Biochemical and Biophysical Research Communications 444 (2014) 682–686

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Aberrant histone acetylation contributes to elevated interleukin-6 production in rheumatoid arthritis synovial fibroblasts

Takuma Tsuzuki Wada [a,b], Yasuto Araki [a,b,*], Kojiro Sato [a], Yoshimi Aizaki [a,b], Kazuhiro Yokota [a], Yoon Taek Kim [c], Hiromi Oda [c], Riki Kurokawa [d], Toshihide Mimura [a,b]

[a] Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University, Saitama 350-0495, Japan
[b] Division of Project, Research Center for Genomic Medicine, Saitama Medical University, Saitama 350-1241, Japan
[c] Department of Orthopaedic Surgery, Faculty of Medicine, Saitama Medical University, Saitama 350-0495, Japan
[d] Division of Gene Structure and Function, Research Center for Genomic Medicine, Saitama Medical University, Saitama 350-1241, Japan

ARTICLE INFO

Article history:
Received 29 January 2014
Available online 7 February 2014

Keywords:
Rheumatoid arthritis
Synovial fibroblasts
Interleukin-6
Epigenetics
Histone modifications
Curcumin

ABSTRACT

Accumulating evidence indicates that epigenetic aberrations have a role in the pathogenesis of rheumatoid arthritis (RA). However, reports on histone modifications are as yet quite limited in RA. Interleukin (IL)-6 is an inflammatory cytokine which is known to be involved in the pathogenesis of RA. Here we report the role of histone modifications in elevated IL-6 production in RA synovial fibroblasts (SFs). The level of histone H3 acetylation (H3ac) in the IL-6 promoter was significantly higher in RASFs than osteoarthritis (OA) SFs. This suggests that chromatin structure is in an open or loose state in the IL-6 promoter in RASFs. Furthermore, curcumin, a histone acetyltransferase (HAT) inhibitor, significantly reduced the level of H3ac in the IL-6 promoter, as well as IL-6 mRNA expression and IL-6 protein secretion by RASFs. Taken together, it is suggested that hyperacetylation of histone H3 in the IL-6 promoter induces the increase in IL-6 production by RASFs and thereby participates in the pathogenesis of RA.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease which is characterized by persistent synovitis and progressive joint destruction [1]. RA synovial fibroblasts (SFs) produce the inflammatory cytokine interleukin (IL)-6 that is important in the pathogenesis of RA [2]. IL-6 is a 21 kDa glycoprotein which consists of 184 amino acids and functions as a pleiotropic cytokine which is involved in the acute phase response and immune regulation [3]. IL-6 induces the activation and differentiation of T cells, immunoglobulin production in B cells, platelet maturation and the production of acute phase proteins, such as C-reactive protein, in hepatocytes. IL-6 deficient mice are resistant to arthritis induction [4]. The remarkable role of IL-6 in the pathogenesis of RA is supported by the striking therapeutic success of Tocilizumab, an IL-6 receptor inhibitor [5].

Multiple lines of evidence suggest that genetic and environmental factors participate in the pathogenesis of RA. Twin studies have shown that the concordance of monozygotic twins (12–15%) is higher than dizygotic twins (3%) in RA [6,7]. The heritability of RA was estimated to be 65% from these studies in twins [8]. A family study reported that the relative risk of RA was 3.0 in offspring, 4.6 in siblings and 6.4 in twins [9]. Serotyping studies demonstrated a significant association between RA and the HLA allele HLA-DRB1. HLA-DRB1 molecules contain a conserved amino acid sequence QKRAA/QRRAA, or "shared epitope", which may contribute to RA susceptibility [10]. Candidate gene studies and genome-wide association studies (GWAS) have identified approximately 60 susceptibility loci for RA [11]. On the other hand, environmental factors such as cigarette smoking, viral infection, *Porphyromonas gingivalis* and silica exposure are suggested to trigger RA [12–15] . However, in spite of these data, the etiological mechanisms of RA are not well understood.

Epigenetic mechanisms including histone modifications have been shown to determine chromatin assembly and influence gene transcription [16–18]. Histone amino-terminal tails are subject to covalent post-translational modifications such as acetylation,

Abbreviations: RA, rheumatoid arthritis; OA, osteoarthritis; SFs, synovial fibroblasts; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; H3ac, acetylation of histone H3; H3K4me3, tri-methylation of histone H3 lysine 4; HAT, histone acetyltransferase.

