MicroRNA-155-IFN-γ Feedback Loop in CD4⁺ T Cells of Erosive type Oral Lichen Planus

Jing-Yu Hu1,2, Jing Zhang1,2, Jing-Zhi Ma3, Xue-Yi Liang1, Guan-Ying Chen1-2, Rui Lu1,2, Ge-Fei Du2 & Gang Zhou1,2

Oral lichen planus (OLP) is a T cell-mediated immune disorder, and we have indicated a Th1-dominated immune response in OLP. MicroRNA-155 (miR-155) could promote Th1 cells polarization. The present study aims to determine the role of miR-155 in immune response of OLP. The expression of miR-155 and the target mRNA was tested by Real-Time PCR. The serum levels of IL-2, 4, 10 and IFN-γ were examined with ELISA. Furthermore, in vitro study was built to observe the function of miR-155 in erosive-type OLP (EOLP). Finally, we determined the expression and correlation of miR-155 and SOCS1 in EOLP CD4⁺ T cells. The results showed miR-155 was high related with the disease severities. Besides, serum IFN-γ was specifically increased in EOLP group, while IL-4 was decreased. In vitro studies showed miR-155 could reinforce IFN-γ signal transducer, and the induction of IFN-γ could also promote miR-155 expression in EOLP CD4⁺ T cells. In addition, miR-155 levels were negatively related with SOCS1 mRNA expression in EOLP CD4⁺ T cells. Our study revealed a positive miR-155-IFN-γ feedback loop in EOLP CD4⁺ T cell, which might contribute to the Th1-dominated immune response. Furthermore, miR-155 could be used for the evaluation and treatment of OLP.

Oral lichen planus (OLP) is one of the most common diseases of oral mucosa, which has been classified as a precancerous lesion by the World Health Organization (WHO). There are six recognized oral manifestations of OLP, i.e. reticular, papular, plaque, atrophic, erosive and bullous lesions, and the erosive type is considered as the most possible premalignant character of OLP. So far, the exact pathogenesis of OLP remains elusive, however, many researchers supported that CD4⁺ T cells were protagonists of the immune response in OLP. The most important function of CD4⁺ T cells was producing a large number of various cytokines, in which, interferon-gamma (IFN-γ) acting via signal transducer and activator of transcription 1 (STAT1) is the key initiator for specification and cell fate commitment for T helper 1 (Th1) cells. Activation of Janus kinases (JAKs) and STAT1 signaling induces the transcription factor T-bet, a master regulator that promotes Th1 cells differentiation. By JAKs-SATAT1 signaling, IFN-γ could inhibit production of anti-inflammatory cytokines like IL-4 and IL-10, while promote secretion of proinflammatory cytokines like IL-2. Our previous study has implicated a predominant role of Th1-type immune response in peripheral blood of OLP. Meanwhile, in circulating of OLP patients, Th1 cell-related cytokines form a special cytokines environment which can be reinforced or attenuated by the epigenetic modifications.

MicroRNAs (miRNAs) are 18- to 25-nucleotide (nt) single-stranded molecules that control nearly 1/3 post-transcriptional gene expression in a epigenetic way. Recently, it has become evident that deregulation of mRNAs induced by miRNAs may affect human immune response, resulting in many pathogenic...
disorders\textsuperscript{14-17}. MiR-155 is encoded within an exon of the non-coding RNA known as B cell integration cluster (Bic) gene\textsuperscript{17}. In many immune diseases such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease, miR-155 was found to have abnormal expression in peripheral blood of the patients\textsuperscript{18-20}. It has been demonstrated that miR-155 greatly involved in the immune mechanism mediated by CD4\textsuperscript{+} T cells. For instance, miR-155 in activated CD4\textsuperscript{+} T cells could promote Th17 cell differentiation, and knocking out of bic gene might lead to a break of Th1/Th2 balance in CD4\textsuperscript{+} T cells\textsuperscript{19-22}. Suppressor of cytokine signaling 1 (SOCS1) was considered as a key target of miR-155 in Th1 cells, which negatively regulated JAKs-SATAT1 signaling. SOCS1 was also the inhibitor of the signal transduction of certain cytokines like IFN-\textgamma and IL-2. In addition, SOCS1 was found to have effects on the differentiation, maturation and function of CD4\textsuperscript{+} T cells\textsuperscript{23-25}.

