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ORIGINAL ARTICLE

CDK5 Regulates PD-L1 Expression and Cell Maturation in Dendritic Cells of CRSwNP

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Abstract— The maturation of dendritic cells is critical for chronic rhinosinusitis with nasal polyps (CRSwNPs), especially eosinophilic chronic rhinosinusitis with nasal polyps (EosCRSwNPs), but the regulation mechanism of dendritic cells (DCs) maturation is still unclear. We identified nasal mucosa of 20 patients with EosCRSwNP, 16 non-EosCRSwNP patients, and inferior turbinate of 14 patients with nasal septum deviation after surgery. The expression of cyclin-dependent kinase 5 (CDK5) and programmed cell death 1 ligand 1 (PD-L1) were detected by immunofluorescent, real-time quantitative PCR, and Western blot in EosCRSwNP. The level of dendritic cell maturation was detected by flow cytometry and immunofluorescence staining after CDK5 expression interference with small interfering RNA (siRNA). The expression of CDK5 and PD-L1 in EosCRSwNP nasal mucosal tissue was significantly higher than that of non-EosCRSwNP and inferior turbinate nasal mucosa tissue, and there was a positive correlation between them. Immunofluorescence staining showed that CDK5 and PD-L1 were co-localized in dendritic cells. Synergistic stimulation of dendritic cells with LPS and TNF-α promotes the maturation of dendritic cells and increases the expression of CDK5 and PD-L1. However, blocking the expression of CDK5 in dendritic cells with siRNAs leads to a blockage of cell maturation. CDK5 can regulate the expression of PD-L1, and its presence is critical for the maturation of dendritic cells. CDK5 may play an important role in the pathogenesis of CRSwNP disease.

KEY WORDS: CDK5; PD-L1; CRSwNP; dendritic cells; maturation; inflammatory.

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INTRODUCTION

Chronic rhinosinusitis (CRS) is a chronic inflammatory disease that mainly occurs in the nasal-sinus mucosa, and has a high incidence rate and severely affects the quality of life of patients [1–3]. CRS is classified into CRS without nasal polyps (CRSsNP) and with nasal polyps (CRSwNP) according to whether it is accompanied by nasal polyps [4]. CRSwNP can be divided into eosinophilic (EosCRSwNP) and non-eosinophilic CRSwNP (non-EosCRSwNP) according to whether the nasal mucosa shows significant eosinophilic inflammation [5, 6]. Studies have found that the symptoms of EosCRSwNP is more difficult to control and it shows worse surgical outcomes

than other types of CRS [4, 7]. Therefore, the study of the pathogenesis and therapeutic strategies of EosCRSwNP is essential to improve the quality of life of CRS patients.

Dendritic cells (DCs) are powerful antigen-presenting cells, it has been known that DCs play a central role in sensing foreign antigens and infectious agents and in initiating appropriate immune responses [8]. They play primary role in the differentiation of naïve T lymphocyte cells in the face of antigens exposure and participate in different immunopathological processes [9, 10]. The study found that a higher proportion of $CD86^+$, $OX40L/PD\text{-}L1^+$ dendritic cells and excessive Th2 response in EosCRSwNPs compared with non-EosCRSwNPs [7]. Although more and more evidence shows the importance of DCs in the development of respiratory immune balance and other respiratory diseases, such as allergic rhinitis and asthma [11, 12], only a few studies have focused on the role of DCs in CRS [13, 14]. And the phenotype and functional characteristics of DCs in different CRS, especially in EosCRSwNP, are not yet clear.

Cyclin-dependent kinase 5 (CDK5) is an important proline-dependent serine/threonine protein kinase. It plays an extremely important role in the formation of a variety of cells and physiological activities such as cytoskeleton, cell cycle, cell proliferation, cell migration, axon guidance, and cell survival [15]. Abnormal expression of CDK5 can lead to neuronal degeneration, abnormal cell growth, cellular inflammatory responses, and changes in cellular metabolism and other physiological diseases [16, 17]. It was found that CDK5/p35 kinase complexes can regulate the phosphorylation of MAPKs (mitogen-activated protein kinase) and then affect the expression of IL-10 in macrophages induced by LPS [18]. TGF-β1 can activate upregulation of CDK5 in dentine cells, thereby activating the ERK1/2 (extracellular regulated protein kinases) signaling pathway and promoting cell differentiation and cellular inflammatory response, but the CDK5 inhibitor roscovitine can block this effect [19]. The above studies indicate that CDK5 may play a very important role in the signal transmission of various immune cells, but so far, the role of CDK5 in CRS disease has not been reported.

