Role of SIRT1 in neutrophil migration during inflammation

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Directed by Professor Young-Min Hyun

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ABSTRACT

Role of SIRT1 in neutrophil migration during inflammation

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Sirtuin 1 (SIRT1) is an enzyme encoded by the *Sirt1* gene and is responsible for deacetylating proteins that regulate cellular processes. In mammals, SIRT1 deactivates the p53 protein by deacetylation and additionally p65 subunit at the lysine 310 residue. Aging is characterized by a chronic, low level of inflammation, and NF-kappa B, as a transcriptional regulator, is the main factor related to inflammation. Hence, SIRT1 is involved in various processes. Neutrophils are involved in the overall immune system, and are one of the types of cells that initially respond to pathogens. Neutrophils possess a significant amount of Ikappa B-alpha, an NF-kappa B inhibitor, in the nucleus of unstimulated cells. We commenced experiments to examine the role of neutrophils in accordance with SIRT1 levels.

Our findings in this study demonstrate the previously undiscovered roles of SIRT1 in neutrophils, such as involvement in phagocytosis and migration patterns from basal migration to chemotactic migration towards bacterial derived molecules. SIRT1 knockout neutrophils were functionally deficient, as compared with wild-type neutrophils *in vitro* and *in vivo*. These results imply that SIRT1 may also play important roles in regulating neutrophils in the innate immune system.

Key words: neutrophil, SIRT1, leukocyte, phagocytosis, migration

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I. INTRODUCTION

Nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase sirtuin-1 (SIRT1) is one of the seven members of the sirtuin family.[1] SIRT1 deacetylates proteins that regulate cellular processes such as apoptosis, DNA repair, stress resistance, inflammation, and drug efflux.[2] The function of SIRT1 in immune cells has been previously reported; SIRT1 deactivates the p53 protein through its deacetylase activity[3] and has an inhibitory function against transcription factor NF-kappa B, which regulates the homeostasis of innate immunity and energy metabolism.[4] SIRT1 regulates NF-kappa B by deacetylating the RelA/p65 subunit at the lysine 310 residue.[5] NF-kappa B recruits immune cells during an immune response by stimulating pro-inflammatory and anti-apoptotic genes.[6] In addition, SIRT1 stimulates autophagy by deacetylation of proteins required for autophagy.[7]

Neutrophils play important roles in innate immunity and respond quickly to early inflammation.[8] They have major roles in the response to bacterial infection in particular, in addition to recruitment to the infected site. CXCR2 and CD11b, a chemokine receptor and an adhesion molecule, have primary roles in neutrophils for removal of bacteria.[9] CXCR2 and CD11b are dependently expressed by FOXO1, a transcription factor inhibited by SIRT1.[10] It has been reported that *in vivo* FOXO1 deficiency negatively affects neutrophil migration from the bone marrow to body circulations and also recruits to the infection site.[11] Thus, FOXO1 regulates neutrophil migration towards chemotaxis and the removal of bacteria *in vitro*.[11] A few SIRT1 functions in leukocytes have been reported. However, the specific roles of SIRT1 in neutrophils remain unclear.

To investigate SIRT1 functions in neutrophils exclusively, a conditional gene-knockout technique was used to eliminate the *Sirt'* gene. The Cre-LoxP system is an ideal method for manipulation of DNA *in vivo*, particularly in mammals.[12-15] This system consists of two components: a Cre recombinase and its recognition site, *lox*P. When a floxed mouse, whose genome contains a ″floxed″ site located in a gene of interest, bred with a Cre mouse expressing the Cre recombinase, Cre-positive, double-floxed mice are created, and the gene located between the *lox*P sites is eliminated (Figure 1).

As SIRT1 plays diverse roles in the immune system, efforts to regulate SIRT1 may help to manage disorders of the immune system. Regulating SIRT1 may become a potential therapeutic approach for curing diseases related to neutrophils since neutrophils are the most abundant white blood cells in the blood stream and the first respond to infections.[16-18]

Herein, we examined the effects of SIRT1 on neutrophil migration and functions such as phagocytosis and calcium regulation.

