Original Article
Effects of acitretin in combination with interferon on human cutaneous T cell lymphoma Hut78 cells

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Abstract: Objective: This study aimed to investigate the effects of acitretin A and/or interferon-α (IFN-α) on the proliferation of human cutaneous T cell lymphoma (CTCL) cells (Hut78) and the IL-15 expression in Hut78 cells. Methods: Hut78 cells were treated with acitretin A and/or IFN-α at different concentrations for 24 h, 48 h or 72 h. The proliferation of Hut78 cells was determined by CCK-8 assay and observation under an invert microscope, and the IL-15 expression was detected by ELISA. Results: The proliferation of Hut78 cells was inhibited by acitretin A or IFN-α in time and dose dependent manners, and combined treatment had more potent capability to inhibit the proliferation of Hut78 cells (P<0.05), suggesting the synergistic effect of acitretin A and IFN-α. Acitretin A and IFN-α were able to independently down-regulate IL-15 expression in Hut78 cells in time and dose dependent manners, and there was synergistic effect on IL-15 expression after combined treatment with acitretin A and IFN-α (P<0.05). Conclusion: Acitretin or IFN-α can inhibit the proliferation of Hut78 cells and down-regulate IL-15 expression in Hut78 cells, and these inhibitions become more obvious after combined treatment.

Keywords: Acitretin, interferon-α, cutaneous T-cell lymphoma, Hut78 cells, interleukin-15, proliferative inhibition

Introduction

Cutaneous T cell lymphoma (CTCL) can be divided into granuloma fungoides and sezary syndrome (SS) [1]. It is a cutaneous malignancy which is highly invasive and can invade the epidermis, lymph nodes and viscera. The incidence of CTCL is increasing over year and it has been the second most common malignancy in non-Hodgkin's lymphoma and thus significantly affects the human health. Granuloma fungoides are also known as mycosis fungoides (MF). Initially, CTCL only invades the skin and presents erythema or plaques. It will become malignant, significantly threatening the life of patients if timely treatment is not performed. At early stage, radiotherapy or chemotherapy may add burden to the mental health of CTCL patient and also affect the physical health. Thus, targeted treatment of CTCL has been a focus in recent studies on the therapies of CTCL. Acitretin is a group of compound with similar structure to natural vitamin A. It can promote the growth and differentiation of epithelial cells and other cell types, but may inhibit the growth of cancer cells [1, 2]. In addition, it may also regulate immune function and inflammation. Some investigators have used acitretin and iso-vitamin A in the treatment of MF, achieving the effectiveness rate of 19%, overall effectiveness rate of 58% and mean time to remission of 13 months [3]. Cheeley et al employed acitretin for the treatment of CTCL in 32 patients, the effectiveness rate was 59% and complete remission was noted in 1 patient [4]. Interferon (IFN) is a glycoprotein produced in human cells after stimulation by virus or other inducers. It can inhibit the replication of virus, has anti-tumor effect and is able to regulate immune function [5]. Currently, interferon α (leukocyte interferon), interferon β (fibroblast interferon), interferon-γ (immune interferon) are used in clinical practice. Both acitretin and IFN are inducers of cell differentiation, and combined use of acitretin and IFN may increase the sensitivity of cancer cells to chemotherapy, elevating the therapeutic efficacy. However, few studies have conducted to investigate acitretin and IFN in the therapy of CTCL. In this study, it was designed to investigate the effects of acitretin and IFN-α on cell growth through in vitro Hut78 cultivation, aiming to clarifying the mechanism

and providing new thoughts for treatment of human cutaneous T cell lymphoma.

Materials and methods

Cell culture, reagents and instrument

Human CTCL Hut78 cells were purchased from the Shanghai Cell Bank of Chinese Academy of Sciences, and maintained in IMDM (Gibco, USA) containing 20% fetal bovine serum (FBS), 100 U/ml penicillin and 100 g/l strepto-mycin (Puzhen Company). Acitretin capsu-les (Chongqing Huabang Company; 10 mg, 2012034), recombinant IFN-α 2b injection (Tianjin Hualida Biotech Co., Ltd, 3×10^6 IU/ml, sa120601), CCK assay kit, human IL-15 ELISA kit (Wuhan Boster Biotech Co., Ltd), Full-wavelength microplate reader (Multiskan 30-1510, Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland), sodium bicarbonate (analytically pure), potassium chloride (analyti-cally pure), potassium dihydrogen phosphate (analytically pure), disodium hydrogen phos-phate (analytically pure) and dimethyl sulfoxide (Beijing Chemical Reagent Company) were used in the present study.

