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N-3-(oxododecanoyl)-L-homoserine lactone suppresses dendritic cell maturation by upregulating the long noncoding RNA NRIR

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N-3-(oxododecanoyl)-L-homoserine lactone (3-O-C12-HSL), a small bacterial signaling molecule secreted by *Pseudomonas aeruginosa* (*P. aeruginosa*), can block dendritic cell (DC) maturation and participate in immune escape, but the underlying mechanism is unclear. We speculate that regulation of DC maturation and function by lncRNAs may be the mechanism by which 3-O-C12-HSL inhibits the immune response. We found that 3-O-C12-HSL increased the expression level of the lncRNA NRIR, impeding monocyte-derived dendritic cell (Mo-DC) maturation. In addition, we observed the effect of NRIR on the expression of CD40, CD80, HLA-DR and IL-6. NRIR overexpression significantly reduced the expression of Mo-DC surface markers, while 3-O-C12-HSL did not significantly reduce the expression of Mo-DC surface markers after NRIR knockdown. These results indicate that 3-O-C12-HSL indeed affects the differentiation and maturation of Mo-DCs through NRIR. IL-6 stimulates T cell proliferation and activation, and we found that high NRIR expression reduced IL-6 levels. However, under NRIR knockdown, 3-O-C12-HSL did not decrease IL-6 expression, suggesting that 3-O-C12-HSL may affect T cell activation through NRIR. This study is the first to elucidate the important role of a lncRNA in the mechanism of 3-O-C12-HSL activity. It also provides new ideas regarding *P. aeruginosa* infection pathogenesis.

Keywords. *Pseudomonas aeruginosa*; dendritic cells; long noncoding RNAs; NRIR

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is one of the most common nonfermentative gram-negative bacteria in the clinic, and it has motive power. It is widely spreading in nature and thrives in almost all environments (Rahme *et al*. 1995; Lee and Zhang 2015). A conditional pathogen causing hospital-acquired infections, *P. aeruginosa* is usually found on and in medical instruments and even disinfectants, thus causing seri-

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ous iatrogenic infections. It generally affects diseased hosts, including patients with cancer, patients suffering traumatic burns, patients with pulmonary cystic fibrosis (CF) and people with low immunity (Chaney *et al*. 2017; Lassek *et al*. 2016). Nosocomial bacterial infection commonly occurs in the lower respiratory tract, which leads to bronchiectasis and chronic obstructive pulmonary disease (COPD) with infection (Parameswaran and Sethi 2012; Wilson *et al*. 2016). In patients with CF, *P. aeruginosa* is the leading cause of chronic pulmonary infection, with a morbidity rate greater than 90% (Koch and Høiby 1993). Pneumonia caused by multidrug resistant *P. aeruginosa* (MDR-PA) infection is very difficult to treat and has a high mortality rate (Morata *et al*. 2012).

Quorum sensing (QS) is a population density-dependent gene regulation mechanism. Bacteria can mediate complex cooperative behaviors through QS (Marques-Rocha *et al*. 2015; Chhabra *et al*. 2003; LeRoy *et al*. 1988). The main components of QS are QS signal synthases, signal receptors and signal molecules (Chhabra *et al*. 2003), through which QS target genes can be activated or inhibited. *P. aeruginosa* exists widely in the environment and has strong adaptability. *P.* aeruginosa can regulate the production of exotoxin, alkaline protease, elastase and other extracellular virulence factors, which may lead to extensive tissue damage and bloodstream dissemination (Bjarnsholt and Givskov 2007; Pearson *et al*. 1994; Pearson *et al*. 1995; Smith *et al*. 2002a, b). All of these depend on the complex multisignal QS system of *P. aeruginosa.* The QS system of *P. aeruginosa* can produce two kinds of signal molecules: *N*-3-(oxododecanoyl)-L-homoserine lactone (3-O-C12-HSL) and *N*-butyryl- L-homoserine lactone (C4-HSL) (Smith *et al*. 2002a, b).

