Roles of PADI4 in the expression of cytokines involved in inflammation and adhesion in differentiated NB4 cells treated with ATRA

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Abstract. Differentiation syndrome (DS) is a common complication in patients with acute promyelocytic leukemia (APL) treated with all-trans-retinoic acid (ATRA). However, the target of ATRA during DS in patients with APL remains to be elucidated. Therefore, the current study aimed to investigate the role of peptidylarginine deiminase 4 (PADI4) in the differentiation of ATRA-induced NB4 APL cells. The results showed that PADI4 was significantly upregulated in peripheral blood samples derived from patients with APL DS compared with patients with APL only. In addition, whether ATRA could enhance the expression levels of PADI4 in NB4 cells *in vitro* was subsequently investigated. The results also showed that PADI4 overexpression promoted the differentiation of NB4 cells treated with ATRA, which was reversed after PADI4 silencing. To uncover the potential mechanisms underlying the above process, PADI4 overexpression induced the secretion of inflammation-related cytokines, such as TNF-α, IL-1β, IL-8, C-C motif chemokine (CCL)2, CCL4, CCR1 and intercellular adhesion molecule-1 in ATRA-treated NB4 cells. However, PADI4 knockdown in the same cells had the opposite effect. The above findings indicated that PADI4 could be involved in the differentiation of ATRA-induced NB4 cells and upregulation of cytokines.

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Introduction

Acute promyelocytic leukemia (APL) is a particular type of acute myeloid leukemia characterized by a balanced reciprocal translocation, resulting in the formation of the oncogenic fusion protein, promyelocytic leukemia-retinoic acid receptor α (PML-RARα) (1,2). It has been reported that PML-RARα, an oncoprotein, acts as a retinoid receptor involved in suppressing retinoic acid-induced myeloid differentiation. Currently, all-trans retinoic acid (ATRA) is considered as an effective drug in treating APL via promoting the degradation of PML-RARα (3). However, patients with long-term exposure to ATRA are more likely to experience hyper-inflammation and differentiation syndrome (DS), thus resulting in poor prognosis among patients with APL (4,5).

DS, also known as retinoic acid syndrome, is characterized by the presence of massive inflammatory differentiating leukemic cells in the bloodstream, which trigger the release of excessive chemokines and cytokines (6). Previous studies demonstrated that ATRA can induce the release of early pro-inflammatory cytokines, such as TNF-α and IL-1β, as well as intercellular adhesion molecule-1 (ICAM-1) in patients with APL complicated with DS (7,8). Therefore, it was hypothesized that the above inflammatory factors could be involved in the pathogenesis of DS.

Previous studies demonstrated that peptidylarginine deiminase 4 (PADI4), contributing to ATRA- and 1α, 25-dihydroxyvitamin D3-induced differentiation of human myeloid leukemia HL-60 cells, is involved in regulating the proliferation of hematopoietic progenitors (9,10). As the only member of the PADI family in the nucleus (11), PADI4 is involved in the pathogenesis of several types of cancer via modulating the transcriptional network for pluripotency (12,13). Another study showed that PADI4 is involved in the differentiation of APL cells (14). However, little is known regarding the role of PADI4 in APL DS. Therefore, the current study aimed to investigate the effect of PADI4 in the pathogenesis of DS. First, the expression levels of PADI4 were detected in patients with APL DS and patients with APL only. Subsequently, *in vitro* experiments were carried out in the NB4 cell line, an APL cell line mimicking ATRA-induced terminal neutrophil maturation, transfected with PADI4 overexpression or silencing plasmids.

Furthermore, the secretion levels of the inflammatory factors TNF-α, IL-1β, IL-8 and ICAM-1, were also determined to uncover the role of PADI4 in ATRA-induced differentiation.

Materials and methods

Patients. A total of three patients (1 male and 2 female; age range 3-8 years) with APL DS admitted to Children's Hospital Affiliated to Shandong University and Jinan Children's Hospital between December 2019 and November 2021 were included in the APL + DS group, while three (1 male and 2 female) age-matched patients with APL, without DS, served as the control group. Patients with APL aged <10 years were enrolled in the present study, while those with other concurrent malignancies or those not willing to receive ATRA therapy were excluded. Patients were diagnosed with APL DS according to the guidelines by the Expert Panel of the European LeukemiaNet, as previously described (15,16). All six patients received inducing therapy with ATRA (25 mg/m^2/day) plus cytarabine and daunorubicin. The parents of each patient signed the informed consent and the study protocol was approved by the Ethical Committee of Children's Hospital Affiliated to Shandong University and Jinan Children's Hospital (approval no. QLET-IRB/P-2021007).

Extraction of monocytes. Peripheral blood samples were collected from six patients with APL with or without DS. The peripheral blood mononuclear cells (PBMCs) were prepared by Ficoll Hypaque gradient centrifugation at 1,000 x g for 20 min at room temperature and were then cryopreserved for the subsequent experiments (17).

Cell culture. NB4 cells, purchased from ATCC, were cultured in 1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Zhejiang Tianhang Biotechnology Co., Ltd.), penicillin (100 U/ml) and streptomycin (100 μg/ml; both from Beyotime Institute of Biotechnology) at 37˚C with 5% CO_2. To induce cell differentiation, NB4 cells were treated with 1 μmol/l ATRA (cat. no. R2625; MilliporeSigma).

