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Altered gene expression profiles of histone lysine methyltransferases and demethylases in rheumatoid arthritis synovial fibroblasts

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ABSTRACT

Objective. *Aberrant histone lysine methylation (HKM) has been reported in rheumatoid arthritis (RA) synovial fibroblasts (SFs). As histone lysine methyltransferases (HKMTs) and demethylases (HKDMs) regulate HKM, these enzymes are believed to be dysregulated in RASFs. The aim of this study is to clarify whether gene expressions of HKMTs and HKDMs are altered in RASFs.*

Methods. *SFs were isolated from synovial tissues obtained from RA or osteoarthritis (OA) patients during total knee joint replacement. The mRNA levels of 34 HKMTs and 22 HKDMs were examined after stimulation with tumour necrosis factor α (TNF-α) in RASFs and OASFs.*

Results. *The gene expression of the 12 HKMTs, including MLL1, MLL3, SUV39H1, SUV39H2, PRDM2, EZH2, SETD2, NSD2, NSD3, SMYD4, DOT1, and PR-set7, that catalyse the methylation of H3K4, H3K9, H3K27, H3K36, H3K79, or H4K20 was higher after TNFα stimulation in RASFs vs. OASFs. The gene expression of the 4 HKDMs, including FBXL10, NO66, JMJD2D, and FBXL11, that catalyse the methylation of H3K4, H3K9, or H3K36 was higher after TNFα stimulation in RASFs vs. OASFs.*

Conclusion. *The study findings suggest that the HKM-modifying enzymes are involved in the alteration of HKM, which results in changes in the gene expression of RASFs.*

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation that results in progressive joint destruction and is difficult to treat effectively (1). RA synovial fibroblasts (SFs), which are also called fibroblast-like synoviocytes (FLS), maintain an activated and aggressive phenotype and play a central role in the aetiology of RA (2). Recent advances have revealed that epigenetic changes are associated with the pathogenesis of RA (3, 4). Epigenetic mechanisms, such as histone modifications, determine chromatin structure and consequently influence gene transcription without any change in the DNA sequence itself (5). We recently reported that compared with osteoarthritis (OA) SFs, RASFs exhibit altered profiles of histone lysine methylation (HKM), including trimethylation of lysine 4 at histone H3 (H3K4me3) and H3K27me3, in some of the genes of the matrix metalloproteinases that are known to be key pathogenic matrix-degrading enzymes (6, 7). OA is an age-related joint degenerative disease that results in pain and disability of the affected joints (8). However, little is currently known about the mechanisms involved in the dysregulation of HKM in RASFs.

HKM is catalysed by histone lysine methyltransferases (HKMTs) or histone lysine demethylases (HKDMs), which methylate or demethylate particular lysine residues in the histone tails, respectively (9). Methylation of H3K4, H3K36, and H3K79 is associated with gene activation, whereas methylation of H3K9, H3K27, and H4K20 is associated with gene repression (10). Our current study investigated the gene expression of HKMTs and HKDMs in RASFs in order to address whether the HKM-modifying enzymes are involved in the dysregulation of HKM.

Materials and methods

Patients and the isolation of SFs

Human synovial tissues were obtained from 7 RA and 7 OA patients during total knee joint replacement at the Saitama Medical University Hospital as previously described (6, 11). All of the RA patients fulfilled the 1987 American College of Rheumatology revised criteria for the diagnosis of RA. This study was approved by the Ethics Committee of Saitama Medical University. Written informed consent was obtained from every patient and all samples were rendered anonymous. After digestion of the synovial tissues, cultured cells (SFs) from passages 4 through 8 were used for the following experiments.

Treatment of SFs with tumour necrosis factor α (TNF-α)

SFs were stimulated with 10 ng/ml recombinant human TNF-α (Peprotech, Rocky Hill, NJ, USA) and cultured for the designated times prior to the quantitative RT-PCR analysis.

