IL-17A Stimulates the Progression of Giant Cell Tumors of Bone

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Keywords: Giant cell tumors of bone, interleukin 17A, IL-17A, proinflammatory cytokine, osteolytic ability, multinucleated giant cells

Abstract

Purpose: Giant cell tumors of bone (GCTB) exhibit aggressive bone lytic behavior. Studies have shown that interleukin 17A (IL-17A) is involved in pathologic bone resorption in various skeletal disorders. Thus, we have investigated the role of IL-17A in GCTBs.

Experimental Design: We evaluated the progression of GCTBs using Campanacci grading and Enneking staging systems in 74 patients with GCTB. The expression of IL-17A and the IL-17A receptor A (IL-17RA) was assessed in GCTB tissues and in both multinucleated giant cells (MNGC) and stromal cells cultured in vitro using immunostaining and reverse transcription PCR (RT-PCR). The effects of IL-17A on the osteolytic activity of the MNGCs and the proliferation of the stromal cells were investigated using the "pit" formation and MTT assays, respectively. The effects of IL-17A on the expression of proosteolytic factors were examined in primary cultured MNGCs and stromal cells using RT-PCR, Western blotting, and gene expression microarrays.

Results: In GCTBs, we detected abundant levels of IL-17A, which were associated with tumor extension and grade. IL-17A is predominantly produced by MNGCs, whereas IL-17RA is expressed by both MNGCs and stromal cells in GCTBs. In the MNGCs, the IL-17A increased the mRNA expression of IL-17A and proosteolytic enzymes, and also enhanced osteolytic ability. In the stromal cells, the IL-17A stimulated cellular proliferation and the expression of proosteolytic factors, including RANKL through myc and STAT3, respectively. In addition, IL-17A stimulated in vivo tumor growth and the extent of angiogenesis in GCTBs.

Conclusion: IL-17A stimulates the progression of GCTBs and might represent a useful candidate marker for progression and as a therapeutic target for GCTBs. Clin Cancer Res; 19(17); 4697–705. ©2013 AACR.

Introduction

Giant cell tumor of bone (GCTB) is a benign, locally aggressive, osteolytic tumor that causes significant bone destruction at the epiphysis of long bones. Although rarely lethal, GCTBs exhibit local recurrence in 8% to 62% of patients following primary surgical treatments, and up to 5% of GCTBs develop pulmonary metastases and spontaneous malignant transformation in 2% of patients (1, 2).

GCTBs are composed of 3 major cell populations: spindle-shaped mononuclear stromal cells (SC), osteoclast-like multinucleated giant cells (MNGC), and the mononuclear cells of monocyte/macrophage origin (3). The stromal cells are the only proliferating population and represent the neoplastic component of GCTBs. The bone-resorption activity of GCTBs is primarily attributed to the MNGCs, the development and activity of which are significantly regulated by stromal cells via a complex network of cytokines and chemokines (1). For instance, the MNGCs and their precursors express the receptor activator of nuclear factor kappa (RANK), through which the stromal cells regulate their formation and osteolytic function by constitutively expressing the RANK ligand (RANKL).

Interleukin-17A (IL-17A), a CD4+ T-cell–derived proinflammatory cytokine, has been implicated in pathologic bone resorption in various skeletal disorders (4, 5). Osteoclasts are the predominant cause of bone resorption during both physiologic and pathologic conditions (6). IL-17A, either derived from the synovial fluids of patients with rheumatoid arthritis or as a recombinant protein, has been shown to exhibit significant ability to transform monocytes into osteoclasts (7–10). Although GCTB is characterized by...
the ubiquitous presence of "osteoclast-like" MNGCs and massive bone destruction (1), the role of IL-17A in the pathogenesis of this disease remains poorly understood. Thus, in this study, we investigated the role of IL-17A in the progression of GCTBs.

Materials and Methods

Patient samples and the evaluation of GCTB progression in patients

Tumor samples and blood were obtained from 74 patients with GCTB, 8 patients with rheumatoid arthritis, and 10 patients with osteosarcoma. Blood samples were also obtained from 20 healthy individuals. All patients with GCTB received extended curettage, with no adjuvant therapy. The clinical characteristics of all of the patients with GCTB are summarized in Table 1. The progression of the GCTBs was evaluated using the Campanacci grading (11) and Enneking staging systems (12). This research was approved by the Research Ethics Board of the General Hospital of People’s Liberation Army (Beijing, China).

