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Aluminum Trichloride Inhibited Osteoblastic Proliferation and Downregulated the Wnt/β-Catenin Pathway

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Abstract Aluminum (Al) exposure inhibits bone formation. Osteoblastic proliferation promotes bone formation. Therefore, we inferred that Al may inhibit bone formation by the inhibition of osteoblastic proliferation. However, the effects and molecular mechanisms of Al on osteoblastic proliferation are still under investigation. Osteoblastic proliferation can be regulated by Wnt/β-catenin signaling pathway. To investigate the effects of Al on osteoblastic proliferation and whether Wnt/β-catenin signaling pathway is involved in it, osteoblasts from neonatal rats were cultured and exposed to 0, 0.4 mM (1/20 IC_{50}), 0.8 mM (1/10 IC_{50}), and 1.6 mM (1/5 IC_{50}) of aluminum trichloride ($AlCl_3$) for 24 h, respectively. The osteoblastic proliferation rates; Wnt3a, lipoprotein receptor-related protein 5 (LRP-5), T cell factor 1 (TCF-1), cyclin D1, and c-Myc messenger RNA (mRNA) expressions; and p-glycogen synthase kinase 3β (GSK3β), GSK3β, and β-catenin protein expressions indicated that $AlCl_3$ inhibited osteoblastic proliferation and downregulated Wnt/β-catenin signaling pathway. In addition, the $AlCl_3$ concentration was negatively correlated with osteoblastic proliferation rates and the mRNA expressions of Wnt3a, c-Myc, and cyclin D1, while the osteoblastic proliferation rates were positively correlated with mRNA expressions of Wnt3a, c-Myc, and cyclin D1. Taken together, these findings indicated that $AlCl_3$ inhibits osteoblastic proliferation may be associated with the inactivation of Wnt/β-catenin signaling pathway.

Keywords Aluminum trichloride · Osteoblastic proliferation · Wnt/β-catenin signaling pathway · Rat osteoblasts

Introduction

Aluminum (Al) is a ubiquitous environmental metal toxicant [1]. Al-containing agents have been extensively utilized in medicine, industry, agriculture, and our daily life with the rapid progress of social economy development [2]. In daily life, the absorption of Al from water and food in human is 0.005 and 0.08–0.5 μg/kg/day. In addition, Al absorbed from industrial air and dialysis solution can reach up to 0.6–8 and 9 μg/kg/day [3]. Although only 0.05–2.2 % of daily Al intake is absorbed in human, it can distribute unequally to all tissues and the Al body burdens will increase as a function of time [4]. Approximately 70 % of the Al accumulates in the bone [5], and the bone is the main target tissue for the Al toxicity [6]. Excessive Al accumulation disrupts bone formation, ultimately causing bone disease which defined as "Al-induced bone disease" (AIBD), including osteodystrophy, osteomalacia, and osteoporosis [7–10]. In AIBD, bone formation inhibition plays a key role, which is characterized by reduced numbers of osteoblasts [11, 12]. Osteoblasts are the main functional cells for bone formation, which can be influenced by proliferation, differentiation, and mineralization of osteoblasts [13, 14]. Our previous research showed the mechanism of Al on the inhibition of osteoblastic differentiation and mineralization

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[15, 16]. But the mechanism of Al on the inhibitation of osteoblastic proliferation remains not clear.

The osteoblastic proliferation is the first stage of bone formation process [17] and is closely related to bone health [18]. The activity of bone formation at the tissue level is dependent on the number of osteoblasts [19]. Current therapeutic strategies to promote osteoblastogenesis in osteoporosis consist in promoting osteoblastic proliferation and osteoblast activity [20, 21]. Some studies showed that Al inhibited osteoblastic proliferation [12, 22], whereas the other studies showed that Al promoted it [23, 24]. Thus, further investigations are indispensable to confirm the effects of Al on the osteoblastic proliferation.

