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CD151 promotes proliferation and migration of SK-NEP-1 cells via the GSK-3β/P21/cyclinD signaling pathway

Jun Wang, Wei Lei, Gang Li, Hui Ma, Hui Guo, Shuang Li*

Urological Surgery, Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430017, China

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ABSTRACT

Wilms'tumor is the most common malignant tumor with a poor clinical prognosis because of metastasis or recurrence among children worldwide. CD151, a member of transmembrane 4 superfamily, has now been confirmed to be involved in tumor progression including the proliferation, migration, invasion and metastasis of tumor cells. GSK-3β/P21/cyclinD signaling pathway plays a critical role in the cell cycle progression, regulating cellular proliferation. In this study, CD151 protein and mRNA levels were examined by western blot and RT-PCR. The proliferation of SK-NEP-1 cells was examined by CCK8 assay and the migration of SK-NEP-1 cells was detected with wound healing assay. Furthermore, p-GSK3β protein, GSK3β protein, p21protein and CyclinD protein were examined by western blot to verify whether CD151 could regulate the Wilms'tumor progression via the GSK-3β/P21/cyclinD signaling pathway. The RT-PCR and western blot results showed that CD151 protein was upregulated in Wilms'tumor cells compared with the control. The results by CCK8 assay and wound healing assay demonstrated that CD151 overexpression promoted the proliferation and migration in SK-NEP-1 cells and CD151 interference showed the opposite effects. Western blot assay revealed that CD151 activated the GSK-3β/P21/cyclinD signaling pathway and upregulated the expression of p-GSK3β protein, p21protein and CyclinD protein. It was also verified that CD151 promotes proliferation and migration of SK-NEP-1 cells through the GSK-3β/P21/cyclinD signaling pathway in this study. The specific aim of the study is to investigate and verify the role of CD151 in Wilm's tumor. Therefore, in-depth study on the molecular mechanisms will provide new strategies and methods for the treatment of Wilm's tumor.

1. Introduction

Wilms' tumor (WT) is one of the most common pediatric tumors among children worldwide, accounting for more than 95% of all childhood renal malignancies [1,2]. Over the past decade, great advances in combined treatments for Wilms' tumor have been achieved with an overall 5-year survival rates of 85% of patients [3,4]. Nevertheless, there are still a number of cases that fail to respond to current multimodality therapy with a poor clinical prognosis because of metastasis or recurrence [5–10]. Hence, it is of great importance to investigate the molecular factors and molecular mechanisms of WT progression in order to improve its diagnosis, prognosis and management.

It is well recognized that acquisition of the proliferation and infiltration of tumor cells requires activation of a molecular programme mediated by complex signaling networks. CD151 is a member of the transmembrane 4 superfamily (TM4SF) that transmits biological signals into other key proteins in the cells (such as kinases), involved in cell adhesion, migration and other pathophysiological processes. CD151 was the first tetraspanin that has now been demonstrated to be involved in tumor progression [11,12]. Increasing evidence from studies in vitro, in vivo and clinical analyses indicates that CD151 supports the growth of various types of tumors [13,14]. It plays a critical role in the proliferation, migration, invasion and metastasis of tumor cells, and its overexpression lead to a poor prognosis for malignant tumors [15–17]. CD151 was verified to participate in nearly all stages of tumor progression. Its involvement in the early stages of tumor development was demonstrated in different biological contexts. For example, CD151 plays a critical role in regulating the proliferation of tumor cells in ductal carcinoma in situ, a pre-invasive form of breast cancer [18]. Numerous studies in vitro and in vivo models of tumor implicate that the effect of CD151 on the further steps of tumor development includes maintenance of tumor neovascularization [19,20], regulation of invasion and metastasis [21,22].

Glycogen synthase kinase 3 (GSK3) is a Ser/Thr protein kinase and

* Corresponding author.
E-mail address: ewysls@163.com (S. Li).

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two members of the mammalian GSK3 family (α and β) are well-known [23]. GSK3β is a multifunctional protein kinase with a wide range of activity regulation and multiple signal transduction pathways involved in tumor formation [24]. Cellular proliferation is mainly regulated by the cell cycle, which has been distributed into four distinct sequential phases (G0/G1, S, G2 and M) [25]. P21 is a negative regulator of the cell cycle that play a repressive role in G1/S progression by inhibiting the activity of cyclin/CDK complexes [26]. GSK3β can mediate the control of cell cycle progression [27]. The evidence showed that overexpressing GSK3β increased the expression of cyclin D1, induced cell entry into the S phase, and facilitated the proliferation of tumor cells [28]. Moreover, a series of studies indicated that the overexpression of GSK3β could directly downregulate the P21 expression by phosphorylation at Thr57, leading to proteasome-mediated degradation [29].