PD-L1 is a ligand for PD-1 (programmed cell death protein 1), a co-stimulatory molecule of the CD28/B7 superfamily, mediating the negative regulatory signal of the immune response [16]. PD-1 has two ligands PD-L1 and PD-L2, although the affinity of PD-L2 is more than three times that of PD-L1, the expression of PD-L1 is more extensive than that of PD-L2 [17]. Studies have found that PD-L1 is expressed on the surface of immune cells such as T lymphocytes and B lymphocytes, DCs, and macrophages as well as various tissues and organs of the human body. PD-L1 mediates $CD4^+$ T cells and $CD8^+$ T cell immune responses [18]. Shi Lili et al. found that TH2 cells were over-differentiated and expression of PD-L1 was increased in dendritic cells in the nasal mucosa of EosCRSwNPs, but PD-L2 expression was normal. Blocking PD-L1 expression in EosCRSwNP dendritic cells can inhibit the production of IL-4, IL-5, and IL-13, promote the production of INF-g, and reduce the proportion of TH2 cells [7]. Therefore, PD-L1 plays a crucial role in dendritic cells inducing differentiation of $CD4^+$ T cells. Studies have found that interferon-γ (IFN-γ) can induce the upregulation of PD-L1 in medulloblastomas to require the mediation of Cdk5, and that the disruption of Cdk5 expression in a medulloblastoma mouse model leads to effective $CD4^+$T cell-mediated tumor rejection [20]. We speculate that CDK5 participates in cellular immunity and inflammatory responses by regulating the expression of PD-L1 in EosCRSwNP.

In the present study, our results demonstrated that CDK5 and PD-L1 expression was significantly increased in EosCRSwNP nasal mucosa. And the degree of maturation of DCs was significantly correlated with the expression level of CDK5. This study has identified that the maturation of DCs in CRSwNP is associated with the expression level of CDK5, especially in EosCRSwNP, which may provide new therapeutic targets and clinical ideas for the treatment of CRSwNP.

MATERIALS AND METHODS

Study Population

Biopsies of patients with EosCRSwNPs ($n = 20$), non-EosCRSwNPs ($n = 16$), and healthy controls ($n = 14$) were obtained from the department of otolaryngology in the Second Hospital of Shandong University (China, approval number: KYLL-(LW)010). Subject exclusion criteria: The study subjects used systemic or topical glucocorticoids within 3 months before surgery; patients with posterior nostril polyps, fungal rhinosinusitis, cystic fibrosis, and patients with acute upper respiratory tract infection within 4 weeks. The mucosal biopsy of the inferior turbinate (IT) was obtained from 14 non-NP patients with deviated septum and those as healthy controls. All tissues of EosCRSwNPs ($n = 20$), non-EosCRSwNPs ($n = 16$), and healthy controls ($n = 14$) were fixed in formalin for histological evaluation, and RNA and protein were extracted for the detection of corresponding gene and protein expression. In our study, CRSwNP was classified as

eosinophils when the percentage of tissue eosinophils exceeded 10% of total infiltrating cells. The patient's specific information is described in detail in Table 1. The approval for this study was obtained from the institutional review boards of the participating hospitals in China.

Reagents

LPS were purchased from Sigma (Cat. No. L2880, Sigma). TNF-α were purchased from DingGuo (Cat. No. YZ-0016, DingGuo). Rabbit polyclonal anti-CDK5 antibody (Cat. No. sc-173, 1:3000 diluted for WB, 1:100 diluted for IF) was from Santa Cruz, and mouse polyclonal anti-CDK5 antibody (Cat. No. BF0121, 1:100 diluted for IF) was from Affinity. Rabbit polyclonal anti-CD123/IL3RA antibody (Cat. No. A3926, 1:100 diluted for IF) and rabbit polyclonal anti-GAPDH antibody (Cat. No. AC027, 1:5000 diluted) was from ABclonal. Mouse polyclonal anti-PD-L1/CD274 antibody (Cat. No. 66248-1-Ig, 1:3000 diluted for WB, 1:100 diluted for IF) was from Proteintech. Fetal bovine serum (FBS) (Cat. No. 10099141) and TRIzol (Cat. No. 15596026) were from Invitrogen.

Immunofluorescence

The sample was treated as we described before, all nasal biopsy specimens (from EosCRSwNPs and non-EosCRSwNPs patients, healthy controls) were fixed in 4% paraformaldehyde and then embedded in paraffin. Tissue blocks were sectioned to a thickness of 4 mm with a Leica microtome (Leica, Wetzlar, Germany). Then the expression of CDK5 and PD-L1 was identified in nasal biopsy sections by immunofluorescence (IF) staining. The paraffin sections were imaged with a confocal microscope (LSM 700, Zeiss).