Osteoblastic proliferation can be regulated by multiple signaling pathways [25–27]. Wnt/β-catenin signaling pathway is an acknowledged one in recent years, which promotes osteoblastic proliferation [28]. It is initiated by Wnt ligands (Wnt3a) binding to a complex receptor composed of members of the Frizzled family and low-density lipoprotein receptor-related protein 5 (LRP-5) [29], leading to phosphorylation (inactivation) of glycogen synthase kinase 3β (GSK3β). Inactivation of GSK3β (p-GSK3β) increases β-catenin levels in the cytosol. The cytosolic β-catenin is transferred to the nucleus and forms complexes with T cell factor (TCF-1), which could modulate the transcription of Wnt-targeted genes [30]. Cyclin D1 and c-Myc are the target genes of Wnt/β-catenin signaling pathway and play a positive role in osteoblastic proliferation [31, 32]. The messenger RNA (mRNA) expressions of cyclin D1 (cell cycle protein) and c-Myc, which are the regulators of osteoblastic proliferation, were decreased by $AlCl_3$ exposure in vivo [33–35]. Thus, it indicates that Wnt/β-catenin signaling pathway is associated with the inhibition of osteoblastic proliferation induced by $AlCl_3$.

In this study, the osteoblasts in logarithmic growth phase were exposed to aluminum trichloride ($AlCl_3$). The osteoblastic proliferation rates, expressions of Wnt/β-catenin signaling pathway key components (Wnt3a, LRP-5, p-GSK3β, GSK3β, β-catenin, and TCF-1), and target genes (cyclin D1 and c-Myc) were detected to explore the effects and relationship of $AlCl_3$ on osteoblastic proliferation and Wnt/β-catenin signaling pathway.

Materials and Methods

Cell Culture and Treatment

The primary osteoblasts were derived from calvarium of 1-day-old SD rats as previously described [15]. The rat calvarium was cut into 1–2-mm^2 pieces and consecutively digested using trypsin (2.5 g/L; Gibco, USA) for 10 min and collagenase II (1.0 g/L; Gibco, USA) for three sequential digestion periods of 15, 30, and 60 min at 37 °C. The supernatant of 15 and 30 min digestions were discarded, and cells obtained from the 60-min digestions were cultured in proliferation medium consisting of α-MEM (Gibco, USA) medium containing 10 % FBS, 2 mM glutamine (Gibco, USA), and 1 % penicillin/streptomycin (Gibco, USA) [36]. Cultures were maintained at 37 °C in a humidified atmosphere of 5 % CO_2-95 % air, and the medium was changed every 2 days until the osteoblasts reached 90 % confluence. The osteoblasts were exposed to 0 (control group), 0.4 mM (low-dose group), 0.8 mM (mid-dose group), and 1.6 mM (high-dose group) of $AlCl_3$ for 24 h which were 0, 1/20 IC_{50}, 1/10 IC_{50}, and 1/5 IC_{50} of $AlCl_3$, respectively. Our previous work had demonstrated that the IC_{50} of $AlCl_3$ on osteoblasts was 8.16 mM (1.089 mg/mL) [37]. Osteoblasts were identificated by morphological observation, ALP staining, and Alizarin red staining according to the previous research [38]. For all experiments, primary osteoblasts used were the third passage [39]. All the study was approved by the Animal Ethics Committee of the Northeast Agricultural University (Harbin, CHN).

Cell Proliferation Rate Assay

The experiment of osteoblastic proliferation rates with $AlCl_3$ exposure were determined by CCK-8 Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), which was used to assess the proliferation potential. Osteoblast suspensions were seeded into the 96-well cell culture plates with growth medium at density of 5×10^4 cells/mL with 100 μL per well. After 24-h incubation, cells were treated with 0, 0.4, 0.8, and 1.6 mM of $AlCl_3$ for 24 h, respectively. Then, each well of the plate was added with 20 μL CCK-8 solution and incubated at 37 °C for 2 h. The cell culture plate optical density (OD) value examined with a microplate reader (Bio-Tek Epoch, USA) at a 450-nm wavelength. Each well in the 96-well cell culture plate was regarded as an independent sample for statistical analysis. All assays were performed in triplicate.

Quantitative Real-Time PCR Analysis

Osteoblasts (5×10^6 cells/mL) were centrifuged at 1500× *g* for 10 min. Wnt3a, LRP-5, TCF-1, cyclin D1, and c-Myc mRNA expressions were detected by quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) [40]. Osteoblasts were harvested and rinsed twice using ice-cold PBS. The total RNA was isolated using Trizol Reagent (Invitrogen, USA) and was analyzed using spectrophotometry at 260 and 280 nm (Pharmacia Biotech, UK). Only samples with an optical density ratio at 260/280 nm >1.8 were used for further analyses. Then, each sample was reversely transcribed into complementary DNA (cDNA) using a reverse transcription kit (Trans Script First-Strand cDNA Synthesis Super Mix,

Trans Gen Blotech, CHN). The gene-specific primers used are shown in Table 1. Gene expressions were examined using SYBR Green fluorescence in qRT-PCR that was performed using ABI PRISM 7500 Real-Time PCR System (Applied Biosystems, CA). The sample was denaturized for 2 min at 50 °C and 10 min at 95 °C and amplified for 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The relative mRNA expressions were normalized to β-actin levels and determined by the $2^{-\Delta\Delta CT}$ method [41]. For cultured cells, cDNA from three different samples for each treatment group was assayed three times in duplicate.

