Original Article
Effect of IL-6 on proliferation of human thyroid anaplastic cancer stem cells

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Abstract: Objective: To investigate the effect of IL-6 on the proliferation of thyroid cancer stem cells. Methods: HTh74 and HTh74R thyroid cancer stem cells were cultured. The proliferation of thyroid cancer stem cells after IL-6 treatment was assessed by the MTT method. The effect of IL-6 on colony formation was observed by colony formation experiments. The expression of OCT4, ABCG2, CD133, and EMT markers was detected by real-time quantitative PCR. Results: IL-6 promoted the proliferation of HTh74 and HTh74R thyroid cancer stem cells and enhanced sphere formation. However, anti-IL-6 inhibited the proliferation of cancer stem cells. IL-6 promoted colony formation by HTh74 and HTh74R cells and enhanced the expression of stem cell genes OCT4 and ABCG2. The expression of EMT markers E-cadherin was significantly decreased but the expression of vimentin and Snail was increased by IL-6 treatment. Conclusions: IL-6 promoted proliferation of thyroid cancer stem cells and colony formation, and increased characteristics of thyroid cancer stem cells and EMT. The proliferative effect of thyroid cancer stem cells depends on activation of the IL6/JAK1/STAT3 pathway. These effects may contribute to the development and metastasis of thyroid cancer.

Keywords: Interleukin-6, thyroid cancer, cancer stem cells, proliferation, clone

Introduction

There is a close link between inflammation and tumors. The inflammatory tumor microenvironment has many roles in tumor progression and metastasis. In the inflammatory state, continuous stimulation by inflammation can induce cell proliferation, increase reactive oxygen species production, cause oxidative DNA damage, induce gene mutation, and lead to carcinogenesis [1-3]. However, studies have demonstrated that the inflammatory tumor microenvironment plays an important role in the pathogenesis of tumors; the effect of inflammatory cytokines on cancer stem cells during tumor development has attracted increasing research attention [4-7].

The inflammatory factor interleukin (IL)-6 not only plays an important role in the immune response and inflammatory reactions but also participates in the development of tumors. A study found that IL-6 is closely related to thyroid disease, but the mechanism of IL-6 in thyroid cancer is still unclear [8, 9]. Anaplastic thyroid cancer (ATC) is a highly aggressive neoplasm resistant to radiation and chemotherapy. In this study, we show the effect of IL-6 on the sphere and colony formation ability of human thyroid anaplastic cancer cells to investigate whether IL-6 induces the proliferation and stem cell characteristics of thyroid cancer stem cells. By exploring the role of inflammation in the pathogenesis of thyroid cancer we can understand its development and proliferation and gain insight into prevention and treatment.

Materials and methods

Cell lines and cell culture

The human ATC cell lines HTh74 and HTh74R were used in the experiments. HTh74R was developed by the continuous exposure of the

HTh74 cell line to doxorubicin at concentrations starting at 10 ng/ml and increasing incrementally to 500 ng/ml. The resistant cell line was maintained in medium containing doxorubicin for more than 6 months. HTh74 and HTh74Rdox cells were cultured in Ham's F-12 medium with L-glutamine, supplemented with 10% fetal calf serum (FCS, v/v), 1% nonessential amino acids (MEM, v/v), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B. HTh74Rdox cells were grown in the same medium with the addition of 0.5 µg/ml doxorubicin. Thyroid cancer stem cells were cultured as described previously [10]. HTh74 and HTh74R cells were placed in DMEM/F12 medium and cultured in suspension at a density of 1×10^5 cells/ml. Cells were treated with different concentrations of IL-6 (0, 1, 5, and 10 ng/ml), and epidermal growth factor (EGF) (20 ng/ml). Basic fibroblast growth factor (bFGF) (20 ng/ml) and B27 (1:50) were added every 2-3 d. The growth of cell spheres was observed after 7 d.

Cell viability assay

Cell viability was analyzed using the MTT method (Sigma, St. Louis, MO, USA). Cells ($4-10 \times 10^3$) were seeded in 96-well plates. Cells were treated with medium containing different doses of IL-6 (Sigma, St. Louis, MO, USA) and/or sIL-6R (Sigma, St. Louis, MO, USA). The cells were incubated with 0.5 mg/ml MTT for 4 h at 37°C. The formazan product was dissolved in dimethylsulfoxide (DMSO), and the absorbance was measured at 490 nm with a plate reader.

In vitro clonogenic assay

Clonogenic assay was performed. HTh74 and HTh74Rdox cells were plated in 6-well plates with a density of 200 cells per well, then cells were treated with varying concentrations of IL-6 (0, 1, 5, 10, and 20 ng/ml). The cells were allowed to form colonies for up to 2 weeks, with the medium replaced every third day. The colonies were analyzed with Giemsa staining and cloning efficiency was measured by counting clone number growing. Triplicate samples were run in 3 independent experiments.

Tumor sphere culture and sphere formation efficiency

Tumor spheres were generated by placing HTh74 and HTh74Rdox cells (1×10^4 cells/ml) into serum-free DMEM/F-12 medium contain-ing B27, bFGF and EGF. Every 2-3 d, B27, bFGF and EGF were added. After 7 d, the number of tumor spheres (large diameter >50 µm) was counted. The sphere-forming efficiency (SFE) was calculated as the number of sphere-like structures formed in 7 d divided by the original number of cells seeded and is expressed as the percentage mean (±SD). Three independent experiments were performed.