Cell Culture and siRNA Transfection

Human dendritic cells (hDCs) were purchased from BeNa (Cat. No. BNCC341107), it was grown and maintained in high-sugar medium supplemented with 10% FBS. Cells were plated at a density of 3×10^5 cells per 60 mm dish and cultured for 24 h. Then cultured cells were transfected with *CDK5* siRNAs by jetPRIME® Transfection Agent (Cat. No. PT-114-15, Polyplus) according to the manufacturer's instructions. *CDK5* siRNAs were obtained from Biotech of Shanghai. Sequence of *CDK5* siRNAs are as follows: UAUGACAGAAUCCCAG CCCTT, GGGCUGGGAUUCUGUCAUATT [21]. In order to increase transfection efficiency, DCs were transfected twice. Briefly, DC cells were transfected with 30 nM siRNAs using jetPRIME® Transfection Agent. The simple statement is that the second transfection was performed 24 h after the end of the first transfection.

RNA Extraction and Quantitative Real-Time PCR

Total RNA of hDCs or frozen nasal tissues in RNA later were extracted using TRIzol. RNA reverse transcription was performed by RT reactions with PrimeScript™ RT reagent Kit with gDNA Eraser according to the manufacturer's protocol (Cat. No. RR047A, Takara). And mRNA levels were detected by SYBR Green gene expression assays. Quantitative real-time PCR (qPCR) was performed with the following primers: CDK5 forward primer, CGCCGCGATGCAGAAATACGAGAA, reverse primer, TGGCCCCAAAGAGGACATC (439 bp); *PD-L1* forward primer, GGACAAGCAGTGACCATCAAG, reverse primer, CCCAGAATTACCAAGTGAGTCCT (235 bp); *GAPDH* forward primer, GGGAAACTGTGGCGTGAT, reverse primer, GAGTGGGTGTCGCTGTTGA (216 bp). Amplification and detection were run with an initial cycle of 95 °C for 10 s followed by 40 cycles of 95 °C for 5 s, 58 °C for 20 s, and 64 °C for 20 s. All PCR reactions were performed in triplicate. Negative control samples (without template) were processed in the same way as the experimental group. The specificity of the amplifications was verified by melting curve analysis. Relative gene expression was calculated using the comparative $2^{-\triangle\triangle Ct}$ method with the housekeeping gene (GAPDH) as a reference.

Western Blot

Human DCs or frozen nasal tissues were homogenized in ice-cold RIPA lysis buffer (Cat. No. P0013B, Beyotime) with 1 mM PMSF and 1× protease inhibitor cocktail (Roche). After, the sample is centrifuged at 4 °C and the supernatant is collected. The samples which contained 50 μg protein were separated on a 12% SDS-PAGE gel and transferred to PVDF membrane. The PVDF membranes were incubated with corresponding primary antibodies overnight at 4 °C, followed by incubation with the appropriate secondary antibodies (Bio-Rad) for 1 h at 37 °C, the signals were detected with the ECL system (Cell Signaling Technology).

Flow Cytometry

Human DCs ($3 \times l0^5$ cells/well) were seeded in six-well plates and transfected as described above. Then cells were treated with LPS and TNF-α after transfection for

Table 1. Patients' Characteristics

	Nasal mucosa healthy control	EosCRSwNPs	Non-EosCRSwNPs
Sample sizes	14	20	16
Median age, years (IQR)	36.2 (9–56)	45(26–69)	38.3 (18–61)
Gender, male/female	8/6	9/12	9/7
Atopy, *n*/*N*	1/14	2/20	1/16
Asthma, *n*/*N*	0	1/20	0
Median CT score (IQR)	0	8.60 (5–14)***	7.75 (3–14)***
Median endoscopy score (IQR)	0	6 (3–9)***	5.25 (3–8)***
Eosinophilia[a]	1/14	20/20	0

The level of significance (p) was obtained from the Student's t test. p value of < 0.05 was considered statistically significant. Gender comparison was performed using the χ^2 test
CT computed tomography, *IQR* interquartile range
*Means the p value is < 0.05, while symbol. **Means the p value is < 0.01, ***Means the p value is < 0.001
[a] The percentage of eosinophils exceeding 10% was categorized as eosinophilia or neutrophilia

24 h. For phenotypic characterization of cells, cells were labeled (30 min, 0 °C) with PECP/Cy5.5-HLA-DR, PE-CD86, APC-CD80, or APC/Cy7-CD83 directed against lineage markers HLA-DR, CD86, CD80, and CD83. Cells were washed three times with cold PBS, and then cytometric analyses were performed on a FACSCalibur (BD Biosciences).

Statistical Analysis

Data were shown as mean ± SD from at least three independently performed experiments. Comparisons of parameters between two groups were analyzed using unpaired Student's t test. Statistical significance was analyzed by a two-tailed paired t test by using GraphPad Prism Software 6.0 (GraphPad Software, La Jolla, CA). A p value < 0.05 was considered statistically significant. Each data point was expressed as mean (standard deviation).