Transfection and PADI4 silencing. The PADI4 overexpression plasmid, pPADI4, and the corresponding control vector were kindly given by Dr Wang Lin from the Shandong Academy of Medicinal Sciences (Jinan, China). To silence PADI4 expression, cells were transfected with small interfering RNA (siRNA) clones targeting PADI4 (siPADI4; sequence, 5'-GCCAACCAGAGCUGUGAAATTUUU CACAGCUCUGGUUGGCTT-3'). The scrambled negative control sequence (NC-siRNA), 5'-UUCUCCGAACGUGUC ACGUUUCUCCGAACGUGUCACGU-3', served as control. The sequences were synthesized by Shanghai GenePharma Co., Ltd. For cell transfection, 20 μg pPADI4/empty vector was electroporated into NB4 cells (1x10^7) using the Electro square porator 830 (BTX Instrument Division, Harvard Apparatus Inc.) with two 500 V pulses of 10 msec pulse length and pausing for 1 min between pulses at room temperature. For PADI4 silencing, 5 μl of 20 μmol/l siRNA was electroporated into NB4 cells (1x10^7) treated with ATRA with two 250 V pulses of 8 msec pulse length and pausing for 30 sec between pulses at room temperature. After incubation on ice for 10 min, RPMI 1640 medium supplemented with 15% FBS was added to the mixture, followed by incubation for an additional 36 h at 37˚C with 5% CO_2. The transfection was confirmed by western blot in the subsequent analysis

Flow cytometry. To investigate the potential effect of PADI4 on cell differentiation, NB4 cells were divided into the control, ATRA, ATRA + siPADI4 and ATRA + NC-siRNA groups. Cells at a density of 1x10^6 were first washed with PBS supplemented with 1% FCS and 0.01% sodium azide, and were then incubated in FCS at 4˚C for 30 min. Subsequently, cells were supplemented with FITC-conjugated anti-human CD11b antibody (cat. no. 562793; BD Biosciences) followed by incubation at 25˚C for 45 min. The cells were then fixed with 1% paraformaldehyde and analyzed on FACSVerse flow cytometer using FACSDiva 6.0 software (BD Biosciences).

ELISA. ELISA was utilized to determine the expression levels of TNF-α, IL-1β, IL-8, C-C motif chemokine ligand (CCL)2, CCL4 and C-C motif chemokine receptor (CCR) 1 in each group using the corresponding commercial kits, according to the manufacturer's instructions. The commercially available kits for TNF-α (cat. no. EHC103a), IL-1β (cat. no. EHC002b) and IL-8 (cat. no. EHC008) were purchased from Neobioscience Technology Co., Ltd., while those for CCL2 (cat. no. DCP00), CCL4 (cat. no. DMB00) and CCR1 (cat. no. CT63246) were from Jinan Quantum Trading Co., Ltd.. Finally, the absorbance at a wavelength of 532 nm was measured using a microplate reader (Synergy HT; BioTek Instruments Inc.).

Reverse transcription-quantitative (RT-q) PCR. Total RNA was extracted from 1x10^6 NB4 cells and PBMCs using TRIzol® reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Subsequently, total RNA was reverse transcribed into cDNA using the Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions on the Eppendorf PCR system. PCR amplification was performed using the SYBR Green Real-time PCR Master Mix (ToYoBo, Japan) according to manufacturer's instructions on Stratagene Mx3000P Real-Time PCR system (Agilent Technologies, Inc.). Each PCR reaction contained 2X real-time PCR Master Mix, 1 μl of each primer and 1 μl cDNA, in a total volume of 10 μl. The specific primer sequences for PADI4 were as follows: 5'-GTTTAGGGTCAG ACAGTCCTGG-3', 5'-AGATGTGAGTAGTGGCACATGC'. The primer sequences for GAPDH were: 5'-GTCTCCTCT GACTTCAACAGCG-3', 5'-ACCACCCTGTTGCTGTAG CCAA-3'. The thermocycling conditions used were as follows: 95˚C for 10 sec; 40 cycles of 60˚C for 5 sec and 72˚C for 10 sec; and 65˚C for 30 sec. Finally, the amplification results were analyzed using the $2^{-\Delta\Delta Cq}$ method, as previously described (18).

Western blot analysis. NB4 cells were washed twice with ice cold PBS and were then lysed using RIPA lysis buffer (Applygen Technologies, Inc.). The protein concentration was determined using the BCA method. Subsequently, protein samples (20 μg) were separated by 10% SDS-PAGE and were then transferred onto a PVDF membrane (MilliporeSigma). The membranes were blocked with 5% skimmed milk in TBST containing 0.05% Tween-20 for 1 h at room temperature and incubated

with antibodies against PADI4 (dilution, 1:500; cat. no. p4874; MilliporeSigma), ICAM-1 (dilution, 1:500; cat. no. ER131207) and GAPDH (dilution, 1:3,000; cat. no. ET1601-4; both from HUABIO, Inc.) overnight at 4˚C. Subsequently, the membranes were incubated with HRP-conjugated secondary antibodies (dilution, 1:20,000; cat. no. HA1001; HUABIO, Inc.) at room temperature for 1 h. The protein levels were quantified by densitometry and normalized to the corresponding GAPDH expression levels. Finally, the protein bands were visualized using the iBright CL750 CL1500 system (Thermo Fisher Scientific Inc.) and the band intensity was measured using ImageJ software (Version 1.49, NIH).