Quantitative real-time RT-PCR

This study used the previously reported standard procedure for all of the quantifications (12, 13). Briefly, total RNA was extracted from the SFs with an RNeasy kit (Qiagen, Tokyo, Japan) and then reverse-transcribed using Superscript III (ThermoFisher Scientific, Kanagawa, Japan) to synthesise cDNA. PCR was performed using Power SYBR Green PCR Master Mix (ThermoFisher Scientific) for 35 cycles in the StepOnePlus Real-Time PCR System (ThermoFisher Scientific). All PCR results were normalised to *18SrRNA*. The PCR primers were designed using MacVector software (MacVector, Apex, NC, USA) and made by ThermoFisher Scientific. Supplementary Tables I and II show the primers used in the study. All reactions were performed in duplicate.

Statistical analysis

The differences between the groups were evaluated by a Mann-Whitney *U*-test. All data are presented as the mean ± SEM. A p-value <0.05 was considered statistically significant. All analyses were performed using JMP 6.0 Software (SAS Institute, Tokyo, Japan).

Results

HKMT gene expression in RASFs

To address which of the HKMT gene expressions were altered in the RASFs, we investigated the mRNA levels of 34 human HKMTs (listed below) after TNF-α stimulation (Fig. 1 and Supplementary Fig. 1).

H3K4. H3K4 is methylated by the KMT2 family (MLL1, MLL2, MLL3, MLL4, MLL5, SET1A, SET1B, and ASH1L) and the KMT7 family (SET7/9), as well as by SMYD3 and PRDM9. *MLL1* and *MLL3* gene expressions were significantly higher at 24, and 48 h, respectively, after TNF-α stimulation in the RASFs *versus* the OASFs.

H3K9. H3K9 is methylated by the KMT1 family (SUV39H1, SUV39H2, G9A, GLP, SETDB1, and SETDB2) as well as by PRDM1, PRDM2, and PRDM4. *SUV39H1*, *SUV39H2*, and *PRDM2* gene expressions were significantly higher at 48, 24 and 48, and 8h, respectively, after the TNF-α stimulation in the RASFs *versus* the OASFs.

H3K27. H3K27 is methylated by the KMT6 family (EZH1 and EZH2). *EZH2* gene expression was significantly higher at 48 h after the TNF-α stimulation in the RASFs *versus* the OASFs.

H3K36. H3K36 is methylated by the KMT3 family (SETD2 and NSD1) as well as by NSD2, NSD3, SMYD1, SMYD2, SMYD3, SMYD4, and SMYD5. *SETD2*, *NSD2*, *NSD3*, and *SMYD4* gene expressions were significantly higher at 8 and 24, 48, 24, and 24 and 48 h, respectively, after the TNF-α stimulation in the RASFs *versus* the OASFs.

H3K79. Gene expression of the H3K79-catalysing KMT4 family, *DOT1*, was significantly higher at 24 h after the TNF-α stimulation in the RASFs *versus* the OASFs.

H4K20. H4K20 is methylated by the KMT5 family (PR-Set7, SUV4-20H1, and SUV4-20H2) and the KMT7 family (SET7/9). *PR-Set7* gene expression was significantly higher at 48 h after the TNF-α stimulation in the RASFs *versus* the OASFs.

HKDM gene expression in RASFs

To clarify which of the HKDM gene expressions were altered in the RASFs, we examined the mRNA levels of 22 human HKDMs (listed below) after the TNF-α stimulation (Fig. 2 and Supplementary Fig. 2).

H3K4. H3K4 is demethylated by the KDM1 family (LSD1 and AOF1), the KDM2 family (FBXL10), and the KDM5 family (JARID1A, JARID1B, JARID1C, and JARID1D) as well as by JARID2 and NO66. *FBXL10* and *NO66* gene expressions were significantly higher at 48, and 24 and 48 h, respectively, after the TNF-α stimulation in the RASFs *versus* the OASFs.