Preparation of acitretin solution

Acitretin was weighed in dark and then dis-solved in DMSO into 10 mmol/l. After steriliza-tion by filtration, acitretin solution was alloquot-ed into 1.5-ml Eppendorf tube and then stored at -20°C in dark. Before use, acitretin solution was diluted with IMDM into 0.1 μmol/l, 1.0 μmol/l and 10 μmol/l. Acitretin solution was prepared immediately before use.

Preparation of IFN-α solution

IFN-α solution is colorless and transparent. IFN-α was dissolved in IMDM into 5000 IU/ml, 10000 IU/ml or 20000 IU/ml.

Treatment

Under an inverted microscope, cells were observed. Cells in logarithmic growth phase were divided into different groups: blank con-trol group (IMDM alone), negative control group (single cell suspension), DMSO group (0.1% DMSO) and treatment groups. Treatment gr-oups were further subdivided into acitretin group, IFN-α group and acitretin + IFN-α group.

The concentration of acitretin was 0.1 μmol/l, 1.0 μmol/l and 10 μmol/l, [5] and the concen-tration of IFN-α was 5000 IU/ml, 10000 IU/ml and 20000 IU/ml [6]. In our pilot study, 10 μmol/l acitretin was found to have significant inhibition on the cell group. To avoid the influ-ence of acitretin on the IFN-α induced inhibi-tion, following combinations were used: 1.0 μmol/l acitretin + 5000 IU/ml IFN-α; 1.0 μmol/l acitretin + 10000 IU/ml IFN-α; 1.0 μmol/l acitretin + 20000 IU/ml IFN-α. Cells in each group were added into 3 wells, and the final vol-ume of each well was 100 μl. The cell density was 4×10^4 cells/ml. Cells were seeded into 96-well plates and incubated at 37°C in an environment with 5% CO_2 for 24 h. Then, cells were treated with acitretin and/or IFN-α for 24, 48 and 72 h.

Observation of cells by inverted microscope

After treatment for 24 h, 48 h and 72 h, cells were observed under an inverted microscope and photographed.

Detection of cell proliferation by CCK8 assay

10 μl of CCK-8 solution was added, followed by incubation for 1 h. The absorbance was mea-sured at 450 nm. Detection was done three times. The inhibition rate (cytotoxic index, CI) was calculated as follow: CI (%) ($1-A_{treatment}/A_{control}$) ×100%. Positive CI refers to the killing effect and negative CI refers to the proliferation promotive effect.

Detection of IL-15 expression by ELISA

At treatment, the supernatant was removed, followed by centrifugation. Then, 90 μl of super-natant was added to the plate with 3,3', 5,5'-Tetramethylbenzidine (TMB) blank con-trol. The standards at 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml and 15.6 pg/ml (90 μl) were added, and in blank control, diluent was added. After incuba-tion at 37°C for 90 min, IL-15 antibody was added to each well (90 μl; except for TMB blank control), followed by incubation at 37°C for 60 min. Following washing in 0.01 M PBS, avidin-peroxidase complex (ABC) (90 μl) was added to each well (except for TMB blank control), fol-lowed by incubation at 37°C for 30 min. After washing in 0.01 M PBS 5 times, visualization was done by addition of TMB (90 μl), followed

Table 1. Effects of acitretin A and/or IFN-α on the proliferation and IL-15 expression of Hut78 cells (x±s)