Western Blot Analysis

p-GSK3β, GSK3β, and β-catenin protein levels were determined by Western blot analysis [40]. The total protein of osteoblasts (5×10^6 cells/mL) was extracted using a bone protein extraction kit (Beijing Tiandz, Inc. Beijing, China), and the total protein concentration was quantified by the BCA assay (Beyotime, China). The protein in an aliquot of the sample was separated by polyacrylamide gels, electro-transferred onto PVDF membranes, and blocked with 5 % non-fat milk in Tris-buffered saline with Tween 20 (TBST) buffer for 2 h. Then, the membranes were incubated using anti-GSK3β, anti-p-GSK3β, and anti-β-catenin (Santa, USA) at dilutions of 1:400 in 5 % non-fat milk overnight at 4 °C and washed three times using TBST, for 20 min each time. Subsequently, the PVDF membranes were incubated with an appropriate secondary antibody at 37 °C for 2 h and then washed three times using TBST. Finally, protein level was determined using the enhanced chemiluminescent (ECL) reagent (Beyotime, China). To assess the presence of comparable amount of proteins in each lane, the membranes were stripped finally to detect the β-actin. Quantitative analysis was carried out using Gel-Pro analyzer 4 image analysis system. All assays were performed in triplicate.

Statistical Analysis

Results are expressed as mean ± standard deviation (SD) throughout the text. Data were analyzed by one-way analysis of variance (ANOVA), using SPSS 22.0 software (SPSS Incorporated, Chicago, IL, USA). Significant changes were classified as follows: $^*P < 0.05$ was considered significant, and $^{**}P < 0.01$ was considered markedly significant. Three independent measurements were performed in triplicate, and the representative graphs were shown.

Results

$AlCl_3$ Suppressed Osteoblastic Proliferation

To investigate the effects of $AlCl_3$ on osteoblastic proliferation, the proliferation rates were determined by CCK-8 Kit. As shown in Fig. 1, $AlCl_3$ exposure significantly decreased the osteoblastic proliferation rates as compared to the control group ($P < 0.01$). This result indicates that $AlCl_3$ exposure inhibits osteoblastic proliferation.

$AlCl_3$ Inactivated the Wnt/β-Catenin Signaling Pathway

To examine the effects of $AlCl_3$ on Wnt/β-catenin pathway, the key components of Wnt/β-catenin pathway were initially examined. p-GSK3β, GSK3β, and β-catenin protein expressions were detected by Western blot. The relative intensity of p-GSK3β is normalized by GSK3β protein levels. The ratio of p-GSK3β/GSK3β and the levels of β-catenin protein decreased in $AlCl_3$-treated groups and were lower in $AlCl_3$-treated group than those in the control group ($P < 0.01$) (Fig. 2a, b). As well as, Wnt3a, LRP-5, and TCF-1 mRNA expressions were detected by qRT-PCR. As shown in Fig. 3a–c, Wnt3a, LRP-5, and TCF-1 mRNA expressions decreased in $AlCl_3$-treated groups and were markedly lower than those in the

Table 1 Primer sequences and amplification lengths of destination fragments

Gene	Primer sequence	Primer length (bp)	Product length (bp)
Wnt-3a	Up 5′AGAGTCTCGTGGCTGGGTGGAC3′ Low 5′GTTGGGCTCGCAGAAGTTAGG3′	21 21	108
LRP-5	Up 5′AAGGGTGCTGTGTACTGGAC3′ Low 5′AGAAGAGAACCTTACGGGACG3′	20 21	120
TCF-1	Up 5′CACCCACCCGTCCTTGAT3′ Low 5′GCTTCTTCGCCTCCTTCT3′	22 22	166
c-Myc	Up 5′CTACTTGGAGGAGACATGGTG3′ Low 5′TGGAGGTGGAGCAGACG3′	21 21	211
Cyclin D1	Up 5′AAAGGGTCACCACCAGCTTA3′ Low 5′ACAGGGCAGACTGTGTGGAT3′	20 20	177
β-Actin	Up 5′AGGGAAATCGTGCGTGACAT3′ Low 5′CCTCGGGGCATCGGAA3′	20 16	163