As a QS signal molecule of *P. aeruginosa*, 3-O-C12-HSL is recognized as helpful for *P. aeruginosa* evasion of the host's immune defense. 3-O-C12-HSL is a multifunctional immunoregulatory factor that can affect the host's immune response. 3-O-C12-HSL can induce epithelial cells and fibroblasts to generate corresponding proinflammatory cytokines, such as IL-6 and IL-8, and cyclooxygenase-2 (Cox-2) to induce inflammatory reactions; excessive inflammatory reactions can lead to host tissue damage and promote *P. aeruginosa* infection (Zhu *et al*. 2008; Smith *et al*. 2002a, b; Mayer *et al*. 2011). Studies have shown that the functions of many kinds of immune cells, such as neutrophils, lymphocytes and macrophages, can be regulated by 3-O-C12-HSL, altering host immune defense (Turkina and Vikstrom 2019). In vitro, 3-O-C12-HSL can inhibit the secretion of tumor necrosis factor-a (TNF-a) by mouse macrophages and induce a strong inflammatory reaction in mouse skin (Smith *et al*. 2002a, b; Telford *et al.* 1998). 3-O-C12-HSL can also reduce the secretion of LPS-stimulated IL-12 and TNF-α and promote the expression of the anti-inflammatory cytokines IL-10 and HLA-G in macrophages (Glucksam-Galnoy *et al.* 2013), evading the host immune defense by affecting the ratio of proinflammatory factors to anti-inflammatory factors. Other studies also showed that 3-O-C12-HSL has immune suppressive activity against human peripheral blood mononuclear cells (PBMCs) (Chhabra *et al*. 2003; Hooi *et al.* 2004). In LPS-stimulated bone marrow-derived dendritic cells (BM-DCs), 3-O-C12-HSL inhibited the production of IL-12 (Skindersoe *et al.* 2009) to inhibit the dendritic cell (DC) maturation and T cell differentiation into Th1 cells. 3-O-C12-HSL can also inhibit LPS-induced maturation of monocyte-derived dendritic cells (Mo-DCs) (Li *et al*. 2015). DCs have strong antigen-presenting ability, which can promote T cell development, which is crucial for the immune response. However, the immune regulation mechanism of 3-O-C12-HSL in DCs has not been fully determined.

Long noncoding RNAs (lncRNAs) can regulate the maturation and function of DCs. It has been certified that the maturation and function of DCs affect gene expression, as well as cell proliferation and differentiation, while lncRNAs can regulate gene expression, cell proliferation and differentiation at the transcriptional and posttranscriptional levels (Wang *et al*. 2014). As a result, it is not difficult to speculate that lncRNAs can regulate DC maturation and function. In fact, it has already been proven that lncRNAs can regulate the maturation of DCs. Cao Xuetao *et al.* screened differentially expressed lncRNAs after treating Mo-DCs with LPS and found that the candidate lncRNA linc-DC could inhibit the secretion of the cytokine IL-12 by Mo-DCs, weaken the antigen-presenting ability of Mo-DCs and affect the proliferation and activation of $CD4^+$ T cells, suggesting that lncRNAs could regulate the maturation of Mo-DCs (Wang *et al*. 2014). At the same time, Mitchell Guttman *et al.* also found that at least 20 lncRNAs were upregulated during DC maturation, suggesting that lncRNAs are involved in DC maturation and function (Fitzgerald and Caffrey 2014).

Based on the above research, we hypothesize that lncRNAs participate in the process of 3-O-C12-HSL inhibiting DC maturation. In this study, we observed changes in lncRNA expression levels after 3-O-C12-HSL treatment of DCs and the effects of lncRNAs on DC maturation and differentiation.

2. Materials and methods

2.1 *Cell purification and culture*

PBMCs contain T cells, B cells, DCs and other immune cells that play an important role in immune function. We used Ficoll-Paque gradient centrifugation to separate PBMCs from normal human peripheral blood and used Miltenyi Biotech reagents to purify $CD14^+$ monocytes from PBMCs. The monocytes (1×10^6 cells/ml) were added to 6-well plates, 2 ml per well, and then induced by IL-4 (1000 U/ml, Peprotech, Rehovot, Israel) and GM-CSF (500 U/ml, Peprotech, Rehovot, Israel). The cells were then cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) containing 10% FBS (Life, Carlsbad, CA) at 37°C in an incubator containing 5% CO_2. On the 4^{th} day, half of the medium was changed. On the 6th day of the cell culture, Mo-DCs were obtained. The Mo-DCs were divided into three groups: LPS (50 ng/ml, Sigma-Aldrich, St. Louis, MO) was added to the LPS group, 3-O-C12-HSL (25 μM, Sigma-Aldrich, St. Louis, MO) was added to the 3-O-C12-HSL group in the presence of LPS (50 ng/ml), and PBS (Sigma-Aldrich, St. Louis, MO) was added in the same volume to the PBS group. Three replicate wells were prepared for each group, and all groups were cultured in a 37°C incubator for 18 h. Afterwards, we collected the culture medium and Mo-DCs. 3-O-C12-HSL is not functional when added alone and must be combined with LPS. Although LPS can stimulate Mo-DC maturation, 3-O-C12-HSL reduces this stimulation and thus plays a role in inhibiting Mo-DC maturation. Therefore, it should be emphasized that all the 3-O-C12-HSL groups in our experiment were 3-O-C12-HSL (25 μM) + LPS (50 ng/ml).