Herein, our aim was to determine the expression of miR-155 in peripheral blood of OLP patients, and analyze the relationship of miR-155 with the cytokines. Furthermore, through regulating miR-155 expression, observations in vitro were built to examine their effects on OLP CD4\textsuperscript{+} T cells proliferation and the levels of cytokines. Finally, certain target of miR-155 would be predicted and confirmed.

**Results**

**The levels of miR-155 and cytokines in peripheral blood of OLP.** The expression of miR-155 increased in peripheral blood of EOLP patients compared with the control (\(p < 0.05\)), in addition, the expression of miR-155 in EOLP group was significant higher than that in NEOLP group (\(p < 0.05\)) (Fig. 1A). However, no difference was found between NEOLP group and the controls. Furthermore, the correlation analysis revealed that the miR-155 expression was highly related with the RAE scores which represented the severities of OLP (\(p < 0.01, r = 0.855\)) (Fig. 1B), and the correlation coefficient was much higher in EOLP patients (\(p < 0.01, r = 0.882\)) (Fig. 1C).

The cytokines profiles in the serum of OLP patients showed that just like the expression pattern of miR-155, the IFN-\gamma levels of EOLP group increased (\(p < 0.01\)), and were higher than that of NEOLP group (\(p < 0.05\)) (Fig. 2B). On the contrary, the IL-4 levels decreased in EOLP group (\(p < 0.05\)), and were much lower than that of NEOLP group (\(p < 0.05\)) (Fig. 2A). Moreover, there was descend of IL-4 levels...
in NEOLP group compared with the control ($p < 0.05$) (Fig. 2A). For the other two cytokines, the levels of IL-2 only increased in NEOLP group ($p < 0.05$) (Fig. 2D), IL-10 levels in the two OLP groups both declined ($p < 0.05$, $p = 0.01$), and there was no difference between them (Fig. 2C).

The impacts of miR-155 regulation on CD4+$^+$ T cell proliferation as well as the levels of IFN-γ and IL-4 in the supernatant. As shown in Fig. 3, when miR-155 was inhibited by antagomir-155, the proliferation of EOLP CD4+$^+$ T cell was declined at the time point of 24 h ($p < 0.01$) and 36 h ($p < 0.01$) compared with the negative control group (ANNC group). In addition, the levels of IFN-γ were decreased in the supernatant ($p < 0.05$), but the levels of IL-4 were increased ($p < 0.05$). when miR-155 was promoted by agomir-155, the proliferation of EOLP CD4+$^+$ T cell was raised at all the four time point (12 h, $p < 0.05$; 24 h, 36 h, 48 h, $p < 0.01$) compared with the negative control group (ANC group). Moreover, the levels of IFN-γ and IL-4 in the supernatant both decreased (IFN-γ, $p < 0.05$; IL-4, $p < 0.01$) (Fig. 4).

The levels of miR-155 in EOLP CD4+$^+$ T cell induced with IFN-γ. After induced by IFN-γ for 24 h, the expression of miR-155 in EOLP CD4+$^+$ T cell was increased compared with the blank control group (Fig. 5).

The expression of miR-155 and SOCS1 mRNA in EOLP CD4+$^+$ T cell. After browsing the potential targets, SOCS1 was found to have one and only one conserved site combined with miR-155. For the first batch of samples, the mean value of SOCS1 mRNA expression in EOLP CD4+$^+$ T cell was higher than that of healthy controls, but there was no statistical difference between them (data not shown). Owing to the insufficient of samples, we supplemented the sample number of both EOLP group and the control group. As shown in Fig. 6A,B, it demonstrated that in EOLP group, the expression of miR-155 of CD4+$^+$ T cell was increased ($p < 0.01$), but the expression of SOCS1 mRNA was decreased ($p < 0.05$). Furthermore, the correlation analysis revealed that the expression of miR-155 in CD4+$^+$ T cell was high related with the RAE scores of EOLP patients ($p < 0.01$), and the correlation coefficient reached up to 0.917 (Fig. 6C). In addition, it demonstrated a negative correlation between the SOCS1 mRNA expression and the miR-155 levels ($p < 0.01$, $r = -0.541$) (Fig. 6D).