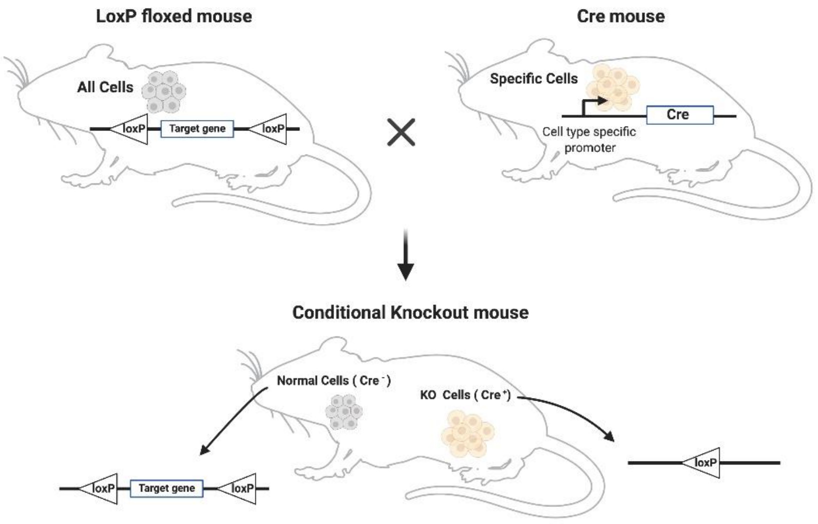


Figure 1, Diagram showing how to generate the conditional knockout mice.

II. MATERIALS AND METHODS

1. Animal

129-Sirt1^{tm3Fwa}/DsinJ(Sirt1^{loxP}) and B6-129P2-Lyz2$^{tm1(cre)lfo}$/J (LysMcre) were provided by Sang-Myeong Lee (Chonbuk National University, Jeonbuk, South Korea). Each mouse was bred to create SIRT1 conditional knockout in the specific cells which have *Lyz2* gene. The following method is appropriate for 8-10 weeks old mice. All animal studies were approved by the Animal Care and Use Committee of the Yonsei University College of Medicine.

2. Neutrophil preparation

Bone marrow cells were isolated from mouse femur and tibia bone marrow. Negative selection was performed to purify neutrophils from bone marrow cells using EasySep Mouse Neutrophil Enrichment Kit(Stemcell Technologies, Vancouver, Canada) and instructions were referred to.

3. RT-PCR

Total RNA was extracted from SIRT1 knockout neutrophil and wild-type neutrophil using Trizol Reagent(Thermo Fisher Scientific, Waltham, Massachusetts, USA). Poly(A) RNA was primed by oligo(dT)(Roche, Basel, Switzerland), and performed reverse transcription using AMV reverse transcriptase(Roche, Basel, Switzerland) at 42°C. cDNA amplification was processed using mouse SIRT1 specific primer (5’ primer, 5’-CAG ACC CTC AAG CCA TGT TT-3’; 3’ Primer, 5’-ACA CAG AGA CGG CTG GAA CT-3’).

4. Phagocytosis assay

Bone marrow cells from both the SIRT1 neutrophil conditional knockout mouse and wild-type mouse were stimulated by 1 μM of fMLP at 37°C for 30 minutes. 1 mg/ml of FITC-conjugated dextran was treated to each stimulated neutrophils to be uptaken at 37°C for 30 minutes. Neutrophils were stained using anti-CD11b, anti-Ly6G antibody to be selected from other immune cells. Selected neutrophils with antibodies were distinguished into FITC-dextran uptaken neutrophil and non-uptaken neutrophil. Percentage of dextran uptaken neutrophil was compared with each SIRT1 knockout neutrophil and wild-type neutrophil.

5. Intracellular calcium ion measurement

SIRT1 knockout neutrophils and wild-type neutrophils were isolated by negative selection described above. Neutrophil was labeled with 2 μM of Fluo-4 AM(Thermo Fisher Scientific, Waltham, Massachusetts, USA), calcium indicator, at 37°C for 30 minutes. After washed three times, 5 x 10^5 of neutrophils was resuspended in HBSS not including calcium ion. Cells were seeded into the confocal dish, and incubated at room temperature for 30 minutes for de-esterificiation. Cells not adhered to the confocal dish were washed out three times with HBSS without containing calcium ion. Fluorescent signals were observed under a 40X objective lens of Nikon Ti2 fluorescent microscope. Phase-contrast images and fluorescent images were conducted every 10 seconds. After 2 minutes of basal calcium level measurements, ionomycin(Sigma-Aldrich, St. Louis, Missouri, USA) was added to trigger maximum calcium influx. Fluorescent level was analyzed using Volocity software (Quorum Technologies, Lewes, United Kingdom).

6. Migration assay

Confocal dishes were coated with bovine plasma fibronectin(10 μg/ml; Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 30 minutes at 37°C.[19] Plates were then rinsed with L-15 media three times.