The isolation and primary culture of osteoclast-like multinucleated giant cells and stromal cells

The GCTB samples were minced (within 4 hours of surgical resection) in a Petri dish using ophthalmic scissors and incubated at 37°C in Dulbecco’s modified Eagle medium (DMEM; Invitrogen) with 0.5 mg/mL collagenase IV (Sigma) and 200 μg/mL DNase I (Sigma) at 37°C for 1 hour. The resulting single-cell suspensions were washed 3 times with complete DMEM (supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin; all from Invitrogen). The cells were resuspended and centrifuged at 700 rpm for 1 minute. The large, heavy MNGCs were enriched in the precipitates and were carefully collected.

The supernatants, which predominantly contained stromal cells and mononuclear cells, were also collected for the further culture of the stromal cells. To separate the MNGCs, the cells in the precipitates were stained with 5 μg/mL Hoechst 33342 (Sigma) at 37°C for 90 minutes. Because of their huge size and multiple nuclei, the Hoechst-stained MNGCs could be clearly separated using their increased Hoechst signal and forward scattering by fluorescence-activated cell sorting. The purified MNGCs were immediately cultured in complete DMEM at 37°C in humidified air with 5% CO2. To identify the osteoclastic features of the cultured primary MNGCs, the cells were stained using a Tartrate-resistant acid phosphatase (TRAP) staining kit (Cellgarage), and the TRAP-positive cells were observed on day 2 by light microscopy (Supplementary Fig. S1A). The stromal cells were obtained by continuous culture, as previously described (refs. 13, 14; Supplementary Fig. S1B).

Isolation of peripheral blood mononuclear cells

For analysis T-helper (TH)17 cells, blood samples were obtained from 3 patients with GCTB and 5 healthy control individuals. Peripheral blood mononuclear cells (PBMC) were purified from peripheral blood by centrifugation, using a Ficoll–Hypaque gradient (Amersham Pharmacia Biotech).

Cytokine assay and flow cytometric analysis

The IL-17A in the serum and the media of primary cultured cells were measured using the human IL-17A ELISA Kit (R&D), according to the manufacturer’s protocols. To detect the IL-17A or IL-17A receptor A (IL-17RA)–positive cells in MNGCs and stromal cells, the cells were labeled with phycoerythrin-conjugated IL-17 monoclonal

Table 1. Patient characteristics

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<td>Yes</td>
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<tr>
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<td>52 (70)</td>
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antibodies (mAb) or phycoerythrin-conjugated IL-17RA mAbs (eBioscience), according to the manufacturers’ protocols. T\textsubscript{i}17 cells in blood were detected according to Shen’s methods (15).

**Immunostaining**

Western blot analysis and immunohistochemistry (IHC) were conducted as previously described (16). To detect the expression of IL-17A and IL-17-AR in MNGCs and stromal cells, cells were seeded into 8-well chamber slides (BD Science). After 24 hours, the expression of IL-17A and IL-17-RA was detected using routine immunofluorescence methods.

**Bone resorption assay**

The MNGCs were seeded onto dentine slices (ALPCO Diagnostic) and incubated in the presence or absence of IL-17A (Genescript) at 37 °C in humidified air with 5% CO\textsubscript{2} for 2 days. The slices were rinsed with PBS, incubated overnight in 1 mol/L ammonium hydroxide, and stained with 1% toluidine blue in 0.5% sodium tetraborate solution. The number of resorptive areas or ‘pits’ per low-power field on each bone slice was counted using reflective light microscopy.

**Gene expression microarray and real-time quantitative RT-PCR analysis**

For the microarray assay, the IL-17RA-positive stromal cells (IL-17RA\textsuperscript{+} SCs) were treated with dimethyl sulfoxide or 10 ng/mL IL-17A (Genescript) for 48 hours. The RNA was then amplified using the Illumina TotalPrep RNA amplification kit and hybridized onto Illumina HumanHT-12v4 expression BeadChips, according to the manufacturer’s protocol. The hybridization data were acquired using an iScan Bead Array scanner (Illumina) and preprocessed using quantile normalization (in compliance with MIAME guidelines). From microarray results, we selected more than 1.5-fold changed genes. Analysis of the pathways was conducted using Ingenuity Pathway Analysis 8.6 software (Ingenuity Systems).