Reverse transcription polymerase chain reaction (RT-PCR) and real-time quantitative PCR (qPCR)

Total RNA was extracted using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. RT-PCR was performed as described previously. Real-time qPCR was performed with an iCycler iQ real-time PCR detector system (Bio-Rad) using ABsolute QPCR SYBR Green Fluorescein Mix (Applied Thermo Fisher Scientific, Inc., Schwerte, Germany) according to the manufacturer's instructions. The cycling conditions were as follows: initial enzyme activation at 95°C for 15 min, followed by 50 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s.

The relative expression levels of each gene in real time were analyzed using the $2^{-\Delta\Delta Ct}$ method and normalized to the expression of the housekeeping gene GAPDH. The 18S rRNA gene was used as a second housekeeping gene. All primers were obtained in powder form from Invitrogen, Inc. Each sample was analyzed in duplicate from 3 independent sets of RNA preparations. The primer sequences, product sizes, cycle numbers and annealing temperatures are listed in **Table 1**.

Western blot analysis

Thyroid cancer stem cells following different treatment were lysed with RIPA buffer. The proteins were treated with 5× sample buffer containing dithiothreitol and boiled for 5 min. Equal amounts of protein (30 µg) was subjected to 12.5% SDS polyacrylamide gel and separated proteins were transferred to NC membranes. The membranes were blocked in 5% skim milk for 1 h at room temperature (RT). The immunoblots were incubated overnight at 4°C with anti-JAK1, anti-phosphorylated JAK1, anti-STAT3, anti-phosphorylated STAT3 (Tyr705 and Ser727) and anti-OCT4, (all antibodies were from Cell Signaling Technology, MA, USA).

Table 1. Primer sequences for the amplification of various genes by PCR

Gene	Primer sequence	Annealing temperature (°C)	Product size (bp)
IL-6	F: 5'-ATGAA CTCCT TCTCC ACAAG CGC-3'	60	630
	R: 5'-GAAGAGCCCT CAGGC TGGAC TG-3'		
IL-6R	F: 5'-CATTG CCATT GTTCT GAGGT TC-3'	60	251
	R: 5'-AGTAG TCTGT ATTGC TGATG TC-3'		
GP130	F: 5'-CATGCTTTGGGTGGAATGGAC-3'	58	326
	R: 5'-CATCAACAGGAAGTTGGTCCC-3'		
OCT4	F: 5'-GACAACAATGAGAACCTTCAGGAG-3'	55	216
	R: 5'-CTGGCGCCGGTTACAGAACCA-3'		
ABCG2	F: 5'-AGTTCCATGGCACTGGCCATA-3'	53	379
	R: 5'-TCAGGTAGGCAATTGTGAGG-3'		
CD133	F: 5'-CTGGGGCTGCTGT TTATTATTCTG-3'	55	234
	R: 5'-ACGCCTTGTCCTTGGTAGTGTTG-3'		
E-cadherin	5'-AAGGAGGCGGAGAAGAGGAC-3'	55	213
	5'-CGTCGTTACGAGTCACTTCAGG-3'		
Vimentin	5'-AAGAGAACTTTGCCGTGGAA-3'	58	246
	5'-TCCAGCAGCTTCCTGTAGGT-3'		
Snail	5'-GAGGCGGTGGCAGACTAG-3'	55	208
	5'-GACACATCGGTCAGACCAG-3'		
β-actin	F: 5'-ACCAACTGGGACGACATGGAGAAA-3'	58	192
	R: 5'-TAGCACAGCCTGGATAGCAACGTA-3'		
18S RNA	F: 5'-CTCAACACGGGAAACCTCAC-3'	58	110
	R: 5'-CGCTCCACCAACTAAGAACG-3'		

F: Forward primer; R: Reverse primer.

Figure 1. Expression of IL-6, IL-6R, and GP130 in HTh74 and HTh74R cells. Hela cells as positive control.

Antibodies in 5% TBST at a dilution of 1:1000, 1:1000, 1:2000, 1:1000, 1:1000 and 1:1000, respectively. Next day, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology, MA, USA) for 2 h at room temperature. The immunoreactive bands were detected with chemiluminescence substrate kit (ProteinSimple, CA, USA) under the Fluor Chem FC2 system.

Cell transfection with siRNA STAT3

Thyroid cancer stem cells were transfected with siRNA STAT3 or a negative control (GenePharma Co., Ltd, Shanghai, China) using Lipofectamine RNAiMAX (GenePharma Co., Ltd, Shanghai, China) as described by the manufacturer's instructions. Cell cultures were incubated for 24 h with various concentrations of siRNA before IL6 treatment. Proteins were extracted 24 h after addition of IL6. The expression of STAT3, or pSTAT3 (Tyr705) (both STAT3 and pSTAT3 antibodies were from Cell Signaling Technology, MA, USA) were detected by western blot as above.

Statistical analysis

Statistical analyses were performed with SPSS 16.0 software. The PCR results were subjected to ANOVA followed by Bonferroni's post hoc test. The values are expressed as the mean ± SD. $P < 0.05$ was considered significant.

Results

Expression of IL-6, IL-6R and GP130 in thyroid cancer cells

With HeLa cells as a positive control, RT-PCR showed that HTh74 and HTh74R cells did not express IL-6R but did express IL-6 and GP130 (**Figure 1**).

