Research Article

LINC00887 Fosters Development of Clear Cell Renal Cell Carcinoma via Inhibiting CD8+ T Cell Immune Infiltration

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Background. lncRNAs affect adaptive and innate immunity of cancer via mediating functional states of immune cells, genes, and pathways. Nonetheless, little is known about the molecular mechanism of lncRNA-mediated CD8+ T cell immune infiltration in progression of clear cell renal cell carcinoma (ccRCC). We designed this work to investigate the role of LINC00887 in regulating CD8+ T cell immune infiltration in ccRCC.

Methods. Correlation between LINC00887 and immune factors and the expression level of LINC00887 in ccRCC were analyzed by bioinformatics methods (TCGA-KIRC database, “edgeR” package, “clusterProfiler” package, and “CIBERSORT” package). LINC00887 expression in ccRCC was examined via RT-qPCR. The cytokilling capacity of CD8+ T cells was evaluated by the lactate dehydrogenase assay. The apoptotic ability of CD8+ T cells was measured by flow cytometry. The chemotactic ability of CD8+ T cells was evaluated by chemotaxis assay. The chemotactic ability of CD8+ T cells was revealed by chemotaxis assay. CXCR3, CXCL9, and CXCL10 levels were assessed by RT-qPCR. Results. As suggested by bioinformatics analysis, LINC00887 was markedly upregulated in ccRCC patients and associated with expression of immune-suppression molecule, thereby abating the immune infiltration level of CD8+ cells in tumor tissue. As revealed by cellular assay, LINC00887 was upregulated in ccRCC cells, and knockdown of LINC00887 resulted in a decreased PD-L1 expression, increased CD8+ T cell toxicity, decreased apoptotic levels, and enhanced chemotaxis. Moreover, we found that LINC00887 exhibited inhibitory effect on immune infiltration of CD8+ cells in clinical tissues. Conclusions. The results of this study suggested that LINC00887 promoted ccRCC progression by inhibiting immune infiltration of CD8+ T cells, providing new insights into pathogenesis of ccRCC and suggesting LINC00887 being a promising immunotherapy target for ccRCC.

1. Introduction

Renal cell carcinoma (RCC) ranks third in common cancers of urinary system [1]. According to histopathological classification, RCC has three main subtypes: papillary renal cell carcinoma, clear cell renal cell carcinoma (ccRCC), and chromophobe renal cell carcinoma. Among them, ccRCC is the most frequent histological subtype, accounting for 80-90% [2]. Clinically, due to no evident symptoms at early stage and insufficient specific diagnostic markers, 20-30% of ccRCC patients have been in advanced-stage and developed metastasis when diagnosed [3]. Metastatic ccRCC is not sensitive to radiotherapy and chemotherapy and that new targeted drugs have poor effects on patients with this type of ccRCC. Therefore, it is urgent to develop appropriate therapies to improve the clinical efficacy [4]. It is encouraging to note that recent use of immune checkpoint therapy is effective in patients with metastatic ccRCC [4]. For example, McDermott et al. [5] found pembrolizumab as a first-line therapy for advanced-stage ccRCC patients with an objective
response rate of 36.4%, a median PFS of 7.1 months, and an OS rate of 88.2% and 70.8% at 12 and 24 months. Results from CheckMate 214 further established that the combination of ipilimumab and nivolumab was associated with higher response rates (RR) (39% in the ITT population), complete response rates (8%) and duration of response compared to sunitinib [6]. All above indicated that immunotherapy has a good effect on RCC patients. Although the above studies showed that immunotherapy has a good therapeutic effect on patients, the therapeutic effect of immunotherapy varies in different patients. Currently, it is believed that the effect of tumor immunotherapy is greatly affected by immune escape of tumor cells [7]. CD8+ T cells infiltrated by tumor are the main effect or cells mediating anti-tumor immune response [8]. However, the immune infiltration of CD8+ T cells is usually negatively regulated by various conditions in tumor microenvironment, so that tumor cells can evade immune surveillance [9]. In conclusion, finding the key factors affecting CD8+ T cell infiltration is of great value for optimizing treatment of ccRCC patients.

