

Changes in sirtuin 2 and sirtuin 3 mRNA expressions in rheumatoid arthritis

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Abstract

Objective: Sirtuins (SIRT) play a prominent role in metabolism, apoptosis, aging, inflammation, and epigenetics. Inflammation, apoptosis, and epigenetics are pathogenic issues in rheumatoid arthritis (RA). This study aimed to evaluate SIRT2 and SIRT3 mRNA expressions in patients with RA.

Material and Methods: Fifty-four patients with RA and 26 healthy controls were enrolled. Disease activity was determined using the disease activity score (DAS) 28-erythrocyte sedimentation rate (ESR) (score of >2.6 was considered to be active). SIRT2 and SIRT3 mRNA expressions in the extracellular plasma were investigated by real-time PCR.

Results: SIRT3 mRNA expression was higher in the RA group than in the healthy control group (4.64 fold, $p < 0.001$), whereas SIRT2 mRNA expression was relatively lower in the RA group than in the healthy control group (0.55 fold, $p = 0.109$). However, SIRT2 (1.73 fold, $p = 0.065$) and SIRT3 (3.58 fold, $p = 0.051$) mRNA expressions were relatively higher in patients with active RA than in those with inactive RA.

Conclusion: In RA, SIRT3 mRNA expression is increased, whereas SIRT2 mRNA expression is decreased. Conversely, SIRT2 and SIRT3 mRNA expressions increase in active RA. Therefore, the fate of each SIRT may differ in RA.

Keywords: Rheumatoid arthritis, sirtuin 2, sirtuin 3

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that is characterized by synovial and systemic inflammation and joint destruction; however, its pathogenesis and cure remain to be completely elucidated (1, 2). It is well known that activated synovial fibroblasts (SFs) play a key role in the pathogenesis of RA (1, 2). It is currently accepted that epigenetic modifications activate SFs and contribute to the inflammatory phenotype in SFs (3-5).

Class III histone deacetylase (HDAC) is also known as sirtuins (SIRT), and the mammalian SIRT family has seven members (SIRT1-SIRT7). SIRT are nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylases, and class I, II, and IV HDACs are zinc dependent (6, 7). SIRT are involved in epigenetics via histone modifications, and the pathogenic effects of SIRT on metabolic, cardiovascular, and neurological processes have been widely evaluated previously (6, 7). In addition to epigenetic effects, SIRT alter several intracellular signaling pathways (8, 9).

It has been reported that SIRT inhibitors decrease the activity of nuclear factor-kappa B (NF- κ B) and phosphorylation of the inhibitor of kappa kinase B-alpha, thereby inhibiting the production of tumor necrosis factor alpha (TNF- α) and interleukin (IL)-6 in macrophage cell cultures (8). It has also been documented that the inhibition of SIRT1 and SIRT2 decreases the toll-like receptor (TLR)-induced activation of macrophages and the production of pro-inflammatory cytokines and other mediators by blocking the activation of mitogen-activated protein kinase (MAPK) and MAPK/extracellular signal-regulated kinase (9). In summary, accumulated data (8, 9) indicate that SIRT can affect both the innate and adaptive immune responses.

Engler et al. (10) demonstrated that SIRT1 modulates the activity of RA SFs. SIRT2 protein and mRNA levels are reported to be significantly decreased in joint tissues of mice with collagen-induced arthritis (CIA) (11). In contrast, SIRT6 protein levels are reported to be moderately increased in the same arthritis experimental model (12). The effect of SIRT on epigenetics, intracellular pathways, and cytokines suggests that they play a role in the pathogenesis of RA (8, 9). This study aimed to evaluate SIRT2 and SIRT3 mRNA expressions in patients with RA and to determine their potential effects on the disease phenotype.



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Submitted: 12 July 2016

Accepted: 2 November 2016

Available Online Date: 11.04.2017

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Material and Methods

Participants

Fifty-four patients with RA and 26 healthy controls (HCs) were included in this study. Patients with RA were diagnosed according to the 2010 ACR-EULAR criteria (13). The study protocol was approved by the institutional ethics committee, and all the participants provided informed consent before enrolling in the study. Individuals aged <18 years, >80 years, or pregnant were excluded.

Detailed history of all participants was obtained, and systemic and rheumatological examinations were performed. Use of corticosteroids and disease-modifying anti-rheumatic drugs was also recorded.