Discussion

The miRNAs in peripheral blood were easy to be collected and detected, thus, many researches aimed to find disease-related miRNAs in circulation to assist the diagnosis and study the remote and systemic
Figure 3. The proliferation activity as well as the IFN-γ and IL-4 levels in supernatant of EOLP CD4+ T cells transfected with antagomir-155. (A) The significant differences of IFN-γ and IL-4 levels were tested by independent-samples t test, and results are represented as box plots. AN represented the antagomir-155 transfected group, and ANNC was the corresponding negative controls. Statistical significances are shown in the blanks. (B) The solid dots and or cube separately showed the mean value of proliferation activity of EOLP CD4+ T cells in two groups at different time point, and the lines stretching from the dot or cube indicated extreme values. For each time point, independent-samples t test was used to determine differences between the two groups, in addition, ”*” , ”**” represent $p < 0.05$ and $p < 0.01$, respectively.

Figure 4. The proliferation activity as well as the IFN-γ and IL-4 levels in supernatant of EOLP CD4+ T cells transfected with agomir-155 (the details was illustrated as Fig. 3).
regulation of miRNA^{16,17,26–28}. Through screening the sera, Nylander E had ever drawn a pessimistic prediction that there was no specific LP-associated miRNA profile in peripheral blood^{29}. However, in 2013, our group first reported that miR-125a was down-regulated in peripheral blood of OLP patients^{5}. In the present study, the result showed that miR-155 was up-regulated in peripheral blood of EOLP patients. Furthermore, miR-155 was found to be high related to the RAE scores of OLP patients (p < 0.01, r = 0.855), and the relationship coefficient was higher when the subjects was limited to EOLP patients (p < 0.01, r = 0.882). It implicated that miR-155 might be a positive candidate to assess the severities of EOLP patients.

We and some researchers had proved that there was chasm about the immune mechanisms of EOLP and NEOLP in peripheral blood, like the expression of transcription factor, the activation of T cells...
and the levels of cytokines. Furthermore, clinically, these two types of OLP showed great difference in treatment, outcome and the malignant risk. As the inflammatory bowel disease had two predominant types of Crohn's disease and ulcerative colitis, in our opinion, NEOLP and EOLP might be considered as two types of diseases but not just different manifestations of OLP. The current data represented that the expression of miR-155 in EOLP group was higher than in NEOLP group; likewise, the level of IFN-γ increased in EOLP group, and was elevated compared with NEOLP group. But nearly to the opposite to miR-155 or IFN-γ, the IL-4 level decreased in EOLP group, and was much lower than that of NEOLP group. The serum levels of IL-2 and IL-10 were distinguished between OLP groups with the controls, but no distinction was presented between EOLP group and NEOLP group. Our data further proved that NEOLP and EOLP were quite different in cytokine levels and miRNA expression, and that the immune response of EOLP tended to be much more Th1-dominated than that of NEOLP. In addition, it also revealed that the expression of miR-155 in peripheral blood might be associated with the levels of IFN-γ and IL-4.