Statistical analysis. All statistical analyses were performed using SPSS 13.0 software (SPSS, Inc.). All data are expressed as the mean ± standard deviation. Significant differences between two groups were analyzed using unpaired Student's t-test, while those among multiple groups with one-way ANOVA followed by a Bonferroni's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

PADI4 is upregulated in APL DS samples and ATRA-treated NB4 cells. The mRNA and protein expression levels of PADI4 in PBMCs derived from patients with APL DS were significantly higher compared with those in patients with APL only (Fig. 1A and B). Compared with the baseline levels, the mRNA expression levels of PADI4 were notably increased in NB4 cells treated with ATRA for 24, 48 and 72 h (P<0.05; Fig. 2A). Similarly, PADI4 was significantly upregulated at 24, 48 and 72 h in ATRA-treated NB4 cells compared with untreated cells (Fig. 2B). These *in vivo* and *in vitro* experiments indicated that ATRA could upregulate PADI4.

PADI4 serves a crucial role in the ATRA-induced differentiation of NB4 cells. As shown in Fig. 3A, PADI4 was successfully knocked down in NB4 cells transfected with the corresponding siRNA clones. No statistically significant difference was observed in the differentiation capacity of NB4 cells in the ATRA + NC-siRNA group compared with the ATRA group (P>0.05; Fig. 3A). The differentiation ability of NB4 cells was assessed by evaluating the synthesis of CD11b, a marker of granulocytic differentiation. Flow cytometric analysis revealed that cell treatment with ATRA induced NB4 cell differentiation. However, the ATRA-induced NB4 cell differentiation was reversed following PADI4 silencing (Fig. 3B and C). Electroporation-mediated PADI4 overexpression further promoted the differentiation of NB4 cells compared with ATRA-treated NB4 cells (P<0.05; Fig. 3D-F). However, the differentiation ability of ATRA-treated NB4 cells transfected with empty vector was notably attenuated compared with the ATRA group. Taken together, the above findings indicated that PADI4 could play a significant role in the ATRA-induced differentiation of NB4 cells.

PADI4 promotes the secretion of inflammatory factors. ELISA was performed to assess the secretion levels of the inflammatory factors TNF-α, IL-1β, IL-8, CCL2, CCL4 and CCR1 in NB4 cells that were reported to be closely related

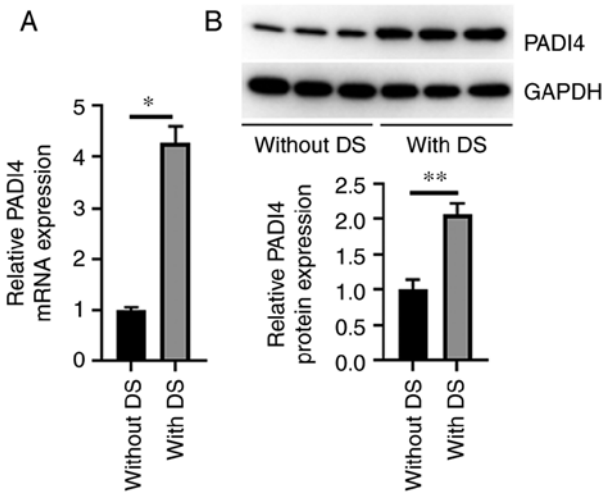

Figure 1. PADI4 mRNA and protein was upregulated in the PBMCs of APL patients complicated with DS. (A) Expression of PADI4 mRNA was significantly increased in the peripheral blood of APL patients with DS vs. APL patients without DS. (B) Comparison of PADI4 protein in PBMCs between APL patients with DS and those without DS symptoms. *P<0.05 and **P<0.01, vs. APL without DS group. PADI4, peptidylarginine deiminase 4; PBMCs, peripheral blood mononuclear cells; DS, differentiation syndrome; APL, acute promyelocytic leukemia.

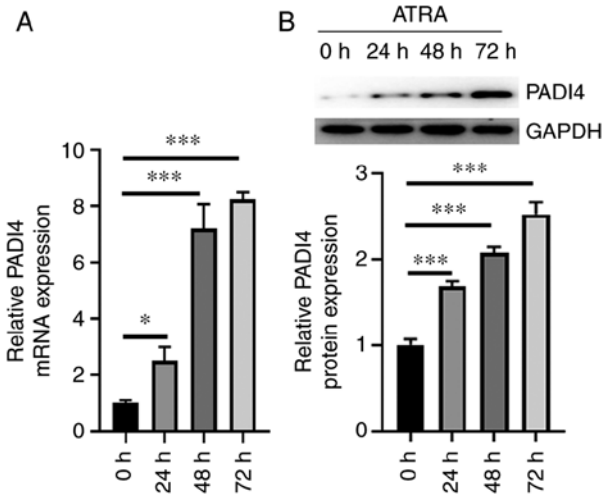

Figure 2. PADI4 expression showed a time-dependent manner in ATRA-induced NB4 cells. (A) Following treatment with ATRA, the expression of PADI4 mRNA was detected by reverse transcription-quantitative PCR, which showed significant increase at 24 h, 48 h and 72 h vs. 0 h. (B) Expression of PADI4 protein in NB4 cells treated with ATRA at 0 h, 24, 48 and 72 h, respectively. *P<0.05 and ***P<0.001 vs. 0 h. PADI4, peptidylarginine deiminase 4; ATRA, all-trans-retinoic acid.