H3K9. H3K9 is demethylated by the KDM1 family (LSD1), the KDM3 family (JMJD1A and JMJD1B), and the KDM4 family (JMJD2A, JMJD2B, JMJD2C, and JMJD2D) as well as by JMJD1C, PHF8, and JHDM1D. *JMJD2D* gene expression was significantly higher at 48 h after the TNF-α stimulation in the RASFs *versus* the OASFs.

H3K27. Gene expressions of the H3K27-catalysing HKDMs, which included the KDM6 family (*UTX* and *JMJD3*), *UTY*, and *JHDM1D*, were comparable in the RASFs and the OASFs.

H3K36. H3K36 is demethylated by the KDM2 family (FBXL10 and FBXL11), the KDM4 family (JMJD2A, JMJD2B, and JMJD2C), and NO66. *FBXL10*, *FBXL11*, and *NO66* gene expressions were significantly higher at 48, 48, and 24 and 48 h, respectively, after the TNF-α stimulation in the RASFs *versus* the OASFs.

H4K20. Gene expression of the H4K20-catalysing HKDM, *PHF8*, was similar in the RASFs and the OASFs.

Discussion

The present study demonstrated that gene expression of HKMTs and HKDMs dynamically changed after activation in the RASFs. We identified 12 out of 34 HKMTs and 4 out of 22 HKDMs in which the gene expression was upregulated after TNF-α stimulation in the RASFs. Examination of the gene expressions for these enzymes in the SFs from a normal donor revealed that the expression was lower than that observed in the RASFs (data not shown). It has been reported that EZH2, which is an H3K27-catalysing HMT, was highly expressed in RASFs (14). Our current data are consistent with the findings of this study. Unexpectedly, we found that the dysregulated HKM-modifying enzymes catalysed most of the histone lysine residues in the RASFs. However, the gene expression of HKDMs that only catalyse H3K27 and H4K20, both of which are repressive histone markers, was not altered in the RASFs. This suggests that various HKMs are involved in the pathogenesis of RA.

The gene expression of the altered HKM-modifying enzymes was only upregulated after stimulation with TNF-α in the RASFs. Since SFs were cultured without any cytokines *in vitro* after isolation from the synovial tissues, the gene expression of the HKM-modifying enzymes might have been lower than that which would be expected in the presence of cytokines. Thus, it is possible that exposure to TNF-α leads to the upregulation of the expres-