1 x 10^5 of neutrophils were added into 200 μl of L-15 supplemented with 10% of FBS, 1% of antibiotics. Neutrophils were incubated in the presence of 1 μg/mL of fMLP for 5 minutes at 37°C and allowed to be adhered to the confocal dish, and not properly attached neutrophils were washed out with L-15 media. For neutrophil live cell staining, 1 μM of CellTracker Red CMTPX dye (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used. Temperature was maintained at 37°C, CO_2 level was also maintained to 5% throughout the experiments using a live-cell imaging chamber(Live Cell Instrument, South Korea). Images were acquired for 30 minutes under a 20X objective lens using a Nikon Ti2 inverted microscope. Images were taken every 10 seconds. Migration of neutrophils were traced using Volocity software(Quorum Technologies, Lewes, UK).

7. Measurement of cytokines

Neutrophil lysate was used to profile intracellular cytokine levels. Lysate were prepared using PRO-PREP protein extraction solution (intron biotechnology, Seongnamm, South Korea). The proteome Profiler mouse cytokine array panel A (R&D Systems, Minneapolis, Minnesota, United States) was used to detect the cytokine expression level in neutrophils. Antibody based cytokine array detects BLC, C5/C5a, G-CSF, GM-CSF, I-309, Eotaxin, sICAM-1, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-12p70, IL-16, IL-17, IL-23, IL-27, IP-10, I-TAC, KC, M-CSF, JE, MCP-5, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, SDF-1, TARC, TIMP-1 TNF-α, TREM-1. Experiment was performed following instruction. Quick spots (Western Vision software, Salt lake city, Utah, USA) software was used for analysis.

8. Data analysis

All results are expressed as the mean plus or minus SEM. The differences between all groups were analyzed by the student *t* test. All statistics were performed using the Prism program version 7.0(GraphPad Software, San Diego, California, USA).

Ⅲ. RESULTS

1. Generation of neutrophil-specific SIRT1 knockout mice

To study the effects of SIRT1 functions in neutrophils, 129-Sirt1^{tm3Fwa}/DsinJ (Sirt1^{loxP}) and B6-129P2-Lyz2$^{tm1(cre)lfo}$/J (LysMcre) mice were crossed to create a conditional SIRT1 knockout in *Lyz*2 positive cells. To confirm the knockout, we checked the mRNA expression levels of each SIRT1 knockout neutrophils and SIRT1 wild-type neutrophils. The mRNA expression level of SIRT1 knockout neutrophils was reduced by about 80 percent, as compared to SIRT1 wild-type neutrophils (Figure 2A, 2B). As the level of SIRT1 mRNA was decreased, the SIRT1 protein expression level was also decreased as well.

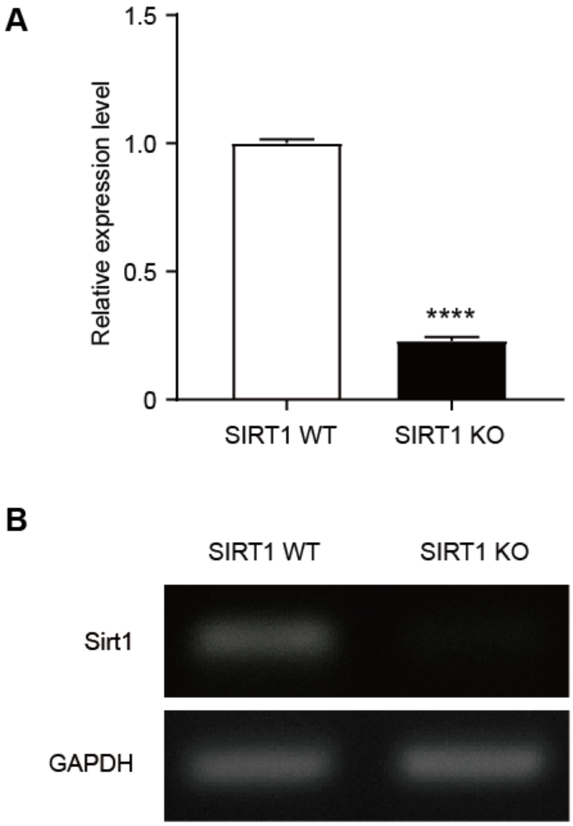


Figure 2. The relative mRNA expression levels. (A) mRNA expression level in SIRT1 wild-type and SIRT1 knockout neutrophils. Relative mRNA expression level was calculated by delta-delta Ct method. *****p*<0.0001. (B) *Sirt*1 expression levels are presented by gel electrophoresis. Glyceraldehyde 3-phosphate dehydrogenase was used as a house keeping gene to prove same amount of cDNA was loaded.