to the inflammation process (19,20). The secretion levels of TNF-α, IL-1β, IL-8, CCL2, CCL4 and CCR1 were significantly increased in the supernatant of cultured NB4 cells in the ATRA group compared with those in the control group (all P<0.05; Fig. 4A). However, the secretion levels of TNF-α, IL-1β, IL-8, CCL2, CCL4 and CCR1 were notably reduced in the ATRA + siPADI4 group compared with the ATRA group (all P<0.05). By contrast, no significant differences were observed in the levels of the above inflammatory factors in the ATRA + NC-siNRA group compared with the ATRA group. In PADI4 overexpressing cells, the expression of TNF-α, IL-1β,

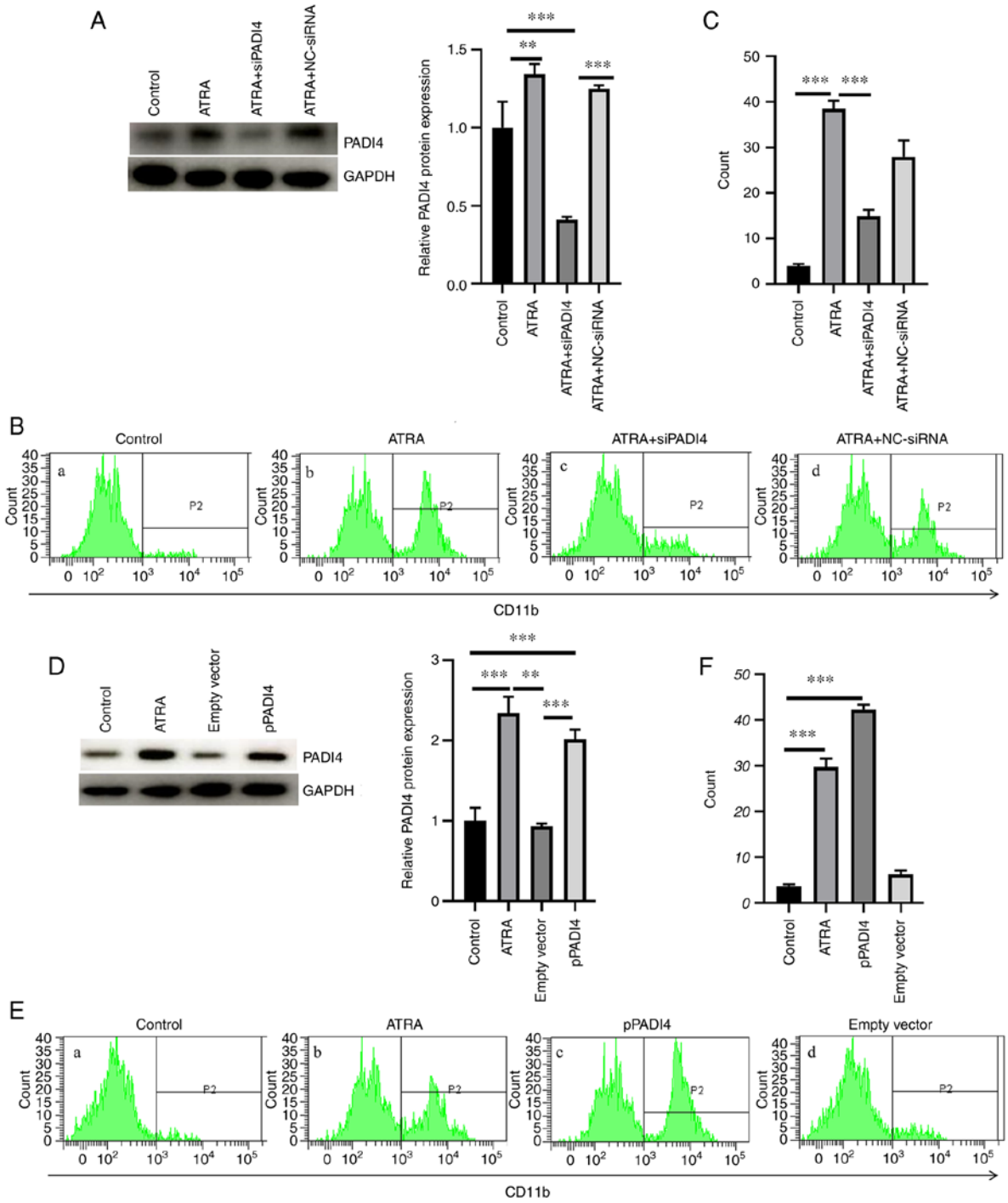

Figure 3. PADI4 promoted NB4 cell differentiation. (A) Relative PADI4 expression in ATRA group showed significant increase vs. control (P<0.05), but the expression was significantly inhibited following siPADI4 (P<0.05). (B) Flow cytometry indicated that silencing of PADI4 inhibited the ATRA-induced differentiation in NB4 cells compared with the ATRA group. The area of interest is in the P2 quadrant. (C) The semi-quantitative analysis data for the NB4 count in each group. PADI4 over-expression promoted the differentiation of NB4 cells based on (D) western blot analysis and (E) flow cytometry. The area of interest in the P2 quadrant. (F) The semi-quantitative analysis data for the NB4 count in each group after PADI4 over-expression. **P<0.01 and ***P<0.01. PADI4, peptidylarginine deiminase 4; ATRA, all-trans-retinoic acid; si, small interfering; pPADI4, PADI4 overexpression plasmid.

