤霉素培养基培养2~3周, 至筛选出稳定细胞, 冻存以供后续实验。

1.5 miR-382-5p模拟物(mimics)和抑制剂(inhibitors)转染NB4细胞

取对数生长期细胞, 以1×10^{6}个/孔接种于6孔板中, 根据lipofectamine说明书加入mimics、inhibitors和相应量的转染试剂。转染24 h后加入新鲜培养基, 48 h后换液, 加1 μmol/L ATRA诱导细胞分化。

1.6 qRT-PCR(quantitative Real-time polymerase chain reaction)

采用RNAiso Plus提取总RNA, 分光光度法测定计算提取的总RNA含量及浓度, RNA样本的D_{260}/D_{280}介于1.8~2.2, D_{260}/D_{230}大于1.9时继续进行检测。使用逆转录试剂盒将RNA反转录为cDNA, 进行qRT-PCR检测miR-382-5p和*PTEN*, *U6*和*β-actin*作为相应内参, 采用$2^{-\Delta\Delta Ct}$的方法计算相对表达量, 引物序列见表1。

1.8 流式细胞术检测CD11b表达

细胞经1 μmol/L ATRA作用24、48 h后, 离心收集细胞并用预冷的磷酸盐缓冲液(phosphate buffered solution, PBS)洗涤3次。100 μL PBS重悬细胞, 加入5 μL CD11b-PE荧光抗体轻混匀, 冰上避光孵育30 min。经PBS洗涤3次后, 流式细胞仪检测各组CD11b表达情况。

1.9 瑞士–吉姆萨染色

收集细胞并用预冷PBS洗3次, 适量PBS重悬细胞。取10 μL细胞悬液进行涂片和瑞氏染液染色。待晾干后显微镜下观察各组细胞形态。

1.10 数据分析

采用实验数据以$\bar{x}\pm s$形式表示, 用GraphPad Prism 5软件进行两样本均数t检验, 分析显著性差异。$P<0.05$为差异有统计学意义。

2 结果

2.1 ATRA对急性髓系白血病细胞PTEN的影响

用1 μmol/L的ATRA分别处理NB4、HL-60、THP-1细胞24、48 h, 同时设置阴性对照组(DMSO组), 提取细胞蛋白。Western blot结果显示, ATRA能够诱导急性髓系白血病细胞向中性粒细胞分化, PTEN随细胞表面分化标志物CD11b的增加而增加(图1)。

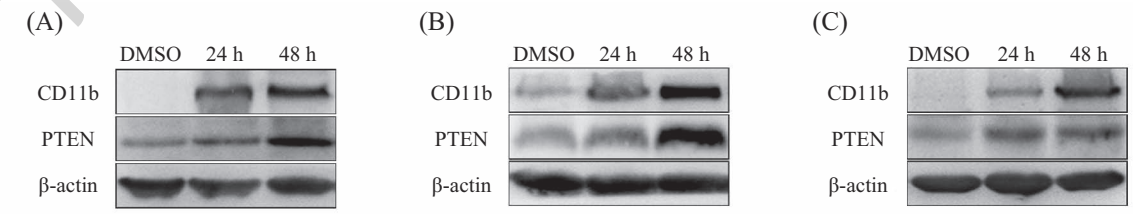
2.2 *PTEN*过表达慢病毒感染细胞的情况

为了探究PTEN在ATRA诱导AML细胞分化中的作用, 我们构建了带Flag标签的*PTEN*过表达慢病毒载体(MOI=50)并分别感染NB4、HL-60、THP-1细胞。用嘌呤霉素(5 mg/mL)筛选7天后观察感染效率(图2A), 继续筛选至Western blot显示已经成功过表达PTEN(图2B)为止, 表明已经获得稳定过表达的

表1 引物序列

Table 1 Primer sequences

基因 Genes	正向引物(5′→3′) Forward primer (5′→3′)	反向引物(5′→3′) Reverse primer (5′→3′)
MiR-382-5p	ATC CGT GAA GTT GTT CGT GG	TAT GGT TGT AGA GGA CTC CTT GAC
U6	CTC GCT TCG GCA GCA CA	AAC GCT TCA CGA ATT TGC GT
PTEN	AAG ACA AAG CCA ACC GAT AC	AAG ACA AAG CCA ACC GAT AC
β-actin	TGA CGT GGA CAT CCG CAA AG	CTG GAA GGT GGA CAG CGA GG