Groups	24 h		48 h		72 h	
	CI (%)	IL-15 (ng/L)	CI (%)	IL-15 (ng/L)	CI (%)	IL-15 (ng/L)
DMSO	0.08±0.005	606.23±9.41	0.04±0.003	701.30±8.27	0.03±0.001	760.27±11.18
Negative control	-	613.81±8.24	-	691.36±10.25	-	780.68±12.33
Acitretin A						
0.1 µmol/L	11.21±0.89[a]	548.46±5.66[a]	27.89±2.11[a]	486.15±5.33[a]	35.69±1.98[a]	399.01±4.92[a]
1.0 µmol/L	19.01±3.23[a]	497.61±6.25[a]	36.33±3.56[a]	448.25±4.39[a]	45.88±2.26[a]	358.36±2.62[a]
10 µmol/L	25.99±2.33[a]	461.32±4.22[a]	44.82±2.25[a]	402.63±6.33[a]	60.95±4.32[a]	293.14±4.23[a]
IFN-α						
5000 IU/ml	12.23±1.58[b]	535.69±6.55[b]	19.75±1.33[b]	472.95±5.66[b]	27.96±2.25[b]	380.72±6.52[b]
10000 IU/ml	18.12±1.26[b]	488.03±5.33[b]	28.22±2.01[b]	439.97±2.32[b]	40.32±1.66[b]	338.02±3.72[b]
20000 IU/ml	24.33±1.86[b]	450.73±5.68[b]	41.86±3.24[b]	395.09±5.42[b]	49.28±3.01[b]	280.07±3.55[b]
1.0 µmol/L acitretin A +						
+ 5000 IU/ml IFN-α	17.89±2.58[a]	491.95±6.34[a]	38.20±1.35[a]	444.33±5.95[a]	43.52±2.21[a]	342.68±6.22[a]
+ 10000 IU/ml IFN-α	29.66±1.32[a,c]	450.30±5.22[a,c]	51.09±1.76[a,c]	380.28±4.95[a,c]	69.49±2.12[a,c]	275.71±5.32[a,c]
+ 20000 IU/ml IFN-α	40.33±1.55[a,d]	394.79±4.89[a,d]	69.02±3.09[a,d]	339.32±5.02[a,d]	80.11±3.21[a,d]	194.68±3.33[a,d]

Note: n = 3. a: $P<0.05$ vs DMSO; b: $P<0.05$ vs negative control; c: $P<0.05$ vs 1.0 µmol/L acitretin A + 10000 IU/m IFN-α; d: $P<0.05$ vs 1.0 µmol/L acitretin A + 20000 IU/ml IFN-α.

by incubation at 37°C for 20 min in dark. Then, 90 µl of TMB was added to stop the reaction, and the blue solution became yellow. The absorbance was adjusted on the basis of that in blank control, and then detection of absorbance was done at 450 nm in a microplate reader.

Statistical analysis

All the experiments were repeated at least 3 times. Quantitative data are expressed as mean ± standard deviation (x±s). Statistical analysis was performed with SPSS version 13.0 (SPSS, Chicago, IL, USA), and comparisons were done with one way analysis of variance followed by LSD test. A value of $P<0.05$ was considered statistically significant.

Results

Effects of acitretin and IFN-α on the proliferation of Hut78 cells

Acitretin group: Acitretin significantly inhibited the proliferation of Hut78 cells as compared to negative control group in dose and time dependent manners. The inhibition was the most obvious after treatment with 10.0 µmol/l acitretin for 72 h ($P<0.05$ vs other groups) (**Table 1**).

IFN-α group: IFN-α dramatically inhibited the proliferation of Hut78 cells as compared to neg-ative control group in dose and time dependent manners. The inhibition was the most obvious after treatment with 20000 IU/ml IFN-α for 72 h ($P<0.05$ vs other groups) (**Table 1**).

Combination of acitretin and IFN-α: 1.0 µmol/l acitretin combined with IFN-α at different concentrations dramatically inhibited the proliferation of Hut78 cells as compared to negative control group, and the inhibition became more obvious with the increase in IFN-α dose and the prolongation of treatment. The inhibition was the most obvious after treatment with 1.0 µmol/l acitretin and 20000 IU/ml IFN-α for 72 h ($P<0.05$ vs other groups) (**Table 1**).

The CI in 1.0 µmol/L acitretin A + 5000 IU/ml IFN-α group was comparable to that in 1.0 µmol/L acitretin A group and 5000 IU/ml IFN-α group at 24, 48 and 72 h (P > 0.05); the CI in 1.0 µmol/L acitretin A + 10000 IU/ml IFN-α group was significantly different from that in 1.0 µmol/L acitretin A group and 10000 IU/ml IFN-α group at 24, 48 and 72 h; the CI in 1.0 µmol/L acitretin A + 20000 IU/ml IFN-α group was also markedly different from that in 1.0 µmol/L acitretin A group and 20000 IU/ml IFN-α group at 24, 48 and 72 h ($P<0.05$) (**Table 1**).