Determination of disease activity

In the RA group, the disease activity status was determined by the disease activity score (DAS) 28-erythrocyte sedimentation rate (ESR) (14). Patients with DAS28-ESR of >2.6 were considered to have active RA.

Laboratory analysis

Blood samples of all participants were collected in tubes containing ethylenediaminetetraacetic acid after overnight fasting. Plasma samples for RNA isolation were stored at -70°C until further analysis. Routine laboratory evaluation of complete blood cell count, fasting blood glucose, lipid profile, hepatic and renal function, ESR, *C-reactive protein (CRP) levels*, rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) antibodies were performed using standard laboratory methods for all the participants.

Total RNA isolation and qRT-PCR

Total RNA was isolated from plasma samples using a genomic extraction kit (QIAamp RNA Blood Mini kit; Qiagen, Hilden, Germany). Equal amounts of RNA from the samples were reverse transcribed to cDNA using SuperScript First-Strand cDNA Synthesis kit (Invitrogen; San Diego, CA, USA). SIRT2 and SIRT3 (Qiagen; Hilden, Germany) mRNA expressions were quantified and normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH). SIRT2, SIRT3, and GAPDH mRNA expressions were measured using a Rotor-Gene SYBR green-based real-time PCR with a real-time PCR system (Rotor-Gene Q; Qiagen, Hilden, Germany). For analyzing qRT-PCR results, the $2^{-\Delta\Delta Ct}$ (ΔCt =target gene-reference gene) formula was used (15). Changes in gene expressions were reported as fold changes relative to those in controls. Fold changes were analyzed using the RT2 Profiler PCR Array Data Analysis Version 3.5 online software (<http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php>).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences, version 21.0 (IBM Corp.; Armonk, NY, USA). Quantitative data with normal distribution were reported as mean±standard deviation. The normal distribution of the variables was evaluated by the Kolmogorov-Smirnov test, and the logarithmic transformations were used to normalize data with skewed distributions. Statistical differences among the groups were determined using the Student's *t*-test. Categorical variables were compared using the chi-square test. Correlation analyses were performed with the Pearson correlation coefficient. Variables were adjusted for age by the analysis of covariance (ANCOVA). *P* values of <0.05 were considered to be statistically significant.

Results

Demographic and laboratory data for the RA and HC groups are presented in Table 1. In the RA group, the mean DAS28-ESR was 3.2±1.5, and the mean disease duration was 8.2±7.9 years. In the RA group, 39 patients received corticosteroids, 29 received methotrexate, 19 received antimalarial drugs, and 10 received sulphasalazine.

SIRT3 mRNA expression was higher in the RA group than in the HC group (*p*<0.001, Table 2). However, SIRT2 mRNA expression was similar in both the HC and RA groups (*p*=0.109). SIRT2 and SIRT3 mRNA expressions were relatively higher in patients with active RA (*n*=28) than in those with inactive RA (*n*=26) (*p*=0.065 and *p*=0.051, respectively; Table 3). SIRT2 and SIRT3 mRNA expressions were not significantly correlated with ESR and CRP levels (*p*>0.05 for all).

SIRT2 and SIRT3 mRNA expressions were lower in RF-positive patients (*n*=34) than in RF-negative patients (*n*=20) and were also lower in anti-CCP-positive patients (*n*=33) than in anti-CCP-negative patients (*n*=21) (Tables 4 and 5). However, SIRT3-mRNA expression decreases in RF-positive patients and SIRT2-mRNA expression decreases in anti-CCP-positive patients were statistically significant (*p*=0.012 and *p*=0.028, respectively; Table 4, 5). Conversely, SIRT2 and SIRT3 mRNA expressions showed no significant effect on the disease duration (*p*>0.05).

SIRT3 mRNA expression was higher in patients who received corticosteroids (*n*=39) than in those who did not (*n*=15) (*p*<0.05), whereas SIRT2 mRNA expression was not significantly different (*p*>0.05). The use of methotrexate, sulphasalazine, and antimalarial drugs did not appear to alter SIRT2 and SIRT3 mRNA expres-

Table 1. Demographic data of the HC and RA groups

	HC (n=26)	RA (n=54)	<i>p</i>
Age (years)	43.8±12.5	49.7±16.1	0.085
Sex (F/M)	21/5	43/11	0.590
BMI (kg/m ²)	27.4±4.8	27.5±5.5	0.798
Ever smokers [n (%)]	7 (26.9)	11 (20.4)	0.512