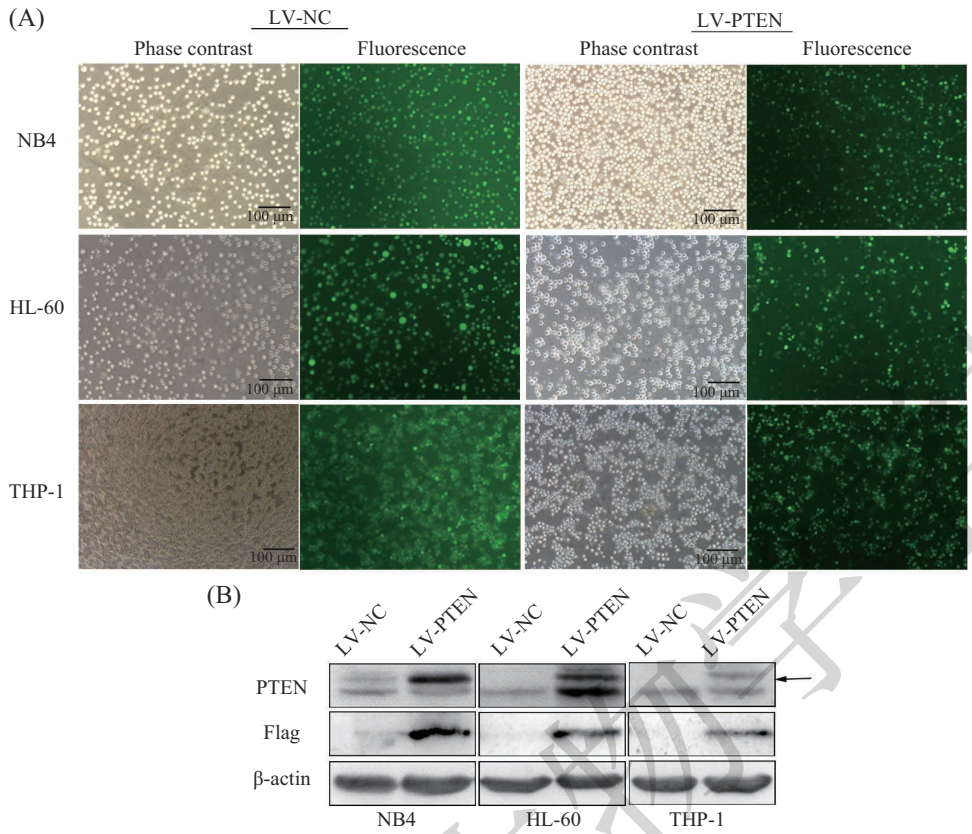


NB4细胞(A)、HL-60细胞(B)和THP-1细胞(C)中CD11b和PTEN的蛋白水平。

The protein expression of CD11b and PTEN were detected by Western blot in NB4 cells (A), HL-60 cells (B), THP-1 cells (C).

图1 ATRA对急性髓系白血病细胞PTEN的影响

Fig.1 Effects of ATRA on protein expression of PTEN in AML cells nverted microscope



A: 荧光显微镜观察感染效率。Fluorescence: 蓝色激发光下观察瞬时感染细胞GFP绿色荧光蛋白; Phase contrast: 相同视野的白光对照。B: NB4、HL-60、THP-1细胞中过表达带Flag标签的PTEN的Western blot检测。 LV-NC: 对照病毒载体感染组; LV-PTEN: *PTEN*过表达病毒载体感染组。

A: efficiency of infection were observed under fluorescence microscope. Fluorescence: observation of transient infected cell line under blue light; Phase contrast: observation of the same field under white light. B: Western blot showed stably expression of PTEN with Flag in NB4, HL-60 and THP-1 cells. LV-NC: comparison lentivirus vector infection group; LV-PTEN: *PTEN* overexpression lentivirus.

图2 *PTEN*过表达慢病毒感染细胞的情况

Fig.2 Effects NB4, HL-60 and THP-1 cells were infected with *PTEN* overexpression lentivirus

细胞株。

2.3 PTEN对ATRA诱导细胞分化的影响

用1 μmol/L的ATRA分别处理稳定过表达*PTEN*的NB4、HL-60、THP-1细胞24、48 h, 同时设置空载病毒组做为对照组(LV-NC组), 提取细胞蛋白。Western blot结果显示, 经1 μmol/L ATRA处理后, 过表达*PTEN*的NB4细胞相比空载病毒组分化明显增强(图3A), 而HL-60、THP-1细胞则无明显差异(图3B和图3C)。细胞形态学显示, 过表达*PTEN*的NB4细胞表示出细胞核/质比例减小、核凹陷的分化趋势; 经ATRA处理48 h后, 细胞核明显变小凹陷, 向中晚幼粒细胞分化更明显(图3D)。流式细胞术检测分化标志物CD11b的结果进一步说明了过表达PTEN促进了ATRA诱导的NB4细胞分化(图3E)。