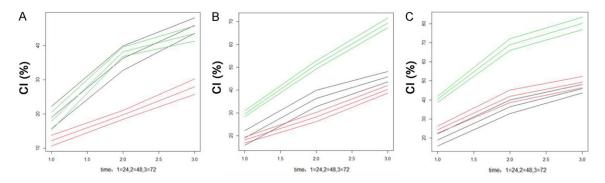The CI after treatment with acitretin A and/or IFN-α is shown in **Figure 1**. The inhibitory effect

Figure 1. Red line: treatment with IFN-α alone (A: 5000 IU/ml IFN-α; B: 10000 IU/ml IFN-α; C: 20000 IU/ml IFN-α); black line: treatment with 1.0 μmol/l acitretin A alone; green line: combined use of IFN-α and acitretin A (A: 5000 IU/ml IFN-α + 1.0 μmol/l acitretin; B: 10000 IU/ml IFN-α + 1.0 μmol/l acitretin; C: 20000 IU/ml IFN-α + 1.0 μmol/l acitretin). Three lines represent the interference. 1: 24 h; 2: 48 h; 3: 72 h.
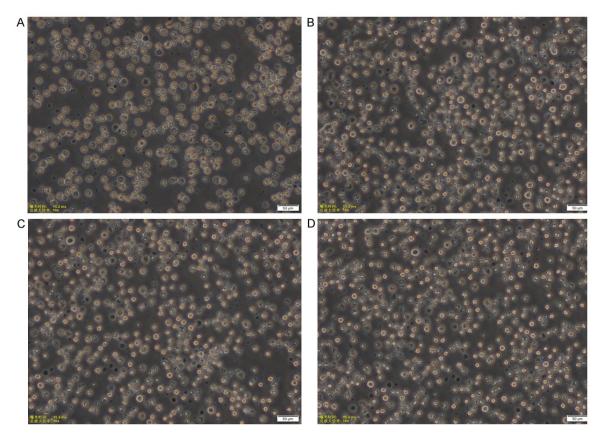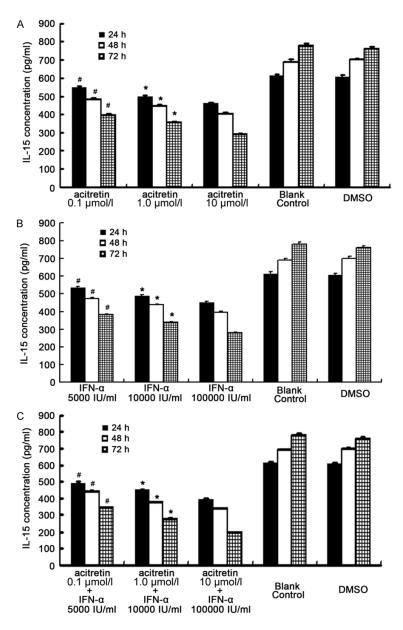

Figure 2. Cell morphology under inverted microscope. A. Untreated Hut78 cells (100×); B. Hut78 cells treated with 1.0 μmol/l acitretin for 48 h (100×); C. Hut78 cells treated with 10000 IU/ml IFN-α for 48 h (100×); D. Hut78 cells treated with 1.0 μmol/l acitretin and 10000 IU/ml IFN-α for 48 h (100×).

was poor after treatment with 5000 IU/ml IFN-α; the CI in 1.0 μmol/l acitretin group was similar to that in 5000 IU/ml IFN-α + 1.0 μmol/l acitretin group. In addition, treatment with 1.0 μmol/l acitretin alone seemed to acquire a high inhibition over time as compared to combined treatment (**Figure 1A**). However, when the IFN-α concentration was 10000 IU/ml or 20000 IU/ml, combined treatment showed a better inhibition as compared to monotreatment. Furthermore, with the increase in IFN-α dose, the inhibitory effect after treatment with IFN-α alone tended to be higher than in acitretin groups (**Figure 1B, 1C**).

Figure 3. IL-15 expression in different groups. A. Acitretin group; B. IFN-α group; C. Combination of acitretin and IFN-α.

observed, cells had a poor refraction, and the number of cells reduced significantly with the prolongation of treatment (Figure 2).