* Corresponding author at: Department of Rheumatology and Applied Immunology, Faculty of Medicine, Saitama Medical University, 38 Morohongo, Moroyama-chou, Iruma-gun, Saitama 350-0495, Japan. Fax: +81 49 295 4849.
E-mail address: arakiya@saitama-med.ac.jp (Y. Araki).

http://dx.doi.org/10.1016/j.bbrc.2014.01.195
0006-291X/

methylation, phosphorylation and ubiquitination [19]. The acetylation of histone H3 (H3ac) and tri-methylation of histone H3 lysine 4 (H3K4me3) are associated with the activation of gene transcription [20]. Recently, there have been increasing reports on epigenetic alterations in RA, although most of them have focused on DNA methylation or microRNAs (miRNA) [21]. We have hypothesized that another epigenetic gene-regulator, histone modification, is associated with the pathogenesis of RA. In the present study, we sought to clarify the role of histone modifications in the elevated IL-6 production that takes place in RASFs. The level of H3ac in the IL-6 promoter was significantly higher in RASFs than in osteoarthritis (OA) SFs. Additionally, curcumin, a histone acetyltransferase (HAT) inhibitor, decreased the level of H3ac in the IL-6 promoter, IL-6 mRNA and IL-6 protein secretion in RASFs.

2. Materials and methods

2.1. Materials

Anti-H3ac and an isotype control IgG were purchased from Upstate. Anti-H3K4me3 was obtained from Abcam. Human tumor necrosis factor (TNF)-α was purchased from Miltenyi Biotech. Sodium butyrate, phenyl methylsulfonyl fluoride (PMSF), micrococcal nuclease (MNase), and curcumin were obtained from Sigma.

2.2. Preparation and culture of SFs

Synovial tissues were obtained from RA and OA patients during total knee joint replacement at the Saitama Medical University Hospital. All of the RA patients fulfilled the American College of Rheumatology 1987 revised criteria for RA. This study was approved by the ethics committee of Saitama Medical University and a written informed consent was obtained from every patient in this study. Synovial tissues were minced into small pieces and incubated with 1.5 mg/ml collagenase and 0.04% hyaluronidase for 2 h at 37 °C as previously described [22]. After overnight culture, nonadherent cells were removed and SFs from passages 4 through 8 were used in this study.

2.3. Real-time quantitative RT-PCR (RT-qPCR)

Total RNA was extracted from SFs using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. 2 μg of total RNA were used to synthesize cDNA using SuperScript III reverse transcriptase (Invitrogen). PCR was conducted in 20 μl of total volume with 0.2 μM primers using Power SYBR Green PCR Master Mix (Applied Biosystems) for 40 cycles in a StepOne Plus Real-Time PCR System (Applied Biosystems). For standardization, 18s ribosomal RNA (18s rRNA) was amplified simultaneously. The sequences of the primers used are available upon request.

2.4. Enzyme-linked immunosorbent assay (ELISA)

SFs (1×10^5 cells) were cultured in DMEM containing 0.5% FBS in 6-well plates for 24 h. The supernatants were collected at 0, 24, 48, and 72 h after stimulation with 10 ng/ml TNF-α. The concentrations of the IL-6 protein were measured using an ELISA kit (PeproTech) according to the manufacturer's instructions and corrected by the cell number.

2.5. Chromatin immunoprecipitation (ChIP) assay

SFs (5×10^5 cells) were incubated with digestion buffer (50 mM Tris–HCl pH 7.6, 1 mM $CaCl_2$, 0.2% Triton X-100, 5 mM sodium butyrate and 0.5 mM PMSF) containing protease inhibitor cocktail (Roche) along with 0.2 U MNase for 10 min at 37 °C. The chromosomal DNA was sonicated using SONIFIER W-150 (Branson), dialyzed with RIPA buffer for 2 h at 4 °C, and incubated with Dynabeads Protein G (Invitrogen) and antibody (anti-H3ac or anti-H3K4me3) overnight at 4 °C. After treatment with 0.2 mg/ml RNase A and 1 mg/ml proteinase K, the immunoprecipitated DNA was used for analysis by real-time PCR using Taqman universal PCR master mix (Applied Biosystems). The sequences of the primers and the probe used are available upon request.

2.6. Treatment of RASFs with curcumin

RASFs were pretreated with 20 μM curcumin for 2 h and followed by stimulation with 10 ng/ml TNF-α. The cells were harvested at 8 h for the analyses using RT-qPCR and ChIP assay. The supernatants were replaced with DMEM containing 0.5% FBS at 8 h and collected at 24 h for ELISA.

2.7. Statistical analysis

The differences between the groups were determined by a Mann–Whitney *U* test or a Wilcoxson's signed rank test. All the results are expressed as the means ± SEM. A p value of <0.05 was defined as statistically significant.

3. Results

3.1. IL-6 mRNA expression was significantly higher in RASFs than in OASFs

Previous reports have shown that the inflammatory cytokine IL-6 is involved in the pathogenesis of RA [2]. We examined whether IL-6 production was increased in RASFs. We isolated SFs enzymatically from synovial tissues of RA and OA patients and compared IL-6 gene expression in RASFs and OASFs. IL-6 mRNA was significantly higher by 3.1-fold in RASFs than OASFs (Fig. 1A). GAPDH mRNA was not significantly different between RASFs and OASFs (Fig. 1A). ELISA was used to measure the IL-6 protein in supernatants secreted by RASFs and OASFs. Although not statistically significant, IL-6 protein production was higher in RASFs than in OASFs (Fig. 1B). These results indicate that IL-6 production is substantially enhanced in RASFs and may thus contribute to synovial inflammation in RA.