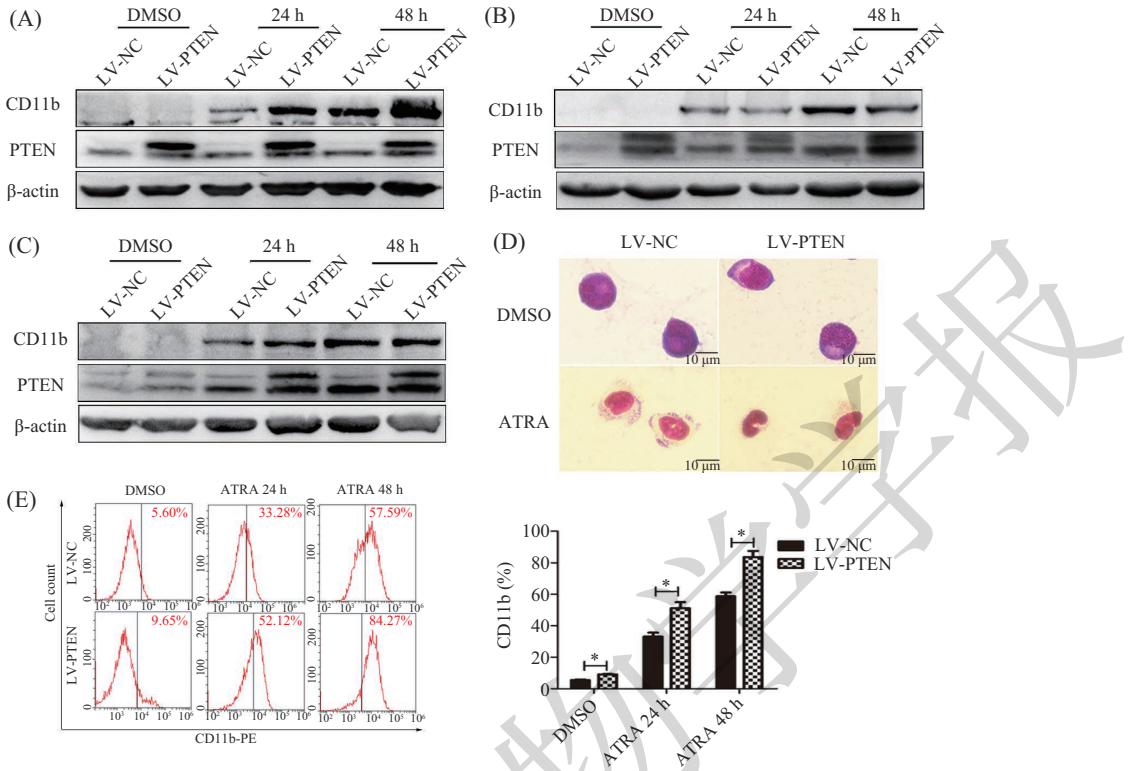
SF1670是一种特异且高效的PTEN抑制剂, 联合1 μmol/L SF1670和1 μmol/L ATRA抑制了ATRA诱导的NB4细胞分化(图4A)。细胞形态学显示, 联合用药组的细胞核凹陷程度较ATRA组降低(图4B), 流式细胞术检测CD11b与Western blot结果相符(图4C)。

2.4 ATRA抑制NB4细胞中miR-382-5p的表达

用1 μmol/L的ATRA分别处理NB4、HL-60、THP-1细胞24、48 h, 同时设置阴性对照组(DMSO组), 提取RNA, qRT-PCR检测miR-382-5p的表达。结果显示: 随着ATRA诱导细胞分化, NB4细胞中miR-382-5p的表达量逐渐降低, 差异有统计学意义(图5A); HL-60(图5B)和THP-1(图5C)细胞中无明显差异。

2.5 NB4细胞中miR-382-5p对PTEN的影响

TargetScan预测miR-382-5p与*PTEN*的结合位点, 如图6A所示。采用对数期生长的NB4细胞, 分别转染miR-382-5p模拟物(mimics)和miR-382-5p抑