IL-6 promotes the formation of HTh74 and HTh74R sphere cells in undifferentiated thyroid cancer

The effects of IL-6 on the formation of sphere cells in the HTh74 and HTh74R thyroid cancer cell lines were observed in the culture medium when these cell lines were cultured with 100 ng/ml sIL-6R and different concentrations of
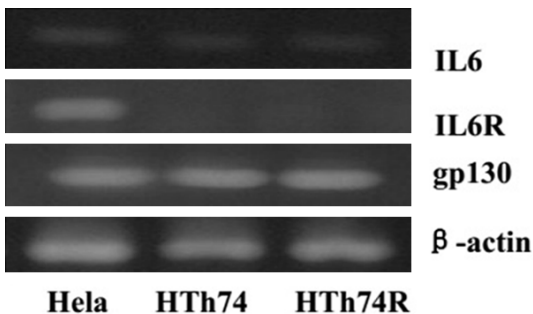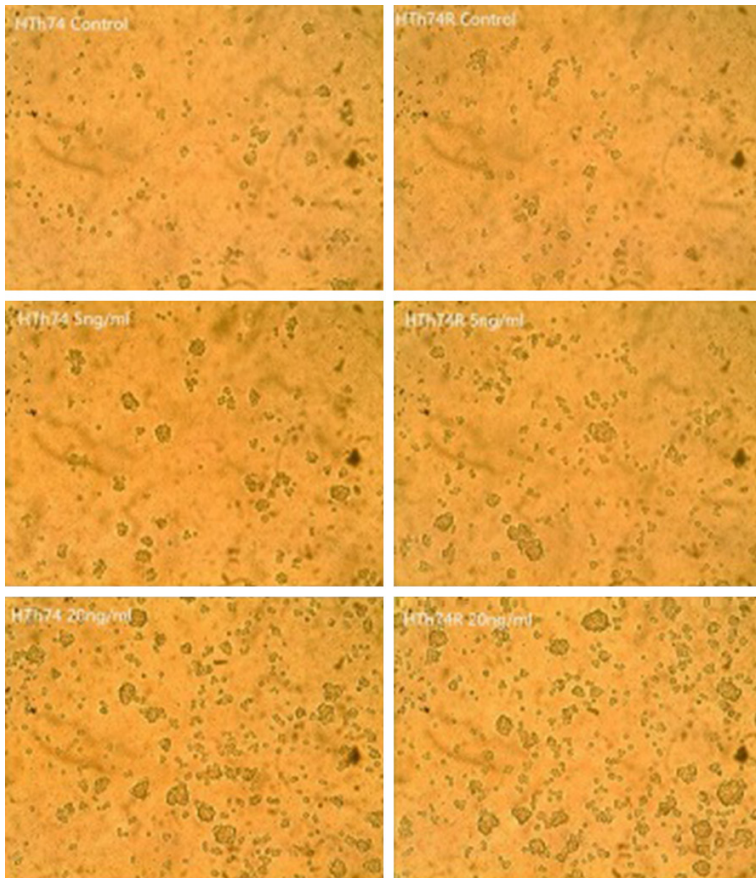
Figure 2. Effect of various concentration of IL-6 (0, 5, 20 mg/ml) on sphere formation in HTh74 and HTh74R cells after treatment with IL6 for 7 days.
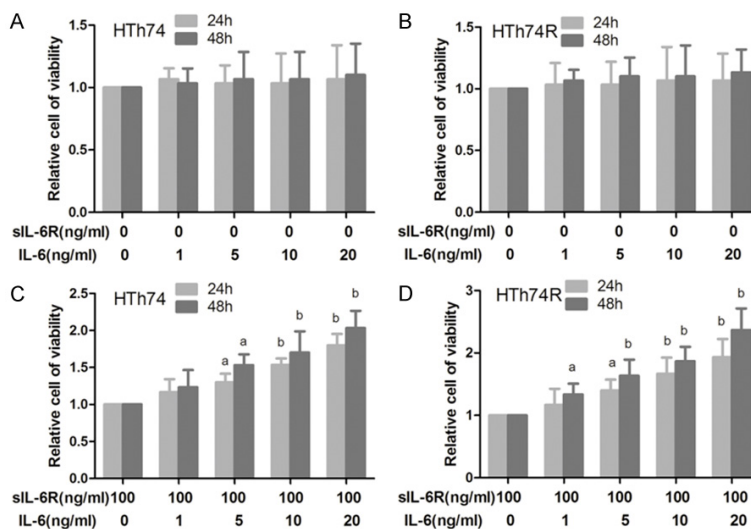

Figure 3. IL-6 promoted the proliferation of HTh74 and HTh74R stem cells. a, $P<0.05$; b, $P<0.01$.

number of sphere cells increased. In addition, the sphere cell formation rate of HTh74R cells was higher than that of HTh74 cells (15% *vs.* 11%) (**Figure 2**).

IL-6 promotes the proliferation of HTh74 and HTh74 cancer stem cells in undifferentiated thyroid cancer

The proliferation of HTh74 and HTh74R cancer stem cells was assessed by an MTT assay. In the absence of sIL-6R, although the concentration of IL-6 increased, HTh74 and HTh74R cancer stem cells showed no significant increase in proliferation (**Figure 3A** and **3B**). When 100 ng/ml sIL-6R was added to the culture medium, the proliferation of HTh74 and HTh74R cancer stem cells increased significantly in a dose- and time-dependent manner with increasing IL-6 concentration, regardless of whether the culture duration was 24 h or 48 h (**Figure 3C** and **3D**).

Anti-IL-6 inhibits the proliferation of HTh74 and HTh74R cancer stem cells in undifferentiated thyroid cancer

We further used anti-IL-6 to block cell proliferation for 48 h and found that with increasing concentrations of anti-IL-6, the proliferation of HTh74 and HTh74R cancer stem cells decreased significantly in a dose-dependent manner (**Figure 4**).