IL-8, CCL2, CCL4 and CCR1 were significantly enhanced compared with control cells (P<0.05). However, there was no significant difference in the secretion levels of TNF-α, IL-1β, IL-8, CCL2, CCL4 and CCR1 between the ATRA and PADI4 overexpression group (P>0.05; Fig. 4B). Overall, these results suggested that PADI4 could be involved in TNF-α, IL-1β, IL-8, CCL2, CCL4 and CCR1 upregulation in ATRA-treated NB4 cells.

PADI4 upregulates ICAM-1. To determine whether PADI4 could affect the expression of ICAM-1, the protein expression levels of ICAM-1 were detected in NB4 cells transfected with 0, 5, 15 and 20 μg pPADI4. Following transfection for 48 h, the protein expression levels of ICAM-1 were assessed using western blot analysis. Additionally, to evaluate whether PADI4 silencing could restore the expression levels of ICAM-1, NB4 cells were transfected with siRNA clones targeting PADI4.

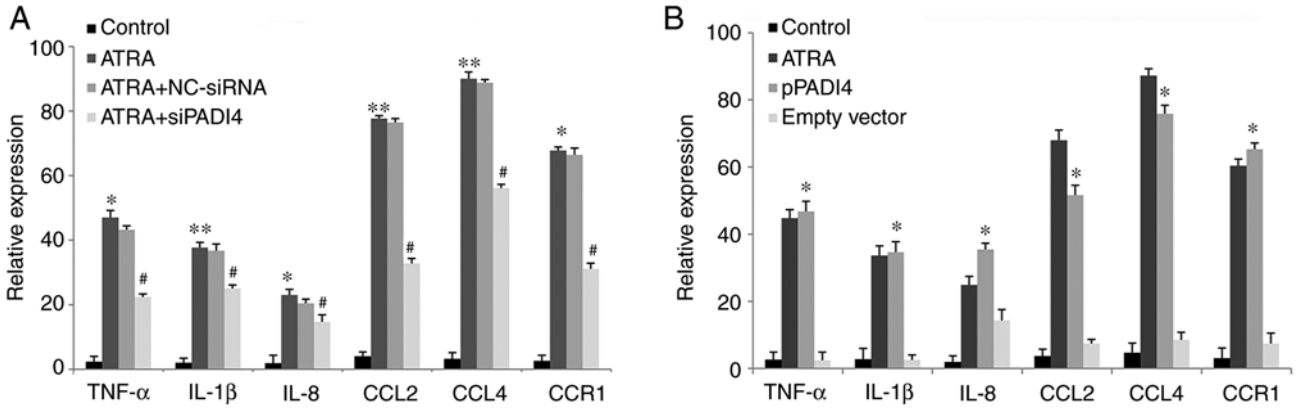

Figure 4. PADI4 regulated the inflammatory molecules in ATRA-induced NB4 cells. (A) Comparison of cytokines between the ATRA-induced NB4 cells and the ATRA-induced NB4 cells subject to PADI4 silencing. (B) Comparison of cytokines between the ATRA-induced NB4 cells and the ATRA-induced NB4 cells subject to PADI4 overexpression. *P<0.05 and **P<0.01 vs. control group; #P<0.05 vs. NC-siRNA. PADI4, peptidylarginine deiminase 4; ATRA, all-trans-retinoic acid; si, small interfering; CCL, C-C motif chemokine; pPADI4, PADI4 overexpression plasmid; NC, negative control.