3.2. H3ac and H3K4me3 in the IL-6 promoter were significantly higher in RASFs than in OASFs

It has been demonstrated that epigenetic mechanisms, including histone modifications, alter chromatin structure and have an affect on trasnscriptional activity. To determine whether histone modifications in the IL-6 promoter were associated with elevated IL-6 gene expression in RASFs, we examined H3ac, which is correlated with active gene transcription, in the IL-6 promoter with a ChIP assay and quantitative PCR in both RASFs and OASFs. The level of H3ac was significantly higher in the proximal IL-6 promoter (from −101 to −16) in RASFs than OASFs, whereas that of H3ac was comparable in the distal IL-6 promoter (from −586 to −528) (Fig. 2A). The level of H3ac was not significantly different in the GAPDH promoter between RASFs and OASFs. (Fig. 2C). We wondered whether other active histone marker profiles were similar to H3ac in the IL-6 promoter in RASFs. We subsequently found that the level of H3K4me3 was significantly higher in the proximal and distal IL-6 promoters in RASFs than OASFs (Fig. 2B). The levels of H3ac and H3K4me3 were higher in the proximal IL-6 promoter than in the distal IL-6 promoter in RASFs. The level of H3K4me3

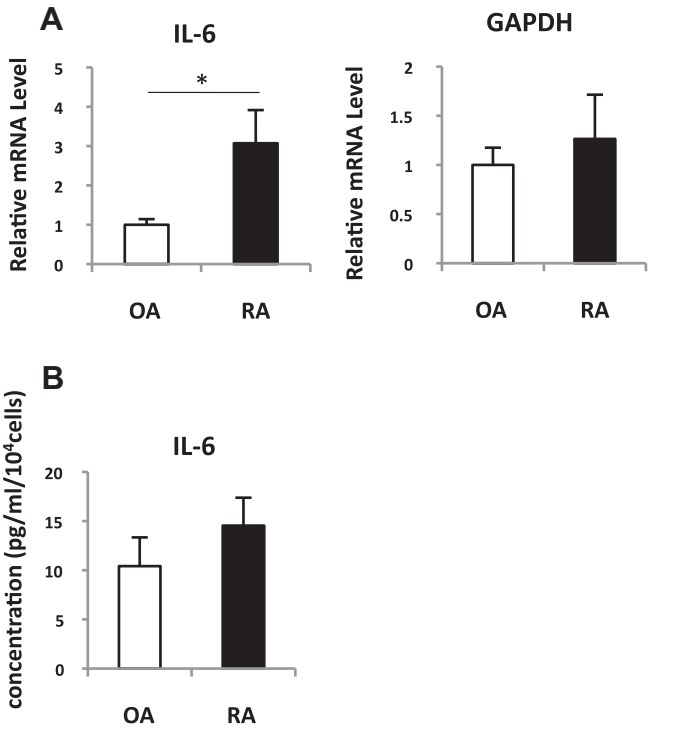

Fig. 1. IL-6 production increases in RASFs. (A) IL-6 and GAPDH mRNAs were examined by quantitative RT-PCR in RASFs ($n = 19$) and OASFs ($n = 21$). Values were expressed as the fold increase versus the value in OASFs. (B) The IL-6 protein in the supernatant of RASFs ($n = 10$) and OASFs ($n = 11$) was measured by ELISA. Values were expressed as the concentration (pg/ml) per 10^4 cells. The data are the means ± SEM. $^*p < 0.05$ (Mann–Whitney U test).

was not significantly different in the GAPDH promoter (Fig. 2D). These results suggest that the chromatin structure is open in the IL-6 promoter, resulting in IL-6 mRNA elevation in RASFs.

3.3. IL-6 mRNA expression in RASFs significantly increased after stimulation with TNF-α

We considered that the high levels of H3ac and H3K4me3 in the IL-6 promoter affected the IL-6 gene expression after activation in RASFs. Therefore, we investigated whether IL-6 transcription was activated in response to TNF-α in RASFs. IL-6 mRNA significantly increased at 4, 24 and 72 h after incubation with 10 ng/ml TNF-α in RASFs compared with OASFs (Fig. 3A), while GAPDH mRNA did not change after stimulation with TNF-α (Fig. 3A). IL-6 protein secretion also significantly increased at 24 and 72 h after stimulation with TNF-α in RASFs compared with OASFs (Fig. 3B). These data imply that the open chromatin in the IL-6 promoter results in robust IL-6 production by RASFs in response to TNF-α.