2. SIRT1 regulates cytoplasmic calcium levels in neutrophils, influencing the regulation of homeostasis

Calcium ions are indispensable for most cellular processes. They affect the physiological and biochemical processes by playing important roles in signal transduction pathways by acting as a second messenger.[20] Calcium ions in neutrophils manage neutrophil activation[21] and migration by regulating actin polymerase.[22] The neutrophil is one of the most motile cells and the first leukocyte to react when inflammation occurs. Thus, the regulation of neutrophil activation and motility are important.

We measured cytoplasmic calcium ion levels using an indirect method to examine the homeostasis of neutrophils. Cytoplasmic calcium ions were labeled using Fluo-4 AM, a calcium indicator. After sufficient de-esterification, images were taken every 10 seconds, and maximum calcium efflux from the cells was triggered by the addition of ionomycin two minutes after the start of imaging (Figure 3A). The calcium levels were measured by quantifying the green fluorescence level of each cell. The SIRT1 knockout neutrophils showed lower maximum calcium efflux levels, as compared with wild-type neutrophils (Figure 3B).

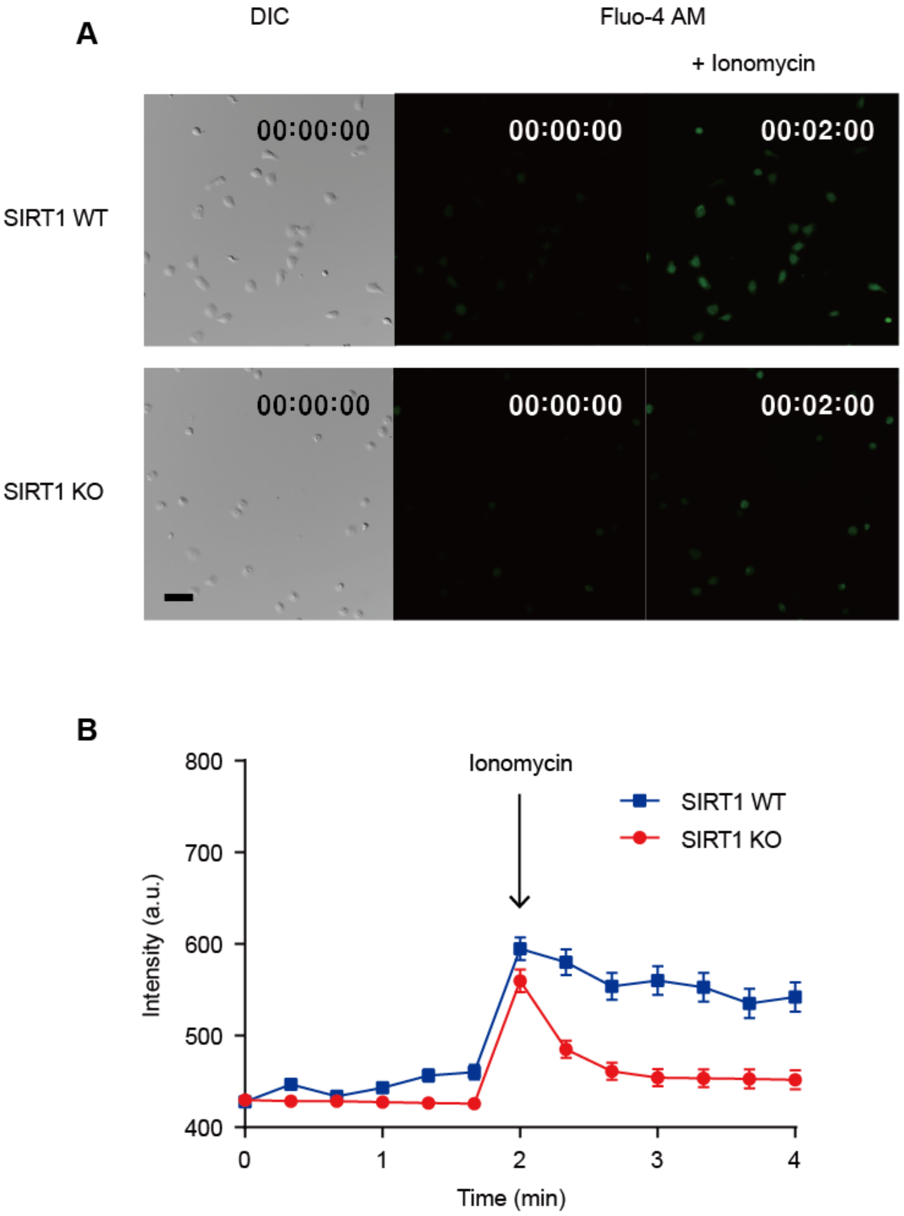


Figure 3. The measurement of Ca^{2+} levels using the Fluo-4 AM calcium indicator, under the fluorescent microscopy. (A-B) Fluo-4 AM labeled