2.2 *Microarray and computational analysis*

Briefly, the total RNA of samples (LPS group cells, 3-O-C12-HSL group cells and PBS group cells) was extracted and used to synthesize double-stranded cDNA, which was then labeled and hybridized with an LncRNA Expression Microarray (Arraystar, Rockville, USA). Then, the unbound probes were washed away. The fluorescence intensity of the microarray scanner chip was scanned by an Agilent DNA microarray scanner, and then the scanned image was input into Agilent feature extraction (v11.0.1.1) software for expression data analysis and grid alignment. The expression data were standardized by quantile standardization and Agilent GeneSpring GX v12.1 software. Finally, the hybridization results were analyzed by using Agilent GeneSpring GX v12.1 software.

2.3 *Transfection of lentiviral vectors for overexpression and knockdown of NRIR*

To overexpress NRIR in Mo-DCs, we constructed a lentiviral vector, PGMLV-CMV-H_NRIR-EF1-ZsGreen1, based on the overexpression vector PGMLV-6465. A mock lentiviral vector, EF1-ZsGreen1, was used as an overexpression lentivirus negative control. To knockdown NRIR in Mo-DCs, we constructed an shRNA lentiviral vector based on the RNAi vector pGMLV-SC5. The target sequence for NRIR was the same as reported previously (Hiroto *et al*. 2014), 5′-CGATGCATGGGAA-GACTAA-3′. The recombinant H_NRIR-shRNA (PGMLV-SC5) was used to knock down NRIR, and Scramble-SC5 lentivirus was used as an shRNA lentivirus negative control. All lentiviral vectors and negative controls were designed and synthesized by Genomeditech (Shanghai, China). $CD14^+$ monocytes were separated as described above. On the 4th day, half of the medium was changed, and at the same time, all the Mo-DCs were divided into 4 groups: the NRIR overexpress group, Ctrl overexpress group, NRIR RNAi group and the Ctrl RNAi group. The four groups were infected with the corresponding lentiviral vector at a multiplicity of infection (MOI) of 20. At 48 h after transfection, the culture medium was centrifuged and replaced with fresh culture medium. On the same day (6th day), each of the four groups was divided into three groups and stimulated with PBS, LPS or 3-O-C12-HSL as described above.

2.4 *Flow cytometry analysis of Mo-DC surface markers*

On the 7th day, the Mo-DCs were stained with anti-CD40-PE-cy7, anti-CD80-PE and anti-HLA-PE-Texas Red probes (eBioscience) at 4°C for 15 min, and then the expression of surface antigens on Mo-DCs was analyzed by flow cytometry. Additionally, we performed FACS analysis by using flow cytometry (BD FACS AriaIII).

2.5 *Laboratory analysis of IL-6 secretion*

Cells were collected from all groups and centrifuged at 2500×g for 15 min at 4°C, and the cell supernatant was used to assay the level of IL-6 via the immunonephelometric method on a Cobas 6000 automated analyzer (Roche Diagnostics, Basel, Switzerland).

2.6 *Quantitative RT-PCR analysis*

The expression of NRIR in cells was detected by RT-PCR. Total RNA was extracted from cells by TRIzol (Invitrogen). A NanoDrop 2000 spectrophotometer (Thermo Scientific) was used to evaluate the quantity, purity and integrity of the RNA. The detected RNA was then used to synthesize cDNA with a PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara) in accordance with the manufacturer's procedures. Then, NRIR expression levels were analyzed by RT-PCR using a ViiA™ Real-Time PCR System (ABI) and SYBR® - *Premix Ex Taq*™ II (Takara) according to the manufacturers' instructions using the cDNA as a template. The internal control was Actin. The relative expression of RNAs was calculated using the comparative Ct method. Primer sequences are listed in table 1.

2.7 *P. aeruginosa-infected patients and relatively healthy controls*

Study design: A retrospective cohort analysis was conducted with 20 patients presenting at the Traditional Chinese Medicine Hospital of Guangdong Province from March to May 2021 with nonurinary tract *P. aeruginosa* infections and there were 20 healthy controls. This study was approved by the Ethics Committee of Guangdong Provincial Hospital of Traditional Chinese Medicine and followed the ethical guidelines of the Declaration of Helsinki.