IL-6 promotes the colony formation ability of HTh74 and HTh74R cells

The cloning experiments showed that IL-6 could significantly promote colony formation by HTh74 and HTh74R cells. As the dose of IL-6 increased,

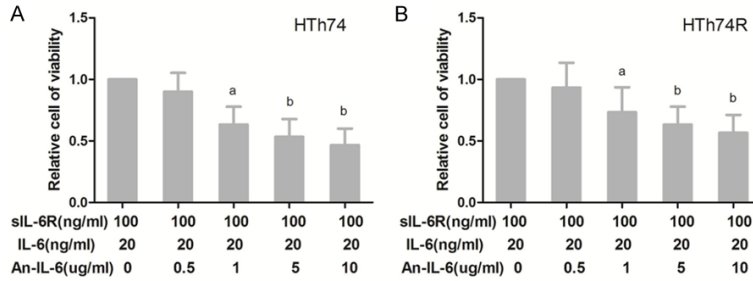IL-6 for 7 d. As the IL-6 concentration increased, the sphere cell formation rate increased, the size of the sphere cells increased, and the

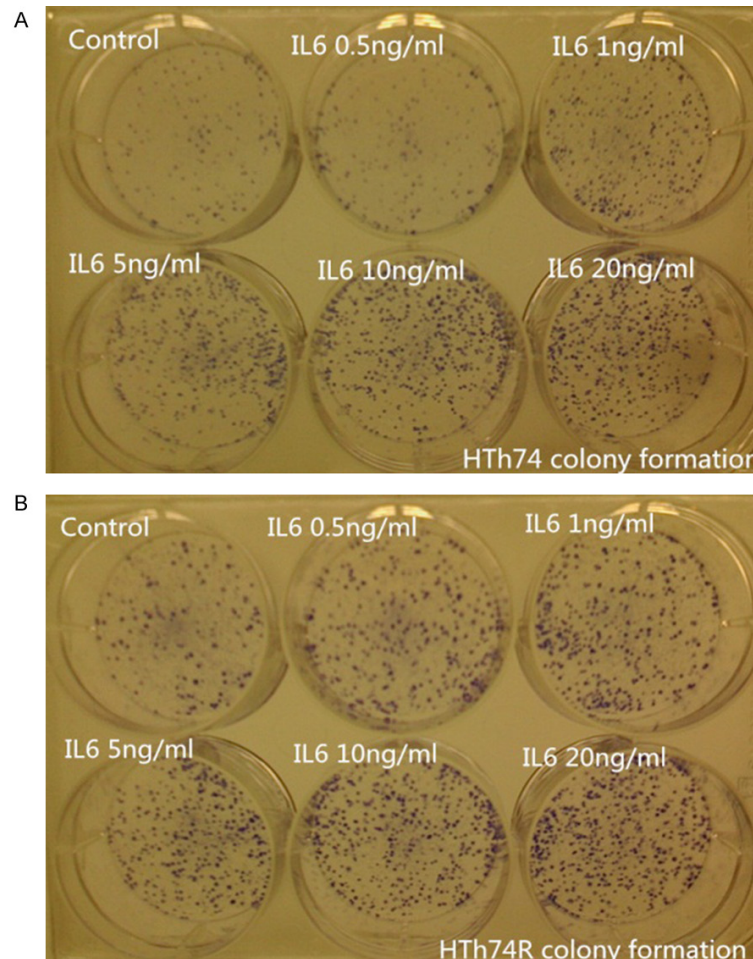**Figure 4.** Anti-IL-6 inhibited the proliferation of HTh74 and HTh74R stem cells. a, $P<0.05$; b, $P<0.01$.

Figure 5. IL-6 promoted clone formation of HTh74and HTh74R cells.

and ABCG2 mRNA in HTh74 and HTh74R cancer stem cells and promoted the expression of CD133 mRNA in HTh74R cells (**Figure 6**).

Effect of IL-6 on the expression of epithelial-mesenchymal transition (EMT) markers in thyroid stem cells

The expression of E-cadherin, vimentin, and Snail was detected by real-time qPCR. After the secondary passaged cells were treated with or without IL-6 for 3 d, total RNA was isolated, and cDNA was amplified by reverse transcription. Real-time qPCR was performed to determine gene expression. After IL-6 stimulation, the expression of E-cadherin was significantly decreased, and the expression of vimentin and Snail was increased (**Figure 7**).

Effect of IL6 on JAK1/STAT3/OCT4 pathway in thyroid cancer stem cells

To evaluate the effect of IL6 on the JAK1/STAT3/OCT4 pathway, phosphorylation of JAK1/STAT3 and OCT4 levels were analyzed in thyroid cancer stem cells treated with/without IL6. Western immunoblot analysis revealed that IL6 dramatically increased the phosphorylation of JAK1/STAT3 (Tyr705 and Ser727). OCT4 level also was increased. When IL6 was added together with anti-IL6 antibody, it abolished the increase in phosphorylation of JAK1/STAT3, and OCT4 levels induced by anti-IL6 antibody (**Figure 8**).

the colony formation rate increased significantly, and the colony formation rate of HTh74R cells was higher than that of HTh74 cells (23% to 19%) (**Figure 5**).

IL-6 promotes gene expression in HTh74 and HTh74R cancer stem cells

The real-time qPCR results showed that IL-6 significantly promoted the expression of OCT4