Detection of IL-15 expression by ELISA

Acitretin group: Acitretin at 0.1 μmol/l-10.0 μmol/l was able to significantly inhibit the IL-15 expression in Hut78 cells as compare to negative control group, and the reduction became more obvious with the increase in acitretin dose and the prolongation of treatment. The lowest IL-15 expression was found after treatment with 10.0 μmol/l acitretin for 72 h. In negative control group, DMSO group, 0.1 μmol/l acitretin group, 1.0 μmol/l group and 10.0 μmol/l group, the IL-15 expression was 613.81 pg/ml, 595.26 pg/ml, 548.46 pg/ml, 497.61 pg/ml and 461.32 pg/ml, respectively, at 24 h, 691.36 pg/ml, 673.25 pg/ml, 486.15 pg/ml, 448.25 pg/ml and 402.63 pg/ml, respectively, at 48 h and 780.68 pg/ml, 762.24 pg/ml, 399.01 pg/ml, 358.36 pg/ml and 293.14 pg/ml, respectively, at 72 h. Acitretin treatment significantly inhibited the IL-15 expression as compared to control group ($P<0.05$) (Figure 3A).

Cell morphology under an inverted microscope

Under an inverted microscope, cells in negative control group had good growth and even size, some cells formed round or oval clusters, cells had full cytoplasm, cell membrane was complete and had favorable refraction, and the nucleus localized at the center of a cell. After treatment with acitretin and/or IFN-α, cell growth became slow with significant change in cell morphology: cells shrunk and disrupted, cell debris without original morphology was

IFN-α group: IFN-α was able to significantly inhibit the IL-15 expression in Hut78 cells as compare to negative control group, and the reduction became more obvious with the increase in acitretin dose and the prolongation of treatment. The lowest IL-15 expression was found after treatment with 20000 IU/ml IFN-α for 72 h. In negative control group, 5000 IU/ml IFN-α group, 10000 IU/ml IFN-α group, and 20000 IU/ml IFN-α group, the IL-15 expression was 608.62 pg/ml, 535.69 pg/ml, 488.03 pg/ml and 450.73 pg/ml, respectively, at 24 h,

702.73 pg/ml, 472.95 pg/ml, 439.97 pg/ml and 395.09 pg/ml, respectively, at 48 h, and 778.53 pg/ml, 380.72 pg/ml, 338.02 pg/ml and 280.07 pg/ml, respectively, at 72 h. IFN-α treatment significantly inhibited the IL-15 expression as compared to control group (P<0.05) (**Figure 3B**).

Combination of acitretin and IFN-α: Acitretin in combination with IFN-α was able to significantly inhibit the IL-15 expression in Hut78 cells as compare to negative control group, and this reduction became more obvious with the increase in dose and the prolongation of treatment. The lowest IL-15 expression was found after treatment with 1.0 μmol/l acitretin and 20000 IU/ml IFN-α for 72 h. In negative control group, DMSO group, 1.0 μmol/l acitretin + 5000 IU/ml IFN-α group, 1.0 μmol/l acitretin + 10000 IU/ml IFN-α group and 1.0 μmol/l acitretin + 20000 IU/ml IFN-α group, the IL-15 expression was 616.02 pg/ml, 595.75 pg/ml, 491.95 pg/ml, 450.30 pg/ml and 394.79 pg/ml, respectively, at 24 h, 683.22 pg/ml, 667.32 pg/ml, 444.33 pg/ml, 380.28 pg/ml and 339.32 pg/ml, respectively, at 48 h and 765.13 pg/ml, 734.25 pg/ml, 342.68 pg/ml, 275.71 pg/ml and 194.68 pg/ml, respectively, at 72 h. Treatment with both acitretin and IFN-α significantly inhibited the IL-15 expression as compared to control group (P<0.05) (**Figure 3C**).

Discussion

CTCL is a class of extranodal non-Hodgkin's lymphoma caused by clonal proliferation of homed T cells in the skin. CTCL has a chronic course, but significantly affects the quality of life of patients. Of CTCL, MF and SS are the most common clinical types [7]. In recent years, the incidence of CTCL is increasing and it has been the second most common malignancy in non-Hodgkin's lymphoma [8, 9]. To date, no specific therapies have been developed for MF and SS. Although some stttrategies are found to be effective for the therapy of MF and SS, no monotherapy has shown its advantage in the survival rate [10, 11]. Generally cutaneous treatment and non-immunosuppressive therapy are widely employed for the therapy of CTCL. However, chemotherapy is the treatment of choice for progressively advanced CTCL. The routine chemotherapy aims to kill cancer cells, inhibit their proliferation and induce their death, but most cytotoxic drugs used in chemotherapy have a poor selectivity to cancer cells. Thus, they not only kill cancer cells, but also affect the growth of normal cells [12]. Moreover, there is the possibility of drug resistance after chemotherapy. Thus, the efficacy of chemotherapy is still not optimal, [12] and therapy of CTCL significantly adds mental and physical burdens to patients. It is imperative to develop strategies for the early therapy of CTCL.