Given the role of CD151 in tumor progression, we hypothesized that CD151 are involved in both the cell proliferation and infiltration in Wilms' tumors. In this study, role of CD151 and GSK-3β/P21/cyclinD signaling pathway in SK-NEP-1 cells were investigated, which included cell proliferation and infiltration.

2. Materials and methods

2.1. Cell culture

Human SK-NEP-1 cell lines, purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China, were cultured in 85% McCoy's 5 A Medium/15% FBS(Merck; Sigma-Aldrich, Inc., San Francisco, CA, USA), supplemented with antibiotic and antimycotic agents (100units/ml penicillin, 100 μg/ml streptomycin; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in Forma Series II 3110 Water-Jacketed CO2 Incubators (Thermo Fisher Scientific Massachusetts, America)with standard culture conditions (37℃, 5% CO2 and 95% humidity).

2.2. CCK8 assay

Cell viability was detected by a Cell Counting Kit-8 (CCK8) assay (Promega, Madison, WI, USA) according to the manufacturer's protocol. The cells were digested, counted, and prepared into a cell suspension at a concentration of 5×10^4 cells/ml in 96-well plates. Add 100 μL of cell suspension per well and 96-well cell culture plates were incubated in an incubator with standard culture conditions (37 ℃, 5% CO2 and 95% humidity) for 24 h; Discard the medium and wash for two times with PBS. The untreated group, the vector control group and the overexpression group were transfected with the empty vector and the CD151 overexpression vector respectively. Change the medium after 6 h and 96-well cell culture plates were incubated at 37 ℃, 5% CO 2 incubator for 24 h. 10 μL of CCK8 solution was added to each well and incubated for 3 h in the incubator. The absorbance was measured at 450 nm and the OD value of each well was read by a microplate reader.

2.3. Wound healing assay

Briefly, cells (1×10^5) were seeded in 6-well plates and incubated overnight. A wound was created with a 100 μL pipette tip at 70% confluence. The cells were then washed for two times with PBS and incubated in serum-free medium at 37 ℃ for 24 h. Images were obtained under Leica DMI4000B microscope (Leica, Wetzlar, Germany). The wound gaps were measured per time-point.

2.4. RNA extraction and quantitative real-time PCR

Total RNA was isolated from SK-NEP-1 cells, matched WT cells with TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, all samples were treated with TRIzol followed by chloroform. The mixture was centrifuged at 14,000 rpm for 10 min at 4 ℃ and 700 μl 75% ethanol was added to the aqueous layer. Finally, the purified RNA was diluted with 30 μl of RNase-free water. For the evaluation of mRNA, synthesis of cDNA was performed using a RNA PCR kit (Takara, Otsu, Shiga, Japan) and quantitative real-time PCR was carried out with the SYBR premix Ex TaqII kit (Takara) according to the manufacturer's instructions. All reactions were performed in triplicate. The 2-ΔΔCt method [30] was adopted and applied to calculate the relative quantities of each gene. GAPDH was used as an endogenous control.

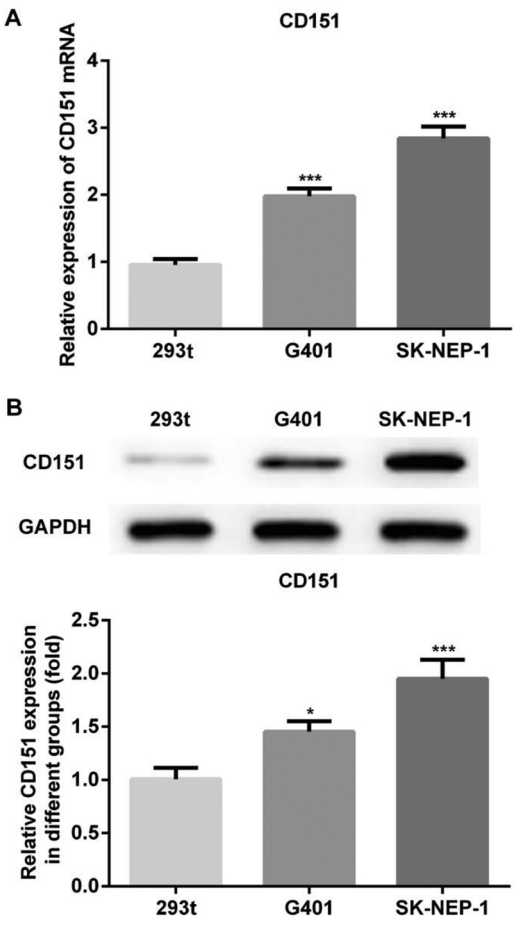

Fig. 1. CD151 mRNA and protein levels in G401 and SK-NEP-1 cells were significantly increased compared to the control group. The protein and mRNA levels of 293 T, G401 and SK-NEP-1 cells were measured by PCR (A) and western blotting (B), then quantified and statistically analyzed. GAPDH was used as the endogenous control. *P < 0.05, ***P < 0.001 vs. control group.