neutrophils attached on confocal dish. Measuring the calcium ion level has been replaced by measuring the intensity of the green fluorescence (Fluo-4 AM). Fluorescence intensity was measured under the fluorescent microscope every 10 seconds. After 2 minutes of basal Ca^{2+} level measurement, 2 μM of ionomycin was treated to trigger maximum Ca^{2+} influx. (A) Scale bar = 30 μm. (B) a.u.: arbitrary unit.

3. Phagocytosis ability was reduced in SIRT1 knockout neutrophils

Phagocytosis is one of the important functions for removing bacteria. Pathogens are engulfed by pseudopods that enter the plasma to create phagosomes.[23] Pseudopodial extension is accompanied by actin polymerization,[24,25] which is regulated by calcium signaling.[26, 27]

FITC-conjugated dextran was used for engulfment by neutrophils because it aggregates easily. Aggregated FITC dextran was taken up and detected under a microscope (Figure 4A). The SIRT1 knockout neutrophils showed decreased activity of phagocytic ability, as compared with SIRT1 wild-type neutrophils (Figure 4B). It should be considered that SIRT1 is also involved in the neutrophil's phagocytic process and the role of SIRT1 in regulating intracellular calcium levels can also affect on phagocytosis.

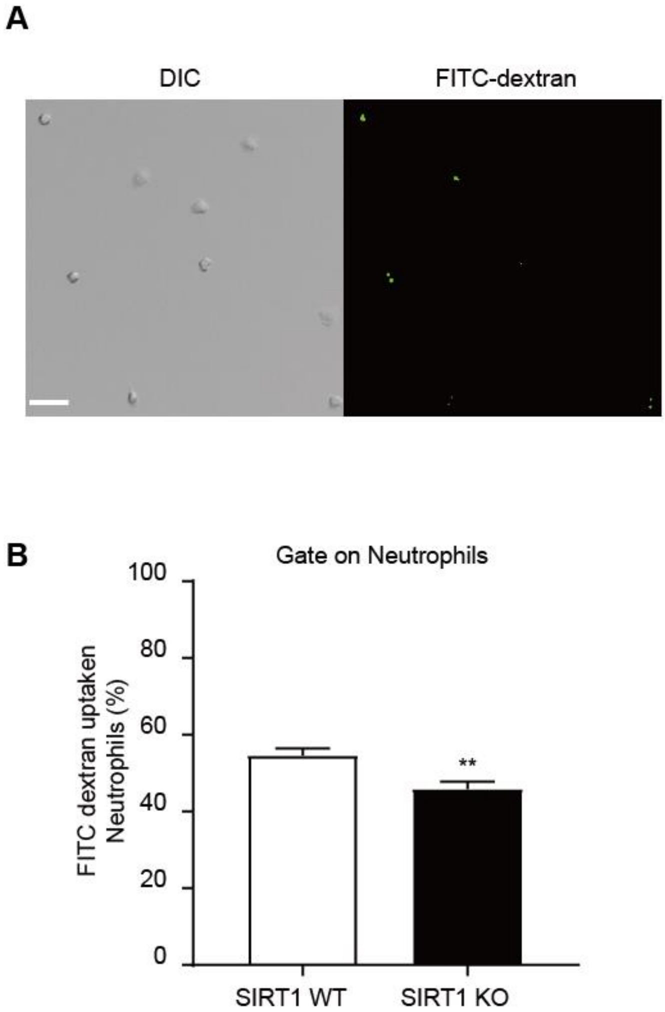
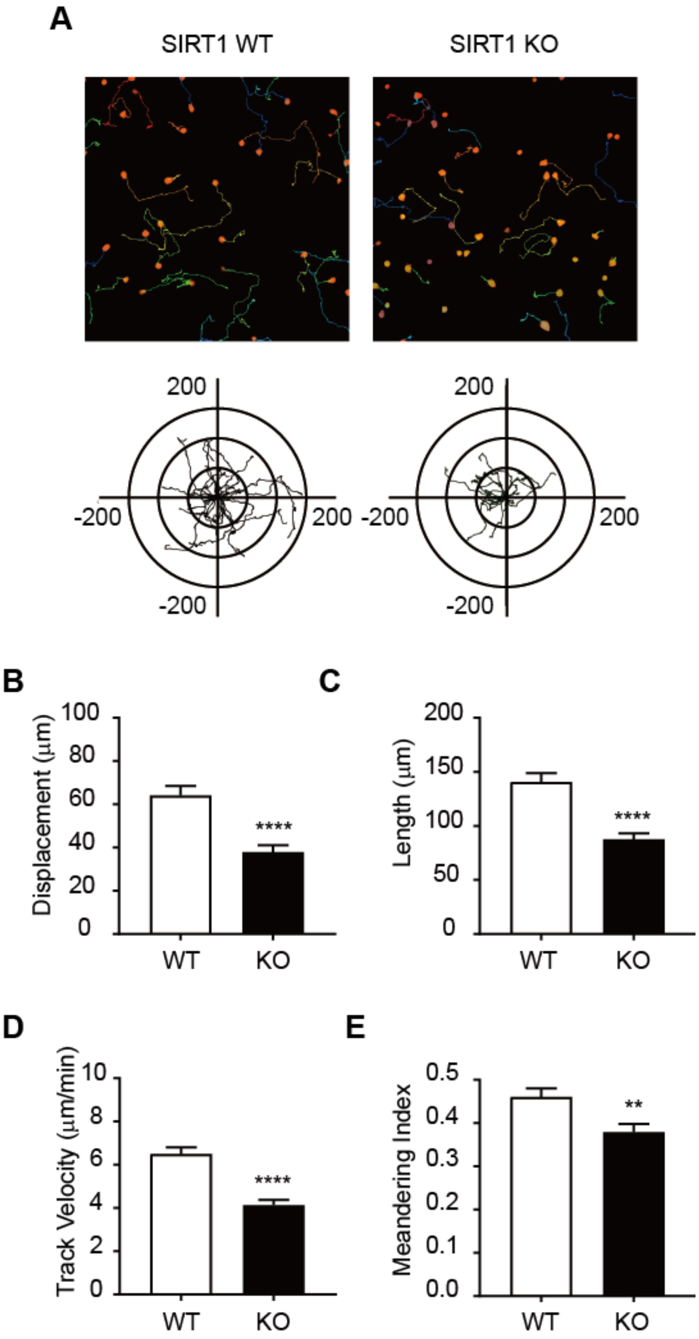