IFN-γ could promote Th1 cell differentiation by induction of T-bet, a transcription factor critical to Th1 cell differentiation, and Th1 cells could produce IFN-γ, which formed a positive feedback to reinforce Th1-dominated immune response. A second way in which IFN-γ was thought to contribute to Th1 immune response functioned by inhibiting the proliferation of Th2 as well as the secretion of IL-4. IL-4 could play the same role in Th2-dominated immune response. Both IFN-γ and IL-4 should exert their function by binding to their receptors on the membrane and being assisted by JAKs/STAT pathway for IFN-γ and JAKs/STAT6 for IL-4. The receptor of IFN-γ (IFN-γR) had two chains, IFN-γRα and IFN-γRβ, while IFN-γR3 was related to the process of signal transduction, in addition, committed Th1 cells could regulate the expression of IFN-γR3 and help CD4+ T cells to resist the anti-proliferation effect of IFN-γ. In most references, miR-155 was reported to inhibit the Th2 cell differentiation and the production of IL-4 by targeting c-MAF, a distinct transcription factor of Th2 cells which was crucial for IL-4 gene transcription. However, there was still debating like Banerjee et al. announcing that miR-155 could target IFN-γRα to inhibit IFN-γ signal transduction.

The current study showed that in the supernatant, IFN-γ levels decreased while IL-4 levels increased in the presence of antagonir-155, and the proliferation activity of EOLP CD4+ T cells was abated at 24h and 36h post-transfection. This part seemingly agree on the mainstream attitude, as miR-155 was suppressed, the pent-up signal transduction of IFN-γ could not lead to Th1 cell differentiation, and the production of IFN-γ was decreased either. On the contrary, the weakening signal transduction of IFN-γ enhanced the IL-4 secretion and Th2 differentiation. The past work had demonstrated a Th1-dominated immune response in EOLP CD4+ T cells, and this may be why the proliferation activity was decreased at 24h and 36h. In the early 12h, for the compensation of attenuated IFN-γ signal transduction, there was less effect on anti-proliferation of IFN-γ, thus, the proliferation activity showed no difference with the negative control group. However, for the last 12h (36-48h), the increasing Th2 cells gradually made up for the decreasing differentiation of Th1 cells, and the immune condition might have been changed.

In conclusion, we found miR-155 was highly expressed in the peripheral blood of EOLP patients, and the expression of miR-155 in EOLP group was higher than in NEOLP group; likewise, the level of IFN-γ increased in EOLP group, and was elevated compared with NEOLP group. The serum levels of IL-2 and IL-10 were distinguished between OLP groups with the controls, but no distinction was presented between EOLP group and NEOLP group. Our data further proved that NEOLP and EOLP were quite different in cytokine levels and miRNA expression, and that the immune response of EOLP tended to be much more Th1-dominated than that of NEOLP. In addition, it also revealed that the expression of miR-155 in peripheral blood might be associated with the levels of IFN-γ and IL-4.
EOLP, and SOCS1 was considered to be the most possible target of miR-155 involved in the feedback loop.

Materials and Methods

This experiments followed the principles outlined in the Declaration of Helsinki in the use of human samples and were approved by the Ethics Committee of School and Hospital of Stomatology, Wuhan University with approved NO 2011051. All participating subjects gave their informed consents.

Patients and controls. The patients involved in this study were clinically and pathologically diagnosed as OLP according to the definition of OLP made by the WHO. In term of the manifestation, they were divided into two groups: erosive type OLP (EOLP) group and non-erosive type OLP (NEOLP) group. Age and gender matched healthy volunteers were recruited as the controls. In the first stage, 10 EOLP patients, 10 NEOLP patients and 10 controls was recruited, and in the second stage, 7 more EOLP patients and 3 more controls were replenished. Table 1 displays the clinical details. The subjects neither had any systemic disorders (such as cardiovascular disease, diabetes mellitus, etc) nor any soft tissue lesions in the oral mucosa. Smokers and severe alcoholics were excluded. Besides, patients on immunotherapy, receiving any medical treatment of OLP (local or systematic) within 3 months or having medicines affecting RNA synthesis and transcription in 6 months should not be included. All patients recruited in this study had been treated as needed following the sample collection.

Evaluation of the severities of OLP patients. RAE (reticular, atrophic and erosive) scoring system recommended by our previous study, which has shown much practicality and efficiency, was used to assess the severity of OLP in different clinical forms (Table 2).