The proliferative effect of IL6 is blocked by STAT3-siRNA

To analyze whether the proliferative effect of IL6 is mediated by activation of the STAT3 signaling pathway, the pathway was blocked using siRNA directed against STAT3. STAT3-siRNA
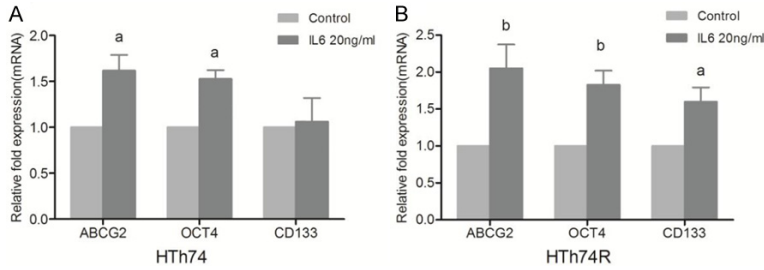
Figure 6. IL-6 promoted the expression of ABCG2, OCT4, and CD133 in HTh74 and HTh74R stem cells. a, $P<0.05$; b, $P<0.01$.
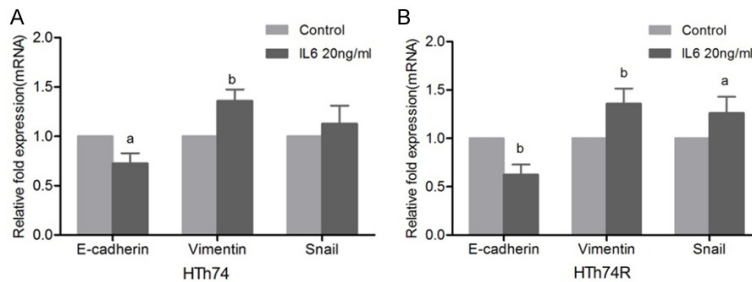

Figure 7. Effects of IL-6 on E-cadherin, vimentin, and Snail expression in thyroid cancer stem cells after 3 d. a, $P<0.05$; b, $P<0.01$.

cancer, and thyroid cancer stem cells may arise due to genetic mutations in normal thyroid stem cells [14]. On the other hand, a few cancer stem cells can renew and differentiate [15]. Thyroid cancer stem cells are believed to be an important cause of tumor resistance, recurrence, and metastasis [16]. Therefore, exploring the biologic characteristics of thyroid cancer stem cells and implementing effective therapeutic measures in thyroid cancers is very important.

Chronic thyroid inflammation plays an important role in the pathogenesis of thyroid cancer. Inflammation may be involved in the development of thyroid cancer. Research has shown that the incidence of thyroid nodules increased significantly in the autoimmune inflammatory state of the thyroid gland [17].

interference decreased the expression of STAT3 proteins. Activation of STAT3 by IL6 resulted in a diminished phosphorylation of STAT3. However, transfection of thyroid cancer stem cells with specific siRNA reduced the effect of IL6 on phosphorylation of STAT3, whereas pretreatment with control siRNA did not affect it. Finally, we investigated whether blocking the STAT3 pathway affects cell growth after IL6 treatment. As revealed by MTT assay, transfection of thyroid cancer stem cells with STAT3-siRNA was slightly increased by IL6. This indicates that the proliferative effect of IL6 depends on activation of the STAT3 pathway (**Figure 9**).

Discussion

Thyroid cancer is one of the most common endocrine tumors and has rapidly increasing incidence rates. At present, the pathogenesis of thyroid cancer is still an active area of clinical research. The relationship between thyroid cancer and stem cells has attracted increasing attention. The study of thyroid cancer stem cells has provided a new perspective for the prevention and treatment of thyroid cancer [11-13].

Research has suggested that on one hand, an imbalance of thyroid stem cells may lead to

The expression of IL-6 has been shown to be significantly increased in autoimmune thyroid disease and thyroid cancer [18]. Furthermore, significantly higher levels of IL-6 were observed in patients with benign and malignant thyroid disease than in healthy individuals, supporting the association between thyroid disease and an underlying inflammatory processes [9]. IL-6 plays an important role in the pathogenesis of thyroid disease [19].

IL-6 can signal by two different pathways: the classical signaling pathway, through membrane bound IL-6R; and the IL-6 trans-signaling pathway, through endogenous soluble IL-6R (sIL-6R). IL-6 promotes cell growth and proliferation mainly through JAK1/STAT3 [20, 21].

Our study confirmed that HTh74 and HTh74R anaplastic thyroid cancer did not express IL-6 receptor. Cell proliferation was unchanged by the presence of IL-6 alone in the culture medium but was mediated by the combination of IL-6 and sIL-6R, showing that thyroid cancer stem cells were regulated by IL-6 trans-signaling. In addition, the combination of IL-6 and sIL-6R significantly increased the proliferation of
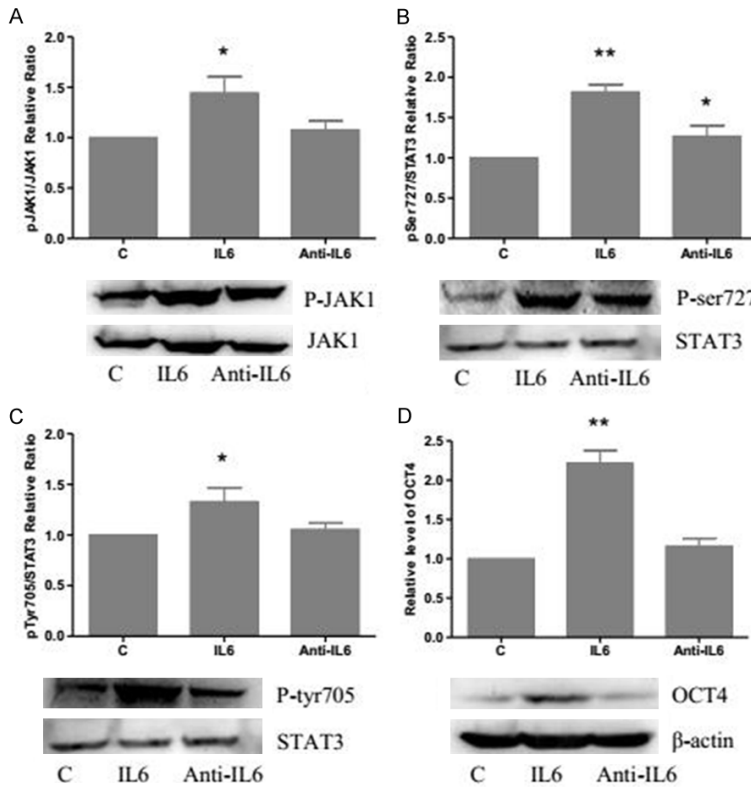
Figure 8. IL6 increased phosphorylation of JAK1/STAT3 and OCT4 level. Thyroid cancer stem cells were treated under different conditions (C, sIL6 100 ng/ml; IL6, sIL6 100 ng/ml + IL6 20 ng/ml; Anti-IL6, sIL6 100 ng/ml +IL6 20 ng/ml + Anti-IL6 10 µg/ml) for 24 h. A. JAK1 phosphorylation was analyzed by western blot as indicated in Materials and Methods. Similar results were obtained in three independent experiments. B, C. STAT3 phosphorylation at Ser727 and Tyr705 were analyzed by western blot. D. OCT4 levels were analyzed by western blot.
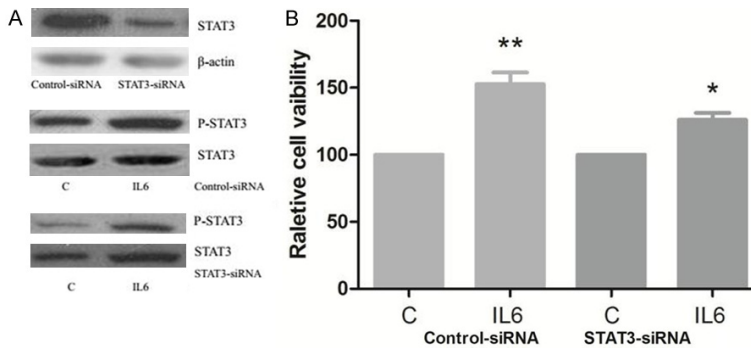

