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Aberrant IGF1–PI3K/AKT/MTOR signaling pathway regulates the local immunity of oral lichen planus

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ABSTRACT

Objectives: Oral lichen planus (OLP) is a T cell-mediated immune-related chronic disease, featured by accumulation of T cells and apoptosis of keratinocytes. Insulin-like growth factors 1 (IGF1) signaling, in combination with its downstream PI3K/AKT/MTOR cascade, plays pivotal roles in the regulation of inflammation and immune response. Meanwhile, TRB3 acts as a connective protein in the pathway. This study investigated the possible function of IGF1–PI3K/AKT/MTOR pathway in the local immunity of OLP.

Methods: The expression of phosphorylated IGF1R (p-IGF1R) and TRB3 in lesional tissues of OLP was measured. The effects of T cells pretreated with PI3K inhibitor LY294002, MTOR antagonist rapamycin and exogenous IGF1 on the cell proliferation and apoptosis, as well as supernatant inflammatory cytokine levels were detected in co-culture system of activated T cells and oral keratinocytes, respectively.

Results: The expression of p-IGF1R and TRB3 in OLP lesions was significantly increased when compared with controls ($P < 0.001$). Rapamycin-treated T cells displayed enhanced apoptosis rate and promoted proliferation of their keratinocytes in the co-culture system. Notably, abnormal expression of IFN-γ and IL-4 were detected in supernatant of T cell alone and co-culture system in response to pharmacological modulators of IGF1–PI3K/MTOR pathway.

Conclusions: The aberrant IGF1–PI3K/AKT/MTOR signaling may participate in the immunoregulatory mechanism of OLP, via regulation on the crosstalk between T cells and keratinocytes, as well as imbalanced cytokine networks.

1. Introduction

Oral lichen planus (OLP) is an immune-related chronic disease that affects 1–2% of the general adult population (Alrashdan et al., 2016; Roopashree et al., 2010). OLP, whose malignant transformation rate ranges from 0.07% to 5.8%, has been categorized as a potentially malignant disorder by World Health Organization (WHO) (van der Waal, 2009; Smh et al., 2017). In OLP lesions, the most typical histopathological feature is the accumulation of T lymphocytes in the superficial lamina propria of oral mucosa (Payeras et al., 2013). It is widely accepted that the interactions between T cells and basal keratinocytes participate the immunopathogenesis of OLP (Payeras et al., 2013). Specifically, T cells are activated after antigen recognition, which may then trigger the apoptosis of basal keratinocytes, and release cytokines to attract additional T cells into the OLP lesions. These infiltrated T cells may affect the antigen presentation and aggravate local inflammation in turn (Gupta and Jawanda, 2015). Our previous study revealed that dysregulated cytokines including interleukin (IL)-4 and interferon (IFN)-γ secreted by activated pathogenic T cells could regulate the apoptosis of keratinocytes (Zhou et al., 2012a). The IFN-γ/IL-4 ratio has been considered as an indicator of Th1/Th2 cytokine balance, which has been reported to be increased in serum and lesions of patients with OLP (Wang et al., 2015).

Insulin-like growth factors 1 (IGF1) signaling, initiated from the activation of IGF1 receptor (IGF1R), plays critical role in the regulation of cell growth and survival (Smith, 2010a). IGF1 is a key pathway in inflammation and immune response (Smith, 2010a). High-affinity binding of IGF1 and IGF1R was reported on activated T cell, which could induce the proliferation of T cells (Smith, 2010a). Moreover, IGF1 could regulate the development and functions of $CD4^+$ T cells (Smith,

Abbreviations: OLP, oral lichen planus; PI3K, phosphoinositide 3-kinases; AKT, protein kinase B; MTOR, mammalian target of rapamycin; LPS, lipopolysaccharide

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2010a). Overexpression of IGF1R has been implicated in various immune inflammatory diseases, including Crohn's disease, Graves' disease, rheumatoid arthritis and experimental autoimmune encephalomyelitis (Vardatsikos et al., 2013; Zhang et al., 2014; Zhou et al., 2017). Notably, our group previously revealed an upregulation of *IGF1* mRNA level in peripheral T cells of patients with OLP (Tan et al., 2016). Therefore, aberrant of IGF1 and its related pathways were hypothesized to be involved in the pathogenesis of OLP.

Phosphatidylinositol 3-kinase (PI3K)/AKT/MTOR cascade, one of the critical nodes in the IGF-1 signaling pathway, is activated after the phosphorylation of IGF1R (Smith, 2010b; Gusscott et al., 2016). As a negative modulator of AKT, tribbles homolog 3 (TRB3) is also a stress-induced mediator of insulin signaling (Gu et al., 2018; Hua et al., 2015; Johnston et al., 2015). It has been established that TRB3 level was overexpressed in response to metabolic stress, peculiarly elevated IGF1 (Johnston et al., 2015). Both IGF1 and TRB3 are implicated in the control of autophagic flux, which exert pivotal roles in cell survival and death, tissue homeostasis, as well as inflammatory regulation (Hua et al., 2015). Increased activity of autophagy related PI3K/AKT/MTOR pathway has been found in abundant T-cell-related diseases (Bertacchini et al., 2015; Song et al., 2016; Blachly and Baiocchi, 2014). Our previous studies have demonstrated activated AKT/MTOR and autophagy in the OLP lesional tissues and local T cells (Zhang et al., 2017). However, to date, the expression of IGF1 receptor and TRB3, as well as the effects of the IGF1–PI3K/AKT/MTOR signaling cascades in the pathogenesis of OLP have not been elucidated.

In present study, the expression of p-IGF1R and TRB3 at protein levels were investigated in OLP lesional tissues. In addition, a co-culture experiment of lipopolysaccharide (LPS)-treated keratinocytes with activated T cells was conducted to investigate the effects of IGF1 and PI3K/AKT/MTOR pathways on the immune environment of OLP.