Serum and EOLP CD4+ T cell isolation. Fourteen milliliter peripheral blood sample was drawn from each subject. Two milliliter blood (without anticoagulation) was kept in room temperature for 1h, and then centrifuged at 3000rpm for 10 min, and stored the supernatant serum in –20°C. Another ten milliliter blood (with anticoagulation) was diluted with equivalent phosphate buffers (PBS) and the sample was transferred to the centrifuge tubes with lymphocytes separation medium (tbdscience Biotech Ltd, Tianjin, China). Isolation of peripheral blood mononuclear cells from EOLP patients was performed by Ficoll-Paque density gradient centrifugation, and then a human CD4 T lymphocyte enrichment set-DM

| Clinical form | Scoring |
|---------------|---------|
| Reticular     | 0 = no white striations; 1 = presence of white striations or keratotic papules |
| Atrophic      | 0 = no lesion; 1 = lesions < 1 cm2; 2 = lesions from 1 to 3 cm2; 3 = lesions > 3 cm2 |
| Erosive       | 0 = no lesion; 1 = lesions < 1 cm2; 2 = lesions from 1 to 3 cm2; 3 = lesions > 3 cm2 |
| Totala        | $\Sigma R + \Sigma (A \times 1.5) + \Sigma (E \times 2.0)$ |

Table 1. Clinical characteristic of OLP patients and the controls.

|                      | EOLP group | NEOLP group | Controls | added EOLP cases | added controls |
|----------------------|------------|-------------|----------|-----------------|---------------|
| Number               | 10         | 10          | 10       | 7               | 3             |
| Age(year)            |            |             |          |                 |               |
| Mean                 | 44         | 41          | 49       | 46              | 48            |
| Range                | 40–63      | 24–63       | 37–55    | 44–50           | 45–52         |
| Gender               |            |             |          |                 |               |
| Female               | 5          | 5           | 5        | 4               | 2             |
| Male                 | 5          | 5           | 3        | 3               | 1             |
| Disease duration (month) |          |             |          |                 |               |
| Mean                 | 29.1       | 11          | 27.9     |                 |               |
| Range                | 2–72       | 3–30        | 22–40    |                 |               |

Table 2. The RAE scoring system for OLP. “The oral cavity was divided into 10 sites: upper/lower labial mucosa, right buccal mucosa, left buccal mucosa, dorsal tongue, ventral tongue, floor of mouth, hard palate mucosa, soft palate/tonsillar pillars, maxillary gingiva and mandibular gingival. The total score was recorded by summation of the scores of all 10 areas multiplied by a weighted factor of 1.5 (atrophic lesion) or 2.0 (erosive lesion).
(BD IMag™ Becton, Dickinson and Company, NJ, USA) was applied for negative selection of CD4+ T lymphocyte. The isolated CD4+ T cells were maintained with RPMI 1640 culture medium containing 20% fetal bovine serum (Gibco® Life Technologies, CA, USA) at 2 × 105 cells/ml.

**IFN-γ induction and miR-155 regulation in EOLP CD4+ T cell.** Recombinant Human IFN-γ (PeproTech, NJ, USA) was reconstituted in water to a concentration of 1.0 mg/ml. Agomir-155 (a modified RNA oligomer specifically increase miR-155 activity), antagonir-155 (a modified antisense RNA oligomer specifically reduce miR-155 activity) and their negative control reagents were prepared as 20 μmol/l working solution (Shanghai GenePharma Co., Ltd, Shanghai, China). For each sample, added 2 ml EOLP CD4+ T cells cultured solution (cell viability > 95%) to two wells of 12-well plate to make sure each well containing 2 × 105 CD4+ T cells, meanwhile, sixteen wells in 96-well plate were filled with 6000 CD4+ T cells, and expanded the volume to 200 μl with Opti-MEM® I Reduced-Serum Medium (Gibco® Life Technologies, CA, USA). Then 10 ng Recombinant Human IFN-γ was appended into one well with CD4+ T cells in 12-well plate, and agomir-155, antagonir-155 as well as their negative control regents were transacted into the CD4+ T cells of the sixteen wells in 96-well plate with proper Lipofectamine 2000 Reagent (Life Technologies, CA, USA) to make sure that the concentration of agomir-155 and its negative control regents was 50 nM, and for antagonir-155 and its negative control regents, the concentration was 100 nM. The IFN-γ induced CD4+ T cells were marked as IFN-γ induced group, and CD4+ T cells transacted with agomir-155, antagonir-155 and their negative control regents were marked as A group, AN group, ANC group, ANNC group, respectively. After 24 h, IFN-γ induced CD4+ T cells as well as the supernatants from A group, AN group, ANC group and ANNC group were collected.