Long noncoding RNAs (lncRNAs) are involved in regulating cellular biological functions like energy metabolism changes, angiogenesis, proliferation, and migration in tumors [10]. It has been previously reported that lncRNAs participate in innate and adaptive immune responses through a complex regulatory network [11]. Huang et al.’s study [12] reported that lncRNA NKILA sensitized T cells to activation-induced cell death, thus promoting tumor immune escape. lncRNA-MM2P promotes macrophage M2 polarization, which in turn promotes immune escape of tumor cells [13]. In lung cancer cells, overexpressed lncRNA NEAT1 inhibits T cell infiltration in tumor tissues by downregulating chemokines such as CXCL10 and CCL5 [14]. These results indicated the potential of lncRNAs in tumor immune regulation, but there are still many tumor immune-related lncRNAs that have not been explored. Therefore, further exploration of immune-related genes regulating CD8+ T cells in ccRCC can provide new targets for the immunotherapy in ccRCC. We found that LINC00887 is a key factor affecting tumor immune escape, and it may play a role as a target of immunotherapy.

LINC00887 (NR_024480.1), with a length of 3,021 nucleotides, is composed of three exons [15]. Previous reports have shown that LINC00887 can affect the development of different cancers. For example, LINC00887 expression is upregulated in glioma, and knocking out LINC00887 can inhibit the proliferation of cancer cells [16]. LINC00887 expression is also upregulated in nasopharyngeal carcinoma, and overexpression of LINC00887 can significantly accelerate proliferative ability of nasopharyngeal carcinoma cells [17]. It was also found that LINC00887 was remarkably overexpressed in RCC and significantly facilitated RCC cell proliferation in vitro [18]. Although these studies indicate the key role of LINC00887 in cancer development, no literature has explored LINC00887 and its role in ccRCC immunity. Thus, we aimed to elucidate the impacts of LINC00887 on ccRCC via regulating CD8+ T cell immune infiltration.

Herein, bioinformatics analysis identified that LINC00887 had a potential in inhibiting immune invasion of CD8+ T cells in tumor tissues, and experiments confirmed that LINC00887 knockdown in ccRCC cells could significantly increase chemotaxis and cytotoxicity of T cells to tumor cells. This study initially revealed the crucial role of LINC00887 in immunoregulation of CD8+ T cells, providing a new reference for the study of immune escape mechanism of ccRCC.

2. Materials and Methods

2.1. Collection of Patient Samples. 10 pairs of ccRCC tissues and corresponding normal paracancer tissues were collected from patients receiving ccRCC surgery in our hospital. Inclusion criteria are as follows: ccRCC was confirmed by histopathological section, and no other treatment was received before surgery. Patients were excluded if they complicated with other tumors or other organic lesions [16]. Immediately after excision, the tissues frozen in liquid nitrogen were prepared for subsequent usage. The protocol involving human sample collection was approved by the Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital Ethics Committee. Written informed consent was acquired from patients.

2.2. Bioinformatics Analysis. The lncRNA differential analysis was carried out using the "edgeR" package [19] (logFC > 2, FDR < 0.05) (normal: 72, tumor: 539). Target genes were identified through literature review. Then, the target genes were grouped and subjected to differential analysis with "edgeR" package (logFC > 2, FDR < 0.05). The "clusterProfiler" package for KEGG and GO analyses were carried out for differentially expressed genes (DEGs). Based on mRNA (FPKM) data, the infiltration of 22 kinds of immune cells in tumor samples were scored using the "CIBERSORT" package. The cells with all infiltration scores being 0 were filtered out, and samples with P > 0.05 were also filtered out. The correlation between target genes and immune cell infiltration scores was analyzed. The difference of immune cell infiltration score was analyzed by grouping target genes. Pearson’s analysis was performed to determine correlation between LINC00887 expression and mRNA expression of immunosuppressive molecules PD-L1 (CD274) and PD-1 (PDCD1). Then, according to SNV data of samples, the mutation landscape oncoplot of LINC00887 high- and low-expression groups in ccRCC tumor samples was drawn using the R package GenVisR [20].

2.3. Cell Culture. Human normal renal cell line HK-2 and ccRCC cell lines Caki-1, 786-O, and A498 were provided by the American Type Culture Collection (ATCC, USA). A498 and Caki-1 were cultivated in Dulbecco’s modified Eagle medium (DMEM) (Sigma, USA). 786-O was cultured in RPMI-1640 medium (Sigma, USA). HK-2 was incubated in DMEM/F12 medium (Sigma, USA). All the mediums contained 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) at 37°C with 5% CO₂.