3.4. Curcumin significantly reduced H3ac in the IL-6 promoter and IL-6 production in RASFs

It is reasonable to expect that the high levels of H3ac in the IL-6 promoter would be associated with the elevated IL-6 production in RASFs. Histone acetylation is increased by the catalytic activity of HATs, including the CREB binding protein (CBP)/p300. Curcumin, a yellow pigment found in turmeric, specifically inhibits CBP/p300 [23]. It has been reported that curcumin reduces histone acetylation with a consequent decrease of gene expression [24]. We thus examined whether curcumin decreased the level of H3ac in the proximal IL-6 promoter after TNF-α stimulation in RASFs. In curcumin-pretreated RASFs, the level of H3ac in the IL-6 proximal promoter was significantly decreased at 8 h after stimulation with 10 ng/ml TNF-α (Fig. 4A). Correspondingly, pretreatment of RASFs by curcumin significantly reduced IL-6 mRNA expression and protein secretion after TNF-α stimulation (Fig. 4B and C). On the other hand, in curcumin-pretreated RASFs, the level of H3ac in the GAPDH promoter and GAPDH mRNA did not change after TNF-α stimulation (Fig. 4A and B). These results suggest that

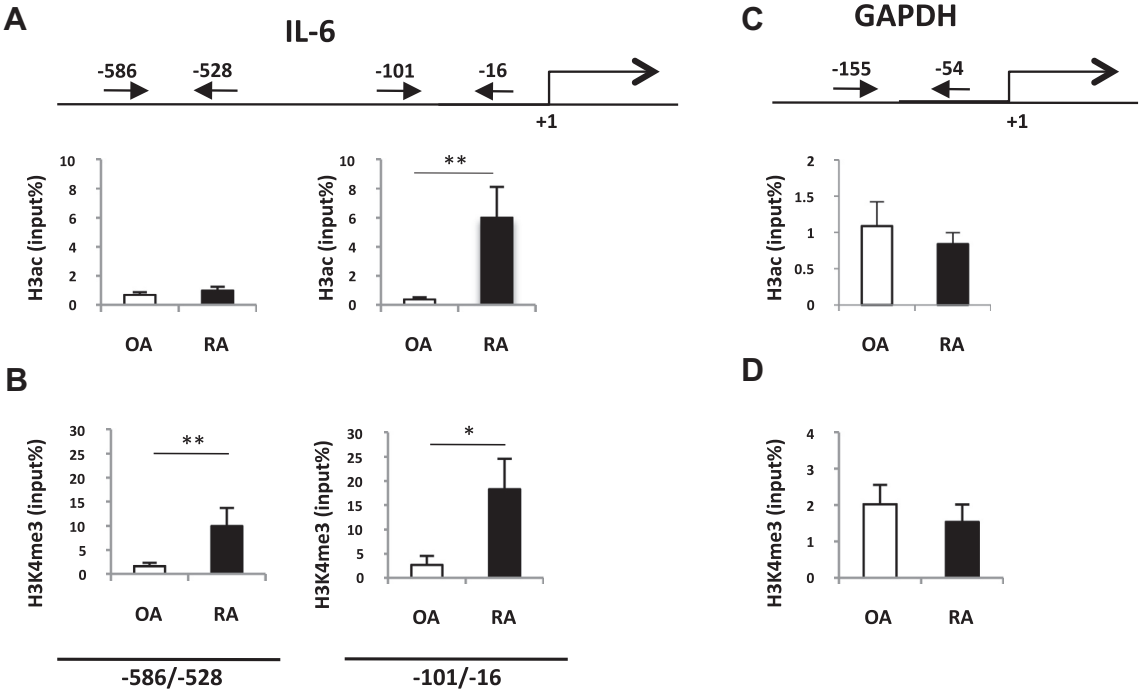

Fig. 2. The levels of H3ac and H3K4me3 increase in the IL-6 promoter in RASFs. (A and B) The levels of H3ac (A) and H3K4me3 (B) in the proximal (from −586 to −528) and distal (from −101 to −16) IL-6 promoter were analyzed by ChIP and quantitative PCR in RASFs (H3ac: $n = 11$; H3K4me3: $n = 7$) and OASFs (H3ac: $n = 11$; H3K4me3: $n = 6$). (C and D) The levels of H3ac (C) and H3K4me3 (D) in the GAPDH promoter were analyzed by ChIP and quantitative PCR in RASFs (H3ac: $n = 11$; H3K4me3: $n = 7$) and OASFs (H3ac: $n = 11$; H3K4me3: $n = 6$). The data are the means ± SEM. $^*p < 0.05$, $^{**}p < 0.01$ (Mann–Whitney U test).