**Proliferation assay of EOLP CD4+ T cells.** Cell counting kit-8 (CCK8) was applied to estimate the proliferation of CD4+ T cells (KeyGEN BioTECH Co., Ltd, Nanjing, China). For each sample, two wells filled with CD4+ T cells in 96-plate from A group, AN group, ANC group, ANNC group, respectively were taken. Then added 20 μl WST-8 to each well, and build a consecutive observation with the time point of 12 hour, 24 hour, 36 hour and 48 hour. At each timing point, the OD value of each well was recorded using Synergy 2 Multi-Mode Reader (BioTek Instruments, Inc, VT, USA).

**RNA extraction and reverse transcription.** Total RNA was extracted from peripheral blood (the last two milliliter with anticoagulation) using miRNeasy Mini Kit (QIAGEN GmbH, Düsseldorf, Germany). Likewise, the RNA of EOLP CD4+ T cells were extracted with miRNeasy Micro Kit (QIAGEN GmbH, Düsseldorf, Germany). Concentration and purity were then determined by NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc, Dubuque, USA). cDNA was synthesized from the total RNA using miScript Reverse Transcription Kit (QIAGEN GmbH, Düsseldorf, Germany). The conditions were under 60 min at 37 °C and 5 min at 95 °C.

**Quantitative real-time polymerase chain reaction (Real-Time PCR) analysis for miR-155 and SOCS1 mRNA.** The analyses of miR-155 expression were done with miScript SYBR® Green PCR Kit (QIAGEN GmbH, Hilden, Germany) using ABI 7500 Real-Time PCR system (Applied Bio-systems Inc, Foster City, CA, USA) in a one-step real-time PCR 96-well optical plate. The PCR initial activation step was at 95 °C for 15 min, followed by 15 s at 94 °C for denaturation, 30 s at 55 °C for annealing, and 34 s at 70°C for extension. The cycle number was set as 35 cycles. MiR-155 expression was normalized to endogenous RNA-U6 as internal control. The forward primer and reverse primer of U6 were synthesized as GCCCTTAGCGTGAAGATGG and CTTTGGTATCGTGGAAGGACTC, respectively, in addition, the reverse primer were synthesized as TGTGCGGAAGTGCGTGT and TTTGCGGCCCATGCTTCTC, respectively. The analyses of SOCS1 mRNA expression in EOLP CD4+ T cells were determined with FastStart Universal SYBR Green Master (ROX) (Roche Applied Science, Basel, Switzerland) according to the manufacturer's instructions. SOCS1 mRNA expression was normalized to endogenous hGAPDH mRNA as internal control. The forward primer of SOCS1 and hGAPDH mRNA were synthesized as GCCCTTAGCGTGAAGATGG and CTTTGGTATCGTGGAAGGACTC, respectively, in addition, the forward primer were synthesized as TGTGCGGAAGTGCGTGT and TTTGCGGCCCATGCTTCTC, respectively. The threshold cycle (Ct) of three replicates and two replicates for internal control per sample was used to calculate 2−ΔΔCt.

**Enzyme-linked immunosorbent assay (ELISA).** ELISA kit (R&D Systems, Minneapolis, MN, USA) was used to test the levels of interleukin 2 (IL-2), IL-4, IL-10 and IFN-γ in serum as well as the supernatant of CD4+ T cells according to the manufacturer’s instructions.