HC: healthy control; RA: rheumatoid arthritis; F: female; M: male; BMI: Body Mass Index

Table 2. Sirtuin mRNA expressions in the HC and RA groups

	HC (n=26)	RA (n=54)	Fold changes	<i>p</i>
	$2^{-\Delta Ct}$	$2^{-\Delta Ct}$		
SIRT2	0.0675	0.0369	0.546	0.109
SIRT3	0.0560	0.2600	4.637	<0.001

SIRT: sirtuin; HC: healthy control; RA: rheumatoid arthritis

Table 3. Effect of disease activity on sirtuin mRNA expressions in the RA group

	Inactive RA (n=26)	Active RA (n=28)	Fold Changes	<i>p</i>
	$2^{-\Delta Ct}$	$2^{-\Delta Ct}$		
SIRT2	0.067	0.116	1.729	0.065
SIRT3	0.177	0.633	3.575	0.051

SIRT: sirtuin; RA: rheumatoid arthritis

Table 4. Sirtuin mRNA expressions in RF-negative and RF-positive patients with RA

	RF-negative (n=20)	RF-positive (n=34)	Fold Changes	<i>p</i>
	$2^{-\Delta Ct}$	$2^{-\Delta Ct}$		
SIRT2	0.1133	0.0743	0.655	0.523
SIRT3	0.5740	0.2272	0.395	0.012

SIRT: sirtuin; RF: rheumatoid factor; RA: rheumatoid arthritis

Table 5. Sirtuin mRNA expressions in anti-CCP-negative and anti-CCP-positive patients with RA

	Anti-CCP - (n=21)	Anti-CCP + (n=33)	Fold changes	<i>p</i>
	$2^{-\Delta Ct}$	$2^{-\Delta Ct}$		
SIRT2	0.1181	0.0628	0.5319	0.028
SIRT3	0.3146	0.2173	0.6908	0.331

SIRT: sirtuin; RF: rheumatoid factor; RA: rheumatoid arthritis

sions ($p > 0.05$ for all). Similarly, body mass index and smoking were not correlated with SIRT2 and SIRT3 mRNA expressions ($p > 0.05$ for both), and their mRNA expressions were not directly correlated with any laboratory and clinical parameters.

Discussion

In this study, increased SIRT3 mRNA expression was observed in patients with RA. Moreover, we found that SIRT2 and SIRT3 mRNA expressions were increased in active RA compared with those in inactive RA.

The epigenetic phenomena encompass DNA methylation, histone modifications, and microRNA expressions, and all those epigenetic mechanisms are documented to be altered in RA. The abovementioned three epigenetic mechanisms are sensitive to external stimuli and affect gene expression, thus leading to an association between environmental stimuli and genetic factors. Acetylation is the best-characterized histone modification. Histone acetylation is catalyzed by histone acetyltransferases (HATs), whereas histone deacetylation is catalyzed by HDACs. HDACs suppress gene transcription via chromatin condensation, whereas HATs enhance it (16, 17). A reduced HDAC/HAT ratio has been previously reported in SFs and PBMCs of patients with RA (16-18).

Class III HDACs are members of the SIRT family. Neiderer et al. (19) reported an increased SIRT1 mRNA expression in the synovial tissues of patients with RA. Similarly, Lee et al. (12) documented that SIRT6 mRNA expression is increased in the synovial tissues of mice with CIA. Conversely, SIRT2 mRNA expression has been reported to be decreased in CIA models (11). In our study, an increased SIRT3 mRNA expression was observed in patients with RA. These results suggest that each SIRT may play different roles in RA. Wendling et al. (20) reported that SIRT1 mRNA expression is significantly lower in patients with RA compared with HCs.

Subcellular localizations and functions are different for each SIRT. SIRT1, SIRT6, and SIRT7 are found in the nucleus; SIRT3, SIRT4, and SIRT5 are found in the mitochondria; and SIRT2 is primarily found in the cytoplasm (21). The NAD⁺/NADH ratio affects the activity of SIRTs (22). Cells provide the necessary energy for aerobic glycolysis, which occurs in the cytoplasm, and/or oxidative phosphorylation, which occurs in the mitochondria (22). Therefore, the selective manner in which each inflammatory cell provides energy may differently alter each SIRT because cellular localizations differ. Pro-inflammatory Th₁₇ cells provide energy

by glycolysis, whereas anti-inflammatory T_{reg} cells provide energy by oxidative phosphorylation (22). Moreover, a metabolic switch from oxidative phosphorylation to glycolysis has been reported in activated macrophages and dendritic cells, similar to in the Warburg effect observed in cancer cells (22-24). This evidence suggests that the expression of each SIRT may be different in each inflammatory disease because different inflammatory cells are involved in different diseases and in the different stages of a disease.