RESULTS

Increased Expression of CDK5 and PD-L1 in Nasal Mucosa of CRSwNPs

In order to detect the expression level of CDK5 and PD-L1 protein in NPs, we performed Western blot analysis. Result showed that CDK5 and PD-L1 protein expression was significantly higher in nasal mucosa of NPs patients compared with control, especially in EosCRSwNPs (Fig. 1a). Through the gray value scan of Western blot results, it was found that the expression level of CDK5 was approximately 1.3-fold higher in non-EosCRSwNPs and 2-fold higher in EosCRSwNPs relative to control tissues (Fig. 1b, c). To further confirm this result, we extracted the mRNA of the corresponding tissues for reverse transcription and performed real-time quantitative PCR. The results showed that the mRNA levels of CDK5 and PD-L1 were increased in both EosCRSwNPs and non-EosCRSwNPs compared with the normal control group, and the increase in EosCRSwNPs is even more pronounced (Fig. 1d, e). In order to examine the correlation of PD-L1 with CDK5 in nasal mucosa, correlation analysis is performed and the results showed that PD-L1 expression was positively correlated with CDK5 expression (Fig. 1f).

The Expression of CDK5 and PD-L1 Increased and Co-localized in DCs of CRSwNPs

To further investigate the expression pattern of CDK5 and PD-L1 in CRSwNPs tissues, we performed immunofluorescence staining of tissue samples. The results showed that compared with the control group, the expression of CDK5 and PD-L1 was significantly increased in CRSwNPs, and the expression of CDK5 and PD-L1 protein increased more significantly in EosCRSwNPs (Fig. 2a). In addition, we also found that the expression level of PD-L1 is correspondingly increased in cells with high expression of CDK5. In contrast, the expression of PD-L1 is low in cells as low expression level of CDK5, and CDK5 and PD-L1 are co-localized in cells (Fig. 2a). To further determine the cell types specifically expressed by CDK5 and PD-L1, we double-stained CD123 and CDK5 or PD-L1, in which CD123-positive DCs are designated as pDCs (plasmacytoid DCs). The results showed that CDK5 and PD-L1 were mainly expressed in dendritic cells of CRSwNP, that is, CDK5 and PD-L1 were co-located in DCs of CRSwNP (Fig. 2b).