**Statistical analyses.** Data were presented as mean ± SD, and statistical significance was defined as p value < 0.05. The One-Way ANOVA and Pearson's correlation test were performed by SPSS 13.0 for windows software (SPSS, Inc, Chicago, USA).
Target prediction. Using TargetScan (version 6.2; http://www.targetscan.org), we looked up the potential targets of miR-155.30

References
1. Gale, N., Pitch, B. Z., Sidransky, D. & Eveson, J. Epithelial precursor lesions in Pathology and Genetics of Head and Neck Tumors in World Health Organization Classification of Tumours (eds Barnes, L. et al.) 177–179 (IARC Press, 2005).
2. McFarlan, B. E. & Healy, C. M. The reported prevalence of oral lichen planus: a review and critique. J Oral Pathol Med. 37, 447–453 (2008).
3. Casparis, S. et al. Oral lichen planus (OLP), oral lichenoid lesions (OLL), oral dysplasia, and oral cancer: retrospective analysis of clinicopathological data from 2002-2011. Oral Maxillofac Surg. 19, 149–156 (2015).
4. Van der Meiij, E. H., Mast, H. & Van der Waal, I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective five-year follow-up study of 192 patients. Oral Oncol. 43, 742–748 (2007).
5. Hu, Y. J. et al. Increasing CCL5/CXCR5 on CD4+ T cells in peripheral blood of oral lichen planus. Cytokine. 62, 141–145 (2013).
6. Zhou, G. et al. Increased IFN-gamma expression on peripheral blood T cells in oral lichen planus correlated with disease severity. J Clin Immunol. 32, 794–801 (2012).
7. Lu, R. et al. Expression of T-bet and GA-TA-3 in peripheral blood mononuclear cells of patients with oral lichen planus. Arch Oral Bio. 56, 499–505 (2011).
8. O'Shea, J. J., Lahesmaa, R., Vahedi, G., Laurence, A. & Kanno, Y. Genomic views of STAT function in CD4(+) T helper cell differentiation. Nat Rev Immunol. 11, 239–250 (2011).
9. Nakayamada, S. et al. Early Th1 cell differentiation is marked by a Th1 cell-like transition. Immunity. 35, 919–931 (2011).
10. Ilia, S., Goulieinos, G. N., Samonis, G. & Galanakis, E. Polymorphisms in IL-6, IL-10-TNF-alpha, IFN-gamma and TGF-beta1 Genes and Susceptibility to Acute Otitis Media in Early Infancy. Pediatr Infect Dis J. 33, 618–621 (2014).
11. Gein, S. V. & Sharavieva, I. L. Effect of rotation and immobilization stress on IL-1beta, IL-2, IL-4, and IFN-gamma production by splenocytes under opiate receptor blockade in vivo. Dokl Biol Sci. 354, 69–71 (2014).
12. Aune, T. M., Collins, P. L. & Chang, S. MicroRNAs in autoimmunity and inflammatory bowel disease: crucial regulators in immune response. Autoimmun Rev. 11, 305–314 (2012).
13. Duttagupta, R. et al. Genome-wide maps of circulating miRNA biomarkers for ulcerative colitis. PLoS One. 7, e31241 (2012).
14. Zonari, E. et al. Association study of genes controlling IL-12-dependent IFN-gamma immunity: STAT4 alleles increase risk of Crohn's Disease. J Immunol. 188, 58–66 (2012).
15. Palomba, S. et al. Circulating immunomicro-RNAs in patients with uveal melanoma developing metastatic disease. Mol Immunol. 58, 182–186 (2013).
16. Outtugupta, R. et al. Genome-wide maps of circulating miRNA biomarkers for ulcerative colitis. PLoS One. 7, e31241 (2012).
17. Naka, S. K., Ebrahimi, M., Wahlin, Y. B., Boldrup, L. & Nylander, K. Changes in miRNA expression in sera and correlation to disease duration of disease in patients with multifocal mucosal lichen planus. J Oral Pathol Med. 41, 86–89 (2012).