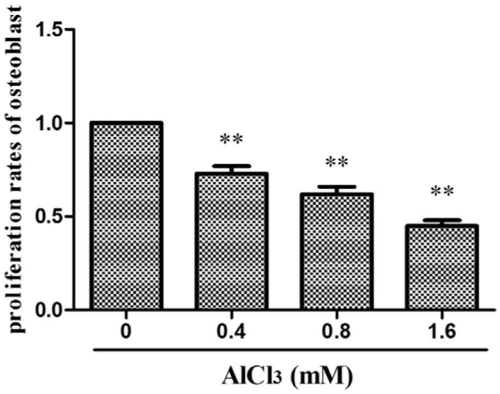

Fig. 1 Effects of $AlCl_3$ on the osteoblastic proliferation rates in rat. Osteoblasts were cultured with proliferation medium for 6 days, then incubated with various concentrations of $AlCl_3$ (containing 0, 0.4, 0.8, and 1.6 mM of $AlCl_3$) for 24 h. The proliferation rates of osteoblasts were determined by CCK-8 method. Data are expressed as means ± SD. $^{**}P < 0.01$ versus control group

control group ($P < 0.01$). These results indicate that $AlCl_3$ inactivates the Wnt/β-catenin signaling pathway.

$AlCl_3$ Suppressed Cyclin D1 and c-Myc mRNA Expressions

Cyclin D1 and c-Myc, which can modulate osteoblastic proliferation, are the target genes of Wnt/β-catenin signaling pathway. As shown in Fig. 4a, b, $AlCl_3$ exposure significantly decreased cyclin D1 and c-Myc mRNA expressions as compared to the control group ($P < 0.01$). These results indicate that $AlCl_3$ downregulates the Wnt/β-catenin signaling pathway and inhibits osteoblastic proliferation.

The Correlation Analysis Among $AlCl_3$ Concentration, Osteoblastic Proliferation Rates, and mRNA Expressions of Wnt3a, c-Myc, and Cyclin D1

The $AlCl_3$ concentration was negatively correlated with osteoblastic proliferation rates and mRNA expressions of Wnt3a, c-Myc, and cyclin D1. The correlation coefficients were −0.991 ($P < 0.01$), −0.948 ($P < 0.01$), −0.874 ($P < 0.01$), and −0.864 ($P < 0.01$), respectively. And the osteoblastic proliferation rates were positively correlated with mRNA expressions of Wnt3a, c-Myc, and cyclin D1 in osteoblasts. The correlation coefficients were 0.944 ($P < 0.01$), 0.836 ($P < 0.01$), and 0.831 ($P < 0.01$), respectively (Table 2).

Discussion

In this study, several important osteoblast observations were obtained. Firstly, we found that osteoblastic proliferation rates decreased, indicating that $AlCl_3$ inhibited osteoblastic proliferation. Subsequently, $AlCl_3$ exposure downregulated the Wnt/β-catenin signaling pathway and decreased the mRNA expressions of cyclin D1 and c-Myc. Moreover, the $AlCl_3$ concentration was negatively correlated with osteoblastic proliferation rates and mRNA expressions of Wnt3a, c-Myc, and cyclin D1. And the osteoblastic proliferation rates were positively correlated with mRNA expressions of Wnt3a, c-Myc, and cyclin D1. All above results suggested that the antiproliferation effect of $AlCl_3$ on osteoblasts might be associated with the downregulation of Wnt/β-catenin signaling pathway.