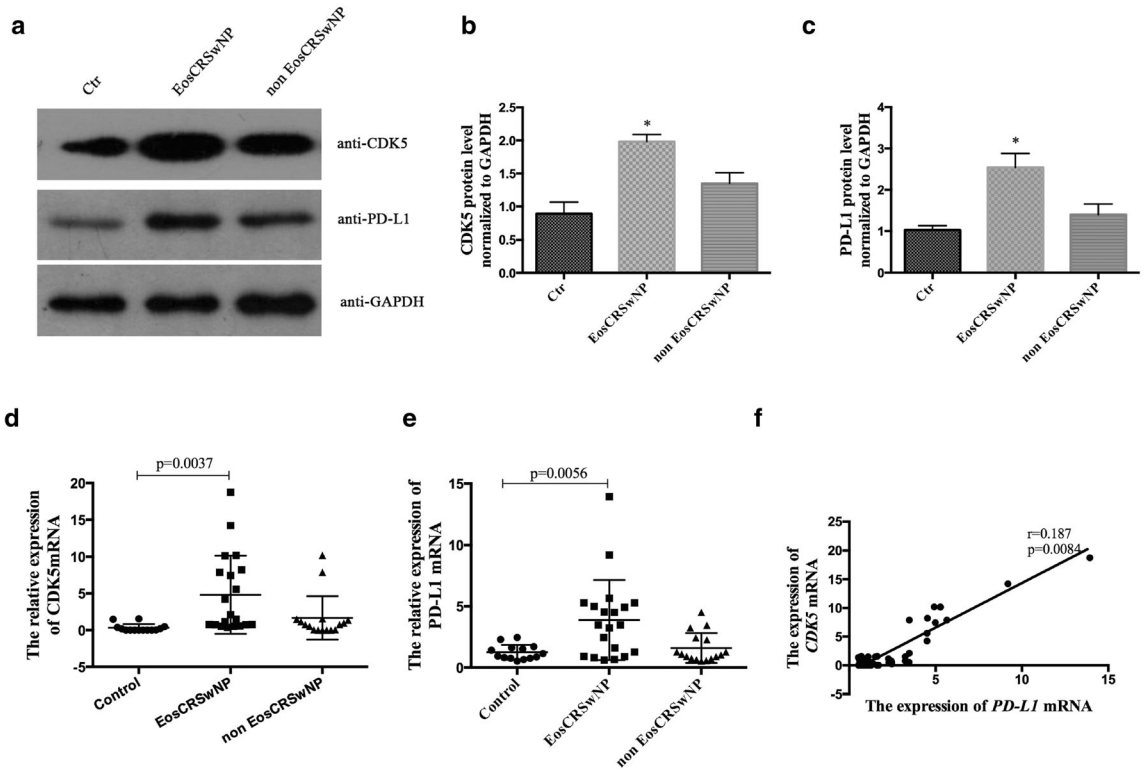

Fig. 1. Increased expression of CDK5 and PD-L1 in nasal mucosa of CRSwNPs. **a** Western blotting was used to analyze the expression of CDK5 and PD-L1 in the total nasal mucosal tissue protein of controlled, EosCRSwNP and non-EosCRSwNP, where GAPDH served as a control. **b**, **c** The relative expression levels of CDK5 and PD-L1 protein were assessed. **d**, **e** The relative mRNA levels of *CDK5* and *PD-L1* in the total nasal mucosal tissue of controlled, EosCRSwNP, and non-EosCRSwNP were detected by real-time quantitative PCR. **f** Significant positive correlations between *PD-L1* and *CDK5* mRNA expression were detected in all samples by real-time quantitative PCR ($n = 50$). The bar graphs and the table show quantification of the results, with each value represents the mean ± SD of three independent experiments. Statistical significance is shown using the Student's *t* test analysis; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Knockdown of CDK5 Affects the Expression of PD-L1 and the Maturation of DCs

To test the effect of CDK5 on PD-L1 expression, we knocked down CDK5 by transfecting small interfering RNA of CDK5 in DCs, soon after DCs were induced with TNF-α and LPS. We first observed the morphology of DCs by light microscopy. The results showed that the DCs stimulated by LPS and TNF-α showed obvious mature characterization compared to control cells. The number of pseudopods that protruded from the cells increased significantly and cells showed more diverse cell shapes. However, it is interesting that the induction of cells by LPS and TNF-α disappears after CDK5 knockdown. In order to further observe the expression changes of CDK5 and PD-L1, we performed immunofluorescence staining. The results showed that compared with the control group, the expressions of CDK5 and PD-L1 were significantly increased after stimulation with LPS and TNF-α, and they are still co-located. After CDK5 knockdown, the expression of PD-L1 was also significantly reduced (Fig. 3a). To further confirm this result, we extracted cellular mRNA and performed real-time quantitative PCR. The results showed that compared with the control group, LPS and TNF-α stimulated cells could cause increased expression of CDK5 and PD-L1, but CDK5 knockdown blocked this effect (Fig. 3b, c). Our Western blot results further confirm this conclusion (Fig. 3c).

To examine the effect of CDK5 on the maturation process of DCs, we used flow cytometry to detect changes of DCs cell maturation. The results showed that compared with the control group, DCs induced by LPS and TNF-α exhibited a higher proportion of HLA-DR$^+$/CD83$^+$ cells; however, the proportion of HLA-DR$^+$/CD83$^+$ DCs cells was significantly reduced induced by LPS and TNF-α after the knockdown of CDK5 (Fig. 4a, b), labeling cells with the CD80 and CD86 antibodies showed the same results and which were not shown in this.