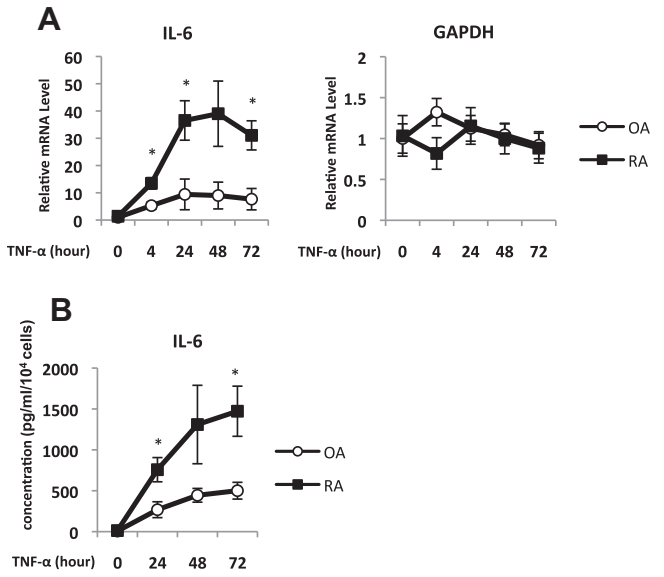

Fig. 3. TNF-α enhances IL-6 production in RASFs. (A) IL-6 and GAPDH mRNAs were examined at 0, 4, 24, 48, and 72 h after stimulation with 10 ng/ml TNF-α by quantitative RT-PCR in RASFs ($n = 8$) and OASFs ($n = 6$). The values are expressed as the fold increase versus the value in OASFs at 0 h. (B) The concentrations of the IL-6 protein in the supernatant of RASFs ($n = 7$) and OASFs ($n = 8$) were measured by ELISA at 0, 24, 48 and 72 h after stimulation with 10 ng/ml TNF-α. Values are expressed as the concentration (pg/ml) per 10^4 cells. The data are the means ± SEM. $^*p < 0.05$ (Mann–Whitney *U* test).

high level of H3ac in the IL-6 promoter specifically increases IL-6 production in RASFs.

4. Discussion

This study shows that increased H3ac in the IL-6 promoter was associated with elevated IL-6 production in RASFs. The chromatin structure was in an open conformation in the IL-6 promoter and IL-6 gene transcription was highly responsive to TNF-α. Curcumin decreased the level of H3ac in the IL-6 promoter and suppressed IL-6 production in RASFs. These results suggest that hyperacetylation of histone H3 in the IL-6 promoter contributes to both constitutive and TNF-α-induced IL-6 production by RASFs. Taken together, epigenetic processes are evidently involved in the pathogenesis of RA and thus may serve as potential targets for the development of RA therapeutics.

Gene transcription depends on the nuclear recruitment of transcription factors and the accessibility of transcription factors to genomic DNA. The latter is determined by epigenetic mechanisms which generate conformational changes in chromatin. NF-κB and p38 MAPK have been shown to bind to the IL-6 promoter and regulate IL-6 gene transcription in RASFs [25,26]. The present study shows that active histone modifications such as H3ac and H3K4me3 are present in the IL-6 promoter of RASFs and that IL-6 mRNA expression and protein production are increased by stimulation with TNF-α. These results imply that decondensation of the chromatin structure results in the increased recruitment of transcription factors such as NF-κB and p38 MAPK to the IL-6 promoter, resulting in an increase in transcription of the IL-6 gene.

DNA methylation and RNA interference by miRNA are well known as epigenetic mechanisms other than histone modifications. Accumulating evidence has shown that DNA methyation and miRNA are involved in the pathogenesis of RA. Global genomic DNA hypomethylation and low DNA (cytosine-5-)-methyltransferase 1 (DNMT1) expression provide RASFs with an active and aggressive phenotype [27]. DNA hypermethylation in the DR3 promoter has