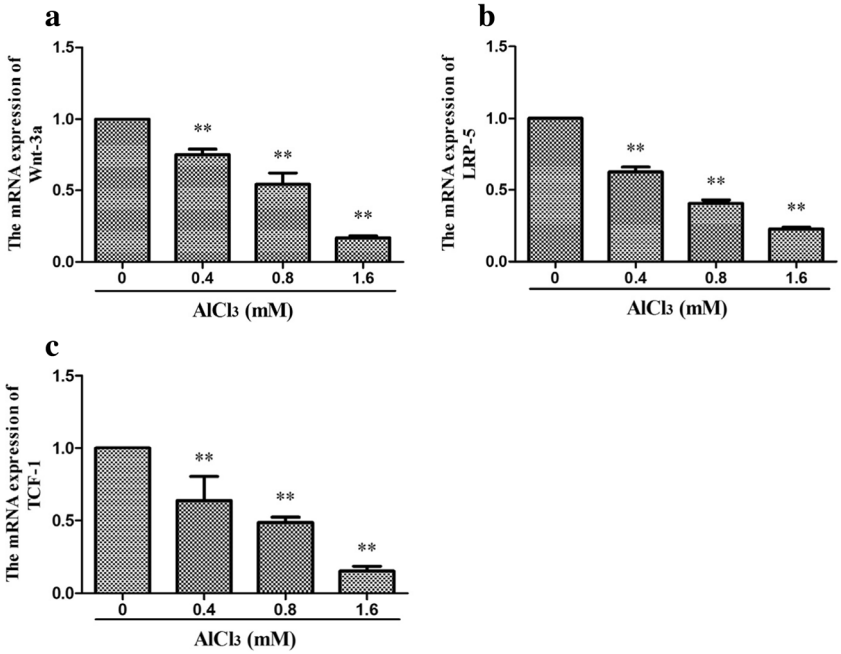

Fig. 2 Effects of $AlCl_3$ on the Wnt3a, LRP-5, and TCF-1 mRNA expressions in primary rat osteoblasts. Osteoblasts were cultured with proliferation medium for 6 days, then incubated with various concentrations of $AlCl_3$ (containing 0, 0.4, 0.8, and 1.6 mM of $AlCl_3$) for 24 h. Wnt3a, LRP-5, and TCF-1 mRNA expressions were detected by qRT-PCR. β-Actin served as an internal control. Data are expressed as means ± SD. $^{**}P < 0.01$ versus control group

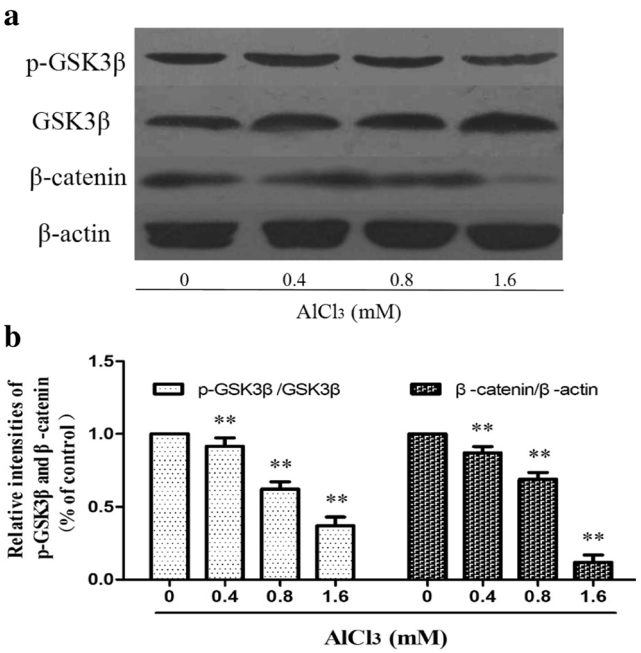

Fig. 3 Effects of $AlCl_3$ on the p-GSK3β, GSK3β, and β-catenin protein expressions in primary rat osteoblasts. Osteoblasts were cultured with proliferation medium for 6 days, then incubated with various concentrations of $AlCl_3$ (containing 0, 0.4, 0.8, and 1.6 mM of $AlCl_3$) for 24 h. **a** The p-GSK3β, GSK3β, and β-catenin protein expressions were detected by Western blotting. **b** The relative intensities of p-GSK3β and β-catenin, which were normalized with total GSK3β and β-actin protein levels. β-Actin served as an internal control. Data are expressed as means ± SD. $^{**}P < 0.01$ versus control group

Osteoblastic proliferation plays an important role for bone formation [7]. Excessive Al deposition in bone inhibits osteoblastic proliferation and leads to AIBD [12, 42]. In this study, we chose the third-passage osteoblasts, and the proliferation medium was changed every 2 days until the osteoblasts reached 90 % confluence. This process was totally 6 days, which was during the logarithmic growth phase of osteoblasts. Osteoblastic proliferation stage lasted for 12 days [34]. Subsequently, the osteoblasts were treated with 0, 1/20 IC_{50}, 1/10 IC_{50}, and 1/5 IC_{50} of $AlCl_3$, respectively. According to the IC_{50} of $AlCl_3$ detected under 24 h, therefore the osteoblasts were treated with $AlCl_3$ for 24 h.