Retinoic acid is an immunomodulator and has similar structure to vitamin A. Retinoic acid can selectively bind to the isomer of retinoid X receptor (RXR) to affect the cell differentiation and induce cell apoptosis [13]. In addition, retinoic acid has wide biological activities to regulate the growth and differentiation of epithelial cells and other cell types [14, 15] and may exert therapeutic effects on several skin cancers [16]. Acitretin is the second generation of retinoic acid and its chemical structure is all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoic acid. Clinically, acitretin is usually used to treat psoriasis and keratosis related skin disease [17, 18]. In recent years, its anti-tumor effect has been paid increasing attention to [19]. In systemic treatment, acitretin has been used for the therapy of skin diseases including cutaneous lymphoma for several decades [20]. In addition, acitretin has wide biological activities and is unable to inhibit the immune function, which makes acitretin attractive in the therapy of CTCL. Some types of cancers have a low sensitivity to acitretin, and thus it is necessary to develop combined therapy with acitretin for CTCT. IFN-α is produced by leukocytes and lymphoblasts after stimulation by virus or cancer cells [21]. IFN-α may also mediate the angiogenesis and immune reaction and exert anti-proliferative and apoptosis-inducing effects [22, 23]. Actually, IFN-α has been used in the clinical treatment of cancers including chronic myeloid leukemia (CML), T-cell lymphoma, B-cell lymphoma, melanoma, renal cell carcinoma, Kaposi's sarcoma (KS) and hairy cell leukemia (HCL) [24, 25]. Recombinant human IFN-α was initially used to treat CTCL in 1984 by Bunn et al due to its anti-tumor activity [26]. IFNα-2b is also widely employed for the therapy CTCL. In addition, it is effective to control MF at early stage and also the first line drug for the therapy of MF at advanced stage and SS [27]. However, its effect on CTCL and the specific mechanism underly-

ing its anti-tumor effect on CTCL are still unclear.

Although some studies have reported that acitretin A and IFN-α have adverse effects in the treatment of CTCL, [28, 29] evident side effects are not observed in the clinical treatment of MF with them according to our experience. Moreover, the clinical efficacy is favorable with the complete remission rate of 100%. The side effects are mainly the fever soon after the intramuscular injection of IFN-α, which, however, will resolve within following 3 days. Oral acitretin A may cause dry mouth and increase in blood lipid. The dose of acitretin A causing side effects is very close to its effective dose. Thus, the presence of mild dry mouth is indicative of effectiveness of acitretin A. However, the dose of acitretin A should be reduced if increase in blood lipid is present. Of note, these side effects of acitretin A are acceptable for patients with lymphoma as a malignancy. Acitretin A is a treatment of choice for psoriasis, maintenance therapy is needed, but adverse effects are not evident. Thus, patients usually have a good compliance to the combined use of acitretin A and IFN-α. Currently, no study reports the therapy of CTCL with both IFN-α and acitretin and the specific mechanism is also poorly understood. In this study, the effects of IFN-α and/or acitretin on the proliferation of Hut78 cells and IL-15 expression in these cells were investigated.

A characteristic of malignancy is the uncontrollable proliferation of cancer cells. In the present study, CCK8 assay was employed to detect the proliferation of Hut78 after treatment with IFN-α and/or acitretin. CCK8 assay has higher sensitivity, better repeatability, better stability and higher clinical relevance in the evaluation of cell proliferation as compared to MTT assay. Thus, CCK8 assay has been a common method in the evaluation of cell proliferation. CCK8 assay and observation of cell morphology showed IFN-α and acitretin could independently inhibit the proliferation of Hut78 cells in dose and time dependent manners, and the highest CI was the observed after 72-h treatment. CCK8 assay showed IFN-α and acitretin could independently inhibit the proliferation of Hut78 cells, and combined use of IFN-α and acitretin exerted synergistic effects to inhibit the proliferation of CTCL cells.