Figure 4. SIRT1 knockout neutrophils had reduced ability of phagocytosis, as compared to wild-type neutrophils. (A-B) Neutrophils were activated with fMLP and incubated with FITC conjugated dextran. (A) DIC and fluorescent images of FITC conjugated dextran uptaken neutrophils. Scale bar = 30 μm. (B) FITC conjugated dextran uptaken neutrophils were counted via flow cytometry analysis. Percentage of dextran uptaken neutrophils gated on total neutrophil counts. $^{**}p<0.01$.

4. Migration of SIRT1 knockout neutrophils was decreased

Neutrophils are very motile and are the first cells to migrate towards an inflammation site. Neutrophil recruitment, the initial part of the inflammation process, is very essential during the acute phase of the immune response, especially for bacterial infection. We attempted to conduct *in vitro* migration assays to determine whether SIRT1 knockout and wild-type neutrophils have different migration patterns. Neutrophils were stained with Celltracker CMTPX dye, and the migration patterns were captured every 10 seconds using fluorescence microscopy. The migration tracks of neutrophils are shown by tracking lines (Figure 5A, upper panels). The center-zeroed tracks on coordinate planes clearly show that SIRT1 knockout neutrophils were less motile than SIRT1 wild-type neutrophils (Figure 5A, lower panels). We additionally analyzed neutrophil characteristics such as displacement, length, track velocity, and meandering index. All factors listed above were reduced in SIRT1 knockout neutrophils (Figure 5B-E). Hence, we could assume that SIRT1 may have important roles in neutrophil migration.


A
SIRT1 WT
SIRT1 KO
200
-200
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-200
200
-200
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-200
B
Displacement (μm)
100
80
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20
0
****
WT
KO
C
Length (μm)
200
150
100
50
0
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WT
KO
D
Track Velocity (μm/min)
10
8
6
4
2
0
****
WT
KO
E
Meandering Index
0.5
0.4
0.3
0.2
0.1
0.0
**
WT
KO

Figure 5. Neutrophil migration was decreased in SIRT1 knockout neutrophils *in vitro*. (A) Migration of neutrophils on fibronectin-coated fMLP-treated confocal dish. Each tracking line represents migratory path of individual cell(upper panel). Center-zeroed tracks of wild-type or SIRT1 knockout neutrophils(lower panel). (B-E) Data are expressed as the mean plus or minus SEM. Statistics were analyzed by the student t test. **p<0.01, ****p<0.0001. Displacement(B), length(C), track velocity(D), meandering index(E).