2.4. Cell Transfection. The sh-NC and sh-LINC00887 were designed by GenePharma (China). A498/786-O experimental cells (1.2 × 10⁵ cells/well) were harvested and seeded onto 6-well plates. And then the cells were randomly divided into two groups which transfected with sh-NC or sh-LINC00887,
respectively. The cells were transfected using Lipofectamine 2000™ (Invitrogen, USA) following the manufacturer’s suggestions, with transfection efficiency ensured by qRT-PCR after transfection for 48 h.

2.5. RT-qPCR. Total RNA was separated with TRIzol reagent (Invitrogen, USA). Reverse transcription was carried out by PrimeScript RT Reagent Kit, and RT-qPCR analysis was performed with SYBR Green PCR Master Mix (Takara, Japan) on the CFX96 assay system. The reaction conditions were 40 cycles of 96°C for 1 min, 96°C for 10 s, 60°C for 5 s, and 72°C for 30 s. Primers are as follows: LINC00887 forward: 5′-GAGGCTGAAATTTGCTGAAAGTC-3′ and reverse: 5′-ATTGCGAGGGTGACAG-3′; CXCR3 forward: 5′-CCACCTAGCTGTAGCAGACAC-3′ and reverse: 5′-AGGGCTCTCCTGCTAGAAGTT-3′; CXCL9 forward: 5′-CCAGTATGAGAAAGGTTCGC-3′ and reverse: 5′-AGGGCTGAGGGCAAATTTGTT-3′; CXCL10 forward: 5′-GTGCGATTCAAGGAGTACCTC-3′ and reverse: 5′-TGATGGCCCTTGGATCTGAGGT-3′; and GAPDH forward: 5′-ACATCGCTCAGACCATG-3′ and reverse: 5′-TGATGTTAGGTCATAGGAGG-3′.

2.6. Immunohistochemistry (IHC) Assay. Paraffin sections (4 μm) were prepared from human tissues fixed in 10% neutral-buffered formalin. To detect CD8, tissue sections were dewaxed in xylene, rehydrated in diluted ethanol, blocked with 0.3% H2O2, then blocked with normal goat serum, and incubated with primary rabbit anti-CD8 (ab4055, Abcam, UK) at 4°C overnight. The tissues were incubated at room temperature with biotinylated goat anti-rabbit IgG (BP-9100, Vector Labs, USA) for 30 min and then incubated with Vectastain ABC-HRP solution (Vector Labs, USA). The sections were permeabilized with 0.3% Triton X-100 and incubated with primary rabbit anti-CD8 serum, and incubated with primary and secondary antibodies. Then, the tissues were treated with Vectastain ABC-HRP solution (Vector Labs, USA) for 1 h. Finally, immunoreactivity was examined using an ECL kit (Thermo Fisher Scientific, USA).

2.7. Lactate Dehydrogenase (LDH) Cytotoxicity Test. CD8+ T cells were tested for cytotoxicity using CytoTox LDH cytotoxicity Assay Kit (Promega, USA). In short, the isolated effecter CD8+ T cells were cocultivated with ccRCC cells in different proportions at 37°C for 24 h. The conditioned medium was transferred to 96-well plates three times and cultured in reaction mixture for 30 min at room temperature [21]. After adding stop buffer, we measured OD490 value. The formula for cytotoxicity calculation of CD8+ T cells was as follows: cytotoxicity (%) = [(LDH activity of experimental group (O/D490)/(maximum LDH activity (OD490))] × 100%.

2.8. Apoptosis Detected by Flow Cytometry. T cells were first cocultured with tumor cells in a 5:1 ratio, followed by the detection of apoptotic cells using the Annexin V-FITC apoptosis detection Kit (Thermo Fisher Scientific, USA). Briefly, the treated cells were plated onto 24-well plates and cultivated for about 24 h. They were then stained for 15 min with Annexin V-FITC and PI solution. FACSCalibur analysis was used to determine the percentage of apoptotic cells.