2. Method and materials

2.1. Participants and tissue specimens

A total of 19 patients with OLP (aged 23–64 years, 12 nonerosive type and 7 erosive type) from the Department of Oral Medicine, School and Hospital of Stomatology, Wuhan University, and 14 age-gender matched healthy volunteers (aged 22–58 years) were recruited in present study. Then, tissue specimens were collected from lesion sites of patients with OLP and healthy oral mucosa from healthy individuals receiving orthogenetic surgery, respectively. All patients were clinically diagnosed and histopathologically confirmed as OLP according to the diagnostic criteria of OLP made by WHO (van der Meij and van der Waal, 2003). Disease severity was evaluated using the RAE (reticular, atrophic and erosive) scoring system proposed in our previous study (Zhou et al., 2012a). These healthy controls had neither any visible lesions in oral mucosa nor any history of systemic disorders. In addition, all participants did not receive any topic or systemic anti-inflammatory or immunomodulatory drugs at least 3 months before sampled. Moreover, all subjects had no history of smoking and alcohol abuse. The experiments were approved by the Ethical Committee Broad of School and Hospital of Stomatology, Wuhan University (No. 2013084), and followed the principles of the Declaration of Helsinki in the use of human samples. Written informed consents were obtained from each subject prior to the study. The clinical data, including gender, age, and clinical forms of OLP were listed in Table 1.

Table 1
Clinical features of the subjects.

	OLP(n = 19)	Control(n = 14)
Gender		
Male	10	8
Female	9	6
Age(years)		
Range	23-64	22-58
Mean ± SD	45.11 ± 12.40	40.21 ± 11.01
Clinical form		
Nonerosive	12	
Erosive	7	

2.2. Immunohistochemistry

The fresh samples were fixed in a 4% formaldehyde solution for more than 24 h and then embedded in paraffin. Later, the tissue sections were deparaffinized in xylene and rehydrated via a graded alcohol series. Endogenous peroxidase activity was blocked via 3% H_2O_2 for 15 min at 37 °C and then rinsed with phosphate buffered saline (PBS). For heat-induced antigen retrieval, the sections were submerged into sodium citrate buffer (10 mM, pH = 6.0) with microwave for 10 min. After cooling at room temperature, the sections were washed with PBS and treated with 5% BSA for 1 h at 37 °C to block non-specific bindings. Then, the slides were incubated with anti-human IGF1 receptor (phospho Y1161; 1:100; Abcam, Cambridge, MA, USA) and anti-human TRB3 mAb (1:200; Abcam) overnight at 4 °C in a moist chamber, respectively. A section only treated with PBS, but not antibody, was used as blank control. These slides were treated with HRP polymer conjugated secondary antibody (Zhongshan Golden Bridge Ltd., Beijing, China) for 1 h at 37 °C and then visualized with diaminobenzidine solution (DAB, Zhongshan Golden Bridge Ltd., Beijing, China). Finally, the nucleus was counterstained with hematoxylin and mounting.

In each slide, 5 consecutive staining areas throughout the epithelium were selected randomly and photoed using a digital camera mounted on a microscope (Olympus, Tokyo, Japan) at 400× magnification for immunoreactive score (IRS) analysis. For immunohistochemistry, evaluation of these slides was performed by two independent investigators who were blind to clinical details via Image-Pro Plus 6.0 (IPP, Media Cybernetics, Inc. Silver Spring, MD, USA). The proportion of positive staining cells stained was measured as follows: 0, staining 0–1% of cells in tissues; 1, staining 1–10%; 2, staining 11%–50%; 3, staining 50%–75%; 4, staining > 75%. For intensity, 4 levels were used (0: negative, 1: weak, 2: intermediate, 3: strong). IRS was obtained by multiplying the intensity and proportion of positive staining score for each slide.

2.3. Cell culture

A co-culture system consists of activated Jurkat T cells and keratinocytes was employed to study the effects of abnormal PI3K/AKT/MTOR signaling pathway *in vitro*. To investigate the involvement of PI3K and MTOR signaling pathway of T cells in the co-culture system, two inhibitors, LY294002 targeting PI3K (Alrashdan et al., 2016; Roopashree et al., 2010) and rapamycin targeting MTOR (van der Waal, 2009; Payeras et al., 2013), were employed to stimulate T cells.

Human oral keratinocytes were seeded in a 24-well plate (1×10^5 cells/well) and were cultured in keratinocyte serum-free medium (K-SFM, Gibco Life Technologies, Carlsbad, CA, USA) at 37 °C with a 5% CO_2 humidified atmosphere for 36 h. The cells were then stimulated with 10 μg/ml LPS (L2654, Sigma-Aldrich, St. Louis, MO, USA) for 24 h.

Jurkat T cells were cultured in RPMI 1640 medium (Gibco Life Technologies) for 48 h, supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA), 1 ug/ml anti/CD3mAb (Bioscience, San Diego, CA, USA) and 2 ug/ml anti/CD28 mAb (Bioscience, San Diego, CA, USA). Then the cells were washed and seeded in 24-well culture plates at a density of 1×10^5 cells /ml. They were divided into the following six groups: control group (Blank); MTOR inhibitor rapamycin (100 nM/ml, Selleckchem); PI3K inhibitor LY294002 (10 μM/ml, Selleckchem); human IGF1 (100 ng/ml, Novoprotein), rapamycin + IGF1; LY294002 + IGF1.

After 48 h of treatment, these T cells were washed and separately

co-cultured with activated keratinocytes via Transwell Cell Culture Inserts (Corning Inc, New York, USA) with 0.4 μm pore size. Transwell inserts provide a cell co-culture environment for T cells (2×10^5 cells/200 μl RPMI 1640 medium supplemented with 10% FBS/well, upper compartment) and keratinocytes (2×10^5 cells/600 μl K-SFM/well, lower compartment) to mimics OLP conditions *in vivo*.

2.4. Cell proliferation analysis

After co-cultured for 24 h, keratinocytes in the lower compartment of Transwell inserts undergo cell proliferation analysis using a Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. After incubation at 37 °C with a 5% CO_2 humidified atmosphere for 4 h, keratinocytes were measured at 450 nm wavelength and the cell proliferation activity was represented by the optical density (OD) value. The assay was repeated in triplicate three times.