Inclusion criteria: The *P. aeruginosa*-infected patients were hospitalized, and sputum samples were analyzed. Sputum smears showed that leukocytes phagocytosed gram-negative bacilli, and sputum cultures of *P. aeruginosa* reached a significant number. The clinical manifestations were fever, cough, yellow or yellow-green purulent sputum, viscous sputum and dyspnea. The relatively healthy controls were other inpatients in the same area. The sputum samples of the patients were analyzed, and no leukocyte phagocytosis was found on the sputum smear. No pathogenic microorganisms were found in sputum culture. PBMC were extracted from peripheral blood of patients and relatively healthy controls, TRIzol (Invitrogen) reagent was added, and the samples were stored in a −80°C refrigerator. After all samples were collected, RNA in the PBMC was extracted according to the manufacturer's instructions, and RT-PCR quantitative analysis was carried out according to the previously described steps.

Exclusion criteria: Patients infected by other pathogenic microorganisms were excluded. In addition, pregnant women were excluded.

2.8 *Statistical analysis*

One-way ANOVA was used to compare the differences in NRIR expression between the LPS group, 3-O-C12-HSL group and PBS group. The differences in Mo-DC surface molecules and IL-6 levels between the three groups with or without the NRIR overexpression lentivirus were analyzed by two-way ANOVA. Comparison of NRIR expression between patients with *P. aeruginosa* infection and relatively healthy controls was conducted by an unpaired t test.

3. Results

3.1 *Identification of LPS-modulated and 3-O-C12-HSL-modulated lncRNAs in primary human monocytes*

To identify whether LPS-modulated and 3-O-C12-HSL-modulated lncRNAs affect DC differentiation and function, we isolated $CD14^+$ monocytes from peripheral blood monocytes, exposed them to LPS (50 ng/

Table 1. List of primer sequences used for RT-PCR

Target name	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
NRIR	TCACGATGCATGGGAAGACT	GAGCCAATATCGCACCACTG
Actin	GGCGGCACCACCATGTACCCT	AGGGGCCGGACTCGTCATACT

ml), 3-O-C12-HSL (25 μM) or PBS for 18 h and subsequently subjected them to RNA sequencing. A total of 1386 transcripts annotated as lncRNAs were identified as significantly ($p < 0.05$) modulated in response to 3-O-C12-HSL exposure compared with those in response to LPS exposure (figure 1). Specifically, 548 lncRNAs (i.e., 39.54%) were upregulated, while 838 lncRNAs (i.e., 60.46%) were downregulated. These data demonstrate that after 3-O-C12-HSL acted on Mo-DCs and that the expression profiles of lncRNAs changed markedly. The results suggest that lncRNA may play a significant role in the process of 3-O-C12-HSL affecting Mo-DC maturation and function.

3.2 *Identification of 3-O-C12-HSL-associated lncRNAs*

To study whether lncRNAs contribute to 3-O-C12-HSL-mediated immune escape, we identified that NRIR expression was significantly different from that in the control group, indicating that it could serve as a candidate, through microarray profiling. The results of the lncRNA expression microarray showed that compared to that in the LPS group, NRIR was upregulated in the 3-O-C12-HSL group. Thus, we focused on NRIR and investigated whether NRIR had a functional relationship with 3-O-C12-HSL. We further examined NRIR expression in the PBS group, LPS group and 3-O-C12-HSL group by RT-PCR. The RT-PCR results showed that NRIR expression in the 3-O-C12-HSL group was higher than that in the LPS group (figure 2).

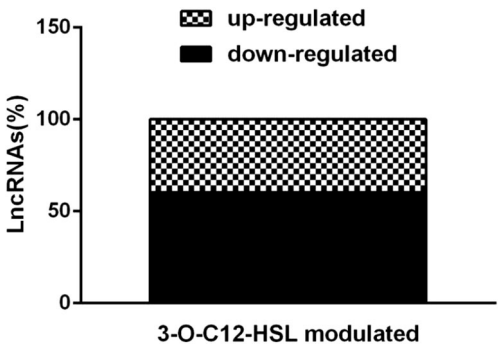

Figure 1. The percentage of lncRNAs up- and downregulated by 3-O-C12-HSL compared with those in the LPS group. Mo-DCs were cultured for 18 h with LPS (50 ng/ml) and 3-O-C12-HSL (25 μM). The sequencing data were analyzed according to materials and methods. Top 50 3-O-C12-HSL modulated up- and downregulated lncRNAs compared to LPS group were listed in supplementary data 1.