2.5. Western blot analysis

Western blot analysis was used to detect the relative protein expression level as described. Total proteins were extracted from SK-NEP-1 cells with RIPA buffer (10 mM Tris-HCl, pH 7.4, 1% Triton X-100, 0.1% SDS) containing protease inhibitors (Beyotime Institute of Biotechnology, China). The total protein concentration was determined using an Enhanced BCA Protein Assay kit (P0010; Beyotime Institute of Biotechnology, Shanghai, China) per as the manufacturer's protocol. A total of 20 μg of protein per lane was separated on a 10% SDS-polyacrylamide gel and then blotted onto polyvinylidene fluoride (PDVF) membranes (Sigma-Aldrich; Merck KGaA). 5% fat-free milk was used to block non-specific protein interactions in 1X TBST buffer at room temperature for 1–1.5 h. The PVDF membranes were incubated with primary antibodies including monoclonal Anti-CD151 antibody (1:1000; SAB1402716; Sigma-Aldrich; Merck KGaA), Anti-phospho-

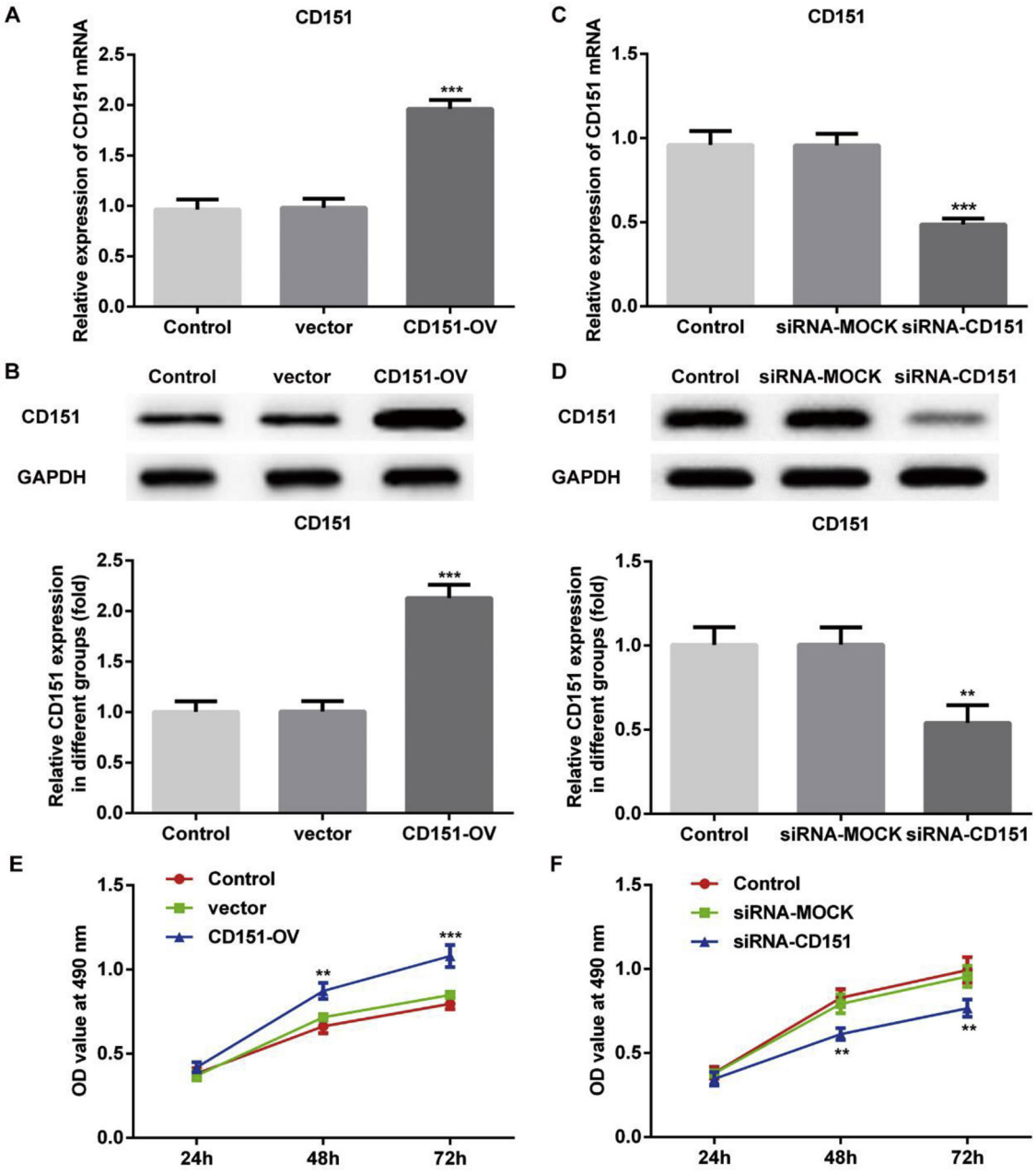

Fig. 2. CD151 overexpression markedly promoted SK-NEP-1 cell proliferation. The CD 151 overexpression upregulated the mRNA (A), protein (B) levels and cell proliferation (E) in SK-NEP-1 cells. Cells were either untreated, or transfected with empty plasmid, transfected with overexpression plasmid of CD151. The CD151 interference downregulated the mRNA (C), protein (D) levels and cell proliferation (F) in SK-NEP-1 cells. Cells were either untreated, or transfected with siRNA-MOCK, transfected with siRNA-CD151. CD151 mRNA and protein levels were measured by PCR and western blotting and GAPDH was used as the endogenous control. The cell viability of SK-NEP-1 cells were measured with CCK8 assay. Data of three independent experiments was quantified and statistically analyzed. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group.

GSK3B (pSer9) antibody (1:1000; SAB4300001; Sigma-Aldrich; Merck KGaA), GSK-3 beta Antibody(1:1000; MAB2506; R&D Systems), Anti-p21 CIP1 antibody produced (1:1000; SAB4500065; Sigma-Aldrich; Merck KGaA), Anti-Cyclin D1(1:10000; ab134175; Abcam) and Monoclonal Anti-GAPDH (1:15000; G8795; Sigma-Aldrich; Merck KGaA) in 5% fat-free milk at 4 °C overnight. On the second day, 1X TBST buffer was used to wash the unbound antibody (10 min each for four times). The PVDF membranes were treated with horseradish peroxidase (HRP)-conjugated secondary antibody (Anti-Mouse IgG; 1:10000; A9917; Sigma-Aldrich; Merck KGaA) diluted (1:2000) by Antibody Diluent Reagent Solution (cat. no. 1956331 A; Thermo Fisher Scientific, Inc.) at room temperature for 1–1.5 h. After washing the PVDF membranes for four times in 1X TBST buffer, protein bands were visualized by an enhanced chemiluminescence (ECL) kit (sc-2048, Sigma-Aldrich, Merck KGaA) following the manufacturer's instructions. Data were analyzed with Image Pro Plus v.6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) for densitometry. GAPDH was used as an endogenous control.

2.6. Statistical analysis

All the experiments were performed at least for three times. Data are presented as the mean ± standard deviation. Data were analyzed by one-way analysis of variance followed by the Schefffe post-hoc test to evaluate the effects of different treatments. Analyses were conducted using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was