2.5. Detection of cell apoptosis with flow cytometric analysis

Meanwhile, the apoptosis rate of T cells in the co-culture system was measured after co-cultured for 24 h using the Annexin V-FITC/PI Apoptosis Detection Kit (KeyGEN Bio-Tech Inc, Nangjing, China), respectively. Both T cells and keratinocytes were washed three times with PBS, and were incubated with Annexin V-FITC and PI in binding buffer for 15 min at room temperature in the dark. Samples were analyzed within 1 h of staining using an Epic Elite ESP Beckman Coulter flow cytometer (Beckman, Fullerton, CA, USA). Data were analyzed by FlowJo software (Treestar, Ashland, OR, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA)

To determine the secretion of pro-inflammatory cytokines, supernatants from co-culture system and T cells alone were collected and assessed using IFN-γ and IL-4 ELISA kits (NeoBioscience biological technical co. Ltd, Shenzhen, China), respectively. The OD at 450 nm wavelength in each well was measured, and the concentration of IFN-γ and IL-4 were calculated by comparison with a standard curve. The assays were repeated in triplicate.

2.7. Statistical analysis

Data are presented as mean ± SD, and statistical significance was considered as $P < 0.05$. Independent-samples *t* test, non-parametric Mann-Whitney U test and Spearman's correlation coefficient were analyzed with SPSS statistical software 17.0 (SPSS, Inc. Chicago, IL, USA).

3. Results

3.1. Upregulated expression of p-IGF1R and TRB3 in OLP lesional tissues

The presence of p-IGF1R (OLP versus controls: 3.47 ± 1.54 versus 0, $P < 0.001$) and TRB3 (OLP versus controls: 3 ± 1.25 versus 0, $P < 0.001$) was dramatically increased in OLP tissues than that in control group. The expression of p-IGF1R and TRB3 was mainly found in the cells of lamina propria, basal layer of epithelium and superficial layer of connective tissue. In addition, p-IGF1R and TRB3 displayed different staining patterns. TRB3 mainly localized in the nucleus, while p-IGF1R generally localized in the membranes (Fig. 1).

In addition, no significant correlation was found between p-IGF1R and TRB3 proteins expression in local tissues ($r = 0.09$, $P = 0.36$) (Fig. 1D). Both p-IGF1R and TRB3 proteins expression in male (p-IGF1R: 3.70 ± 1.77, TRB3: 3.22 ± 1.39) and female OLP patients (p-IGF1R: 3.22 ± 1.30, TRB3: 2.80 ± 1.14) showed no significant differences ($P = 0.79$ and 0.826, Fig. 1E), respectively. Furthermore, no significant correlation was found between the expression of p-IGF1R ($r = 0.05$, $P = 0.83$) or TRB3 protein and the age of patients ($r = 0.22$, $P = 0.36$ Fig. 1G1, G2). To further investigate the correlation of p-IGF1R and TRB3 with disease severity of OLP, we examined the correlations of the expression of these two proteins with RAE scores. However, neither p-IGF1R ($r = -1.43$, $P = 0.45$) nor TRB3 expression ($r = -0.11$, $P = 0.57$) in OLP local tissues had significant correlations with RAE scores (Fig. 1F1, F2).

3.2. Effects of IGF1 and PI3K/ MTOR pathways on co-culture system

3.2.1. Effects of IGF1 and PI3K/ MTOR pathways on apoptosis of T cells in co-culture system

As shown in Fig. 2, a significantly higher proportion of apoptotic T cells was observed in the rapamycin group when compared with the controls and IGF1 group ($Group_{(CON)}$ versus $Group_{(Rapamycin)}$ versus $Group_{(IGF1)}$: 15.10% ± 2.50 versus 22.52% ± 4.09 versus 14.52% ± 3.49, $P < 0.01$). Meanwhile, the apoptosis rate of T cells in the rapamycin + IGF1 group was also higher than that in the IGF1 group ($Group_{(IGF1+LY294002)}$ versus $Group_{(IGF1)}$: 14.44% ± 3.68 versus 14.52% ± 3.49, $P < 0.05$). However, neither LY294002 nor IGF1 stimulation alone had effects on the T-cell apoptosis in co-culture system.

3.2.2. Effects of IGF1 and PI3K/ MTOR pathways on proliferation of keratinocytes in co-culture system

In present study, all treated group displayed increased cell viability of keratinocytes in comparison with control group ($P < 0.01$, Fig. 2C). Notably, keratinocytes co-cultured with T cells pretreated rapamycin had significant elevation of cell proliferation when compared with IGF1-treated and control groups ($Group_{(CON)}$ versus $Group_{(Rapamycin)}$: 1.00 ± 0.02 versus 1.63 ± 0.05, $P < 0.01$; $Group_{(Rapamycin)}$ versus $Group_{(IGF1)}$: 1.63 ± 0.05 versus 1.28 ± 0.04, $P < 0.01$). However, the proliferation of keratinocyte co-cultured with IGF1 and rapamycin co-stimulated T cells was decreased when compared with IGF1-treated and rapamycin group ($P < 0.01$). In contrast, cell viability of keratinocyte co-cultured with T cells in the LY294002 + IGF1 group was significantly increased than that in the LY294002- or IGF1-treated group ($Group_{(LY294002+IGF1)}$ versus $Group_{(LY294002)}$: 1.64 ± 0.04 versus 1.30 ± 0.04, $P < 0.01$; $Group_{(LY294002+IGF1)}$ versus $Group_{(IGF1)}$: 1.64 ± 0.04 versus 1.28 ± 0.04, $P < 0.01$).

3.2.3. Effects of IGF1 and PI3K/ MTOR pathways on inflammatory cytokines secretion

The perturbations of IGF1 and PI3K/ MTOR pathways in T cells co-cultured with keratinocytes could induce a downregulation of IFN-γ level (Fig. 3). Specifically, the expression of IFN-γ reduced in IGF1 and rapamycin treated group when compared with controls ($P < 0.05$). Notably, the IFN-γ level decreased more significantly under the co-stimulation of IGF1 with rapamycin ($P < 0.01$); whereas the inhibition of IFN-γ by IGF1 could be partly remitted by functioning together with LY294002 ($P < 0.01$). Unlike IFN-γ, exogenous IGF1, rapamycin and LY294002 alone had no effects on the production of IL-4 ($P > 0.05$). However, when IGF1 co-stimulated with rapamycin ($P < 0.05$) or LY294002 ($P < 0.01$), the secretion of IL-4 was restricted in comparison with controls.