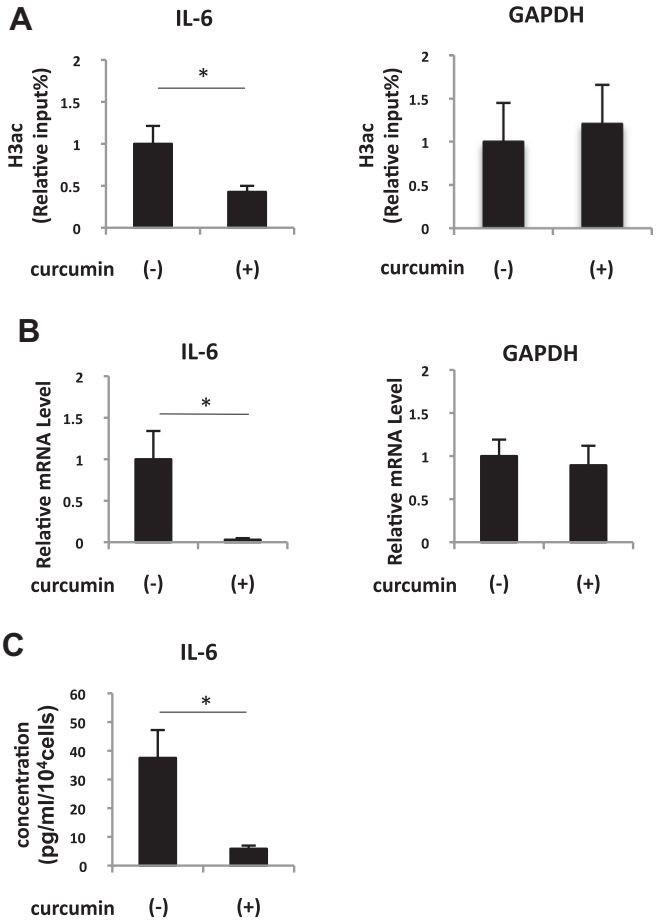

Fig. 4. Curcumin decreases the level of H3ac in the IL-6 promoter and IL-6 production in RASFs. (A and B) RASFs ($n = 5$) were pretreated with or without 20 μM curcumin, stimulated with 10 ng/ml TNF-α, and harvested at 8 h. The levels of H3ac in the IL-6 and GAPDH promoters (A) and IL-6 and GAPDH mRNA (B) were examined by quantitative PCR. Values were expressed as the fold increase versus the value in curcumin-untreated RASFs. (C) RASFs ($n = 8$) were pretreated with or without 20 μM curcumin and stimulated with 10 ng/ml TNF-α. The supernatants were replaced with DMEM containing 0.5% FBS at 8 h and collected at 24 h. The concentrations of IL-6 protein in the supernatant were measured by ELISA. Values are expressed as the concentration (pg/ml) per 10^4 cells. The data are the means ± SEM. $^*p < 0.05$ (Wilcoxson's signed rank test).

been shown to result in resistance to apoptosis in RASFs [28]. Genome-wide analyses have identified differentially methylated genes that are suggested to contribute to RASF phenotypes [29,30] . In addition, miR-155 and miR-146a are also highly expressed in RASFs [31]. The same group showed that expression of miR-203 was higher in RASFs than in OASFs and that miR-203 increased MMP-1 and IL-6 production in RASFs [32]. Compared with DNA methylation and miRNA, the studies of histone modifications have been quite limited in RA. Histone methyltransferase enhancer of zeste homolog 2 (EZH2), which induces tri-methylation of histone 3 lysine 27 (H3K27me3), is overexpressed in RASFs and secreted fizzled-related protein 1 (SFRP1) has been identified as a target gene of EZH2 [33]. Here we report aberrant histone modifications in the IL-6 promoter in RASFs. This is the first report of increased active histone modification markers in the IL-6 promoter in RASFs.

The balance of activities between HATs and histone deacetylases (HDACs) was shown to be shifted toward histone hyperacetylation in RA synovial tissues [34]. Our data also showed hyperacetylation in the IL-6 promoter in RASFs. TNF-α-induced IL-6 gene expression is regulated by a transcriptional complex which consists of CBP/p300 [35]. Therefore we stimulated RASFs with TNF-α after treatment with curcumin which specifically

inhibited CBP/p300, resulting in the decrease in the level of H3ac in the IL-6 promoter, IL-6 mRNA expression and IL-6 protein secretion. In the past few years, the introduction of anti-rheumatic biologics blocking the effects of key inflammatory cytokines, such as TNF-α or IL-6, have dramatically improved the outcomes in RA [36]. However biologics do have several problems, including limited efficacy, a high risk of infection and enormous cost. Therefore, alternative treatments have been sought and epigenetic mechanisms are promising targets. Recently HAT inhibitors have received attention to as a novel epigenetic therapeutic for RA. Curcumin has been shown to exert an anti-inflammatory effect in both murine arthritis models [37,38] and RASFs [39]. A randomized pilot study suggested the efficacy and safety of curcumin in RA patients [40]. Curcumin is thus an attractive candidate for treating RA.

In conclusion, increased histone acetylation conferred elevated IL-6 production on RASFs. Furthermore, the decrease in H3ac reduced both IL-6 mRNA expression and IL-6 protein secretion in RASFs. The results suggest that epigenetic dysregulation is involved in the pathogenesis of RA. Epigenetic mechanisms are implicated to be a promising target for RA therapeutics.

Acknowledgments

This work was supported in part by JSPS KAKENHI Grant Nos. 22591083 (to T.M.), 22890172 (to Y.A.), and 23790544 (to Y.A.) and by a Grant from Ochiai Memorial Award 2013 (to T.T.W.). We gratefully acknowledge Toshiko Ishibashi, Natsuko Kurosawa, and Yukari Yamada for their technical assistance.