5. Cytokine expression levels were differently regulated in SIRT1 knockout mice

Cytokines are signaling molecules that mediate and regulate immune responses by aiding cell-to-cell communication. Cytokines generally function as intercellular messenger molecules that induce specific biological activities and in somet cases, act on the same cells that secrete the molecules (known as autocrine signaling). Knowing the level cytokine expression is useful for identifying how SIRT1 is involved in cellular mechanisms. Therefore, we performed cytokine profiling based on antibody detection in SIRT1 wild-type and SIRT1 knockout neutrophils. The dots in dark colors, numbered from 1 to 5 (1, sICAM-1; 2, IL-1ra; 3, IL-16; 4, SDF-1; 5, TREM-1), are highly expressed in the neutrophils of both SIRT1 wild-type and SIRT1 knockouts under basal conditions (Figure 6A). The expression levels of most cytokines were increased in SIRT1 knockout neutrophils. Cytokine IL-6, SDF-1, TREM-1, IL-ra and sICAM-1 were increased in the order listed, as compared with the wild-type (Figure 6B).

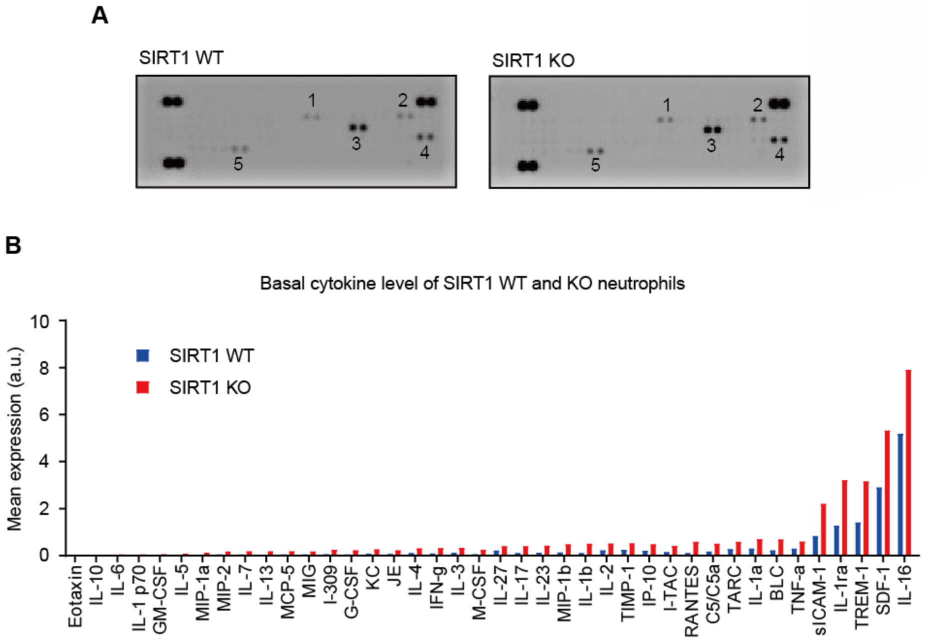

Figure 6. Profiled cytokine expression levels in basal status of SIRT1 wild-type neutrophils and SIRT1 knockout neutrophils. (A) Cytokine assay based on antibody detection method was used for analysis. Each dots represents cytokine expression level. (1; sICAM-1, 2; IL-1ra, 3; IL-16, 4; SDF-1, 5; TREM-1) (B) Basal cytokine expression levels were profiled by measurement of pixel density of each dots.

IV. DISCUSSION

Neutrophils are the most abundant granulocyte accounting for about 60 percent of all granulocytes and comprise the first line of innate immune responses against foreign pathogens. They have crucial roles within the cascade of the innate immune response, including recognition of bacteria, cytokine secretion, and recruitment to the inflamed sites.