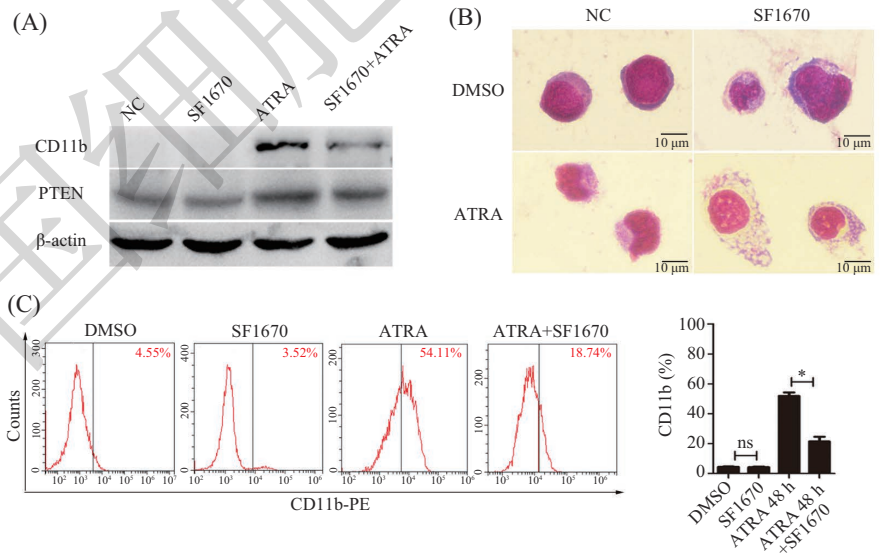


A~C: *PTEN*过表达的NB4细胞(A)、HL-60细胞(B)和THP-1细胞(C)中检测CD11b和PTEN蛋白水平。D: 瑞氏–吉姆萨染色后显微镜油镜观察细胞形态。E: 流式细胞术检测细胞表面标志物CD11b。*P<0.05。

A-C: the protein expression of CD11b and PTEN were detected by western blot in *PTEN* overexpression NB4 cells (A), HL-60 cells (B) and THP-1 cells (C). D: cell morphological changes were imaged with a microscope after stained with Wright-Giemsa solution. E: the expression of CD11b was detected by flow cytometry. *P<0.05.

图3 PTEN促进ATRA诱导的NB4细胞分化

Fig.3 PTEN promoted ATRA-induced differentiation of NB4 cells

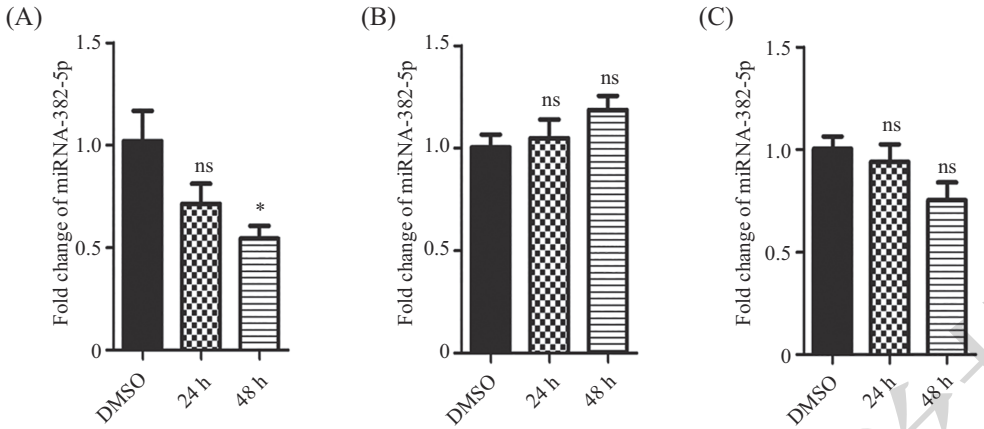


A: ATRA联合SF1670检测PTEN和CD11b的表达。B: 瑞氏–吉姆萨染色后显微镜油镜观察细胞形态。C: 流式细胞术检测细胞表面标志物CD11b, *P<0.05; ns表示无意义。

A: the protein expression of CD11b and PTEN were detected by Western blot after treated with SF1670 combined with ATRA in NB4 cells. B: cell morphological changes were imaged with a microscope after stained with Wright-Giemsa solution. E: the expression of CD11b was detected by flow cytometry. *P<0.05; ns stands for no significance.