For the qRT-PCR analysis, the total RNA of the cells was isolated using the TRIzol reagent (Invitrogen), according to the manufacturer’s protocols. Gene expression was quantified by real-time quantitative RT-PCR using QuantiTect SYBR Green dye (Qiagen). The primer sequences were as follows: MMP-9 (179 bp) forward 5'-TTGACAGGCCAAGAGTGG-3', MMP-9 reverse 5'-GCCATTACCGTCTGCTTAT-3', cathepsin K (127 bp) forward 5'-CCCGCAGTACTGAGACACCC-3', cathepsin K reverse 5'-AAAGCCCACAGGAAACCA-3', IL-17 (154 bp) forward 5'-AACCGA-TCCACCTCCTACTTG-3', IL-17 reverse 5'-TCCTCATCTGCTGATGGGCAACA-3', IL-17RA (268 bp) forward 5'-CCCGCAGTACTGAGACACCC-3', cathepsin K reverse 5'-AAAGCCCACAGGAAACCA-3', IL-17 (154 bp) forward 5'-AACCGA-TCCACCTCCTACTTG-3', IL-17 reverse 5'-TCCTCATCTGCTGATGGGCAACA-3', IL-17RA (268 bp) forward 5'-ATGGA-CAGTCGCAAGACAGGACGTTTTACCTG-3', RANKL (211 bp) forward 5'-AGAGAAAGCAGGAGTATGATGAG-3', GAPDH reverse 5'-TGGCTGCGAGTGAATTCAATGG-3', and GAPDH forward 5'-TGGCTGCGAGTGAATTCAATGG-3'.

**MTT assay**

The cells were seeded into 96-well cell culture plates at 4 × 10\textsuperscript{3} per well density. The next day, the cells were treated with the indicated concentrations of IL-17A for the indicated times. For the inhibitor studies, a myc inhibitor (50 μmol/L 10058-F4, Santa Cruz Biotechnology) or a STAT3 inhibitor (20 μmol/L AG490, Selleckchem) were used as pretreatments at 30 minutes before the IL-17A treatment and the cell proliferation was measured after 48 hours of IL-17A treatment. The cellular proliferation was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide (Sigma), according to the manufacturer’s protocol.

**Animal models**

IL-17A\textsuperscript{+} SCs were isolated from 3 patients with GCTB and transfected with indicated plasmid (IL-17A and short hairpin RNA of IL-17RA plasmid from Origene). Then, 2 × 10\textsuperscript{6} of IL-17A\textsuperscript{+} SCs were injected subcutaneously into the intrascapular region of the neck (8 nude mice/group). Nodules were excised 4 weeks after inoculation.

**Statistical analysis**

Kaplan–Meier survival analysis and χ\textsuperscript{2} test were used for calculating the recurrence rate of GCTB and the association of IL-17A expression with clinical parameters, respectively. All of the other results are expressed as the mean values ± SD and statistical significance was analyzed by unpaired Student t test or one-way ANOVA and Duncan’s multiple range tests using SAS statistical software package version 6.12 (SAS Institute). P ≤ 0.05 was considered to be statistically significant.

**Results**

**IL-17A is significantly increased in GCTB and correlates with progression**

Recent studies have shown that IL-17A is abundantly increased in several tumor types and such increased IL-17A levels stimulate tumor progression (17–19). Consistent with other tumors, the serum levels of IL-17A were significantly increased in patients with GCTB, compared with healthy individuals and the results similar to patients with rheumatoid arthritis (Fig. 1A). Because the T\textsubscript{i}17 cells release IL-17A, we measured the proportion of T\textsubscript{i}17 cells in the blood of patients with GCTB and healthy individuals, respectively. But there was no difference between patients with GCTB and healthy individuals (Fig. 1B). We also measured the IL-17A mRNA expression levels in GCTB, rheumatoid arthritis, and osteosarcoma tissues by qRT-PCR. Our results show that the IL-17A mRNA expression levels found in using GCTB tissues are similar to those in the rheumatoid arthritis tissues but higher than those in the osteosarcoma tissues (Fig. 1C). Next, we investigated the association of IL-17A expression with clinical parameters. In all 74 cases, we found that IL-17A is more frequently overexpressed in lesions (Campanacci grading system) and extracompartamental extension lesions (Enneking staging system; Table 2), suggesting that IL-17A might be involved in GCTB progression. Although no
statistical significance was observed in the recurrence rate between IL-17A-high and IL-17A-low patient groups, the recurrence rate tended to be higher in the IL-17A-over-expressing patients (Fig. 1D).