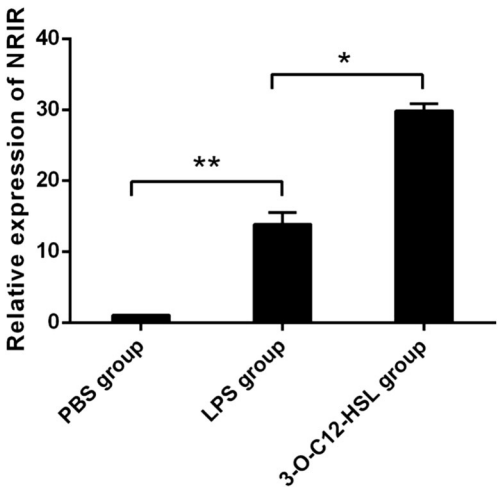

Figure 2. NRIR expression levels were analyzed by RT-PCR in all groups. The PBS group consisted of Mo-DCs cultured for 18 h in the presence of PBS; the LPS group consisted of Mo-DCs cultured for 18 h in the presence of LPS (50 ng/ml); and the 3-O-C12-HSL group consisted of Mo-DCs cultured for 18 h in the presence of 3-O-C12-HSL (25 μM). Results are shown as the mean ± SEM of three experiments. *$p < 0.05$, ** $p < 0.01$.

These data demonstrate that NRIR is involved not only in LPS-induced maturation of Mo-DCs but also in 3-O-C12-HSL blocking the maturation of Mo-DCs, suggesting that NRIR may be involved in the process of 3-O-C12-HSL impeding Mo-DC maturation.

3.3 *Effect of NRIR on Mo-DC surface markers*

To study the role of NRIR in Mo-DC differentiation and maturation, we constructed lentiviral vectors enabling high expression or knockdown of NRIR and then detected the phenotype of Mo-DCs after altering NRIR expression. Flow cytometry showed high NRIR expression significantly decreased the expression of functional molecules on mature Mo-DCs in all the three groups (LPS group, 3-O-C12-HSL group and PBS group), including the costimulatory molecules CD40 and CD80 and the antigen-presenting molecule HLA-DR. When NRIR was knocked down, there was no difference in the expression of these molecules in the LPS and PBS groups, but in the 3-O-C12-HSL group, CD40, CD80, and HLA-DR were not reduced (figure 3). These results suggest that 3-O-C12-HSL inhibits Mo-DC differentiation and maturation through NRIR.