2.9. Transwell Assay. Chemotactic migration of CD8+ T cells was assessed in a 24-well plate with a 5 μm aperture polycarbonate filter (Corning, USA). First, ccRCC tumor tissue was incubated in DMEM containing 10% FBS for 48 h. Next, 600 μL of tumor supernatant was introduced to the lower chamber. Anti-CCL5 (1 μg/mL) and/or anti-CXCL10 (5 μg/mL) was introduced to neutralize antibodies. DMEM containing 10% FBS was the control. Purified CD8+ T cells (purity > 90%) from TILs were counted. Next, 5 × 10^5 CD8+ T cells were supplemented to the upper chamber and cultivated for 2 h at 37°C with 5% CO2. Cells in the lower chamber were calculated by an automatic cell counter [22].

2.10. Western Blot. Total proteins were extracted from cells and subjected to a bicinchonic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific, USA). Protein samples (40 μg/lane) were subsequently analyzed by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis. Thereafter, the proteins were transferred to a polyvinylidene fluoride (Millipore, USA) membrane. The membrane was subsequently blocked in 5% skimmed milk for 2 h at room temperature and then kept in specific primary antibodies overnight (4°C). Main antibodies included rabbit anti-PD-L1 antibody (ab205921, Abcam, UK), rabbit anti-GAPDH antibody (ab9485, Abcam, UK). The following day, it was incubated with secondary antibody conjugated to HRP (HRP-goat anti-rabbit IgG, ab205718, Abcam, UK) for 1 h. Finally, immunoreactivity was examined using an ECL kit (Thermo Fisher Scientific, USA).

2.11. Data Analysis. Statistical analysis was performed with the SPSS 13.0 software (SPSS Inc., USA). Quantitative data from 3 independent assays were expressed as mean ± standard deviation. Student’s t test compared the difference between two groups. One-way analysis of variance compared differences among three or more groups. P value less than 0.05 represents significant difference.

3. Results

3.1. LINCO00887 Is Remarkably Highly Expressed in ccRCC. First, LINCO00887 expression was analyzed in ccRCC using TCGA-KIRC database. LINCO00887 level was dramatically upregulated in ccRCC tissues versus normal tissues (Figure 1(a)). Subsequently, LINCO00887 expression in ccRCC cell lines (A498, Caki-1, and 786-O) and HK-2 cell line was revealed by RT-qPCR, indicating that LINCO00887 was significantly upregulated in ccRCC cells (Figure 1(b)). The above experiments demonstrated a high level of LINCO00887 in ccRCC. LINCO00887 level was relatively high in A498 and 786-O cells, so the two cell lines were selected for subsequent assays.

3.2. LINCO00887 Affects CD8+ T Cell-Mediated Immune Responses. Through differential expression analysis, 577 DEGs were obtained. GO and KEGG analyses demonstrated that these genes were mainly enriched in complement and coagulation cascades, acute inflammatory response, and other signaling pathways (Figures 2(a) and 2(b)). This suggested that LINCO00887 may have a potential link with immunity. Then, CIBERSORT analyzed immune cell infiltration of tumor tissues from the samples. After the unqualified samples and immune cells were filtered out, the relative infiltration levels
of 21 kinds of immune cells in 414 ccRCC tumor tissues were obtained (Figure 2(c)). Then, the difference in the abundance of immune cell infiltration was analyzed between LINC00887 high- and low-expression groups. The results stated that abundance of CD8+ T cell immune infiltration was markedly reduced in LINC00887 high-expression group (Figure 2(d)). We then analyzed the correlation between the LINC00887 level and infiltration abundance of 21 kinds of immune cells. As revealed above, LINC00887 level was notably negatively correlated with abundance of CD8+ T cell infiltration (Figure 2(e)). Therefore, we hypothesized that LINC00887 may inhibit immune infiltration of CD8+ T cells in tumor tissues.