Figure 9. The proliferative effect of IL6 is blocked by STAT3-siRNA in thyroid cancer stem cells. Cells were transfected with various concentrations of STAT3-siRNA or with control siRNA using Lipofectamine RNAiMAX. Twenty-four hours after transfection, cells were treated with IL6 and SiI6r for 24 hours. Protein expression of STAT3 and pSTAT3 was detected by western blots (A). Cell proliferation was measured by MTT assays (B). Differences were significant between the IL6-treated cells transfected with STAT3- siRNA and control siRNA (*, P<0.05; **, P<0.01).

HTh74 and HTh74R cancer stem cells. However, proliferation was inhibited by anti-IL-6.

Furthermore, IL-6 is proven to promote the proliferation of thyroid cancer stem cells, and anti-IL-6 has an antitumor effect; these findings provide a new basis for the targeted treatment of thyroid cancer with anti-IL-6.

Studies have shown that IL-6 is often used to expand hematopoietic and embryonic stem cells [22]. As a stem cell promoter, IL-6 can promote the expansion of cancer stem cells and tumorigenesis in hepatocellular carcinoma [23]. Thus, IL-6 can not only promote the growth of cancer stem cells but can also promote the proliferation and growth of other stem cells. We assessed the formation rate of HTh74 and HTh74R cell spheres; the results showed that the formation, volume, and number of sphere cells increased with increasing IL-6 concentration. The rate of formation also increased significantly. In addition, we confirmed that IL-6 promoted colony formation by thyroid cancer cells, which reflects the characteristics of tumor cell population dependence and proliferative ability. Currently, the study of thyroid cancer stem cells is very limited. We previously [10] reported that metformin can reduce the rate of sphere cell formation in thyroid cancer. Furthermore, metformin can reduce colony formation by thyroid cancer cells.

Thyroid cancer stem cells have been suggested to be the source of proliferation, metastasis, recurrence, and drug resistance in thyroid cancer [24-26]. Our previous studies have confirmed that several markers, such as OCT4, ABCG2, and CD133, have been identified for the character-

ization of cancer stem cells [12, 25]. This study showed that the inflammatory factor IL-6 combined with sIL-6R was able to promote the gene expression of OCT4 and ABCG2 in HTh74 and HTh74R cancer stem cells. These results indicated that IL-6 could enhance the characteristics of cancer stem cells, for example, the greater self-renewal capacity of cancer stem cells and the greater capacity of tumor cells to growth and metastasis.

OCT4 is a key regulator that maintains the pluripotency and self-renewal properties of stem cells [27]. OCT4 promotes tumorigenesis and inhibits the apoptosis of cancer cells [28]. We investigated the effect of IL-6 on the expression of OCT4 in thyroid stem cells and found that IL-6 promoted the expression of OCT4 in thyroid stem cells. One study found that fusion proteins contained the coding sequence of OCT4 fused to activating regions but not those fused to repressing regions act like OCT4, suppressing differentiation and promoting the maintenance of undifferentiated phenotypes. This finding shows that the necessary and sufficient function of OCT4 in promoting pluripotency is to activate specific target genes [29]. Furthermore, OCT4 is essential for inhibiting the apoptosis of embryonic stem cells in response to stress, an effect that may be mediated through the STAT3/survivin pathway [30]. ABCG2 is a member of the ATP-binding cassette (ABC) transporters and is widely expressed in stem cells, even being recognized as a universal stem cell marker; ABCG2 plays an important role in promoting stem cell proliferation and the maintenance of the stem cell phenotype, and it is involved in the metastasis, invasion, and drug resistance of tumors [31-33]. CD133 has been used for the identification and isolation of a putative cancer stem cell population from several human cancers. In addition, studies have found that the stem cell marker CD133 is closely related to EMT in ATC [34, 35].