On the other hand, the presence of IGF1 ($P < 0.01$) and LY294002 alone ($P < 0.01$) suppressed the IFN-γ level in supernatant of T cells cultured alone. But rapamycin alone ($P < 0.01$) and co-stimulation with LY294002 and IGF1 ($P < 0.01$) promoted the secretion of IFN-γ when compared with controls. However, T cells co-stimulated with IGF1 and rapamycin displayed less IFN-γ expression than rapamycin treated alone ($P < 0.01$), but increased in comparison with IGF1 alone ($P < 0.01$). In the supernatant of T cells, stimulation of IGF1, LY294002, IGF1 + rapamycin, as well as IGF1 + LY294002 showed decreased IL-4 expression when compared with controls.

Interestingly, less IFN-γ expression was observed in all groups from

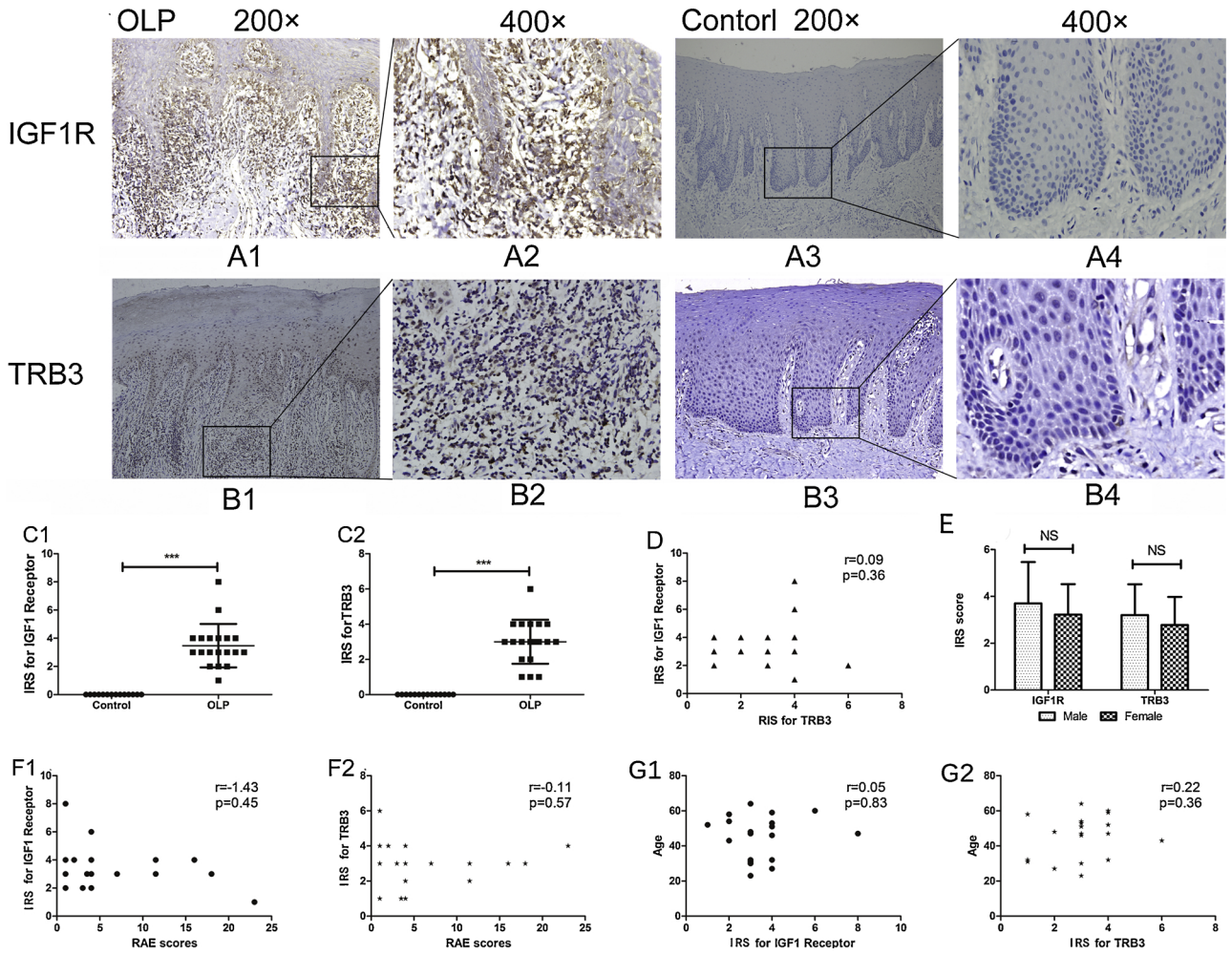

Fig. 1. The expression of p-IGF1R and TRB3 in tissue samples from OLP patients and controls. (A) The expression of p-IGF1R in OLP lesional tissue samples (n = 19) was significantly increased in the OLP group compared with normal controls (n = 14). (B) The expression of TRB3 in OLP lesional tissue samples (n = 19) was significantly increased in the OLP group compared with normal controls (n = 14). Semi-quantitative analysis of p-IGF1R and TRB3 expression in OLP patients and controls using IRS. (D-G) The associations of p-IGF1R and TRB3 expression in OLP patients with their disease severity and different genders or ages, as well as correlation between the two proteins were analyzed via Spearman's correlation test. Magnification: A1, A3, B1, B3: 200×; A2, A4, B2, B4: 400 × . Data were presented as mean ± SD (***P < 0.001, NS: nonsignificant).

the co-culture system (P < 0.01) while IL-4 level was increased in the controls (P < 0.05), rapamycin group (P < 0.01), LY294002 group (P < 0.01), and IGF1 group (P < 0.01) under co-culture condition.