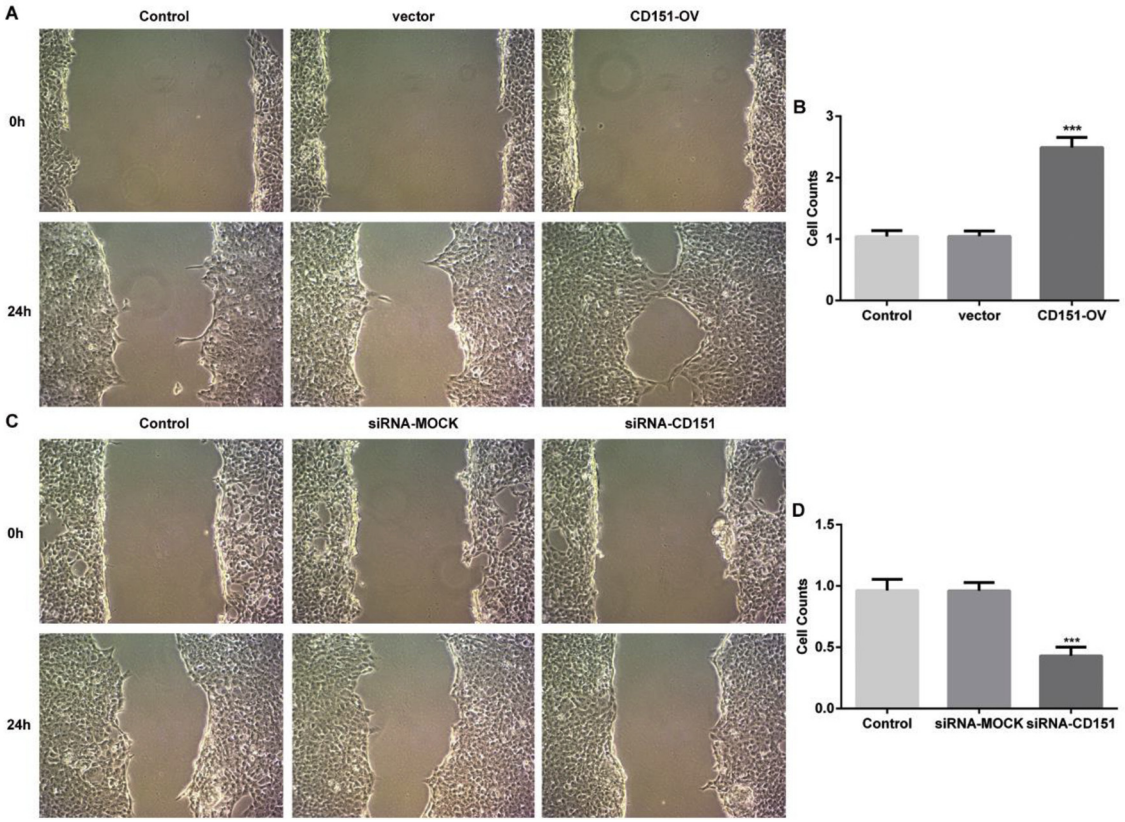

Fig. 3. CD151 overexpression promoted SK-NEP-1 cell migration. The CD151 overexpression promoted SK-NEP-1 cell migration (A, B). Cells were either untreated, or transfected with empty plasmid, transfected with overexpression plasmid of CD151. The CD151 interference inhibited SK-NEP-1 cell migration (C, D). Cells were either untreated, or transfected with siRNA-MOCK, transfected with siRNA-CD151. The SK-NEP-1 cell migration was measured by transwell assay, then quantified and statistically analyzed. ***P < 0.001 vs. control group.

considered to indicate a statistically significant difference.

3. Results

3.1. CD151 protein and mRNA levels in G401 and SK-NEP-1 cells were significantly increased compared to 293 T cells

To analyze whether CD151 was associated with Wilms' tumor, we detected the CD151 mRNA and protein levels in 293 T, G401 and SK-NEP-1 cells by RT-qPCR and western blot. CD151 mRNA levels ($P < 0.001$, Fig. 1A) and protein levels ($P < 0.05$, Fig. 1B) in G401 cells were significantly increased compared to the control group. CD151 mRNA levels ($P < 0.001$, Fig. 1A) and protein levels ($P < 0.001$, Fig. 1B) in SK-NEP-1 cells were significantly increased compared to the control group. Therefore, we selected SK-NEP-1 cells for the following experiments.

3.2. CD151 overexpression significantly promoted SK-NEP-1 cell proliferation

To analyze the effect of CD151 on the proliferation of SK-NEP-1 cells, we detected the CD151mRNA and protein levels by RT-qPCR and western blot following treatment with CD151 overexpression or interference. The cell viability of SK-NEP-1 cells was assessed by CCK8 assay. CD151 overexpression significantly elevated the mRNA level ($P < 0.001$; Fig. 2A) and protein level ($P < 0.001$; Fig. 2B) compared with the control group. CD151 overexpression significantly increased cell viability for 48 h ($P < 0.01$; Fig. 2E) and 72 h ($P < 0.001$; Fig. 2E) compared with the control group. CD151 interference decreased the mRNA level ($P < 0.001$; Fig. 2C) and protein level ($P < 0.01$; Fig. 2D) compared with the control group. CD151 interference significantly decreased cell viability for 48 h ($P < 0.01$; Fig. 2F) and 72 h ($P < 0.01$; Fig. 2F) compared with the control group.

3.3. CD151 overexpression significantly promoted SK-NEP-1 cell migration

To measure the effect of CD151 on the migration of SK-NEP-1 cells, we detected the migratory capacities in SK-NEP-1 cells with wound healing assays. The results revealed a significant decrease in the wound-healing distance in the CD151-overpression SK-NEP-1 cells after 24 h ($P < 0.001$; Fig. 3A and B). Meanwhile, the wound-healing distance of the siRNA-CD151 transfected cells was more extensive compared with the control group ($P < 0.001$; Fig. 3C and D). The phenomenon in this experiment indicated that CD151 could significantly promoted the migration of SK-NEP-1 cells.

3.4. CD151 promoted proliferation and migration of SK-NEP-1 cells via GSK-3β/P21/cyclinD signaling pathway

To determine whether CD151 influenced the proliferation and migration in SK-NEP-1 cells through GSK-3β/P21/cyclinD signaling pathway, we detected the protein level of GSK3β, p-GSK3β, p21 and CyclinD by western blot following treatment with CD151 overexpression or interference. CD151 overexpression significantly elevated the protein levels of p-GSK3β ($P < 0.001$; Fig. 4A), p21 ($P < 0.001$; Fig. 4A) and CyclinD ($P < 0.001$; Fig. 4A) compared with the control group. Meanwhile, CD151 interference decreased protein level of p-GSK3β ($P < 0.01$; Fig. 4B), p21 ($P < 0.001$; Fig. 4B) and CyclinD ($P < 0.01$; Fig. 4B) compared with the control group.