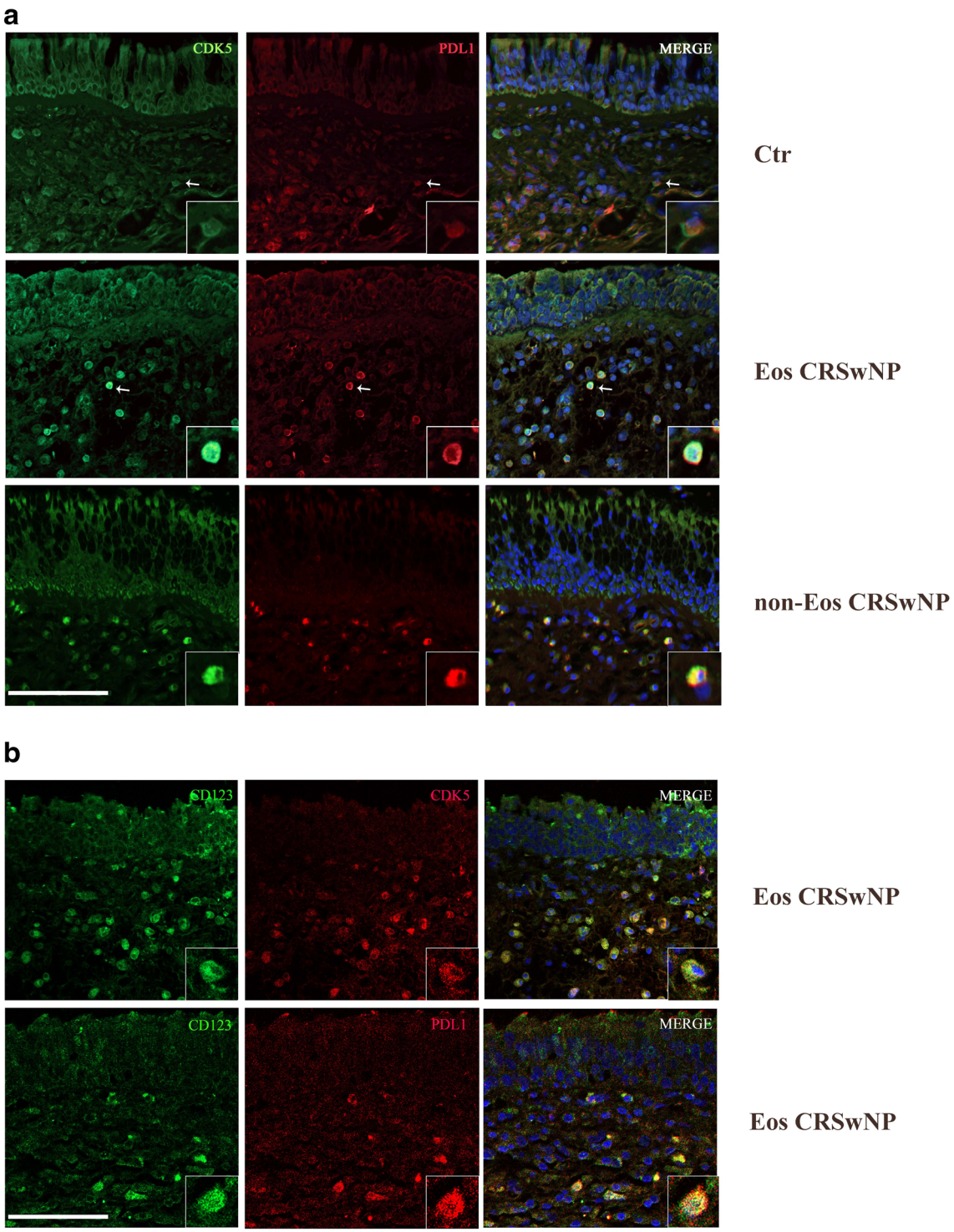

Fig. 2. The expression of CDK5 and PD-L1 in DCs of CRSwNPs. **a** Expression patterns of CDK5 and PD-L1 in nasal mucosal tissue of controlled, EosCRSwNP, and non-EosCRSwNP (×400 magnification; CDK5 stained in green; PD-L1 stained in red; nucleus stained in blue; scale bar = 100 μm). **b** CDK5 and PD-L1 are mainly increased in DCs, and CD123 antibodies are used to specifically label plasmacytoid DCs (×400 magnification; CDK5 or PD-L1 stained in red; CD86 stained in green; nucleus stained in blue; scale bar = 100 μm).