3.2.4. Effects of IGF1 and PI3K/ MTOR pathways on the ratio of IFN-γ/IL-4

In the co-culture system, the ratio of IFN-γ/IL-4 in the combination of IGF1 and rapamycin group was decreased when compared with controls (P < 0.01). Additionally, co-stimulation with IGF1 and LY294002 increased the IFN-γ/IL-4 ratio compared with IGF1 or LY294002 alone (P < 0.01). However, when T cells were cultured alone, the ratio of IFN-γ/IL-4 in rapamycin group was increased compared with controls (P < 0.01), but the elevation was diminished by combination of IGF1 and rapamycin (P < 0.01). In addition, the stimulation of LY294002 together with IGF1 obviously upregulated the ratio of IFN-γ/IL-4 (P < 0.01). Moreover, T cells cultured alone possessed a significant higher ratio of IFN-γ/IL-4 than the co-culture system from all groups (P < 0.01).

4. Discussion

Increased paracrine or autocrine of IGF1 has been proved to be an important regulatory factor implicated in many autoimmune and inflammation diseases (Smith, 2010a). The combination of IGF1 and phosphorylated IGF1R can activate PI3K/AKT/MTOR signaling cascade to transmit modulatory signals from extracellular stimulations (Gusscott et al., 2016). Our previous study revealed an elevated expression of *IGF1* mRNA in the peripheral T cells of OLP (Tan et al., 2016). In addition, we have found the activation of AKT/MTOR pathway in local lesions of OLP (Zhang et al., 2017). In present study, the expression of p-IGF1R and TRB3 was significantly upregulated in lesional tissues of OLP, with no relevant to the genders, ages, as well as disease severity. Thus, increased p-IGF1R in local environment of OLP was in accordance with our previous evidence. Though the TRB3 always serves as a negative modulator of AKT, the overexpressed TRB3 accompanied with induced p-AKT in OLP might be a downstream effect of the IGF1 signaling, due to the fact that TRB3 can be upregulated by IGF1 (Johnston et al., 2015).

The IGF1 signaling pathway has been reported to be a dual regulator of autophagy (Jia et al., 2006; Troncoso et al., 2012). Moreover, our previous study has found the hyperactive AKT/MTOR-autophagy in local T cells of OLP (Zhang et al., 2017). To evaluate the effects of IGF1 and PI3K/AKT/MTOR pathways on OLP, inhibition of autophagy-related PI3K and MTOR signaling combined with exogenous IGF1 was done in T cells in the co-culture system *in vivo*. The MTOR antagonist rapamycin, but not the PI3K inhibitor LY294002, significantly

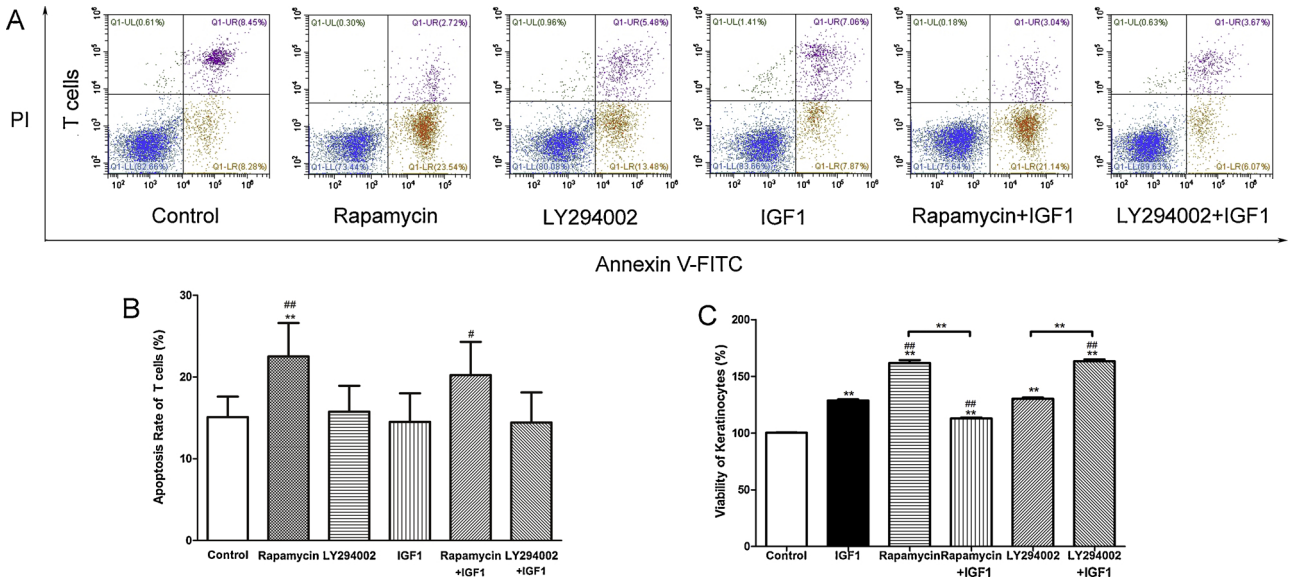

Fig. 2. The apoptosis of T cells and proliferation of keratinocytes in co-culture system. (A and B) The apoptotic cell rate in T cells alone and from the co-culture system, which were divided into the control group (Blank), rapamycin (100 nM/ml), LY294002 (10 μM/ml); human IGF1 (100 ng/ml), rapamycin + IGF1; LY294002 + IGF1. (C) The proliferation of keratinocytes co-cultured with pretreated T cells was measured via CCK-8. Results were expressed as the mean ± SD (∗∗P ≤ 0.01, ∗ P < 0.05, compared to Control; ## P ≤ 0.01, # P < 0.05, compared to IGF1).

increased the apoptosis of T cells in present study; whereas co-stimulation of IGF1 and rapamycin partly restored the pro-apoptosis effects on T cells. Similarly, it has been reported that the inhibiton of MTOR mediated by rapamycin could be antagonized by exogenous IGF1 (Du et al., 2013). Besides, enhanced T-cell apoptosis mediated by rapamycin could promote the proliferation of keratinocytes in co-culture system. Notably, IGF1 exerted antagonium on the regulation of keratinocytes proliferation mediated by rapamycin-treated T cells, but had a synergistic effect on promoting the viability of keratinocytes co-cultured with LY294002-treated T cells. It was presumed that MTOR signaling might dominate the suppression of T-cell apoptosis, which negatively regulated the proliferation of keratinocytes. Furthermore, IGF1 might function as an arbiter to balance the viability of keratinocytes when co-cultured with T cells treated with PI3K/MTOR inhibitors. In OLP lesions, the most typical histopathological feature is large numbers of activated T lymphocytes and substantial degradation of keratinocytes triggered by T cells in oral mucosa, which is well accepted to be one of key events in the occurrence of OLP (Roopashree et al., 2010). Overall, present data indicated that activation of IGF1 and PI3K/AKT/MTOR pathways and their related aberrant autophagy in T cells might modulate the crosstalk between T cells and keratinocytes, and therefore underlie the pathogenesis of OLP.