References

[1] B. Bartok, G.S. Firestein, Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis, Immunol. Rev. 233 (2010) 233–255.
[2] T. Hirano, T. Matsuda, M. Turner, N. Miyasaka, G. Buchan, B. Tang, K. Sato, M. Shimizu, R. Maini, M. Feldmann, et al., Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis, Eur. J. Immunol. 18 (1988) 1797–1801.
[3] T. Kishimoto, The biology of interleukin-6, Blood 74 (1989) 1–10.
[4] S. Ohshima, Y. Saeki, T. Mima, M. Sasai, K. Nishioka, S. Nomura, M. Kopf, Y. Katada, T. Tanaka, M. Suemura, T. Kishimoto, Interleukin 6 plays a key role in the development of antigen-induced arthritis, Proc. Natl. Acad. Sci. USA 95 (1998) 8222–8226.
[5] T. Tanaka, A. Ogata, M. Narazaki, Tocilizumab for the treatment of rheumatoid arthritis, Expert Rev. Clin. Immunol. 6 (2010) 843–854.
[6] K. Aho, M. Koskenvuo, J. Tuominen, J. Kaprio, Occurrence of rheumatoid arthritis in a nationwide series of twins, J. Rheumatol. 13 (1986) 899–902.
[7] A.J. Silman, A.J. MacGregor, W. Thomson, S. Holligan, D. Carthy, A. Farhan, W.E. Ollier, Twin concordance rates for rheumatoid arthritis: results from a nationwide study, Br. J. Rheumatol. 32 (1993) 903–907.
[8] A.J. MacGregor, H. Snieder, A.S. Rigby, M. Koskenvuo, J. Kaprio, K. Aho, A.J. Silman, Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins, Arthritis Rheum. 43 (2000) 30–37.
[9] K. Hemminki, X. Li, J. Sundquist, K. Sundquist, Familial associations of rheumatoid arthritis with autoimmune diseases and related conditions, Arthritis Rheum. 60 (2009) 661–668.
[10] P.K. Gregersen, J. Silver, R.J. Winchester, The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis, Arthritis Rheum. 30 (1987) 1205–1213.
[11] S. Viatte, D. Plant, S. Raychaudhuri, Genetics and epigenetics of rheumatoid arthritis, Nat. Rev. Rheumatol. 9 (2013) 141–153.
[12] L. Klareskog, L. Padyukov, L. Alfredsson, Smoking as a trigger for inflammatory rheumatic diseases, Curr. Opin. Rheumatol. 19 (2007) 49–54.
[13] C. Munz, J.D. Lunemann, M.T. Getts, S.D. Miller, Antiviral immune responses: triggers of or triggered by autoimmunity?, Nat Rev. Immunol. 9 (2009) 246–258.
[14] N. Wegner, R. Wait, A. Sroka, S. Eick, K.A. Nguyen, K. Lundberg, A. Kinloch, S. Culshaw, J. Potempa, P.J. Venables, Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis, Arthritis Rheum. 62 (2010) 2662–2672.
[15] P. Stolt, H. Kallberg, I. Lundberg, B. Sjogren, L. Klareskog, L. Alfredsson, E. s. group, Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study, Ann. Rheum. Dis. 64 (2005) 582–586.
[16] B. Li, M. Carey, J.L. Workman, The role of chromatin during transcription, Cell 128 (2007) 707–719.
[17] Y. Araki, Z. Wang, C. Zang, W.H. Wood 3rd, D. Schones, K. Cui, T.Y. Roh, B. Lhotsky, R.P. Wersto, W. Peng, K.G. Becker, K. Zhao, N.P. Weng, Genome-wide analysis of histone methylation reveals chromatin state-based regulation of gene transcription and function of memory CD8+ T cells, Immunity 30 (2009) 912–925.
[18] N.P. Weng, Y. Araki, K. Subedi, The molecular basis of the memory T cell response: differential gene expression and its epigenetic regulation, Nat. Rev. Immunol. 12 (2012) 306–315.
[19] S.L. Berger, The complex language of chromatin regulation during transcription, Nature 447 (2007) 407–412.
[20] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[21] K. Klein, C. Ospelt, S. Gay, Epigenetic contributions in the development of rheumatoid arthritis, Arthritis Res. Ther. 14 (2012) 227.
[22] K. Yokota, T. Miyazaki, H. Hemmatazad, R.E. Gay, C. Kolling, U. Fearon, H. Suzuki, T. Mimura, S. Gay, C. Ospelt, The pattern-recognition receptor nucleotide-binding oligomerization domain-containing protein 1 promotes production of inflammatory mediators in rheumatoid arthritis synovial fibroblasts, Arthritis Rheum. 64 (2012) 1329–1337.
[23] K. Balasubramanyam, R.A. Varier, M. Altaf, V. Swaminathan, N.B. Siddappa, U. Ranga, T.K. Kundu, Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription, J. Biol. Chem. 279 (2004) 51163–51171.