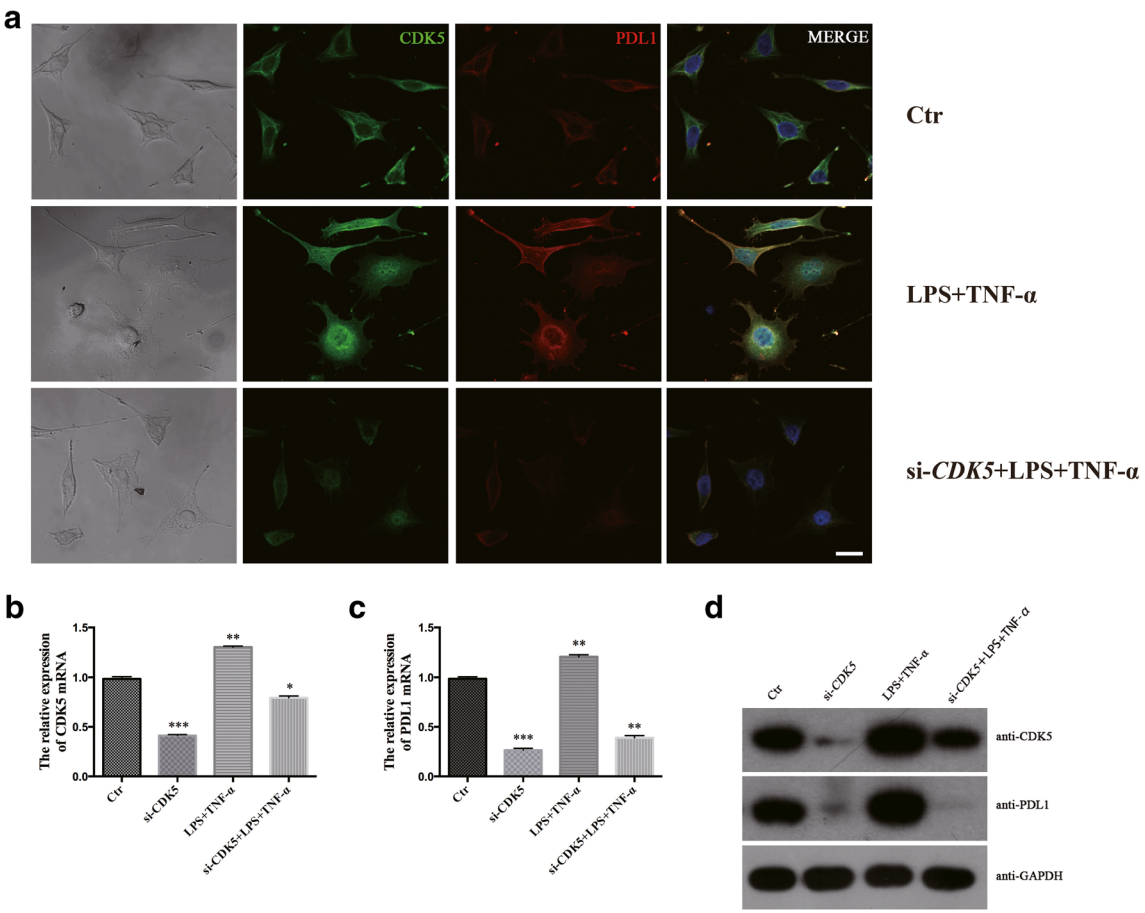

Fig. 3. CDK5 affects the expression of PD-L1 and the maturation of DCs. **a** Immunofluorescence was used to detect the expression of CDK5 and PD-L1 in DCs after induction of LPS and TNF-α, and the effect of si-CDK5 transfection on this process (×400 magnification; CDK5 stained in green; PD-L1 stained in red; nucleus stained in blue; scale bar = 10 μm). **b**, **c** The relative mRNA levels of *CDK5* and *PD-L1* in DCs were detected by real-time quantitative PCR. **d** The relative expression levels of AHR and TNF-α protein were assessed by Western blot in DCs, GAPDH served as a control. The bar graphs and the table show quantification of the results, with each value represents the mean ± SD of three independent experiments. Statistical significance is shown using the Student's *t* test analysis; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

DISCUSSION

CDK5 plays an extremely important role in various physiological activities, although it shares some structural homology with other typical CDK family members, it has own specific activating factor, which has been found to have p35, p39, and CCNI [22–24]. Abnormal expression of CDK5 can cause cell physiology disorders, such as cell cycle blocking or reentry, abnormal cell differentiation, and delayed cell maturation, thereby disrupting the physiological state of the tissue and triggering various related diseases. However, so far, the role of CDK5 in the development of CRSwNPs has not been reported. Our results showed that the expression of CDK5 was significantly increased in CRSwNPs nasal mucosa tissues compared to control mucosa tissues, especially EosCRSwNPs. Therefore, we believe that abnormally elevated CDK5 expression may be an important mechanism of CRSwNP disease. Effective regulation of CDK5 expression may be a key breakthrough point in the treatment of CRSwNP, especially EosCRSwNP.

It is well known that CDK5 can be involved in the regulation of cellular physiological activities by regulating the expression changes of a variety of downstream proteins or protein phosphorylation [25, 26]. PD-L1 is an important immune regulation-related protein, and it is also an important downstream regulatory protein of CDK5. A large number of studies on PD-L1 are mainly focused on the direction of cancer treatment, and there have been great