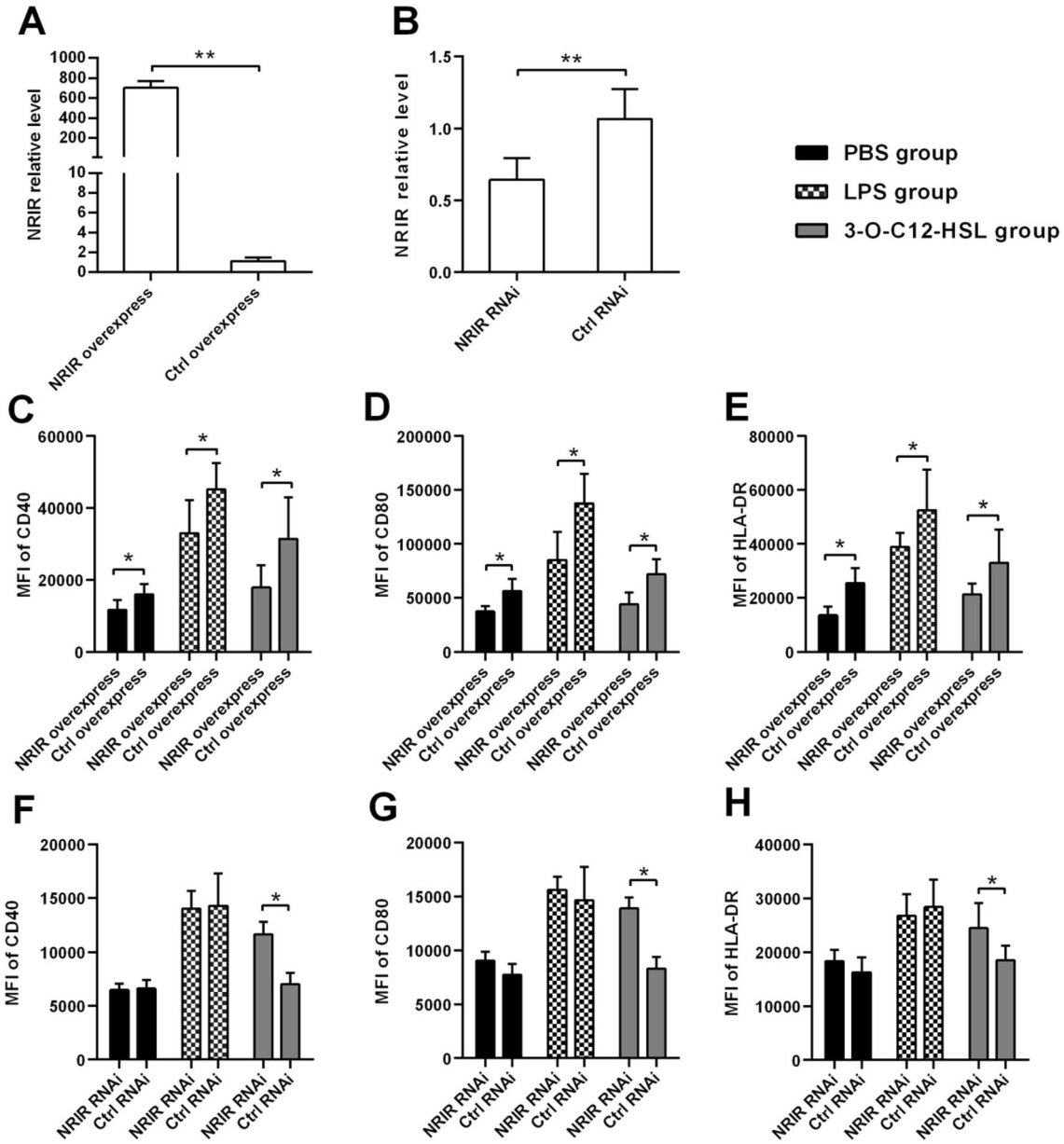

Figure 3. Increasing or decreasing NRIR expression affects the maturation of Mo-DCs. NRIR overexpress, Mo-DCs with the NRIR overexpression lentivirus; Ctrl overexpress, Mo-DCs with the overexpression negative control lentivirus; NRIR RNAi, Mo-DCs with the NRIR shRNA lentivirus; Ctrl RNAi, Mo-DCs with the shRNA negative control lentivirus. (**A**) Relative expression of NRIR in cells with the NRIR overexpression lentivirus. (**B**) Relative expression of NRIR in cells with the NRIR shRNA lentivirus. (**C, D, E**) Mean fluorescence intensity of CD40, CD80 and HLA-DR signals on Mo-DCs with the NRIR overexpression lentivirus as determined by flow cytometry. $*p < 0.05$. (**F, G, H**) Mean fluorescence intensity of CD40, CD80 and HLA-DR signals on Mo-DCs with the NRIR shRNA lentivirus as determined by flow cytometry. $*p < 0.05$.

3.4 *Effect of NRIR on IL-6 expression in Mo-DCs*

To verify whether the selected lncRNA NRIR was effectively related to 3-O-C12-HSL-related immune escape and whether 3-O-C12-HSL acts as an immunosuppressant in vivo, we overexpressed or knocked down NRIR by lentivirus. Then, we measured the IL-6 levels in the supernatants from all groups. IL-6 is an effective lymphocyte activator that can increase antibody production and induce an acute phase reaction. Our findings showed that 3-O-C12-HSL could reduce IL-6 expression induced by LPS, which was consistent with previous reports (Smith *et al*. 2002a, Telford *et al*. 1998). The increased expression of NRIR

also decreased the IL-6 expression in each group (figure 4A). However, when NRIR was knocked down, 3-O-C12-HSL did not decrease IL-6 expression (figure 4B). IL-6 is a T cell activation cofactor, and our data demonstrate that 3-O-C12-HSL may affect T cell activation through NRIR.

3.5 *Differences in NRIR expression in the PBMC of P. aeruginosa-infected patients and relatively healthy people*

Similar to the case for other nonfermenting bacteria, it is difficult to determine whether *P. aeruginosa* isolated from sputum is a colonizing bacterium or a pathogenic bacterium, so additional methods are needed to determine which case is occurring. Therefore, we assume that if a molecular marker level can be increased or decreased in *P. aeruginosa*-infected patients, which would be distinct from that in healthy people, this difference would help us determine whether the positive *P. aeruginosa* culture was colonization or infection. As a result, we performed a comparison experiment to identify differences between *P. aeruginosa*-infected patients and relatively healthy people regarding the NRIR level in PBMC . The results showed that there was significant difference in NRIR expression between the two groups (figure 5). These data demonstrate that NRIR can be used as the biomarker to distinguish between infection and colonization of *P. aeruginosa.*

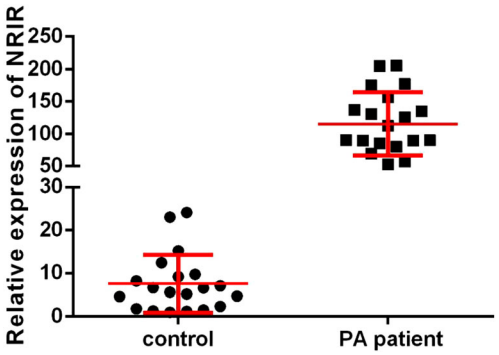

Figure 5. Relative NRIR expression in *P. aeruginosa*-infected patients and relatively healthy controls. There was significant difference in NRIR expression between the two groups. $P < 0.05$.