3.3. LINC00887 Is Associated with the Expression of Immunosuppressive Molecules. Since the above analysis revealed the close relation between LINC00887 and immune cell infiltration in ccRCC, we further explored the correlation between LINC00887 and immunosuppressive molecules. LINC00887 was prominently positively correlated with PD-1 expression (Figure 3(a)) while negatively correlated with PD-L1 expression (Figure 3(b)). Subsequently, expression differences of checkpoint molecules such as CTLA4, LAG3, TGFBI, NOS3, and IDO1 between LINC00887 high- and low-expression groups were analyzed. As a result, CTLA4 and LAG3 were highly expressed in the LINC00887 low-expression group (Figures 3(c) and 3(d)), while TGFBI, NOS3, and IDO1 immunosuppressive genes were highly expressed in the LINC00887 high-expression group (Figures 3(e)–3(g)). SNP analysis results showed that mutations in immune checkpoint molecules such as PBRM1 were more frequent in the group with high LINC00887 expression (Figures 3(h) and 3(i)). In summary, bioinformatics studies revealed the association between LINC00887 and the immunosuppression of ccRCC.
Figure 2: Continued.
Figure 2: Continued.
Figure 2: Continued.

- B cells naive
- B cells memory
- Plasma cells
- T cells CD8
- T cells CD4 memory resting
- T cells CD4 memory activated
- T cells follicular helper
- T cells regulatory (Tregs)
- T cells gamma delta
- NK cells resting
- NK cells activated
- Monocytes
- Macrophages MO
- Macrophages M1
- Macrophages M2
- Dendritic cells resting
- Dendritic cells activated
- Mast cells resting
- Mast cells activated
- Eosinophils
- Neutrophils
3.4. LINC00887 Inhibits the Cell Killing and Chemotactic Abilities of Immune Cells. Bioinformatics analysis exhibited that LINC00887 level was associated with PD-L1 level, combined with the report that the expression of PD-L1 may affect the functions of CD8+ T cells [23], we detected the protein level of PD-L1 in tumor cells, manifesting that knockdown LINC00887 could inhibit the expression of PD-L1 (Figure 4(a)). To further verify the bioinformatics results, CD8+ T cells were isolated using EasySep™ Direct Human CD8+ T Cell Isolation Kit. 786-O and A498 cells were stably transfected with sh-NC and sh-LINC00887, and tumor cells were cocultured with CD8+ T cells. LDH assay was used to measure cytotoxicity of CD8+ T cells to the above cells. As a result, sh-LINC00887 could significantly increase the cell killing ability of CD8+ T cells to ccRCC cells (Figure 4(b)). Then, apoptosis of CD8+ T cells was detected. The sh-LINC00887 treatment could significantly reduce apoptosis of CD8+ T cells (Figure 4(c)). Chemotactic experiments showed that the chemotactic ability of CD8+ T cells was enhanced after sh-LINC00887 treatment (Figure 4(d)). RT-qPCR demonstrated that sh-LINC00887 could promote expression of chemokines CXCL9, CXCL10, and CXCR3 (Figure 4(e)). In summary, cell experiments confirmed that LINC00887 could indeed promote the development of ccRCC by inhibiting immune response mediated by CD8+ T cells.

3.5. LINC00887 Can Repress CD8+ T Cell Immune Infiltration in Clinical Tissues. To verify the impact of LINC00887 on immune infiltration of CD8+ T cells, 10 pairs of ccRCC tissues and corresponding adjacent tissues were gathered and subjected to RT-qPCR for LINC00887 expression quantitation. The results suggested that evident upregulation of LINC00887 in ccRCC tissues compared with paracancerous tissues (Figure 5(a)). Next, patients were assigned into high and low LINC00887 expression groups. Then, IHC assay determined CD8+ cell immune infiltration level in ccRCC tissues. CD8+ cells had a lower infiltration level in ccRCC tissues with high LINC00887 expression (Figure 5(b)). This suggested that LINC00887 showed an inhibitory effect on CD8+ cell immune infiltration in clinical tissues.
Cor = 0.26 (p-value = 4.764e-10)

Cor = -0.16 (p-value = 1.99e-04)

Figure 3: Continued.
Figure 3: Continued.
Figure 3: Continued.
4. Discussion

IncRNAs participate in multiple processes of human cancer, including tumor growth, angiogenesis, metastasis, drug resistance, self-renewal of cancer cells, and tumor microenvironment regulation [24]. Recent studies have uncovered important functions of various aberrantly expressed IncRNAs in ccRCC. For instance, DMDRMR-mediated manipulation of m6A receptor IGF2BP3 to m6A modified CDK4 drives ccRCC progression [25]. IncRNA-LET suppresses ccRCC cell growth by modulating miR-373-3p [26]. LINC00973 is involved in cancer immunosuppression via positive modulation of Siglec-15 in ccRCC [27]. Bioinformatics analysis in this study revealed significant overexpression of LINC00887 in ccRCC and its potential link with immune infiltration.