Sirtuins target and deacetylate lysine residues of histone proteins, leading to epigenetic modifications; however, they also target non-histone substrates such as transcription factors. SIRT1, SIRT2, and SIRT6 have been reported to suppress NF- κ B activation by deacetylating p65 (11, 12, 25, 26). Therefore, they have anti-inflammatory roles. It has been documented that these anti-inflammatory SIRTs decrease the pro-inflammatory cytokine and chemokine levels and ameliorate experimentally induced arthritis (11, 12, 19). In our study, SIRT3 mRNA expression was high in RA. Moreover, increased SIRT1 and SIRT6 mRNA expressions in RA have been previously demonstrated (12, 19). However, the cause of overexpression remains unknown.

In our study, unlike SIRT3, SIRT2 mRNA expressions were not increased in RA. The cellular localizations of SIRT3 and SIRT2 are different. SIRT3 is localized in the mitochondria and is a key regulator of mitochondrial protein acetylation (27, 28). Thus, it may be concluded that increased SIRT3 mRNA expression is caused by mitochondrial stress/destruction. It may also be concluded that increased SIRT3 mRNA expression causes metabolic complications in RA such as insulin resistance, dyslipidemia, accelerated atherosclerosis, and rheumatoid cachexia. A chronic high-fat diet has been demonstrated to affect hepatic SIRT3 activity and levels (29, 30). SIRT3 mRNA expression has been documented to be altered in pancreatic islet cells harvested from patients with type 2 diabetes and mice with experimentally induced diabetes (31).

Decreased SIRT3 production in islet cells results in increased reactive oxygen species (ROS) levels (31). The antioxidant effect of SIRT3 has also been observed in malignant cells (32-34). In an *in vitro* study in cardiomyocytes, Sundaresan et al. (35) reported that the antioxidant role of SIRT3 is associated with Forkhead box class O (FoxO) transcription factors. SIRT3 deacetylates FoxO3a and traps it inside the nucleus to enhance the transcription of FoxO-dependent

antioxidant genes, including manganese superoxide dismutase (MnSOD) and catalase. Increased expression of MnSOD and catalase suppresses ROS levels (35). Thus, the oxidant/antioxidant balance has a prominent role on the pathogenesis of RA (36). The cause of increased SIRT3 mRNA expression may be a response to suppress oxidative stress in RA.

In our study, patients with active RA had increased SIRT2 and SIRT3 mRNA expressions compared with those with inactive RA. Pais et al. (25) documented that lipopolysaccharide-induced inflammation decreases SIRT2 mRNA expressions in the brain. Caton et al. (31) also reported that the incubation of pancreatic islet cells with pro-inflammatory cytokines, including IL-1 β and TNF α , decreases SIRT3 mRNA expression. Conversely, it has been demonstrated that SIRT1 mRNA expression is increased in TNF- α -induced SFs (19). Therefore, it may be concluded that the cause of SIRT2 and SIRT3 mRNA expressions in active RA remains unknown. However, the anti-inflammatory action of SIRT2 and the antioxidant effect of SIRT3 suggest that the increased expression in patients with active RA aimed at suppressing the disease activity.

This present preliminary study has some limitations. First, SIRT2 and SIRT3 mRNA expressions were only analyzed in the plasma, but their expressions can also be analyzed in synovial tissues. Second, SIRT mRNA expression in patients with active RA should be analyzed again after remission because a prospective evaluation could be more informative.

In conclusion, SIRT3 mRNA expression increases in RA. Furthermore, SIRT2 and SIRT3 mRNA expressions increase in active RA. Therefore, the fate of each SIRT may differ in RA.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Muğla Sıtkı Koçman University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.Y., S.S.K.; Design - M.K., S.Y.; Supervision - C.Ş., S.S.K.; Resources - C.Ş.; Materials - M.K., C.Ş.; Data Collection and/or Processing - S.Y.; Analysis and/or Interpretation - M.K.; Literature Search - C.Ş., S.Y.; Writing Manuscript - S.Y., S.S.K.; Critical Review - S.S.K.; Other - C.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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