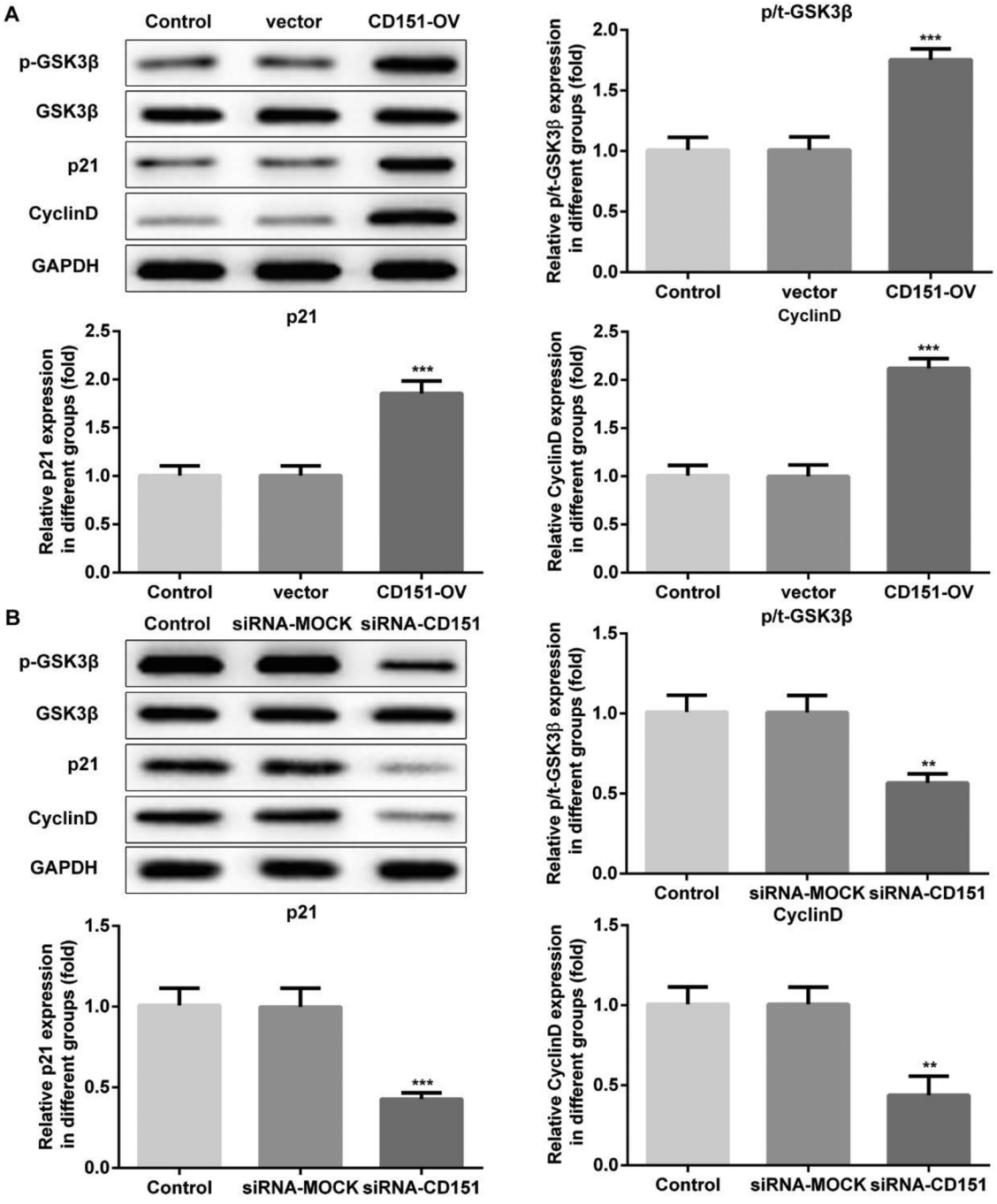

Fig. 4. CD151 promoted proliferation and migration of SK-NEP-1 cells via GSK-3β/P21/cyclinD signaling pathway. The CD 151 overexpression upregulated the protein levels of p-GSK3β, p21 and CyclinD in SK-NEP-1 (A). Cells were either untreated, or transfected with empty plasmid, transfected with overexpression plasmid of CD151. The CD151 interference downregulated the protein levels of p-GSK3β, p21 and CyclinD in SK-NEP-1 (B). Cells were either untreated, or transfected with siRNA-MOCK, transfected with siRNA-CD151. The protein levels were measured by western blotting, GAPDH was used as the endogenous control, then quantified and statistically analyzed. **P < 0.01, ***P < 0.001 vs. control group.

4. Discussion

In this study, we revealed that CD151 was obviously upregulated in Wilms'tumor. Furthermore, we demonstrated that CD151 was a positive regulator of the proliferation and migration of SK-NEP-1 cells because its overexpression significantly promoted SK-NEP-1 cell proliferation and migration, whereas its silencing led to the opposite effect. Finally, we demonstrated that CD151 influences the proliferation and migration of SK-NEP-1 cells through the regulation of GSK-3β/P21/cyclinD signaling pathway.