图4 抑制PTEN阻碍了ATRA诱导的细胞分化

Fig.4 Knocking down PTEN reduced cell differentiation induced by ATRA

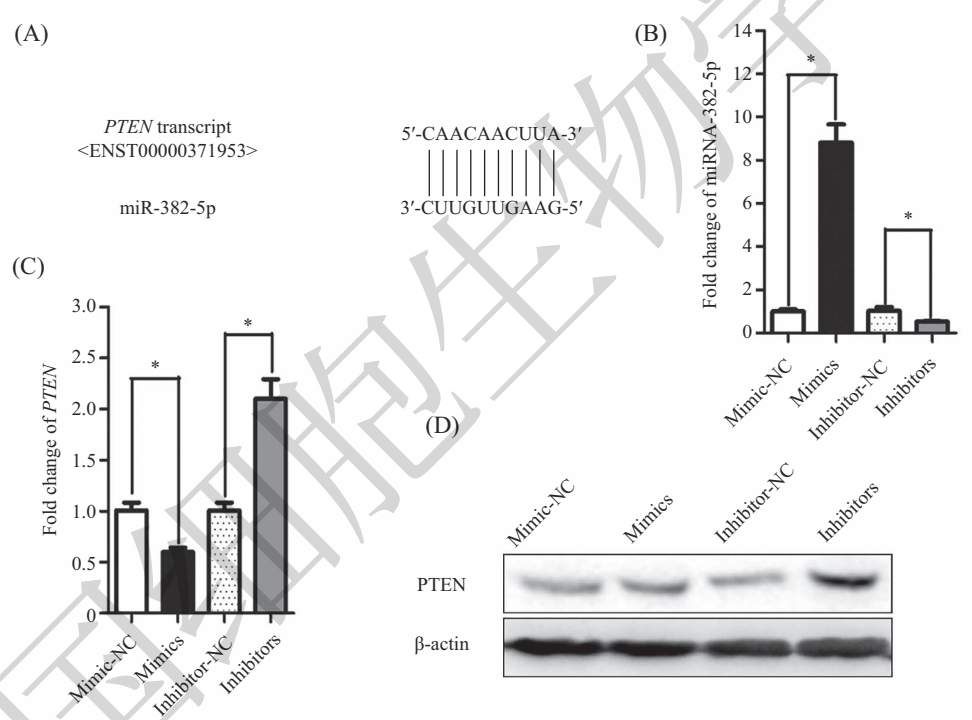


A~C: NB4细胞(A)、HL-60细胞(B)、THP-1细胞(C)中miR-382-5p的表达。*P<0.05, 与对照组相比; ns代表无统计学差异。

A-C: the expression of miRNA-382-5p in NB4 cells (A), HL-60 cells (B) and THP-1 cells (C). *P<0.05 *vs* control group; ns stands for no significance.

图5 ATRA诱导细胞分化后, qRT-PCR检测miR-382-5p的表达

Fig.5 After ATRA treatment, the expression of miRNA-382-5p was detected by qRT-PCR



A: *PTEN*基因与miR-382-5p基因结合位点报告; B: qRT-PCR检测miR-382-5p; C: 转录水平检测*PTEN*; D: Western blot检测PTEN蛋白表达水平。*P<0.05。

A: binding sites of mature miR-382-5p on *PTEN* transcript; B: expression of miR-382-5p was detected by qRT-PCR; C: expression of *PTEN* gene; D: protein level of PTEN. *P<0.05.