Immunologic dissonance mediated by imbalanced cytokine networks, especially Th1/Th2 cytokines, plays pivotal role in the onset and progression of OLP (Wang et al., 2014; Lu et al., 2015). Specifically, IL-4 is a representative cytokine of Th2 cells, which has been extensively investigated in the pathogenesis of OLP (Zhou et al., 2012b). Besides, IFN-γ is a most attractive Th1 cytokine and serves as one of key determinants in local immune responses in OLP (Lu et al., 2015). The expression of IFN-γ was decreased in T cells under stimulation with activated IGF1 and suppressed PI3K/MTOR. Moreover, IGF1 acted collaboratively with rapamycin, but alleviated the effect of LY294002 in the inhibition of IFN-γ level and the ratio of IFN-γ/IL-4. Besides, the IL-4 expression was impaired when stimulated by rapamycin or LY294002 together with IGF1. Many researches including ours had demonstrated increased IFN-γ level and the IFN-γ/IL-4 ratio, as well as declined IL-4 in serum and lesions of patients with OLP, which revealed the Th1-biased immune response in OLP (Zhou et al., 2012a; Hu et al., 2013; Wang et al., 2015). Thus, upregulation of PI3K/MTOR pathway could be strongly interconnected with the IFN-γ–represented Th1-dominated cytokine regulation. Induction of IGF1 signaling in T cells may be an attempt to restore the imbalanced cytokine networks, though failed due to complicated immune response in OLP. The combination of IGF1 activation and MTOR suppression might be a promising target for reengineering immunologic dissonance in OLP.

According to present study, IGF1 and PI3K/MTOR pathways exerted different influences on the cytokine levels secreted by T cell alone and co-culture system. Higher IFN-γ level and IFN-γ/IL-4 ratio, as well as lower IL-4 concentration was observed in all T cells cultured alone when compared with co-cultured ones. Once Th2 cells are activated by IL-4, which in turn produce more IL-4 and function as a positive feedback loop (Raphael et al., 2015). Moreover, IL-4 can negatively modulate the production of IFN-γ (Schroder et al., 2004). Thus the interaction between IL-4 and IFN-γ may have influence on the T-cell cytokine-mediated immune environment. Notably, pharmacological induced autophagy promoted the IFN-γ expression, while IGF1 and pharmacological impaired autophagy could decline the IFN-γ and IL-4 secreted by T cells alone. Besides, IGF1 antagonized the roles of blockage of PI3K and MTOR pathways in regulating IFN-γ production, respectively. Furthermore, the trend of IFN-γ/IL-4 ratio in T cells alone was opposite to the co-culture system when stimulated with rapamycin or LY294002 together with IGF1. As suggested by Harris J et al., pro-inflammatory cytokine IFN-γ is an autophagy inducer, whereas IL-4 can block autophagy flux (Harris, 2011). It is hypostasized that blockage of PI3K/MTOR might result in upregulated autophagy in T cells under the mediation of IGF1 signaling.

In conclusion, the overexpression of IGF1R and TRB3 suggested the aberrant IGF1 and AKT pathways in OLP local environment. Furthermore, IGF1–PI3K/AKT/MTOR signaling participated in the crosstalk between T cells and keratinocytes, and influenced the imbalanced cytokine networks, which may shed a light on the study of pathogenesis and therapeutic strategies of OLP.

Declaration of interests

The authors declare that they have no competing interests.