Wilms'tumor is the most common pediatric renal tumor and the prognosis of WT in clinics depends on many factors, including tumor stage, histological subtype, preoperative tumor volume and the patients' age [31–34]. As the same, the molecular factors about the prognosis of WT patients are of great importance. In the present study, we detected the expression and functions of CD151 in WT, as it plays a key role in tumor development and progression due to its involvement in cell proliferation, apoptosis and migration in serious cancers, such as ductal carcinoma, breast cancer and malignant squamous cell carcinoma [18,35]. Our study first found that the expression of CD151 was much higher in G401 and SK-NEP-1 cells compared to 293 T cells. CCK8 assay and wound healing assay revealed that CD151 overexpression

markedly promoted proliferation and migration in SK-NEP-1 cells while CD151 interference considerably decreased proliferation and migration in SK-NEP-1 cells, further supporting the hypothesis that CD151 acts as a risk factor in Wilms'tumor. Moreover, western blot results revealed that high CD151 promoted proliferation and migration of SK-NEP-1 cells via GSK-3β/P21/cyclinD signaling pathway.

In conclusion, CD151 was identified as a novel regulator of proliferation and migration in SK-NEP-1 cells through GSK-3β/P21/cyclinD signaling pathway. Therefore, this study provided new insights into the possibility of CD151 being a potential therapeutic target in Wilms'tumor.

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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JW, WL, GL, HM, HG and SL made substantial contributions to the conception and design of the study, performed the experiments and analyzed the data. JW, WL, GL, HM, HG and SL managed the literature searches and figure preparations. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