18. Zonari, E. et al. Association of po-lymorphisms in the tumor necrosis factor-alpha and interleukin-10 genes with oral lichen planus: a study in a chinese cohort with Han ethnicity. J Interferon Cytokine Res. 36, 542–550 (2012).
19. Hul, S., Van der Meij, E., Mast, H. & Van der Waal, I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective five-year follow-up study of 192 patients. Oral Oncol. 43, 742–748 (2007).
20. Imaizumi, T. et al. Expression of T-bet and GA-TA-3 in peripheral blood mononuclear cells of patients with oral lichen planus. Arch Oral Bio. 56, 499–505 (2011).
21. O'Shea, J. J., Lahesmaa, R., Vahedi, G., Laurence, A. & Kanno, Y. Genomic views of STAT function in CD4(+) T helper cell differentiation. Nat Rev Immunol. 11, 239–250 (2011).
22. Nakayamada, S. et al. Early Th1 cell differentiation is marked by a Th1 cell-like transition. Immunity. 35, 919–931 (2011).
23. Gein, S. V. & Sharavieva, I. L. Effect of rotation and immobilization stress on IL-1beta, IL-2, IL-4, and IFN-gamma production by splenocytes under opiate receptor blockade in vivo. Dokl Biol Sci. 354, 69–71 (2014).
24. Aune, T. M., Collins, P. L. & Chang, S. MicroRNAs in autoimmunity and inflammatory bowel disease: crucial regulators in immune response. Autoimmun Rev. 11, 305–314 (2012).
25. Duttagupta, R. et al. Genome-wide maps of circulating miRNA biomarkers for ulcerative colitis. PLoS One. 7, e31241 (2012).
26. Zonari, E. et al. Association study of genes controlling IL-12-dependent IFN-gamma immunity: STAT4 alleles increase risk of Crohn's Disease. J Immunol. 188, 58–66 (2012).
27. Hul, S. et al. Association study of genes controlling IL-12-dependent IFN-gamma immunity: STAT4 alleles increase risk of pulmonary tuberculosis in Morocco. J Infect Dis. 210, 611–618 (2014).
28. Huang, W. T. & Lin, C. W. EBV-Encoded miR-BART20-5p and miR-BART8 Inhibit the IFN-gamma-STAT1 Pathway Associated with Disease Progression in Nasal NK-Cell Lymphoma. Am J Pathol. 184, 1185–1197 (2014).
29. Ma, Z. J. & Xia, Q. H. The function of microRNA-155 in immunosystem. Chin J Cell Mol Immunol. 27, 1364–1366 (2011).
30. Wang, P. et al. Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1. J Immunol. 185, 6226–6233 (2010).
31. Imazumii, T. et al. IFN-gamma and TNF-alpha synergistically induce microRNA-155 which regulates TAR2/IP-10 expression in human mesangial cells. Am J Nephrol. 32, 462–468 (2010).
32. DiGiandomenico, A., Wylezinska, L. S. & Hawiger, J. Intracellular delivery of a cell-penetrating SOCS1 that targets IFN-gamma signaling. Sci Signal. 2, ra37 (2009).
Acknowledgements
This work was supported by a Grant (No. 81170972, 81371147) from National Natural Science Foundation of China.

Author Contributions
J.Y.H., J.Z., J.Z.M., R.L., G.F.D. and G.Z. designed research. J.Y.H., J.Z., X.Y.L. and G.Y.C. performed the experiment. G.Z., J.Z.M., R.L. and G.F.D. contributed new reagents/analytic tools. J.Y.H. and J.Z. analyzed data. J.Y.H. wrote the paper and all authors reviewed the manuscript.

Additional Information
Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Hu, J.-Y. et al. MicroRNA-155-IFN-γ Feedback Loop in CD4⁺ T Cells of Erosive type Oral Lichen Planus. Sci. Rep. 5, 16935; doi: 10.1038/srep16935 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/