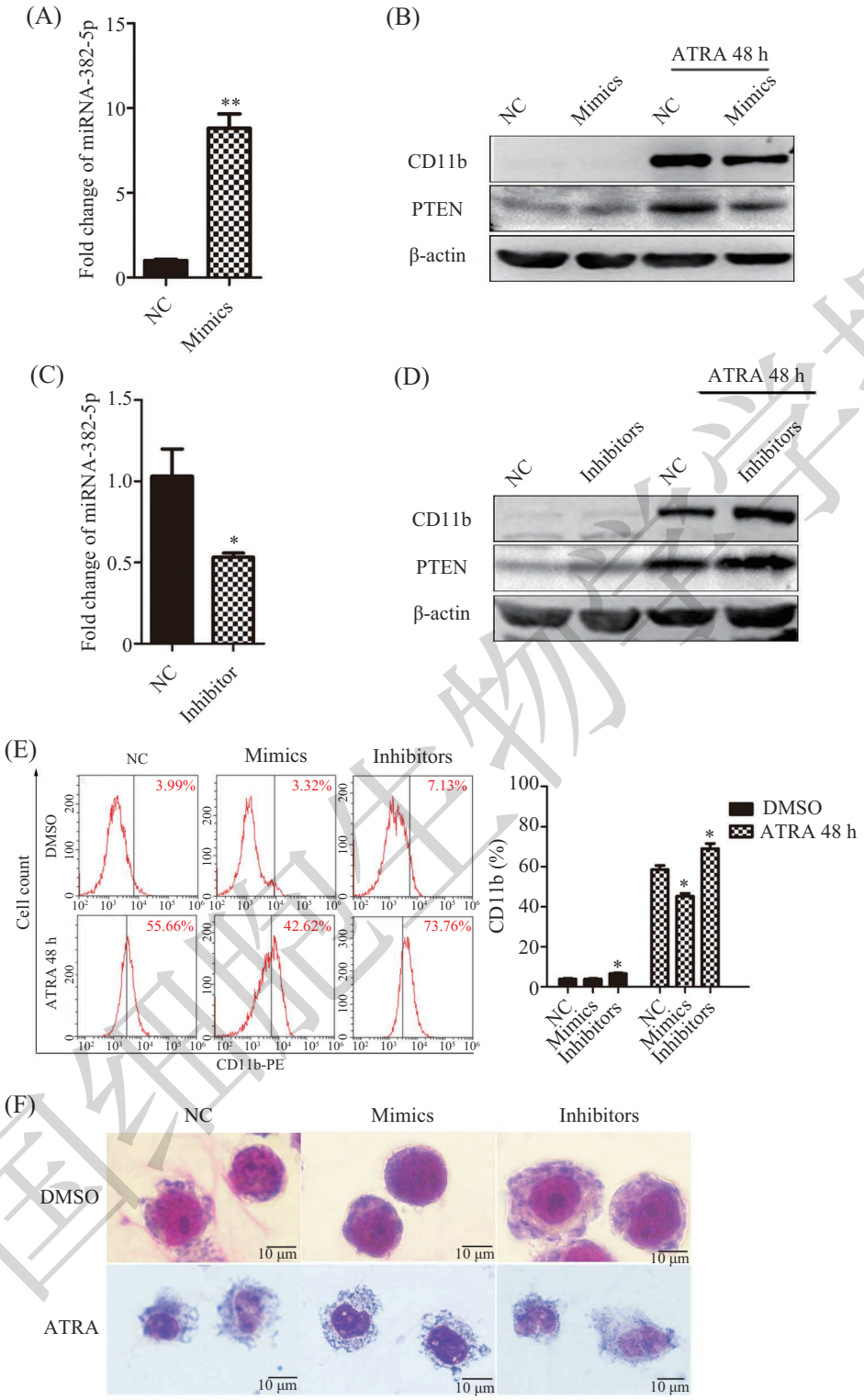
图6 NB4细胞中miR-382-5p对PTEN的影响

Fig.6 Influences of miR-382-5p on PTEN in NB4 cells

制剂(inhibitors), 并转染相应的对照mimics-NC和inhibitors-NC。转染48 h后, qRT-PCR检测转染效率(图6B)及*PTEN*的mRNA表达(图6C); 72 h后Western blot检测PTEN蛋白表达水平(图6D)。

2.6 NB4细胞中miR-382-5p对ATRA诱导分化的影响

NB4细胞分别转染miR-382-5p模拟物(mimics)和miR-382-5p抑制剂(inhibitors), 并转染相应的对照mimics-NC和inhibitors-NC。转染48 h后, 加入1 μmol/L的ATRA处理48 h, 提取蛋白。qRT-PCR检测转染效能(图7A和图7C), Western blot结果显示, 转染miR-382-5p模拟物(mimics)抑制了ATRA诱导的细胞分化(图7B), 而抑制miR-382-5p则促进了分化(图



A: 转染miR-382-5p模拟物(mimics) 48 h后检测miR-382-5p; B: 转染miR-382-5p模拟物(mimics)后, Western blot检测细胞分化标志物CD11b; C: 转染miR-382-5p模拟物(inhibitors) 48 h后检测miR-382-5p; D: 转染miR-382-5p模拟物(mimics)后, Western blot检测细胞分化标志物CD11b; E: 流式细胞术检测CD11b的表达; F: 瑞氏–吉姆萨染色后显微镜油镜观察细胞形态。*P<0.05, **P<0.01, 与对照组相比。

A: the miR-382-5p expression after transfection with miR-382-5p mimics. B: the cell differentiation marker CD11b after transfection with miR-382-5p mimics. C: the miR-382-5p expression after transfection with miR-382-5p inhibitors. D: the cell differentiation marker CD11b after transfection with miR-382-5p inhibitors. E: the expression of CD11b was detected by flow cytometry. F: cell morphological changes were imaged with a microscope after stained with Wright-Giemsa solution. *P<0.05, **P<0.01 *vs* control group.

图7 NB4细胞中miR-382-5p对ATRA诱导分化的影响

Fig.7 Effects of miR-382-5p on ATRA-induced differentiation of NB4 cells

7D)。流式细胞术检测CD11b的表达(图7E)和细胞形态学分析(图7F)的结果与Western blot结果一致, 说明NB4细胞中miR-382-5p抑制了ATRA诱导的细胞分化。