4. Discussion

The purpose of this study was to explore the potential role of lncRNAs in the pathogenesis caused by *P. aeruginosa*-secreted 3-O-C12-HSL. A study showed that *P. aeruginosa* infection changes the expression of lncRNAs in CF respiratory epithelial cells, which may play a potential role in the innate immune system response (Viviane *et al*. 2017). There is also evidence that lncRNAs are involved in the pathogenesis of autoimmune and inflammatory diseases (Koch and Høiby 1993). However, in the pathogenesis induced by *P. aeruginosa*, it has not been found that lncRNAs are related to the immune disorders caused by 3-O-C12-HSL. The work in this paper is based on the possibility

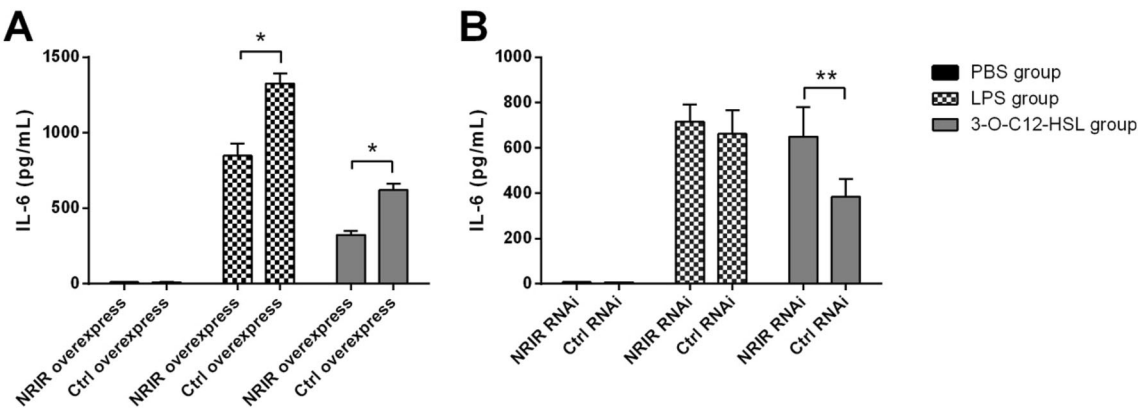

Figure 4. NRIR can affect IL-6 expression in Mo-DCs. NRIR overexpress, Mo-DCs with the NRIR overexpression lentivirus; Ctrl overexpress, Mo-DCs with the overexpress negative control lentivirus; NRIR RNAi, Mo-DCs with the NRIR shRNA lentivirus; Ctrl RNAi, Mo-DCs with the shRNA negative control lentivirus; PBS group, LPS group and 3-O-C12-HSL group, Mo-DCs cultured for 18 h in the presence of PBS, LPS (50 ng/ml) or 3-O-C12-HSL (25 μM), respectively, after transfection with the lentivirus and green fluorescent protein expression. **(A)** The IL-6 level was detected by Roche electrochemiluminescence in cells with high expression of NRIR. **(B)** The IL-6 level was detected by Roche electrochemiluminescence in cells in which NRIR was knocked down. *$p < 0.05$, **$p < 0.01$.

that 3-O-C12-HSL may hinder DC maturation through lncRNAs, thus affecting T cell differentiation and the immune response.

NRIR (negative regulator of interferon response, lncRNA NRIR, also known as lncRNA-CMPK2) (Zheng *et al.* 2017) is located on human chromosome Chr2p25.2 and resides mostly in the nucleus (Hiroto *et al.* 2014). NRIR is induced by interferon (IFN) and is produced in different human and mouse cell types. After NRIR knockdown, some protein-encoded antiviral interferon-stimulated gene (ISGs) was upregulated, and IFN-stimulated hepatitis C virus (HCV) replication in hepatocytes was significantly reduced, suggesting that NRIR expression may affect the antiviral effect of IFN (Hiroto *et al.* 2014). Another study showed that IFN-induced transmembrane proteins (IFITMs) are negatively regulated by NRIR and can inhibit Hantaan virus (HTNV) infection (Zheng *et al.* 2017). In addition, NRIR was also found to be associated with immune disorders in autoimmune diseases, as its levels were significantly elevated in monocytes from systemic sclerosis (SSc) patients and in the blood of systemic lupus erythematosus (SLE) patients (Cao *et al*. 2019; Mariotti *et al*. 2019). In our study, we found that in the process of 3-O-C12-HSL affecting DC maturity, there were many lncRNAs involved, and NRIR was one of the lncRNAs with a significant increase in expression. All these studies indicate that NRIR is involved in the innate immune response, virus infection, autoimmune diseases and bacterial infection.