**IL-17A is predominantly produced by MNGCs, whereas IL-17RA is expressed by both MNGCs and SCs in GCTBs**

As mentioned above, GCTBs are composed of 3 different cell types and further, IL-17A induces signal transduction through its receptor. Thus, we next investigated which cell types expressed IL-17RA and secreted IL-17A in GCTB. As shown in Fig. 2A, IL-17A was abundantly and almost exclusively produced by the MNGCs. In contrast, IL-17RA was expressed by both the MNGCs and the stromal cells. These results were confirmed using cultured primary MNGCs and stromal cells by immunofluorescence staining and flow cytometric methods (Fig. 2B and C). The IL-17A mRNA was more significantly expressed in the MNGCs (Fig. 2D). We also detected high concentration of IL-17A in the conditioned media from the MNGCs compared with the stromal cells (Fig. 2D). Taken together, these data suggest that the main producer of IL-17A in GCTBs is the MNGCs, whereas both of the MNGCs and stromal cells express the receptor, are potential targets of IL-17A.

**IL-17A enhances the osteolytic ability of MNGCs and increases the expressions of IL-17A, MMP-9, and cathepsin K in MNGCs**

MNGCs were reported to act as osteoclast cells, directly causing the massive bone resorption observed in the GCTBs (2, 6, 20). On the basis of the simultaneous presence of IL-17A and IL-17RA on the MNGCs, we investigated the effects of IL-17A on the capacity of the MNGCs to resorb a mineralized matrix, measured with a “pit” formation assay using dentine slices. As shown in Fig. 3A, IL-17A treatment significantly increased the numbers and areas of the MNGC-mediated bone “pits”, compared with the control group. Moreover, we showed...
that IL-17A could increase the mRNA expression of MMP-9 and cathepsin K in the MNGCs (Fig. 3B). Interestingly, we further found that the expression of IL-17A in the MNGCs was also upregulated by IL-17A treatment (Fig. 3B). Collectively, these results suggest that IL-17A enhances the osteolytic ability of MNGCs, not only by increasing the expression of proosteolytic factors but also via an autocrine feedback loop by upregulating its own expression.

**IL-17A stimulates the proliferation and the expression of proosteolytic factors in SCs and promotes angiogenesis in GCTBs**

Recent studies have shown that IL-17A can affect cellular proliferation (21, 22). As expected, the proliferation rate of the IL-17RA⁺ SCs was increased in an IL-17A dose-dependent manner (Fig. 4A). Furthermore, such results were confirmed in xenograft models. Result show that tumor growth is stimulated by IL-17A overexpression; however, knockdown of IL-17RA suppressed IL-17A-stimulated tumor growth in IL-17RA⁺ SCs (Fig. 4B). In contrast, the IL-17A does not affect the proliferation of IL-17RA-negative stromal cells (IL-17RA⁻ SCs; Fig. 4A). In addition, our gene expression microarray results revealed that IL-17A treatment significantly increased the expression of several proosteolytic factors in the IL-17RA⁺ SCs, including RANKL, MMP-2, and MMP-9 (Supplementary Fig. S2A). Because RANKL plays a key role in osteolysis, we further confirmed the effects of IL-17A on RANKL expression at both the mRNA and protein levels and we observed the significantly increased expression of RANKL in the IL-17RA⁺ SCs (Fig. 4C). Notably, the IL-17RA⁻ SCs, which were collected from postsorting cultures and

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**Table 2.** The association of IL-17A expression with clinical parameters

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<tr>
<td>Grade III</td>
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*χ² test.

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**Figure 2.** IL-17A is primarily produced in MNGCs, and the IL-17RA is expressed in both MNGCs and stromal cells. A, immunohistochemical analysis shows that IL-17A is predominantly located in MNGCs, whereas IL-17RA is expressed in MNGCs and some stromal cells. A representative photograph of × 400; the white arrows indicate the MNGCs. B, expression of IL-17A and IL-17RA was assessed in the cultured primary MNGCs and stromal cells using immunofluorescence. C, IL-17A or IL-17RA⁺ positive cells were detected in MNGCs and stromal cells by flow cytometry analysis. The MNGCs and stromal cells were isolated from three patients with osteosarcoma with high IL-17A expression. For IL-17 detection, the cells were permeabilized. D, the IL-17A mRNA and protein levels were measured in the cultured primary cells and their cultured media. The mean values ± SD are shown. **, P < 0.001.
subjected to the same treatment and analysis as the IL-17RA⁺SCs, showed no statistically significant changes in any of the experiments (Fig. 4C). In addition to the proosteolytic factors, we also showed that the proangiogenic factor VEGF was increased by IL-17A treatment in IL-17RA⁺SCs (Supplementary Fig. S2A). Such results were also confirmed by the immunohistochemical analysis of the GCTB tissues. Our IHC results show that the VEGF expression and angiogenesis rates were significantly increased in the IL-17A high-expressing GCTB tissues compared with the IL-17A low-expressing GCTB tissues (Fig. 4D).