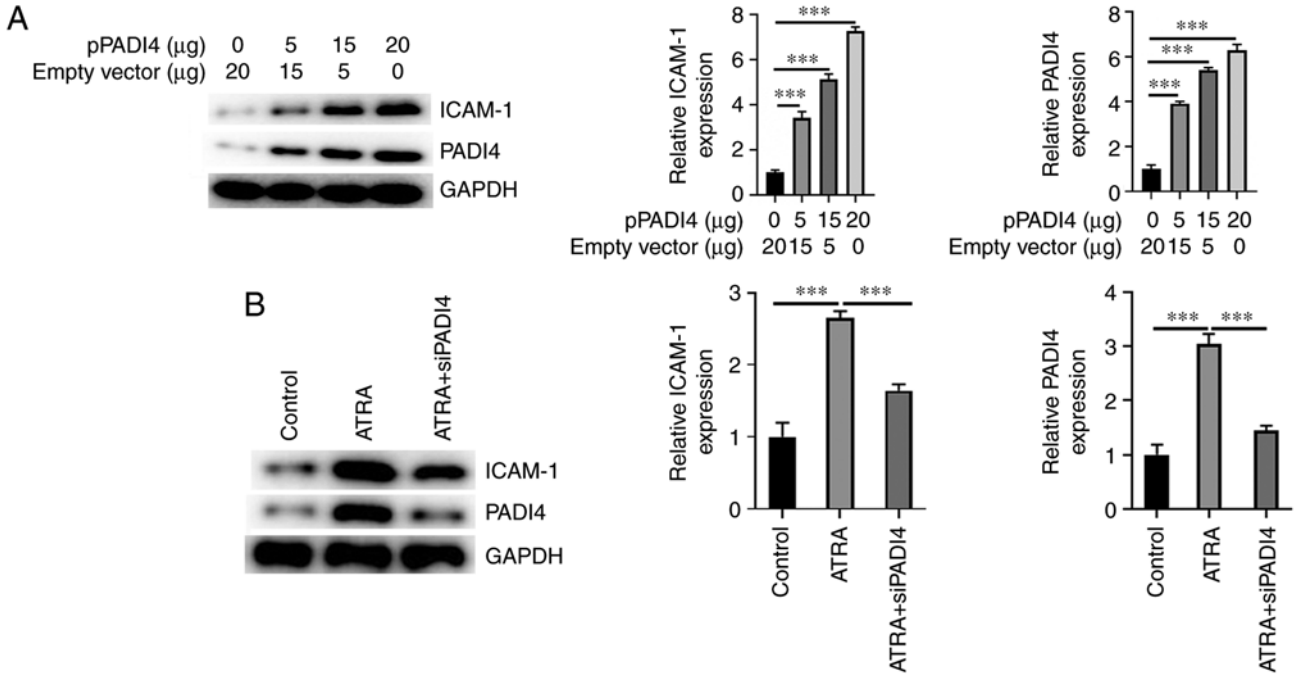

Figure 5. PADI4 induces ICAM-1 expression in ATRA-induced NB4 cells. (A) NB4 cells transfected with 0, 5, 15 and 20 μg of plasmid PADI4 showed upregulation of ICAM-1. (B) NB4 cells subjected to PADI4 silencing showed downregulation of ICAM-1. ***P<0.001. PADI4, peptidylarginine deiminase 4; ICAM-1, intercellular adhesion molecule-1; ATRA, all-trans-retinoic acid; pPADI4, PADI4 overexpression plasmid.

The results showed that the protein expression levels of ICAM-1 were increased in PADI4 overexpressing NB4 cells in a dose-dependent manner (Fig. 5A). As shown in Fig. 5B, ICAM-1 was markedly upregulated in cells in the ATRA group compared with the NC group. However, the above effect was reversed in PADI4-depleted NB4 cells. The aforementioned findings indicated that PADI4 could be involved in ICAM-1 upregulation in NB4 cells.

Discussion

Differentiation therapy based on ATRA and arsenic trioxide has been commonly used for treating APL (3). However, several patient may experience DS (21). Although the pathogenesis of DS remains to be elucidated, mounting evidence has suggested that sustained hyperinflammation serves a critical role in its pathogenesis (16,21,22). The current study showed that PADI4 could serve an essential role in regulating the expression of inflammatory cytokines, such as TNF-α and IL-1β, in ATRA-treated NB4 cells. In addition, PADI4 could also promote the expression of ICAM-1, which was positively associated with the clinical status of DS.

It has been reported that PADI4 is upregulated in particular types of cancer (23,24). Previous studies in solid tumors suggest that PADI4 can bind with the downstream targets of cytokeratin and p53, including *OKL38* and *Elk-1* (13,25,26). Another study also revealed that PADI4 promoted cellular differentiation in patients with hematologic neoplasm: Under

in vitro conditions, PADI4 can trigger NB4 cell differentiation by regulating the PADI4/SOX4/PU.1 signaling pathway (14). In the present study, PADI4 was upregulated in PBMCs and serum samples of patients with APL DS compared with patients without DS. However, our understanding of the effects of PADI4 overexpression during the development of DS in patients with APL remains limited. Therefore, the present study aimed to investigate the effect of PADI4 on ATRA-mediated NB4 cell differentiation *in vitro*, in PADI4-overexpressing and -depleted NB4 cells. The results demonstrated that PADI4 overexpression was involved in ATRA-induced NB4 cell differentiation. By contrast, the ATRA-induced NB4 cell differentiation was inhibited after PADI4 knockdown. These findings indicated that PADI4 could serve a critical role in the ATRA-induced differentiation of NB4 cells.

It has been hypothesized that PADI4 is closely associated with inflammation. In a previous study, PADI4 activation was associated with the exacerbation of kidney ischemia-reperfusion injury by enhancing renal tubular inflammatory responses and neutrophil infiltration (27). In addition, PADI4 silencing in a rat model of hemorrhagic shock can attenuate local inflammatory responses (28). Other studies indicate that PADI4 can be involved in the prevention of bacterial infection by promoting the formation of neutrophil extracellular traps (29,30). In ATRA-induced APL cells, the expression levels of cytokines, such as IL-1β, IL-6, IL-8, TNF-α, L-selectin, lymphocyte function-associated antigen 1 and ICAM-1 are notably increased (31,32). For example, Ninomiya *et al* (33) show that APL cells can migrate into lung tissues in the presence of chemotactic factors secreted by ATRA-induced alveolar epithelial cells, eventually triggering DS after their metastasis to lung tissues and alveolar space. These findings suggest that PADI4 could trigger the pathogenesis and progression of inflammation, while PADI4 inactivation can eliminate or prevent the occurrence of inflammation. In our previous study, PADI4 was considered to participate in the expression of TNF-α and IL-1β in ATRA-treated NB4 cells (8). In the present study, PADI4 was involved in the regulation of inflammation-related cytokines, such as TNF-α, IL-1β, IL-8, CCL2, CCL4, CCR1 and ICAM-1. Following PADI4 silencing, the expression of inflammation-related cytokines was notably decreased compared with the control group.