[1] A.M. Davidoff, Wilms' tumor, Curr. Opin. Pediatr. 22 (3) (2010) 386.
[2] G. Diniz, Histopathological and Molecular Characteristics of Wilms Tumor. Wilms Tumor [Internet], (2016) Chapter 3.
[3] J.S. Dome, C.V. Fernandez, E.A. Mullen, J.A. Kalapurakal, J.I. Geller, V. Huff, et al., Children's Oncology Group's 2013 blueprint for research: renal tumors, Pediatr. Blood Cancer 60 (6) (2013) 994–1000.
[4] J. Su, S.J. Li, Z.H. Chen, C.H. Zeng, H. Zhou, L.S. Li, et al., Evaluation of podocyte lesion in patients with diabetic nephropathy: Wilms' tumor-1 protein used as a podocyte marker, Diabetes Res. Clin. Pract. 87 (2010) 167–175.
[5] E. Szychot, J. Apps, K. Pritchard-Jones, Wilms' tumor: biology, diagnosis and treatment, Transl. Pediatr. 3 (1) (2014) 12–24.
[6] J.C. Routh, P.E. Grundy, J.R. Anderson, A.B. Retik, K.C. Kurek, B7-h1 as a biomarker for therapy failure in patients with favorable histology Wilms' tumor, J. Urol. 189 (4) (2013) 1487–1492.
[7] E.B. Cone, S.S. Dalton, M. Van Noord, E.T. Tracy, H.E. Rice, J.C. Routh, Biomarkers for wilms tumor: a systematic review, J. Urol. 196 (2016) 1530–1535.
[8] C. Qi, Y. Hu, F. Yang, H. An, J. Zhang, H. Jin, et al., Preliminary observations regarding the expression of collagen triple helix repeat-containing 1 is an independent prognostic factor for Wilms' tumor, J. Pediatr. Surg. 51 (2016) 1501–1506.
[9] J. Brok, K. Pritchard-Jones, J.I. Geller, F. Spreafico, Review of phase I and II trials for Wilms' tumour e can we optimise the search for novel agents? Eur. J. Cancer 79 (2017) 205–213.
[10] X. Yu, Z. Li, M.T. Chan, W.K. Wu, The roles of microRNAs in Wilms' tumors, Tumor Biol 37 (2016) 1445–1450.
[11] F. Berditchevski, Complexes of tetraspanins with integrins: more than meets the eye, J. Cell. Sci. 114 (Pt 23) (2001) 4143–4151.
[12] P.A. Lazo, Functional implications of tetraspanin proteins in cancer biology, Cancer Sci. 98 (2007) 1666–1677.
[13] P.M. Sincock, G. Mayrhofer, L.K. Ashman, Localization of the transmembrane 4 superfamily (TM4SF) member PETA-3 (CD151) in normal human tissues: comparison with CD9, CD63, and alpha5beta1 integrin, J. Histochem. Cytochem. 45 (1997) 515–525.
[14] M. Zoller, Tetraspanins: push and pull in suppressing and promoting metastasis, Nat. Rev. Cancer 9 (2009) 40–55.
[15] M. Kohno, H. Hasegawa, M. Miyake, T. Yamamoto, S. Fujita, CD151 enhances cell motility and metastasis of cancer cells in the presence of focal adhesion kinase, Int. J. Cancer 97 (2002) 336–343.
[16] C.W. Chien, S.C. Lin, Y.Y. Lai, B.W. Lin, S.C. Lin, J.C. Lee, et al., Regulation of CD151 by hypoxia controls cell adhesion and metastasis in colorectal cancer, Clin. Cancer Res. 14 (2008) 8043–8051.
[17] S.H. Yoo, K. Lee, J.Y. Chae, K.C. Moon, CD151 expression can predict cancer progression in clear cell renal cell carcinoma, Histopathology 58 (2011) 191–197.
[18] V. Novitskaya, H. Romanska, M. Dawoud, J.L. Jones, F. Berditchevski, Tetraspanin CD151 regulates growth of mammary epithelial cells in three-dimensional extracellular matrix: implication for mammary ductal carcinoma in situ, Cancer Res. 70 (2010) 4698–4708.