3 讨论

血液系统肿瘤主要表现为白血病细胞的恶性增殖和分化阻滞。两个独立小组实验均证明了PTEN在正常造血干细胞和白血病造血干细胞的自我更新能力中发挥关键作用, PTEN的缺失使正常造血干细胞不断消耗, 随之产生白血病初始细胞, 促进白血病的发生[22-23]。分化诱导治疗的药物, 如全反式维甲酸(ATRA)和佛波酯(TPA), 均能在诱导分化的同时上调PTEN的表达[24]。而在急性髓系白血病和急性淋巴细胞白血病中, PTEN的表达均存在不同程度的低表达[25]。这些研究提示, PTEN在血液细胞分化过程中起着重要的作用。因此, 我们用ATRA诱导急性早幼粒细胞株NB4细胞和非急性早幼粒细胞株HL-60和THP-1细胞分化, 发现在诱导细胞分化时PTEN均有不同程度的上调。为了进一步研究PTEN在ATRA诱导细胞分化中的作用, 我们用ATRA处理慢病毒载体稳定过表达PTEN的细胞, 结果显示, 与对照组相比, NB4细胞*PTEN*慢病毒组明显促进细胞分化, 而HL-60和THP-1细胞无明显差异。PTEN的功能与遗传突变相关, 机制包括表观沉默、转录抑制和转录后修饰、microRNA调控等[11]。我们猜想这一差异结果可能与PTEN的调控机制相关。

许多研究表明, microRNA在白血病的发生和发展中起着重要的作用, 相比其他类型的白血病, 急性早幼粒细胞白血病中miR-382-5p的表达明显上调[20-21], 但目前对于其在白血病中的作用机制尚未阐明。在CD34+造血干细胞中过表达miR-382-5p使巨核细胞前体减少而中性粒前体增加[26], 我们猜想急性早幼粒细胞白血病中miR-382-5p的明显上调可能与此相同。为了探究miR-382-5是否与分化相关, 我们用qRT-PCR检测ATRA诱导不同细胞分化过程中miR-382-5p的表达, 结果显示, 在NB4细胞中, ATRA能够下调miR-382-5p的表达。microRNA主要通过与靶基因mRNA的3′非翻译区(UTR)的特异性结合, 在转录后水平调节基因的表达[27]。而大多数microRNA是通过靶向肿瘤抑制因子发挥作用的[28]。我们运用miRBase和miRNAMap数据库进行分析, 预测miR-382-5p与*PTEN*基因有靶向关系。在NB4细胞中转染miR-382-5p的模拟物(mimics)和抑制剂(inhibitors), 转染后加用ATRA诱导分化, qRT-PCR检测转染效率和PTEN mRNA水平, Western blot检测PTEN蛋白水平的改变, 证明miR-382-5p可以靶向调节*PTEN*基因, 并且抑制了ATRA诱导的细胞分化。近年来, miR-382-5p在不同疾病中的功能逐渐被发现, 如促进肝细胞的增殖[29]、促进乳腺癌细胞的侵袭和转移[30]、胃癌中血管生成性miRNA标记[31]和偏头痛发作期间和无痛期的标志物[32]等。由于miR-382-5p在急性早幼粒细胞白血病中特异性高表达, 我们猜想其可能是因为PML/RARα的间接转录诱导作用[33], 这也解释了急性早幼粒细胞白血病中PTEN表达量比其他类型急性髓系白血病中更低的现象[34]。

综上所述, miR-382-5靶向*PTEN*调节ATRA诱导急性早幼粒细胞的分化。本研究进一步阐明了ATRA治疗急性早幼粒细胞白血病的新机制, 为急性早幼粒细胞白血病的诊断和治疗提供了新思路。

参考文献 (References)

1 Kakizuka A, Miller WH, Umesono K, Warrell RP, Frankel SR, Murty VV, *et al*. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. Cell 1991; 66(4): 663-74.

2 de The H, Lavau C, Marchio A, Chomienne C, Degos L, Dejean A. The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. Cell 1991; 66(4): 675-84.

3 Chen Z. PML nuclear bodies mediate the therapeutic response of APL cells. Cancer Discov 2014; 4(3): Of20.

4 Grignani F, Ferrucci PF, Testa U, Talamo G, Fagioli M, Alcalay M, *et al*. The acute promyelocytic leukemia-specific PML-RAR alpha fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. Cell 1993; 74(3): 423-31.

5 Arteaga MF, Mikesch JH, Qiu J, Christensen J, Helin K, Kogan SC, *et al*. The histone demethylase PHF8 governs retinoic acid response in acute promyelocytic leukemia. Cancer Cell 2013; 23(3): 376-89.

6 Degos L, Dombret H, Chomienne C, Daniel MT, Miclea JM, Chastang C, *et al*. All-trans-retinoic acid as a differentiating agent in the treatment of acute promyelocytic leukemia. Blood 1995; 85(10): 2643-53.

7 Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. Blood 2008; 111(5): 2505-15.