However, the current study has some limitations. First, the sample size was small. Second, although the current study revealed the role of PADI4 in the ATRA-induced differentiation of NB4 cells based on overexpression and silencing experiments, the particular mechanism underlying the effect of PADI4 on regulating the expression of IL-1β, TNF-α and ICAM-1 was not discovered. Therefore, further studies are needed to investigate the possible mechanism of the above process.

In summary, the present study demonstrated that PADI4 was upregulated in PBMCs of patients with APL DS and ATRA-treated NB4 cells. In addition, the results showed that PADI4 was involved in the upregulation of cytokines in ATRA-treated NB4 cells, while PADI4 silencing exhibited the opposite effect. The aforementioned findings indicated that PADI4 could be involved in ATRA-induced NB4 differentiation and increased the expression levels of inflammation-related cytokines. Therefore, PADI4 could serve as a novel treatment strategy for treating DS.

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Availability of data and materials

The datasets generated and/or analyzed during the study are available from the corresponding author on reasonable request.

Authors' contributions

QG designed the current study. XS, XM and FL performed the experiments. QG and FL analyzed the data. XY and YW drafted the manuscript and analyzed data. XM and XY interpreted data and revised the final manuscript. XS and QG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted with full adherence to the international norms of medical ethics, as set out in the Helsinki Declaration. The patients' parents gave their informed written consent for enrollment in the study. The study was approved by the Ethics Committee of the Children's Hospital Affiliated to Shandong University (approval no. QLET-IRB/P-2021007).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Deschler B and Lübbert M: Acute myeloid leukemia: Epidemiology and etiology. Cancer 107: 2099-2107, 2006.
2. Wang ZY and Chen Z: Acute promyelocytic leukemia: From highly fatal to highly curable. Blood 111: 2505-2515, 2008.
3. Albanesi J, Noguera NI, Banella C, Colangelo T, De Marinis E, Leone S, Palumbo O, Voso MT, Ascenzi P, Nervi C, *et al*: Transcriptional and metabolic dissection of ATRA-induced granulocytic differentiation in NB4 acute promyelocytic leukemia cells. Cells 9: 2423, 2020.
4. Molinaro A, Zanta D, Moleti ML, Giona F, Conter V, Rizzari C, Biondi A and Testi AM: Challenging management of severe differentiation syndrome in pediatric acute promyelocytic leukemia treated with ATRA/ATO. Mediterr J Hematol Infect Dis 14: e2022027, 2022.
5. Montesinos P, Bergua JM, Vellenga E, Rayón C, Parody R, de la Serna J, León A, Esteve J, Milone G, Debén G, *et al*: Differentiation syndrome in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline chemotherapy: Characteristics, outcome, and prognostic factors. Blood 113: 775-783, 2009.