Present data showed that $AlCl_3$ exposure suppressed osteoblastic proliferation rates. However, some studies showed the opposite results [24, 43]. In neonatal mouse osteoblasts, $AlCl_3$ stimulated osteoblastic proliferation within the concentration range of 10^{-8}–10^{-6} M and inhibited osteoblastic proliferation more than 3×10^{-6} M [23]. Al sulfate could stimulate human osteoblastic TE-85 cell proliferation at the concentration below 50 μM [24]. Thus, these effects of $AlCl_3$ may depend on experimental conditions such as the type of osteoblastic cell and the concentrations of Al^{3+}. The concentrations of Al^{3+} in neonatal mouse osteoblasts and human osteoblasts were markedly lower than that in our cultures. Our results suggested that $AlCl_3$ inhibited osteoblastic proliferation within the concentrations from 0.4 to 1.6 mM.

Wnt3a, a member of the Wnt family, can specifically activate the Wnt/β-catenin signaling pathway and is known as a promoter of the osteoblastic proliferation [36, 37]. In this study, $AlCl_3$ exposure decreased Wnt3a mRNA expression, which induced the inactivation of Wnt/β-catenin signaling pathway. Thus, the inhibitory effect of $AlCl_3$ on Wnt/β-catenin signaling pathway may be induced by downregulation of Wnt3a. Wnt3a activates Wnt/β-catenin signaling pathway by binding to LRP-5 [44]. LRP-5 plays a positive role in the regulation of bone mass [36]. LRP-$5^{-/-}$ mice have a decreased number of osteoblasts and low bone formation [45]. In this study, the mRNA expression of LRP-5 was decreased with $AlCl_3$ treatment in osteoblasts. Similar with our previous study in vivo, we found that 0.4 g/L $AlCl_3$ exposure decreased the mRNA expression of LRP-5 in rat femora [35]. These results demonstrated that the apparently negative effect of osteoblastic proliferation induced by $AlCl_3$ was associated with the decreased expression of LRP-5.

GSK3β is a negative regulator of Wnt/β-catenin signaling pathway [46]. GSK3β, a member of β-catenin destruction complex, regulates cell cycle and growth [47, 48]. It phosphorylates β-catenin to induce degradation of β-catenin by

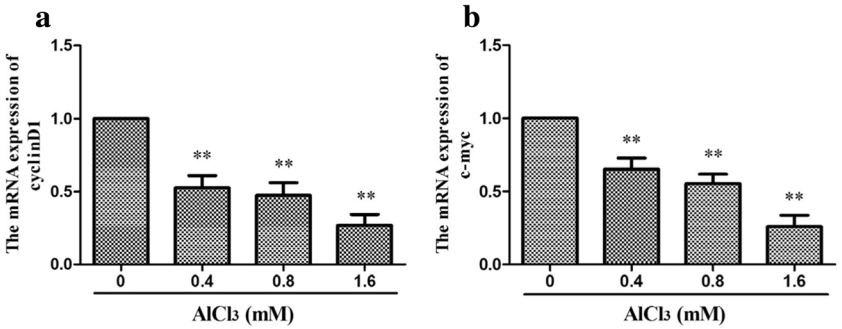

Fig. 4 Effects of $AlCl_3$ on the cyclin D1 and c-Myc mRNA expressions in primary rat osteoblasts. Osteoblasts were cultured with proliferation medium for 6 days, then incubated with various concentrations of $AlCl_3$ (containing 0, 0.4, 0.8, and 1.6 mM of $AlCl_3$) for 24 h. Cyclin D1 and c-Myc mRNA expressions were detected by qRT-PCR. β-Actin served as an internal control. Data are expressed as means ± SD. $^{**}P < 0.01$ versus control group

Table 2 The correlation analysis among $AlCl_3$ concentration, cell proliferation rates, and mRNA expressions of Wnt3a, c-Myc, and cyclin D1