Author contributions

Rui-Jie Ma performed the experiments, analyzed the data and

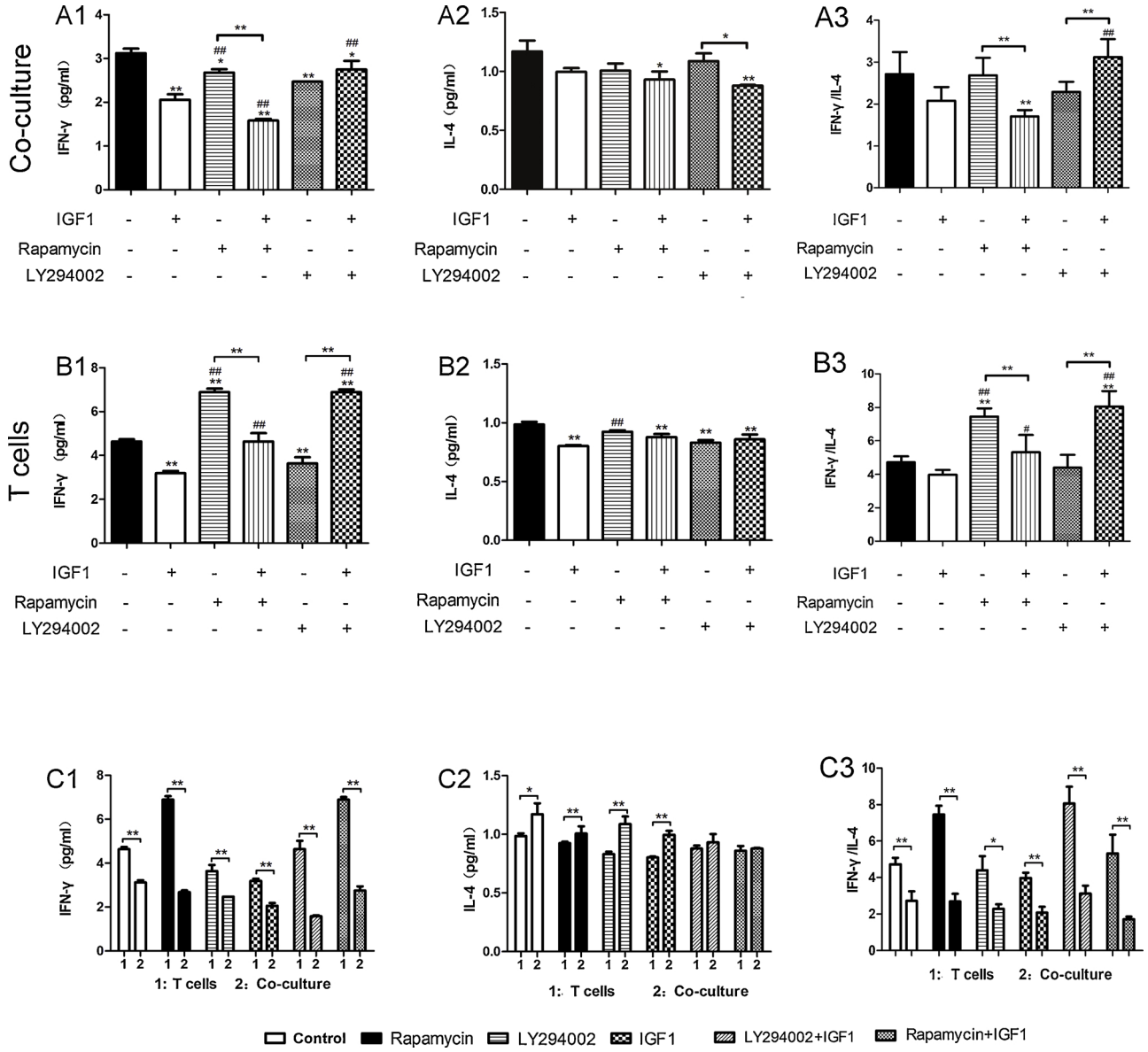

Fig. 3. Effects of blockage of PI3K/MTOR pathway and exogenous IGF1 on inflammatory cytokines in T cells alone and co-culture system. The levels of IFN-γ and IL-4 in supernatants were measured via ELISA. In the co-culture system, after PI3K/MTOR was blocked in T cells the level of IFN-γ was decreased ($Group_{(CON)} > Group_{(IGF1+LY294002)} > Group_{(Rapamycin)} > Group_{(LY294002)} > Group_{(IGF1)} > Group_{(IGF1+Rapamycin)}$: 3.12 ± 0.18 > 2.75 ± 0.35 > 2.68 ± 0.14 > 2.47 ± 0 > 2.06 ± 0.21 > 1.58 ± 0.07, P < 0.01), as well as the IL-4 in IGF1 + rapamycin and IGF1 + LY294002 group ($Group_{(CON)} > Group_{(IGF1+Rapamycin)} > Group_{(IGF1+LY294002)}$: 1.17 ± 0.09 > 0.93 ± 0.07 > 0.88 ± 0.01, P < 0.05). However, the IFN-γ secretion in T cells cultured alone showed a different trend ($Group_{(Rapamycin)} > Group_{(CON)} > Group_{(LY294002)} > Group_{(IGF1)}$: 6.89 ± 0.16 > 4.64 ± 0.10 > 3.64 ± 0.28 > 3.19 ± 0.10, P < 0.01; $Group_{(Rapamycin)} > Group_{(IGF1+Rapamycin)}$: 6.89 ± 0.16 > 4.64 ± 0.38, P < 0.01; $Group_{(IGF1+LY294002)} > Group_{(IGF1)}$: 6.89 ± 0.12 > 3.19 ± 0.10, P < 0.01), so as the IL-4 level ($Group_{(CON)} > Group_{(Rapamycin)} > Group_{(IGF1+Rapamycin)} > Group_{(IGF1+LY294002)} > Group_{(LY294002)} > Group_{(IGF1)}$: 0.99 ± 0.04 > 0.92 ± 0.22 > 0.88 ± 0.05 > 0.86 ± 0.07 > 0.83 ± 0.04 > 0.80 ± 0.04, P < 0.01). Results were expressed as the mean ± SD (*P < 0.05, ** P < 0.01, compared to control group. ## P ≤ 0.01, # P < 0.05, compared to IGF1).

drafted the manuscript. Ya-Qin Tan and Gang Zhou helped to design experiments and revise the manuscript.

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References

Alrashdan, M.S., Cirillo, N., McCullough, M., 2016. Oral lichen planus: a literature review and update. Arch. Dermatol. Res. 308 (8), 539–551. https://doi.org/10.1007/s00403-016-1667-2.

Bertacchini, J., Heidari, N., Mediani, L., Capitani, S., Shahjahani, M., Ahmadzadeh, A., et al., 2015. Targeting PI3K/AKT/mTOR network for treatment of leukemia. Cell. Mol. Life Sci. 72 (12), 2337–2347.

Blachly, J.S., Baiocchi, R.A., 2014. Targeting PI3-kinase (PI3K), AKT and mTOR axis in lymphoma. Br. J. Haematol. 167 (1), 19–32. https://doi.org/10.1111/bjh.13065.

Du, W., Yi, Y., Zhang, H., Bergholz, J., Wu, J., Ying, H., et al., 2013. Rapamycin inhibits IGF-1-mediated up-regulation of MDM2 and sensitizes Cancer cells to chemotherapy. PLoS One 8 (4), e63179. https://doi.org/10.1371/journal.pone.0063179.

Gu, J., Yan, X., Dai, X., Wang, Y., Lin, Q., Xiao, J., et al., 2018. Metallothionein preserves