IL-15 was first identified in the supernatant of epidermal cell line of monkey kidney by Grabstein et al in 1994 and thereafter named [30]. IL-15 has a wide resource. IL-15 expression is detectable in multiple tissues (such as skeletal muscle, heart, liver and spleen) and cell types (such as T lymphocytes, B lymphocytes, monocyte-macrophages, dendritic cells, keratinocytes and microglia). Especially, monocyte-macrophages have a higher expression of IL-15 [31]. IL-15 possesses multiple effects on the lymphocytes [32] and has been identified as a cytokine with similar functions to IL-12 [33]. IL-2 and IL-15 can stimulate the proliferation and activation of lymphocytes. IL-15 may promote the maturation of NK cells and is also essential for the survival of mature NK cells [34]. IL-15 may promote the survival and proliferation of naïve CD8T cells and is also crucial for the survival of memory CD8T cells [35]. IL-15 has been found to be involved in multiple immune reactions and is important for the immunoregulation. Studies have confirmed IL-15 is closely related to the pathogenesis of some diseases. In allergic diseases, IL-15 expression significantly increases, which promotes the proliferation of CD8T cells and NK cells, leading to the elevated susceptibility to allergy [32]. There is evidence showing that IL-15 possesses the anti-tumor effect which is dependent on CD8T cells and NK cells [36]. IL-15 insufficiency is a key cause of reduced number and killing activity of NK cells and CD8T cells, which may be improved by the addition of IL-15 [37]. However, there is still controversy on the role of IL-15 in human cutaneous T lymphocytic lymphoma. There is evidence showing that human acute T lymphocytic leukemia (ATL) and CTCL cells have IL-15Rα, to which IL-15 binds, leading to the cell proliferation [38]. In addition, studies also confirm the over-expression of IL-15 and IL-16 in CTCL cells [39]. Study also reveals that IL-15 may facilitate the progression of cutaneous T lymphocytic lymphoma and prolong the survival time of cancer cells [40]. In early CTCL, the IL-15 content in the skin is at a low level, but its secretion increases with the progression of CTCL. This suggests that cancer cells may secret IL-15. Taken together, IL-15 not only is an important cytokine secreted by CTCL, but may be involved in the progression of CTCL. Thus, IL-15 probably possesses the anti-apoptotic effect on cancer cells [41]. Of note, evidence also reveals that IFN is able to

up-regulate IL-15 expression to exert anti-tumor effect [42]. The relationship among IFN, IL-15 and cancer cells is still unclear. In this study, ELISA was performed to detect IL-15 expression in human Hut78 cells after treatment with acitretin A and/or IFN.

In our study, IL-15 expression was detected in negative control group, acitretin group, IFN-α group and acitretin + IFN-α group after treatment for 24 h, 48 h and 72 h. Results showed both acitretin and IFN-α could independently inhibit the IL-15 expression in Hut78 cells in dose and time dependent manners, and the lowest IL-15 expression was observed after 72-h treatment. Furthermore, acitretin and IFN-α could synergistically inhibit IL-15 expression in Hut78 cells. These findings were inconsistent with previously reported: IFN may act on cells to induce IL-15 expression [42]. There is evidence showing that the anti-tumor effect of IL-15 is ascribed to its activation on natural killer cells and/or CD8+ cells [36]. To date, no study has shown the relationship between acitretin and IL-15. Thus, we speculate that (1) acitretin and IFN-α may induce the apoptosis of Hut78 cells, which overwhelms their induction of IL-15 expression and thus indirectly leads to the reduction in IL-15 expression in Hut78 cells; (2) IFN-α is really able to reduce IL-15 expression. Thus, the definitive effect of acitretin and/or IFN-α on IL-15 expression is required to be validated in animal studies.

The mechanism underlying the acitretin induced proliferative inhibition is still unclear and might be related to the expression of some apoptosis related oncogenes and other growth related cytokines. Our results indicate that acitretin and IFN-α may synergistically inhibit the proliferation of Hut78 cells, which provide a new way for the clinical therapy of CTCL.

Conclusion

In conclusion, both acitretin and IFN-α are able to inhibit the proliferation of Hut78 cells, and their inhibitory effect is dose and time dependent: the higher the dose or the longer the treatment, the more obvious the inhibition is. Acitretin in combination with IFN-α has better ability to inhibit the proliferation of Hut78 cells as compared to acitretin or IFN-α alone, suggesting the synergistic effect. In addition, both acitretin and IFN-α are able to reduce IL-15 expression in Hut78 cells, and the combination of acitretin and IFN-α has more potent ability to inhibit the IL-15 expression, which may be related to the proliferative inhibition of acitretin and IFN-α.

Disclosure of conflict of interest

None.

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