[24] Y. Araki, M. Fann, R. Wersto, N.P. Weng, Histone acetylation facilitates rapid and robust memory CD8 T cell response through differential expression of effector molecules (eomesodermin and its targets: perforin and granzyme B), J. Immunol. 180 (2008) 8102–8108.
[25] C. Georganas, H. Liu, H. Perlman, A. Hoffmann, B. Thimmapaya, R.M. Pope, Regulation of IL-6 and IL-8 expression in rheumatoid arthritis synovial fibroblasts: the dominant role for NF-kappa B but not C/EBP beta or c-Jun, J. Immunol. 165 (2000) 7199–7206.
[26] M. Suzuki, T. Tetsuka, S. Yoshida, N. Watanabe, M. Kobayashi, N. Matsui, T. Okamoto, The role of p38 mitogen-activated protein kinase in IL-6 and IL-8 production from the TNF-alpha- or IL-1beta-stimulated rheumatoid synovial fibroblasts, FEBS Lett. 465 (2000) 23–27.
[27] E. Karouzakis, R.E. Gay, B.A. Michel, S. Gay, M. Neidhart, DNA hypomethylation in rheumatoid arthritis synovial fibroblasts, Arthritis Rheum. 60 (2009) 3613–3622.
[28] N. Takami, K. Osawa, Y. Miura, K. Komai, M. Taniguchi, M. Shiraishi, K. Sato, T. Iguchi, K. Shiozawa, A. Hashiramoto, S. Shiozawa, Hypermethylated promoter region of DR3, the death receptor 3 gene, in rheumatoid arthritis synovial cells, Arthritis Rheum. 54 (2006) 779–787.
[29] K. Nakano, J.W. Whitaker, D.L. Boyle, W. Wang, G.S. Firestein, DNA methylome signature in rheumatoid arthritis, Ann. Rheum. Dis. 72 (2013) 110–117.
[30] J.W. Whitaker, R. Shoemaker, D.L. Boyle, J. Hillman, D. Anderson, W. Wang, G.S. Firestein, An imprinted rheumatoid arthritis methylome signature reflects pathogenic phenotype, Genome Med. 5 (2013) 40.
[31] J. Stanczyk, D.M. Pedrioli, F. Brentano, O. Sanchez-Pernaute, C. Kolling, R.E. Gay, M. Detmar, S. Gay, D. Kyburz, Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis, Arthritis Rheum. 58 (2008) 1001–1009.
[32] J. Stanczyk, C. Ospelt, E. Karouzakis, A. Filer, K. Raza, C. Kolling, R. Gay, C.D. Buckley, P.P. Tak, S. Gay, D. Kyburz, Altered expression of microRNA-203 in rheumatoid arthritis synovial fibroblasts and its role in fibroblast activation, Arthritis Rheum. 63 (2011) 373–381.
[33] M. Trenkmann, M. Brock, R.E. Gay, C. Kolling, R. Speich, B.A. Michel, S. Gay, L.C. Huber, Expression and function of EZH2 in synovial fibroblasts: epigenetic repression of the Wnt inhibitor SFRP1 in rheumatoid arthritis, Ann. Rheum. Dis. 70 (2011) 1482–1488.
[34] L.C. Huber, M. Brock, H. Hemmatazad, O.T. Giger, F. Moritz, M. Trenkmann, J.H. Distler, R.E. Gay, C. Kolling, H. Moch, B.A. Michel, S. Gay, O. Distler, A. Jungel, Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients, Arthritis Rheum. 56 (2007) 1087–1093.
[35] W. Vanden Berghe, K. De Bosscher, E. Boone, S. Plaisance, G. Haegeman, The nuclear factor-kappaB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter, J. Biol. Chem. 274 (1999) 32091–32098.
[36] C. Scheinecker, K. Redlich, J.S. Smolen, Cytokines as therapeutic targets: advances and limitations, Immunity 28 (2008) 440–444.
[37] S.H. Mun, H.S. Kim, J.W. Kim, N.Y. Ko, K. Kim do, B.Y. Lee, B. Kim, H.S. Won, H.S. Shin, J.W. Han, H.Y. Lee, Y.M. Kim, W.S. Choi, Oral administration of curcumin suppresses production of matrix metalloproteinase (MMP)-1 and MMP-3 to ameliorate collagen-induced arthritis: inhibition of the PKCdelta/JNK/c-Jun pathway, J. Pharmacol. Sci. 111 (2009) 13–21.
[38] G. Huang, Z. Xu, Y. Huang, X. Duan, W. Gong, Y. Zhang, J. Fan, F. He, Curcumin protects against collagen-induced arthritis via suppression of BAFF production, J. Clin. Immunol. 33 (2013) 550–557.
[39] C. Park, D.O. Moon, I.W. Choi, B.T. Choi, T.J. Nam, C.H. Rhu, T.K. Kwon, W.H. Lee, G.Y. Kim, Y.H. Choi, Curcumin induces apoptosis and inhibits prostaglandin E(2) production in synovial fibroblasts of patients with rheumatoid arthritis, Int. J. Mol. Med. 20 (2007) 365–372.
[40] B. Chandran, A. Goel, A randomized, pilot study to assess the efficacy and safety of curcumin in patients with active rheumatoid arthritis, Phytother. Res. 26 (2012) 1719–1725.