8 Mi JQ, Li JM, Shen ZX, Chen SJ, Chen Z. How to manage acute promyelocytic leukemia. Leukemia 2012; 26(8): 1743-51.

9 Sanz MA, Montesinos P. How we prevent and treat differentiation syndrome in patients with acute promyelocytic leukemia. Blood

2014; 123(18): 2777-82.
10 Zhu HH, Guo ZP, Jia JS, Jiang Q, Jiang H, Huang XJ. The impact of oral arsenic and all-trans-retinoic acid on coagulopathy in acute promyelocytic leukemia. Leuk Res 2018; 65: 14-9.
11 Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 2012; 13(5): 283-96.
12 Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. Nat Rev Cancer 2011; 11(4): 289-301.
13 Dragojlovic-Munther M, Martinez-Agosto JA. Multifaceted roles of PTEN and TSC orchestrate growth and differentiation of Drosophila blood progenitors. Development 2012; 139(20): 3752-63.
14 Choorapoikayil S, Kers R, Herbomel P, Kissa K, den Hertog J. Pivotal role of Pten in the balance between proliferation and differentiation of hematopoietic stem cells in zebrafish. Blood 2014; 123(2): 184-90.
15 Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004; 303(5654): 83-6.
16 Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. Nucleic Acids Res 2005; 33(4): 1290-7.
17 Karp X, Ambros V. Developmental biology. Encountering microRNAs in cell fate signaling. Science 2005; 310(5752): 1288-9.
18 Garzon R, Pichiorri F, Palumbo T, Visentini M, Aqeilan R, Cimmino A, *et al*. MicroRNA gene expression during retinoic acid-induced differentiation of human acute promyelocytic leukemia. Oncogene 2007; 26(28): 4148-57.
19 Brauer-Hartmann D, Hartmann JU, Wurm AA, Gerloff D, Katzerke C, Verga Falzacappa MV, *et al*. PML/RARalpha-regulated miR-181a/b cluster targets the tumor suppressor RASSF1A in acute promyelocytic leukemia. Cancer Res 2015; 75(16): 3411-24.
20 Li Z, Lu J, Sun M, Mi S, Zhang H, Luo RT, *et al*. Distinct microRNA expression profiles in acute myeloid leukemia with common translocations. Proc Natl Acad Sci USA 2008; 105(40): 15535-40.
21 Jongen-Lavrencic M, Sun SM, Dijkstra MK, Valk PJ, Lowenberg B. MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood 2008; 111(10): 5078-85.
22 Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, *et al*. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature 2006; 441(7092): 518-22.
23 Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, *et al*. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature 2006; 441(7092): 475-82.
24 Hisatake J, O'Kelly J, Uskokovic MR, Tomoyasu S, Koeffler HP. Novel vitamin D(3) analog, 21-(3-methyl-3-hydroxy-butyl)-19-nor D(3), that modulates cell growth, differentiation, apoptosis, cell cycle, and induction of PTEN in leukemic cells. Blood 2001; 97(8): 2427-33.
25 Yang J, Liu J, Zheng J, Du W, He Y, Liu W, *et al*. A reappraisal by quantitative flow cytometry analysis of PTEN expression in acute leukemia. Leukemia 2007; 21(9): 2072-4.
26 Zini R, Rossi C, Norfo R, Pennucci V, Barbieri G, Ruberti S, *et al*. miR-382-5p controls hematopoietic stem cell differentiation through the downregulation of MXD1. Stem Cells Dev 2016; 25(19): 1433-43.
27 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116(2): 281-97.
28 Lujambio A, Lowe SW. The microcosmos of cancer. Nature 2012; 482(7385): 347-55.
29 Bei Y, Song Y, Wang F, Dimitrova-Shumkovska J, Xiang Y, Zhao Y, *et al*. miR-382 targeting PTEN-Akt axis promotes liver regeneration. Oncotarget 2016; 7(2): 1584-97.
30 Ho JY, Hsu RJ, Liu JM, Chen SC, Liao GS, Gao HW, *et al*. MicroRNA-382-5p aggravates breast cancer progression by regulating the RERG/Ras/ERK signaling axis. Oncotarget 2017; 8(14): 22443-59.
31 Seok JK, Lee SH, Kim MJ, Lee YM. MicroRNA-382 induced by HIF-1alpha is an angiogenic miR targeting the tumor suppressor phosphatase and tensin homolog. Nucleic Acids Res 2014; 42(12): 8062-72.
32 Andersen HH, Duroux M, Gazerani P. Serum microRNA signatures in migraineurs during attacks and in pain-free periods. Mol Neurobiol 2016; 53(3): 1494-500.
33 Licht JD. Reconstructing a disease: What essential features of the retinoic acid receptor fusion oncoproteins generate acute promyelocytic leukemia? Cancer Cell 2006; 9(2): 73-4.
34 Noguera NI, Piredda ML, Taulli R, Catalano G, Angelini G, Gaur G, *et al*. PML/RARa inhibits PTEN expression in hematopoietic cells by competing with PU.1 transcriptional activity. Oncotarget 2016; 7(41): 66386-97.