To investigate the effect of IL-6 on EMT markers in thyroid stem cells, we determined the expression of E-cadherin, vimentin and Snail using real-time qPCR. We found that thyrosphere-derived cells expressed the epithelial cell marker E-cadherin and the mesenchymal cell markers vimentin and Snail. After the thyroid stem cells were treated with IL-6 for 3 d, the expression of E-cadherin was significantly decreased, and the expression of vimentin and Snail was slightly increased.

One study indicated that IL-6 was involved in tumor metastasis and EMT in breast cancer by the IL-6/JAK1/STAT3 signaling pathway [36]. Thus, IL-6 is closely related to EMT, and EMT and inflammatory pathways have been targeted for carcinoma treatment [37]. Currently, the relationship between EMT and thyroid cancer stem cells has not been clarified; therefore, EMT of thyroid cancer stem cells needs further research.

STAT3 involved in the pathogenesis of tumor. Overexpression of STAT3 promotes cell proliferation, inhibits apoptosis, and promotes the transformation of cells to cancer cells. Our results indicated IL6/STAT3 can regulate OCT4 expression. Some research suggests that OCT4 may regulate signal transduction pathways of JAK-STAT3, and have an effect on cell proliferation and differentiation in human embryonic stem cells [38, 39]. It is worth mentioning that IL6 regulates CSC-associated OCT-4 mRNA expression through the IL6-JAK1-STAT3 signal pathway, but anti-IL6 antibody can prevented OCT-4 gene expression. This result suggested that this pathway plays a key role in the conversion of non-CSCs into CSCs through regulation of OCT-4 gene expression in breast cancer stem cells [40].

To further analyze whether the proliferative effect of IL6 is mediated by activation of the STAT3 signaling, we found that STAT3-siRNA interference could decrease the expression of the STAT3 proteins in thyroid cancer stem cells. Meanwhile, we investigated if blocking of STAT3 pathway affects cell proliferation after IL6 treatment by MTT assay. Compared with controls, after treatment with STAT3-siRNA, thyroid cancer stem cells markedly decreased. This result shows that the proliferative effect of IL6 depends on activation of the IL6/JAK1/STAT3 pathway.

In summary, the inflammatory factor IL-6 can promote the growth and proliferation of thyroid cancer stem cells. IL-6 promoted the colony formation and EMT of HTh74 and HTh74R cells and thus may contribute to the development and metastasis of thyroid cancer. In addition, the promotion of cell growth and proliferation by IL-6 is mainly mediated by the IL-6/STAT

pathway. Exploring the pathogenesis and treatment of thyroid cancer with IL-6 as the target can provide new insight into the pathogenesis and new directions for the treatment of thyroid cancer.

Disclosure of conflict of interest

None.

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References

[1]   Coussens LM and Werb Z. Inflammation and cancer. Nature 2002; 420: 860-7.

[2]   Lima PC, Moura Neto A, Tambascia MA and Zantut Wittmann DE. Risk factors associated with benign and malignant thyroid nodules in autoimmune thyroid diseases. ISRN Endocrinol 2013; 2013: 673146.

[3]   Rollins BJ. Inflammatory chemokines in cancer growth and progression. Eur J Cancer 2006; 42: 760-767.

[4]   Trivanović D, Krstić J, Djordjević IO, Mojsilović S, Santibanez JF, Bugarski D and Jauković A. The roles of mesenchymal stromal/stem cells in tumor microenvironment associated with inflammation. Mediators Inflamm 2016; 2016: 7314016.

[5]   Wang D and DuBois RN. Immunosuppression associated with chronic inflammation in the tumor microenvironment. Carcinogenesis 2015; 36: 1085-1093.

[6]   Landskron G, De la Fuente M, Thuwajit P, Thuwajit C and Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014; 2014: 149185.

[7]   Wu X, Takekoshi T, Sullivan A and Hwang ST. Inflammation and tumor microenvironment in lymph node metastasis. Cancers (Basel) 2011; 3; 927-944.

[8]   Murai H, Murakami S, Ishida K and Sugawara M. Elevated serum interleukin 6 and decreased thyroid hormone levels in postoperative patients and effects of IL6 on thyroid cell function in vitro. Thyroid 1996; 6: 601-606.

[9]   Provatopoulou X, Georgiadou D, Sergentanis TN, Kalogera E, Spyridakis J, Gounaris A and Zografos GN. Interleukins as markers of inflammation in malignant and benign thyroid disease. Inflamm Res 2014; 63: 667-674.

[10]   Chen G, Xu S, Renko K and Derwahl M. Metformin inhibits growth of thyroid carcinoma cells, suppresses self-renewal of derived cancer stem cells, and potentiates the effect of chemotherapeutic agents. J Clin Endocrinol Metab 2012; 97: E510-520.

[11]   Vicari L, Colarossi C, Giuffrida D, De Maria R and Memeo L. Cancer stem cells as a potential therapeutic target in thyroid carcinoma. Oncol Lett 2016; 12: 2254-2260.

[12]   Peng W, Wang K, Zheng R and Derwahl M. 1,25 dihydroxyvitamin D3 inhibits the proliferation of thyroid cancer stem-like cells via cell cycle arrest. Endocr Res 2016; 41: 71-80.

[13]   Nagayama Y, Shimamura M and Mitsutake N. Cancer stem cells in the thyroid. Front Endocrinol (Lausanne) 2016; 7: 20.

[14]   Derwahl M. Linking stem cells to thyroid cancer. J Clin Endocrinol Metab 2011; 96: 610-613.

[15]   Lin Z, Lu X, Li W, Sun M, Peng M, Yang H, Chen L, Zhang C, Cai L and Li Y. Association of cancer stem cell markers with aggressive tumor features in papillary thyroid carcinoma. Cancer Control 2015; 22: 508-514.