[19] Y. Takeda, Q. Li, A.R. Kazarov, M. Epardaud, K. Elpek, S.J. Turley, et al., Diminished metastasis in tetraspanin CD151-knockout mice, Blood 118 (2011) 464–472.
[20] F. Zhang, J.E. Michaelson, S. Moshiach, N. Sachs, W. Zhao, Y. Sun, et al., Tetraspanin CD151 maintains vascular stability by balancing the forces of cell adhesion and cytoskeletal tension, Blood 118 (2011) 4274–4284.
[21] R. Bari, Q. Guo, B. Xia, Y.H. Zhang, E.E. Giesert, S. Levy, et al., Tetraspanins regulate the protrusive activities of cell membrane, Biochem. Biophys. Res. Commun. 415 (2011) 619–626.
[22] Yánez-Mó, M. Mittelbrunn, F. Sánchez-Madrid, Tetraspanins and intercellular interactions, Microcirculation 8 (2001) 153–168.
[23] J.R. Woodgett, Molecular cloning and expression of glycogen synthase kinase-3/factor A, EMBO J. 9 (8) (1990) 2431–2438.
[24] R. Mancinelli, G. Carpino, S. Petrungaro, C.L. Mammola, L. Tomaipitinca, A. Filippini, et al., Multifaceted roles of GSK-3 in cancer and autophagy-related diseases, Oxid. Med. Cell. Longev. 8 (2017) 1–14.
[25] H. Goto, A. Inoko, M. Inagaki, Cell cycle progression by the repression of primary ciliaformation in proliferating cells, Cell. Mol. Life Sci. 70 (20) (2013) 3893–3905.
[26] J. Cicenas, K. Kalyan, A. Sorokinas, A. Jatulyte, D. Valiunas, A. Kaupinis, Highlights of the latest advances in research on CDK inhibitors, Cancers (Basel) 6 (4) (2014) 2224–2242.
[27] R. Mishra, Glycogen synthase kinase 3 beta: can it be a target for oral cancer, Mol. Cancer 9 (144) (2010).
[28] Q. Cao, X. Lu, Y.J. Feng, Glycogen synthase kinase-3beta positively regulates the proliferation of human ovarian cancer cells, Cell Res. 16 (7) (2006) 671–677.
[29] L. Rössig, C. Badorff, Y. Holzmann, A.M. Zeiher, S. Dimmeler, Glycogen synthase kinase-3 couples AKT-dependent signaling to the regulation of p21Cip1 degradation, J. Biol. Chem. 277 (12) (2002) 9684–9689.
[30] Livak, Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method, Methods 25 (2001) 402–408.
[31] J. Krishnan, J. Pietras, M. Nachmann, G. Brown, Adult Wilms' tumor with a unique presentation of high-grade fever, photophobia, and headache, Rev. Urol. 14 (1-2) (2012) 31–34.
[32] B. Isidor, F. Bourdeaut, D. Lafon, G. Plessis, E. Lacaze, C. Kannengiesser, et al., Wilms' tumor in patients with 9q22.3 microdeletion syndrome suggests a role for ptch1 in nephroblastomas, Eur. J. Hum. Genet. 21 (7) (2013) 784–787.
[33] Y.K. Yadav, U. Sharma, K. Gupta, R. Arora, Squamous predominant teratoid Wilms' tumor, J. Lab. Phys. 4 (1) (2012) 50–52.
[34] E. Pluciennik, M. Nowakowska, W.I. Wujcicka, A. Sitkiewicz, B. Kazanowska, E. Zielińska, et al., Genetic alterations of wwox in Wilms' tumor are involved in its carcinogenesis, Oncol. Rep. 28 (4) (2012) 1417–1422.
[35] Q. Li, X.H. Yang, F. Xu, C. Sharma, H.X. Wang, K. Knoblich, et al., Tetraspanin CD151 plays a key role in skin squamous cell carcinoma, Oncogene 32 (2013) 1772–1783.