Akt2 activity and cardiac function via inhibiting TRB3 in diabetic hearts. Diabetes 67 (3), 507–517.
Gupta, S., Jawanda, M.K., 2015. Oral lichen planus: an update on etiology, pathogenesis, clinical presentation, diagnosis and management. Indian J. Dermatol. 60 (3), 222–229.
Gusscott, S., Jenkins, C., Lam, S., Giambra, V., Pollak, M., Weng, A., 2016. IGF1R derived PI3K/AKT signaling maintains growth in a subset of human T-Cell acute lymphoblastic leukemias. PLoS One 11 (8), e0161158.
Harris, J., 2011. Autophagy and cytokines. Cytokine 56 (2), 140–144.
Hu, J.Y., Zhang, J., Cui, J.L., Liang, X.Y., Lu, R., Du, G.F., et al., 2013. Increasing CCL5/CCR5 on CD4+ T cells in peripheral blood of oral lichen planus. Cytokine 62 (1), 141–145. https://doi.org/10.1016/j.cyto.2013.01.020.
Hua, F., Li, K., Yu JJ, Lv X.X., Yan, J., Zhang, X.W., et al., 2015. TRB3 links insulin/IGF to tumour promotion by interacting with p62 and impeding autophagic/proteasomal degradations. Nat. Commun. 6, 7951.
Jia, G., Cheng, G., Gangahar, D., Agrawal, D., 2006. Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. Immunol. Cell Biol. 84 (5), 448–454.
Johnston, J., Basatvat, S., Ilyas, Z., Francis, S., Kiss-Toth, E., 2015. Tribbles in inflammation. Biochem. Soc. Trans. 43 (5), 1069–1074.
Lu, R., Zhang, J., Sun, W., Du, G., Zhou, G., 2015. Inflammation-related cytokines in oral lichen planus: an overview. J. Oral Pathol. Med. 44 (1), 1–14. https://doi.org/10.1111/jop.12142.
Payeras, M.R., Cherubini, K., Figueiredo, M.A., Salum, F.G., 2013. Oral lichen planus: focus on etiopathogenesis. Arch. Oral Biol. 58 (9), 1057–1069. https://doi.org/10.1016/j.archoralbio.2013.04.004.
Raphael, I., Nalawade, S., Eagar, T., Forsthuber, T., 2015. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 74 (1), 5–17.
Roopashree, M.R., Gondhalekar, R.V., Shashikanth, M.C., George, J., Thippeswamy, S.H., Shukla, A., 2010. Pathogenesis of oral lichen planus - a review. J. Oral Pathol. Med. 39 (10), 729–734. https://doi.org/10.1111/j.1600-0714.2010.00946.x.
Schroder, K., Hertzog, P.J., Ravasi, T., Hume, D.A., 2004. Interferon-gamma: an overview of signals, mechanisms and functions. J. Leukoc. Biol. 75 (2), 163–189. https://doi.org/10.1189/jlb.0603252.
Smh, A., Abushouk, A.I., Attia, A., Elmaraezy, A., Menshawy, A., Ahmed, M.S., et al., 2017. Malignant transformation of oral lichen planus and oral lichenoid lesions: a meta-analysis of 20095 patient data. Oral Oncol. 68, 92–102. https://doi.org/10.1016/j.oraloncology.2017.03.012.
Smith, T.J., 2010a. Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? Pharmacol. Rev. 62 (2), 199–236. https://doi.org/10.1124/pr.109.002469.
Smith, T., 2010b. Insulin-like growth factor-I regulation of immune function: a potential therapeutic target in autoimmune diseases? Pharmacol. Rev. 62 (2), 199–236.
Song, Q., Han, C.C., Xiong, X.P., He, F., Gan, W., 2016. Wei SH et al. PI3K-Akt-mTOR signal inhibition affects expression of genes related to endoplasmic reticulum stress. Genet. Mol. Res. 15 (3). https://doi.org/10.4238/gmr.15037868.
Tan, Y.Q., Zhang, J., Du GF, Lu R., Chen, G.Y., Zhou, G., 2016. Altered autophagy-associated genes expression in T cells of oral lichen planus correlated with clinical features. Mediators Inflamm., 4867368.
Troncoso, R., Vicencio, J.M., Parra, V., Nemchenko, A., Kawashima, Y., Del Campo, A., et al., 2012. Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. Cardiovasc. Res. 93 (2), 320–329. https://doi.org/10.1093/cvr/cvr321.
van der Meij, E.H., van der Waal, I., 2003. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J. Oral Pathol. Med. 32 (9), 507–512.
van der Waal, I., 2009. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 45 (4-5), 317–323. https://doi.org/10.1016/j.oraloncology.2010.02.016.
Vardatsikos, G., Pandey, N.R., Srivastava, A.K., 2013. Insulino-mimetic and anti-diabetic effects of zinc. J. Inorg. Biochem. 120, 8–17. https://doi.org/10.1016/j.jinorgbio.2012.11.006.
Wang, H., Han, Q., Luo, Z., Xu, C., Liu, J., Dan, H., et al., 2014. Oral lichen planus may enhance the expression of Th17-associated cytokines in local lesions of chronic periodontitis. Clin. Oral Investig. 18 (6), 1647–1654. https://doi.org/10.1007/s00784-013-1131-z.
Wang, Y., Zhou, J., Fu, S., Wang, C., Zhou, B., 2015. A study of association between oral lichen planus and immune balance of Th1/Th2 cells. Inflammation 38 (5), 1874–1879. https://doi.org/10.1007/s10753-015-0167-4.
Zhang, G.Q., Yang, S., Li, X.S., Zhou, D.S., 2014. Expression and possible role of IGF-IR in the mouse gastric myenteric plexus and smooth muscles. Acta Histochem. 116 (5), 788–794. https://doi.org/10.1016/j.acthis.2014.01.011.
Zhang, N., Zhang, J., Tan, Y.Q., Du GF, Lu R., Zhou, G., 2017. Activated Akt/mTOR-autophagy in local T cells of oral lichen planus. Int. Immunopharmacol. 48, 84–90. https://doi.org/10.1016/j.intimp.2017.04.016.
Zhou, G., Zhang, J., Ren, X.W., Hu, J.Y., Du, G.F., Xu, X.Y., 2012a. Increased B7-H1 expression on peripheral blood T cells in oral lichen planus correlated with disease severity. J. Clin. Immunol. 32 (4), 794–801.
Zhou, G., Zhang, J., Ren, X.W., Hu, J.Y., Du, G.F., Xu, X.Y., 2012b. Increased B7-H1 expression on peripheral blood T cells in oral lichen planus correlated with disease severity. J. Clin. Immunol. 32 (4), 794–801. https://doi.org/10.1007/s10875-012-9683-2.
Zhou, X., Zhang, Y., Li, Y., Xu, Y., Zhang, L., Li, Y., et al., 2017. Klotho suppresses tumor progression via inhibiting IGF-1R signaling in T-cell lymphoma. Oncol. Rep. 38 (2), 967–974.