Item		AlCl3 concentration	Cell proliferation rates	Wnt3a mRNA expression	c-Myc mRNA expression	Cyclin D1 mRNA expression
AlCl3 concentration	Pearson correlation	1	−0.991**	−0.948**	−0.874**	−0.864**
	Sig. (two-tailed)		0.000	0.000	0.000	0.000
	N	36	36	36	36	36
Wnt3a mRNA expression	Pearson correlation	−0.991**	1	0.944**	0.836**	0.831**
	Sig. (two-tailed)	0.000		0.000	0.001	0.001
	N	36	36	36	36	36
Cell proliferation rates	Pearson correlation	−0.948**	0.944**	1	0.716**	0.694*
	Sig. (two-tailed)	0.000	0.000		0.009	0.012
	N	36	36	36	36	36
c-Myc mRNA expression	Pearson correlation	−0.874**	0.836**	0.716**	1	0.938**
	Sig. (two-tailed)	0.000	0.001	0.009		0.000
	N	36	36	36	36	36
Cyclin D1 mRNA expression	Pearson correlation	−0.864**	0.831**	0.694*	0.938**	1
	Sig. (two-tailed)	0.000	0.001	0.012	0.000	
	N	36	36	36	36	36

**Correlation is significant at the 0.05 level (two-tailed)

*Correlation is significant at the 0.01 level (two-tailed)

phosphorylated β-catenin in osteoblasts [49]. β-Catenin is a vital component in the Wnt/β-catenin signaling pathway [29] and can promote osteoblastic proliferation [50]. The decrease of p-GSK3β/GSK3β induces degradation of β-catenin and inactivation of Wnt/β-catenin pathway [51, 52]. In this study, $AlCl_3$ downregulated p-GSK3β/GSK3β and β-catenin protein levels in osteoblasts. These findings indicate that the inhibitory effect of $AlCl_3$ on osteoblastic proliferation may be mediated by suppression of β-catenin expression. Furthermore, β-catenin is the molecular node of the Wnt/β-catenin pathway; it translocates into the nucleus to bind with transcriptional factor TCF-1 to activate the transcription of target genes [53]. In the study, $AlCl_3$ decreased mRNA level of TCF-1, which would downregulate the expressions of targeted genes (cyclin D1 and c-Myc).

Cyclin D1 and c-Myc are the target genes of Wnt/β-catenin signaling pathway, as well as the regulators of osteoblastic proliferation [34, 50, 54]. Osteoblastic proliferation is closely related with cell cycle progression [46]. Cyclin D1 is a major regulator of the progression of cells into the proliferative stage of the cell cycle [30, 55, 56]. Moreover, aberrant cell cycle progression contributes to uncontrolled cell proliferation [57]. c-Myc is a transcription factor that drives the synthesis of mRNAs [58] and protein [59]. Moreover, c-Myc plays a key role in G1-phase progression and upregulates cyclin D1 [60]. Some studies have demonstrated that the inhibition of Wnt/β-catenin signaling pathway downregulated the expressions of cyclin D1 and c-Myc and then inhibited bone formation in rats [35, 61]. As our results osteoblastserved, $AlCl_3$ treatment decreased the mRNA expressions of cyclin D1 and c-Myc, confirming that $AlCl_3$ inhibited osteoblastic proliferation. In addition, the osteoblastic proliferation rates were positively correlated with mRNA expressions of Wnt3a, c-Myc, and cyclin D1 while negatively with $AlCl_3$ concentration. Taken together, the inhibition of the Wnt/β-catenin signaling pathway, the consequent depression of cyclin D1 and c-Myc mRNA expressions, and the correlation analysis strongly suggested that the Wnt/β-catenin signaling pathway was involved in $AlCl_3$-suppressed osteoblastic proliferation.

Cao et al. cultured osteoblasts under standard differentiation culture conditions (10 % FBS, 50 μg/mL ascorbic acid, and 10 mM β-glycerophosphate) [62] and demonstrated that the inactivation of Wnt/β-catenin signaling pathway inhibited osteoblastic differentiation in Al-treated osteoblasts [15], indicating that the Wnt/β-catenin signaling pathway plays a key role in osteoblastic proliferation and differentiation in $AlCl_3$-treated osteoblasts; it also affected bone formation in $AlCl_3$-treated rats [35]. Taken together, these studies can provide a new approach in the diagnosis and treatment for healing Al-induced diseases through the Wnt/β-catenin signaling pathway.

Conclusions

$AlCl_3$ inhibited osteoblastic proliferation probably through a mechanism involving downregulating the Wnt/β-catenin signaling pathway.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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