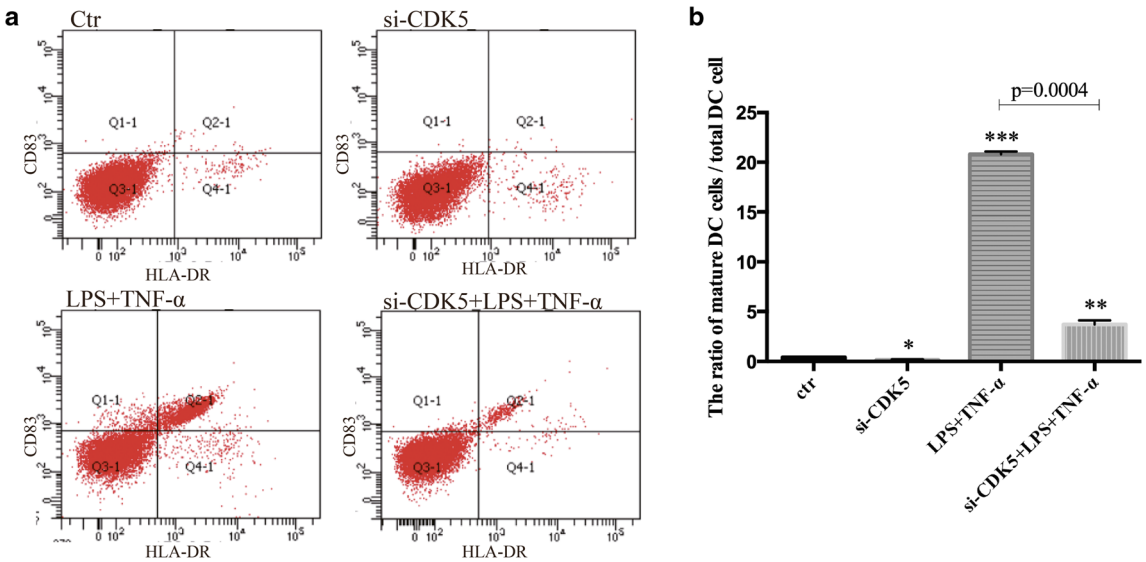

Fig. 4. Knockdown of CDK5 in DCs blocks cell maturation. **a** Flow cytometry was used to detect DCs cell maturation after CDK5 knockdown and LPS and TNF-α induction. Among them, all DCs were labeled with HLA-DR, and CD83 was used to label mature DCs. **b** The histograms show the calculated relative proportions of mature DCs. The bar graphs and the table show quantification of the results, with each value represents the mean ± SD of three independent experiments. Statistical significance is shown using the Student's *t* test analysis; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

breakthroughs and advances, but the role of PD-L1 in CRSwNPs still confuses us. Some researchers have found that the loss of Cdk5 leads to persistent expression of the PD-L1 transcriptional repressor interferon regulatory factors IRF2 and IRF2BP2, resulting in reduced PD-L1 expression [27]. Our results show that changes in the expression of CDK5 directly affect the expression of PD-L1. When the expression of CDK5 is increased, the expression of PD-L1 also increases. On the contrary, when the expression of CDK5 is relatively low, the expression of PD-L1 also decreases accordingly. The expression of CDK5 directly affects the expression of PD-L1. When the expression of CDK5 is increased, the expression of PD-L1 also increases. On the contrary, when the expression of CDK5 is relatively low, the expression of PD-L1 also decreases accordingly. And in CRSwNPs, the expression of CDK5 and PD-L1 all showed a significant increase. We speculated that CDK5 might be involved in the pathogenesis of CRSwNPs by regulating the expression of PD-L1.

Our results showed the expression patterns of CDK5 and PD-L1 in CRSwNPs by immunofluorescence staining on CRSwNPs tissue sections. It showed that the expression positions of the two proteins overlap in DCs of nasal mucosal tissue, which further provides evidence that CDK5 can affect the expression of PD-L1. In addition, we unexpectedly found that CDK5 and PD-L1 are mainly expressed in dendritic cells under the nasal epithelium. Studies have shown that cell maturation of DCs in CRSwNPs is abnormal compared with DCs in healthy nasal mucosa [28]. And it is well known that the high expression of CDK5 in B cell lymphoma, which will lead to signal transduction and protein phosphorylation of STAT3, thereby activating STAT3 activity and promoting cell proliferation and differentiation [29]. To examine the effect of CDK5 expression on the function of DCs, we performed flow cytometry and found that knockdown of CDK5 in DCs cells blocked the maturation of DCs. We believe that CDK5 participates in the pathogenesis of CRSwNPs by affecting the maturation of DCs.

The development and use of targeted drugs for the treatment of chronic rhinitis sinusitis remains an important challenge for patients with chronic rhinitis. The discovery of precise biomarkers is important for the treatment of chronic rhinitis sinusitis. Our results suggest that CDK5 may be involved in the regulation of DCs cell maturation in chronic rhinitis sinusitis by modulating PD-L1 expression. The change of expression level of CDK5 in CRSwNPs tissue cells may have a good effect on the disease treatment of CRSwNPs, especially EosCRSwNPs. Therefore, CDK5 can be used as a novel biomarker for predicting chronic sinusitis targeted drugs, and it is great significance for the study and treatment of chronic rhinitis and sinusitis.

CONCLUSION

Our study showed that the expression of CDK5 and PD-L1 was significantly increased in CRSwNPs, especially in EosCRSwNPs, and CDK5 could regulate the expression of PD-L1 and the process of DCs cell maturation, CDK5 may play an important role in the development of CRSwNPs. This study will provide a new perspective for gene-targeted therapy of CRSwNPs.

AUTHORS CONTRIBUTION

SL, XM, LCC, and ZHL did conception and design; LCC, ZHL, ZLL, JP, ZL, LT, ZXM, SDS, CGH, and XQ conducted analysis and interpretation; SL, XM, LCC, and ZHL drafted the manuscript for important intellectual content; the version submitted for publication was finally approved by SL, XM, LCC, and ZHL.

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COMPLIANCE WITH ETHICAL STANDARDS

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

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