CD8+ T cell infiltration degree can be used as an indicator of tumor prognosis [28]. The immune infiltration of CD8+ T cells is negatively regulated by the tumor microenvironment, leading to tumor cells evading immune surveillance and thus promoting tumor progression [9]. Zhang et al. [29] found that TCL6 correlates with CD8+ T cell infiltration and indicates poor survival in breast cancer. Chen et al. [30] found that GOLM1 is positively connected to...
Figure 4: Continued.
invasive tumor-associated macrophages inhibited by PD-L1 and CD8+ T cells in colorectal cancer tissues, and zoledronic acid combined with anti-PD-L1 therapy reduces CD8+ T cell inhibition and pD-L1+ TAMs infiltration, resulting in inhibition of tumor growth in mice HCC model. Our work found that LINC00887 knockdown in ccRCC could improve the toxicity and chemotactic ability of CD8+ T cells to ccRCC cells. This suggested that LINC00887 promoted ccRCC progression through a CD8+ T cell-mediated immune response.

Herein, LINC00887 was significantly positively correlated with immunosuppressive molecule PD-1, and immunosuppressive molecules TGFβ1, NOS3, and IDO1 also showed higher expression levels in LINC00887 high-expression group. Considering that PD-1 is the main inhibitory receptor of T cells, the higher its expression level, the weaker the effector capacity of T cells [31]. LINC00887 was positively correlated with PD-1 expression, further suggesting that LINC00887 mediated stronger T cell inhibition. TGFβ1 is also a key gene in T cell regulation, and blocking TGFβ can effectively amplify CD8+ T cells, thus enhancing the efficacy of immune checkpoints [32]. In this study, the expression trend of TGFβ1 and LINC00887 was consistent, which may suggest that the two exert a synergistic role in mediating CD8+ T cell immunosuppression. IDO1 is a potential target for tumor immunotherapy. Targeted inhibition of IDO1 expression reverses tumor suppressive immune microenvironment [33]. In our results, IDO1 expression was also relatively high in the LINC00887 group. In the present study, SNP mutation landscape of LINC00887 high- and low-expression groups was analyzed. The results demonstrated that PBRM1 mutation was more frequent in the LINC00887 high-expression group. PBRM1 mutation is known to be closely related to the high angiopoiesis level in renal cancer [34], and PBRM1 mutations reduce the clinical benefit of immune checkpoint blocking therapy in RCC [35]. The results suggested that the oncoenic effect of LINC00887 may be associated with a higher PBRM1 mutation level. Also, NOS3 was reported to serve as an oncoene in gastric cancer [36], which supports the predicted result on the expression relationship between LINC00887 and NOS3 in this study. In a word, the expression trend of LINC00887 is relatively consistent with that of most immunosuppressive genes. This further confirmed that LINC00887 was involved...
in the immunosuppressive regulation of ccRCC and promoted malignant progression of ccRCC through synergistic expression of immunosuppressive molecules.

In conclusion, this study preliminarily illustrated the high level of LINC00887 in ccRCC, and it was negatively connected to the abundance of CD8+ T cell immune infiltration; LINC00887 knockdown led to increased cytotoxicity, weakened apoptosis ability, and enhanced chemotactic ability of CD8+ T cells. However, limitations still exist. First, the results have not been verified in vivo at the animal level. Second, the detailed regulatory mechanism of LINC00887 in regulating the immune response in ccRCC remains to be determined. In the future, we will further verify the results and explore the role of LINC00887 in ccRCC antitumor drug development through animal experiments. Based on above studies, our team believes that LINC00887 can be a biomarker for ccRCC diagnosis and can play a role in future drug development as a target for immunotherapy of ccRCC.

**Data Availability**

The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author on reasonable request.

**Ethical Approval**

The protocol involving human sample collection was approved by the Shengli Clinical Medical College of Fujian Medical University, Fujian Provincial Hospital Ethics Committee.

**Consent**

All authors consent to submit the manuscript for publication.
Conflicts of Interest
The authors declare no conflicts of interest.

Authors’ Contributions
Jinfeng Wu and Rongcheng Lin contributed equally to this work.

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