**IL-17A stimulates cellular proliferation and RANKL expression in IL-17RA⁺SCs through myc and STAT3 signaling, respectively**

We further investigated how IL-17A enhances RANKL expression and stromal cell proliferation. Previous studies have shown that IL-17A directly increases RANKL expression through IL-6 and STAT3 signaling (23). Consistent with those findings, our gene expression microarray results also showed that IL-17A increased IL-6 and STAT3 expression (Supplementary Fig. S2A). Furthermore, we used qRT-PCR and Western blotting confirmed that IL-17A induced STAT3 expression in primary cultured MNGCs (Fig. 4C). In addition, our results show that STAT3 inhibitor treatment can suppress IL-17A–induced RANKL expression in IL-17RA⁺SCs (Supplementary Fig. S3A). The gene array pathway analysis suggested that myc might be responsible for the IL-17A effects on SC proliferation (supplementary Fig. S2B). Indeed, the addition of a myc inhibitor to the culture of IL-17A–treated stromal cells in vitro effectively reduced cellular proliferation (Supplementary Fig. S3B). These data suggest that IL-17A stimulates RANKL expression and cellular proliferation through STAT3 and myc, respectively.

**Discussion**

In this study, we found that IL-17A was significantly increased in patients with GCTBs compared with healthy individuals (Fig. 1A and C). In addition, we showed that increased IL-17A in the GCTBs was from the abnormal secretion of IL-17A in the MNGCs (Fig. 2). In this study, we showed that increased IL-17A is closely correlated with the grade and extension of the GCTBs (Table 2). This result suggesting the IL-17A can be a prognostic marker for GCTB progression.

To identify which cell populations are the targets of IL-17A in the GCTBs, we investigated the expression of IL-17A in the GCTBs. In the IL-17A receptor family, receptor A is main receptor of IL-17A and plays an important regulatory role in expression of genes, including cytokines, chemokines, and tissue remodeling effectors (24). In this study, we identified that IL-17A is expressed by both the MNGCs and the stromal cells (Fig. 2A–C). Our results show that IL-17A might play an important role in GCTB by regulating the function of both MNGCs and stromal cells.

A hallmark of GCTB is its aggressive bone-lytic behavior (25). Both MNGCs and stromal cells are known to play a critical role in the osteolysis of GCTBs. In the GCTBs,
MNGCs exhibit a bone-resorbing function and many osteoclast properties, and they might exceed 50% of the total cell content of the tumor (26). Thus, we conducted a series of experiments to show whether and how does the IL-17A alters the bone-lytic behavior of MNGCs. IL-17A stimulation significantly enhanced the ability of the MNGCs bone resorption in vitro (Fig. 3A) and increased the production of osteolytic factors such as MMP-9 and cathepsin K (Fig. 3B). In the GCBTs, cathepsin K is the primary proteolytic enzyme, which largely degrades the organic components of bone (27). MMP-9 is an osteoclast bone matrix resorption enzyme that stimulates bone resorption by giant cells (28, 29). According to Zhang and colleagues report, the IL-17A induced the expression of cathepsin K and MMP-9 in osteoclasts and that the inhibition of these enzymes expression reduces the bone-resorption efficacy of the osteoclasts (30). These data suggest that IL-17A enhances the osteolytic ability of MNGCs by increasing the expression of MMP-9 and cathepsin K.