[16]   Jung CW, Han KH, Seol H, Park S, Koh JS, Lee SS, Kim MJ, Choi IJ and Myung JK. Expression of cancer stem cell markers and epithelial-mesenchymal transition-related factors in anaplastic thyroid carcinoma. Int J Clin Exp Pathol 2015; 8: 560-568.

[17]   Lumachi F, Basso SM and Orlando R. Cytokines, thyroid diseases and thyroid cancer. Cytokine 2010; 50: 229-233.

[18]   Ruggeri RM, Villari D, Simone A, Scarfi R, Attard M, Orlandi F, Barresi G, Trimarchi F, Trovato M and Benvenga S. Co-expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) in thyroid nodules is associated with co-expression of CD30 ligand/CD30 receptor. J Endocrinol Invest 2002; 25: 959-966.

[19]   Linkov F, Ferris RL, Yurkovetsky Z, Marrangoni A, Velikokhatnaya L, Gooding W, Nolan B, Winans M, Siegel ER, Lokshin A and Stack BC Jr. Multiplex analysis of cytokines as biomarkers that differentiate benign and malignant thyroid diseases. Proteomics Clin Appl 2008; 2: 1575-1585.

[20] Rose-John S, Scheller J, Elson G and Jones SA. Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. J Leukoc Biol 2006; 80: 227-236.

[21] Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci 2012; 8: 1237-1247.

[22] Gammaitoni L, Bruno S, Sanavio F, Gunetti M, Kollet O, Cavalloni G, Falda M, Fagioli F, Lapidot T, Aglietta M and Piacibello W. Ex vivo expansion of human adult stem cells capable of primary and secondary hemopoietic reconstitution. Exp Hematol 2003; 31: 261-270.

[23] Wan S, Zhao E, Kryczek I, Vatan L, Sadovskaya A, Ludema G, Simeone DM, Zou W and Welling TH. Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. Gastroenterology 2014; 147: 1393-1404.

[24] Hardin H, Montemayor-Garcia C and Lloyd RV. Thyroid cancer stem-like cells and epithelial-mesenchymal transition in thyroid cancers. Hum Pathol 2013; 44: 1707-1713.

[25] Zheng X, Cui D, Xu S, Brabant G and Derwahl M. Doxorubicin fails to eradicate cancer stem cells derived from anaplastic thyroid carcinoma cells: characterization of resistant cells. Int J Oncol 2010; 37: 307-315.

[26] Todaro M, Iovino F, Eterno V, Cammareri P, Gambara G, Espina V, Gulotta G, Dieli F, Giordano S, De Maria R and Stassi G. Tumorigenic and metastatic activity of human thyroid cancer stem cells. Cancer Res 2010; 70: 8874-8885.

[27] Tai MH, Chang CC, Kiupel M, Webster JD, Olson LK and Trosko JE. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. Carcinogenesis 2005; 26: 495-502.

[28] Wang YD, Cai N, Wu XL, Cao HZ, Xie LL and Zheng PS. OCT4 promotes tumorigenesis and inhibits apoptosis of cervical cancer cells by miR-125b/BAK1 pathway. Cell Death and Disease 2013; 4: e760.

[29] Hammachi F, Morrison GM, Sharov AA, Livigni A, Narayan S, Papapetrou EP, O'Malley J, Kaji K, Ko MS, Ptashne M and Brickman JM. Transcriptional activation by Oct4 is sufficient for the maintenance and induction of pluripotency. Cell Rep 2012; 1: 99-109.

[30] Guo Y, Mantel C, Hromas RA and Broxmeyer HE. Oct-4 is critical for survival/antiapoptosis of murine embryonic stem cells subjected to stress: effects associated with Stat3/survivin. Stem Cells 2008; 26: 30-34.

[31] Ding XW, Wu JH and Jiang CP. ABCG2: a potential marker of stem cells and novel target in stem cell and cancer therapy. Life Sci 2010; 86: 631-637.

[32] Robey RW, Polgar O, Deeken J, To KW and Bates SE. ABCG2: determining its relevance in clinical drug resistance. Cancer Metastasis Rev 2007; 26: 39-57.

[33] Ejendal KF and Hrycyna CA. Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. Curr Protein Pept Sci 2002; 3: 503-511.

[34] Wu Y and Wu PY. CD133 as a marker for cancer stem cells: progresses and concerns. Stem Cells Dev 2009; 18: 1127-1134.

[35] Liu J and Brown RE. Immunohistochemical detection of epithelialmesenchymal transition associated with stemness phenotype in anaplastic thyroid carcinoma. Int J Clin Exp Patho 2010; 3: 755-762.

[36] Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramirez N, Oberyszyn TM and Hall BM. Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. Oncogene 2009; 28: 2940-2947.

[37] Prud'homme GJ. Cancer stem cells and novel targets for antitumor strategies. Curr Pharm Des 2012; 18: 2838-2849.

[38] Rodda DJ, Chew JL, Lim LH, Loh YH, Wang B, Ng HH and Robson P. Transcriptional regulation of Nanog by OCT4 and SOX2. J Biol Chem 2005; 280: 24731-24737.

[39] Medvedev SP, Shevchenko AI, Mazurok NA and Zakiian SM. OCT4 and NANOG are the key genes in the system of pluripotency maintenance in mammalian cells. Genetika 2008; 44: 1589-1608.

[40] Kim SY, Kang JW, Song X, Kim BK, Yoo YD, Kwon YT and Lee YJ. Role of the IL-6-JAK1-STAT3-Oct-4 pathway inthe conversion of non-stem cancer cells into cancer stem-like cells. Cell Signal 2013; 25: 961-969.