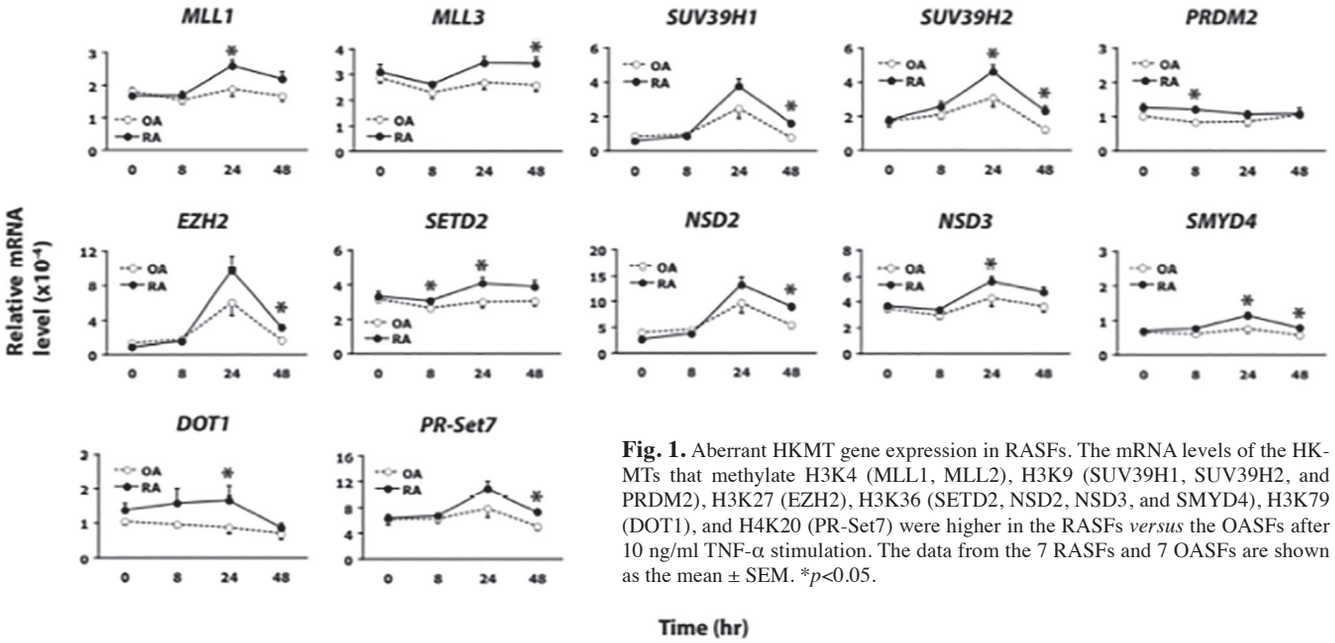

Fig. 1. Aberrant HKMT gene expression in RASFs. The mRNA levels of the HKMTs that methylate H3K4 (MLL1, MLL2), H3K9 (SUV39H1, SUV39H2, and PRDM2), H3K27 (EZH2), H3K36 (SETD2, NSD2, NSD3, and SMYD4), H3K79 (DOT1), and H4K20 (PR-Set7) were higher in the RASFs *versus* the OASFs after 10 ng/ml TNF-α stimulation. The data from the 7 RASFs and 7 OASFs are shown as the mean ± SEM. **p*<0.05.

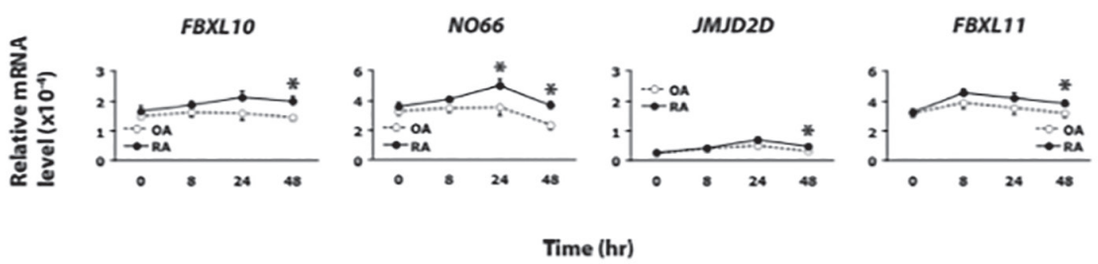

Fig. 2. Aberrant HKDM gene expression in RASFs. The mRNA levels of the HKDMs that demethylate H3K4 (FBXL10, NO66), H3K9 (JMJD2D), and H3K36 (FBXL10, FBXL11, and NO66) were higher in the RASFs *versus* the OASFs after 10 ng/ml TNF-α stimulation. The data from the 7 RASFs and 7 OASFs are shown as the mean ± SEM. **p*<0.05.

sion of the identified HKMTs and HKDMs *in vivo* in the inflammatory joints in RA patients. As the upregulation of these genes was only observed at specific times after the TNF-α stimulation, it is now uncertain whether repression of the enzymes might be a good target for RA treatment. Further studies to assess whether this treatment is useful will need to be undertaken.

In conclusion, this study demonstrated the presence of aberrant gene expression of the HKM-modifying enzymes in RASFs, which may lead to changes in the HKM. Furthermore, results of this study suggest that the HKM-modifying enzymes are involved in the pathogenesis of RA.

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