For next, we investigated the effects of IL-17A on stromal cell proliferation and the expression of proosteolytic factors. Although stromal cells are not directly responsible for resorptive activity, they can enhance the resorption ability of giant cells by expressing and secreting multiple key components of osteolysis (26, 31, 32). In addition, stromal cells represent the major neoplastic and proliferative component of GCTBs (31, 33) and the increased proliferation of stromal cells is closely correlated with the progression of GCTB (34). Our in vitro results revealed that IL-17A can stimulate the proliferation and expression of MMP-2, MMP-9, TGFβ-1, and RANKL in IL-17RA+SCs (Fig. 4A and B and Supplementary Fig. 5A). In vivo experiment also have shown that IL-17A strongly stimulates tumor growth (Fig. 4B). Previous studies have shown that MMP-2 (35), MMP-9...
(14), TGFβ-1 (36), and RANKL (32) are proosteolytic factors, these are secreted by stromal cells and significantly enhance the osteolytic ability of the MNGCs. In particular, RANKL is a key osteolytic regulator because it regulates giant cell formation, osteoclast differentiation, and bone resorption (37). In addition, Branstetter and colleagues reported that the RANKL-inhibitor denosumab induces tumor reduction and bone formation in patients with GCTB (25). Thus, using Western blot analysis further confirmed the increased expression of RANKL by IL-17A in stromal cells and observed the expression of RANKL was significantly increased by IL-17A in the IL-17RA+SCs (Fig. 4B). Apart from bone resorption, MMPs also play critical roles in tumor angiogenesis by increasing VEGF bioavailability (38, 39). Angiogenesis is well known to contribute to tumor progression, and VEGF signaling is essential for osteoclast formation (40). In this study, we demonstrated increased angiogenesis in the IL-17A–high patient (Fig. 4D). In addition, our in vitro experiment shows that IL-17A can significantly stimulate VEGF expression in stromal cells (Supplementary Fig. S2A). These results are consistent with other studies. For instance, Hashizume and colleagues reported that IL-17A could increase VEGF production in rheumatoid arthritis (23) and Numasaki and colleagues reported that IL-17A promotes tumor angiogenesis (41). Taken together, these data suggest that IL-17A stimulates GCTB progression by promoting angiogenesis and at least partially by promoting cellular proliferation and proosteolytic factor expression in stromal cells.

Finally, to elucidate the mechanism by which the MNGC-derived IL-17A regulates RANKL expression and enhances proliferation of IL-17RA+SCs in GCTBs, we conducted gene expression profiling and the gene array pathway analysis (Fig. 4B and Supplementary Fig. S2). Results suggest STAT3 might be involved the RANKL regulation. Using RT-PCR and Western blot analysis further showed that IL-17A could activate the STAT3 pathway and then regulate RANKL expression in the IL-17RA+SCs (Supplementary Fig. S3). This result is consistent with other reports. Hashizume and colleagues also reported that IL-17A could induce RANKL expression through STAT3 signaling (23). Moreover, through gene expression microarray analysis, we found the pathway by which IL-17A enhances stromal cell proliferation. Previous studies have shown that myc is involved in cancer growth. However, no information has been available for the IL-17A–mediated regulation of myc. In this study, we report that the IL-17A mediates stromal cell proliferation through myc signaling (Supplementary Fig. S3A).

The interactions between stromal cells and MNGC are essential for understanding the etiopathogenesis of GCTB (1). The majority of previous studies have focused on and elegantly profiled the mechanisms underlying how stromal cells regulate MNGCs (1). However, whether and how MNGCs regulate stromal cells remains poorly understood. Our current study has revealed the important finding that cytokines secreted by MNGCs can regulate the expression of RANKL expression and the proliferation of stromal cells.

In summary, our study, to the first of our knowledge, has delineated the association of IL-17A with the progression of GCTBs. IL-17A stimulates the progression of GCTBs by promoting angiogenesis, stromal cell proliferation, and osteolysis through increasing the expression of proosteolytic factors in both the MNGCs and the stromal cells. Our results suggest that IL-17A is a useful progression marker of GCTB and blocking IL-17A might represent a new therapeutic strategy for GCTB.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: M. Xu, C.-X. Xu, K.-X. Fan, Y. Wang

Development of methodology: M. Xu, Z.-G. Song, C.-X. Xu, K.-X. Fan, Y. Wang

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Xu, Z.-G. Song, C.-X. Xu, G.-H. Rong, K.-X. Fan, G. Han, W. Wang, W. Chai, Y. Wang

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Xu, Z.-G. Song, C.-X. Xu, K.-X. Fan, J.-Y. Chen, W. Zhang, J.-P. Jia, W. Chai, Y. Wang

Writing, review, and/or revision of the manuscript: M. Xu, C.-X. Xu, K.-X. Fan, Y. Wang

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Xu, Z.-G. Song, C.-X. Xu, G.-H. Rong, J.-Y. Chen, W. Zhang, W.-T. Liang, Y. Wang

Study supervision: C.-X. Xu, Y. Wang

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References


9. van Humburg JP, Asmaiwiidjaja PS, Davelaar N, Mus AMG, Colin EM, Hazes JM, et al. Th17 cells, but not Th1 cells, from patients with early

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rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. Arthritis Rheum 2011;63:73–83.


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