6. Jambrovics K, Uray IP, Keresztessy Z, Keillor JW, Fésüs L and Balajthy Z: Transglutaminase 2 programs differentiating acute promyelocytic leukemia cells in all-trans retinoic acid treatment to inflammatory stage through NF-κB activation. Haematologica 104: 505-515, 2019.
7. Dubois C, Schlageter MH, de Gentile A, Balitrand N, Toubert ME, Krawice I, Fenaux P, Castaigne S, Najean Y, Degos L, *et al*: Modulation of IL-8, IL-1 beta, and G-CSF secretion by all-trans retinoic acid in acute promyelocytic leukemia. Leukemia 8: 1750-1757, 1994.
8. Guo QW, Li F, Song L, Wang YP and Yang XM: Effect of PADI4 on the expression of inflammatory cytokines during NB4 cells differentiation. Zhongguo Shi Yan Xue Ye Xue Za Zhi 29: 1065-1070, 2021 (In Chinese).
9. Nakashima K, Hagiwara T, Ishigami A, Nagata S, Asaga H, Kuramoto M, Senshu T and Yamada M: Molecular characterization of peptidylarginine deiminase in HL-60 cells induced by retinoic acid and 1alpha,25-dihydroxyvitamin D(3). J Biol Chem 274: 27786-27792, 1999.
10. Nakashima K, Arai S, Suzuki A, Nariai Y, Urano T, Nakayama M, Ohara O, Yamamura K, Yamamoto K and Miyazaki T: PAD4 regulates proliferation of multipotent haematopoietic cells by controlling c-myc expression. Nat Commun 4: 1836, 2013.
11. Nakashima K, Hagiwara T and Yamada M: Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. J Biol Chem 277: 49562-49568, 2002.
12. Christophorou MA, Castelo-Branco G, Halley-Stott RP, Oliveira CS, Loos R, Radzisheuskaya A, Mowen KA, Bertone P, Silva JC, Zernicka-Goetz M, *et al*: Citrullination regulates pluripotency and histone H1 binding to chromatin. Nature 507: 104-108, 2014.
13. Chang X and Han J: Expression of peptidylarginine deiminase type 4 (PAD4) in various tumors. Mol Carcinog 45: 183-196, 2006.
14. Song G, Shi L, Guo Y, Yu L, Wang L, Zhang X, Li L, Han Y, Ren X, Guo Q, *et al*: A novel PAD4/SOX4/PU.1 signaling pathway is involved in the committed differentiation of acute promyelocytic leukemia cells into granulocytic cells. Oncotarget 7: 3144-3157, 2016.
15. Sanz MA, Fenaux P, Tallman MS, Estey EH, Löwenberg B, Naoe T, Lengfelder E, Döhner H, Burnett AK, Chen SJ, *et al*: Management of acute promyelocytic leukemia: Updated recommendations from an expert panel of the european LeukemiaNet. Blood 133: 1630-1643, 2019.
16. Tang L, Chai W, Ye F, Yu Y, Cao L, Yang M, Xie M and Yang L: HMGB1 promotes differentiation syndrome by inducing hyperinflammation via MEK/ERK signaling in acute promyelocytic leukemia cells. Oncotarget 8: 27314-27327, 2017.
17. Fuss IJ, Kanof ME, Smith PD and Zola H: Isolation of whole mononuclear cells from peripheral blood and cord blood. Curr Protoc Immunol 7: 7.1.1-7.1.8 (Suppl 85), 2009.
18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
19. Yang Y and Dai M: Expression of PADI4 in patients with ankylosing spondylitis and its role in mediating the effects of TNF-α on the proliferation and osteogenic differentiation of human mesenchymal stem cells. Int J Mol Med 36: 565-570, 2015.
20. Harney SM, Meisel C, Sims AM, Woon PY, Wordsworth BP and Brown MA: Genetic and genomic studies of PADI4 in rheumatoid arthritis. Rheumatology (Oxford) 44: 869-872, 2005.
21. Stahl M and Tallman MS: Differentiation syndrome in acute promyelocytic leukaemia. Br J Haematol 187: 157-162, 2019.
22. Mohammadzadeh Z, Omidkhoda A, Chahardouli B, Hoseinzadeh G, Moghaddam KA, Mousavi SA and Rostami S: The impact of ICAM-1, CCL2 and TGM2 gene polymorphisms on differentiation syndrome in acute promyelocytic leukemia. BMC Cancer 21: 46, 2021.
23. Chang X, Han J, Pang L, Zhao Y, Yang Y and Shen Z: Increased PADI4 expression in blood and tissues of patients with malignant tumors. BMC Cancer 9: 40, 2009.
24. Chang X and Fang K: PADI4 and tumourigenesis. Cancer Cell Int 10: 7, 2010.
25. Yao H, Li P, Venters BJ, Zheng S, Thompson PR, Pugh BF and Wang Y: Histone Arg modifications and p53 regulate the expression of OKL38, a mediator of apoptosis. J Biol Chem 283: 20060-20068, 2008.
26. Zhang X, Gamble MJ, Stadler S, Cherrington BD, Causey CP, Thompson PR, Roberson MS, Kraus WL and Coonrod SA: Genome-wide analysis reveals PADI4 cooperates with Elk-1 to activate c-Fos expression in breast cancer cells. PLoS Genet 7: e1002112, 2011.
27. Ham A, Rabadi M, Kim M, Brown KM, Ma Z, D'Agati V and Lee HT: Peptidyl arginine deiminase-4 activation exacerbates kidney ischemia-reperfusion injury. Am J Physiol Renal Physiol 307: F1052-F1062, 2014.
28. He W, Zhou P, Chang Z, Liu B, Liu X, Wang Y, Li Y and Alam HB: Inhibition of peptidylarginine deiminase attenuates inflammation and improves survival in a rat model of hemorrhagic shock. J Surg Res 200: 610-618, 2016.
29. Knight JS, Luo W, O'Dell AA, Yalavarthi S, Zhao W, Subramanian V, Guo C, Grenn RC, Thompson PR, Eitzman DT, *et al*: Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine models of atherosclerosis. Circ Res 114: 947-956, 2014.
30. Knight JS, Subramanian V, O'Dell AA, Yalavarthi S, Zhao W, Smith CK, Hodgin JB, Thompson PR and Kaplan MJ: Peptidylarginine deiminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. Ann Rheum Dis 74: 2199-2206, 2015.
31. Montesinos P and Sanz MA: The differentiation syndrome in patients with acute promyelocytic leukemia: Experience of the pethema group and review of the literature. Mediterr J Hematol Infect Dis 3: e2011059, 2011.
32. Luesink M, Pennings JL, Wissink WM, Linssen PC, Muus P, Pfundt R, de Witte TJ, van der Reijden BA and Jansen JH: Chemokine induction by all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia: Triggering the differentiation syndrome. Blood 114: 5512-5521, 2009.
33. Ninomiya M, Kiyoi H, Ito M, Hirose Y, Ito M and Naoe T: Retinoic acid syndrome in NOD/scid mice induced by injecting an acute promyelocytic leukemia cell line. Leukemia 18: 442-448, 2004.