To study the effect of NRIR on Mo-DC maturation, we constructed NRIR overexpression and NRIR shRNA lentiviral vectors and transfected them into Mo-DCs. Flow cytometry analysis showed that NRIR overexpression downregulated the expression of CD40, CD80 and HLA-DR on the surface of Mo-DCs; when NRIR was knocked down, the expression of CD40, CD80, and HLA-DR was not reduced in the 3-O-C12-HSL group. CD40 and CD80 are surface markers of mature DCs that are expressed at low levels on immature DCs and upregulated on mature DCs. Highly expressed CD40 and CD80 bind to the corresponding ligands, which provides the necessary secondary signal (costimulatory signal) for T cell activation and immune response induction (Banchereau *et al*. 2000; Banchereau and Steinman 1998). HLA-DR is a major histocompatibility (MHC) class II molecule (Aliseychik *et al*. 2018; Hansson *et al*. 1986) that is highly expressed on the surface of mature DCs (Li *et al*. 2012; Van Vre *et al.* 2008). HLA-DR binds to the T cell receptor (TCR) on the surface of T cells, providing the first signal for T cell activation (Bernard *et al.* 2002). CD40, CD80 and HLA-DR are all markers of DC maturation that can provide activation signals for T cell proliferation. In this study, NRIR overexpression reduced CD40, CD80 and HLA-DR expression on the surface of Mo-DCs, but under NRIR knockdown, 3-O-C12-HSL did not reduce the levels of the three markers on Mo-DCs compared with the shRNA negative control, indicating that 3-O-C12-HSL can inhibit DC maturation through NRIR.

We also found that the multifunctional cytokine IL-6, the levels of which can rapidly increase in acute inflammation, such as occurs during infection, surgery and trauma, had significantly decreased levels in both the supernatant after high NRIR expression. However, under NRIR knockdown, 3-O-C12-HSL did not decrease IL-6 expression. IL-6 can stimulate not only activated B cell proliferation and antibody secretion but also T cell proliferation and activation (Dienz and Rincon 2009; Li *et al*. 2018). Our data show that 3-O-C12-HSL can inhibit the production of IL-6 through NRIR, which suggests that 3-O-C12-HSL may affect T cell proliferation through NRIR.

Since the clinical diagnosis of *P. aeruginosa* infection and colonization is very difficult, we investigated whether molecular markers can be used to help us determine whether *P. aeruginosa* is causing an infection. As we expected, there was significant difference in NRIR levels between *P. aeruginosa*-infected patients and relatively healthy people. Combined with the clinical symptoms, signs and sputum culture results, NRIR can help clinicians to determine whether *P. aeruginosa* is a pathogen or a colonization bacteria. This will help clinicians when to choose antibiotics and when to continue to observe the progress of the disease, which will reduce the abuse of antibiotics. Therefore, NRIR can be used as a biomarker of *P. aeruginosa* infection, and help patients get more appropriate treatment during hospitalization.

In conclusion, our work has shown that 3-O-C12-HSL can change the expression level of NRIR, which can reduce the expression of molecular markers on the surface of DCs. This finding suggests that NRIR can affect DC differentiation and maturation. Therefore, NRIR has a potential effect on DC antigen presentation, which can affect T cell activation. NRIR can inhibit IL-6 production, which can also affect T cell proliferation, thus affecting the host immune response to *P. aeruginosa*. All these data need to be further verified by functional tests, such as mixed lymphocyte tests and molecular mechanism tests. NRIR is an IFN-

stimulated negative regulatory factor, and its generation depends on JAK-STAT signaling (Hiroto *et al*. 2014). However, regarding the process of 3-O-C12-HSL inhibiting the immune response, the mechanism through which NRIR acts and whether it also depends on the JAK-STAT signaling pathway remain to be further studied. The present study is the first demonstration that lncRNAs play an important role in the pathogenesis of 3-O-C12-HSL, and these findings may be helpful for further understanding the role of lncRNAs in *P. aeruginosa* infection pathogenesis.

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