It has been reported that nicotineamide phosphoribosyltransferase (NAMPT), in conjunction with G-CSF, triggers granulopoiesis.[28] SIRT1 is a class III histone deacetylase. NAD+ is essential for the activity of SIRT1 as a deacetylase. Granulopoiesis develops in the bone marrow and is the mechanism that leads to granulocyte production. There are two steps in granulopoiesis; one is ‘granulocyte lineage determination’ which converts oligopotent cells to unipotent cells, such as maturation from common lymphocyte progenitors or common myelocyte progenitors to granulocyte or monocyte, respectively.[29] ‘Committed granulopoiesis’ consists of the maturation stages of unipotent cells (myeloblasts, promyelocytes, myelocytes) into functional cellular metamyelocytes.[30] Each step is regulated by different cytokines, with G-CSF involved in the formation of myeloblasts from granulocyte-monocyte progenitors. Neutrophils have a short circulatory life span and their survival can be modulated by several cytokines, including G-CSF.[31,32] G-CSF is secreted from several immune cells, macrophages, and endothelial cells. Indirectly, we could assume that SIRT1 in other cells could modulate neutrophil function and migration by regulating G-CSF. Therefore, we investigated the G-CSF function in granulopoiesis as it applies to neutrophil differentiation and vice versa.

We investigated the relationship between SIRT1 and neutrophils to determine the effects of SIRT1 on G-CSF-induced granulopoiesis, differentiation of the neutrophils, and the production of functional deficiency. Consequently, we determined several functions of neutrophils using SIRT1 knockout and wild-type neutrophils, and our data showed that SIRT1 knockout neutrophils had decreased functioning in phagocytosis and a reduced intracellular calcium level, which is important for regulating homeostasis. *In vitro* migration assays showed that SIRT1 regulated migration patterns in neutrophils. Additionally, we performed cytokine array analysis to elucidate the downstream processes of SIRT1 to determine which molecules were affected when SIRT1 was knocked out. Our previous data showed that inflammatory processes were down regulated in the absence of SIRT1. Interestingly, cytokine profiling data showed that the expressions of cytokines were increased in SIRT1 knockout neutrophils during basal status for all tested cytokines. Furthermore, we evaluated each increased cytokines. IL-6, SDF-1, TREM-1, IL-1ra and sICAM-1 were mainly expressed in neutrophils during basal status and were greatly increased in SIRT1 knockout neutrophils, as compared to the SIRT1 wild-type neutrophils.

IL-6 is an interleukin that functions as both a pro-inflammatory and an anti-inflammatory cytokine, regulates neutrophil trafficking during acute inflammation, and has a crucial role as a checkpoint regulators by managing chemokine production and leukocyte apoptosis.[33]

SDF-1, also known as CXCL12, binds with CXCR4 and CXCR2, regulates the mobilization of neutrophils from bone marrow to inflammatory sites, and returns neutrophils back from inflammatory sites to bone marrow as well.[34,35]

TREM-1 is a receptor expressed on myeloid cells and regulates chemotaxis in neutrophils.[36] TREM-1 has roles in pro-inflammatory responses and can be stimulated by molecules such as Fc receptor, CD14, and Toll-like receptors. Stimulation of these receptors with TREM-1 can cause myeloid cells to respond to other stimuli.

The next most expressed cytokine was interleukin-1 receptor antagonist (IL-1ra), which functions as an IL-1 inhibitor. The IL-1 family possesses strong pro-inflammatory effects.[37] Neutrophil recruitment to the inflammation site can be reduced with increased IL-1ra.

Lastly, sICAM-1 (soluble intercellular adhesion molecule-1) can exist as a circulating, or unexpressed form of ICAM-1. ICAM-1 is membrane-expressed form of sICAM-1. ICAM-1 is expressed on certain cells, such as macrophages and neutrophils. It has a binding affinity for LFA-1, which is involved with the firm adhesion in neutrophil migration.[38] An increased ICAM-1 level can decrease the motility of neutrophils by allowing for firm adhesion.

As we have discussed, several consequences were brought about from the absence of SIRT1. Understanding of SIRT1 mechanisms in neutrophils is needed to understand related diseases.

V. CONCLUSION

Given that SIRT1 is involved in diverse mechanisms of inflammatory responses during infection, especially from the point of the migration function of immune cells, we could assume that SIRT1 also plays an important role in neutrophils against inflammation. However, the function of SIRT1 in neutrophils was unclear, and there were limited reports that SIRT1 participates in the regulation of autophagy and differentiation of neutrophils.[39] Since the expression level of SIRT1 protein is lower in neutrophils, it was difficult to detect the protein levels of SIRT1 by western blotting and antibody staining. Without knowing how much SIRT1 protein was expressed in neutrophils, our group studied the phenotype of SIRT1 knockout neutrophils; SIRT1 knockout neutrophils were functionally deficient in migration and phagocytosis as compared with wild-type neutrophils. Also, the intracellular calcium levels were lower in the knockout than in the wild-type. The study was limited by the fact that we were unable to quantify the amounts of the SIRT1 protein in neutrophils. We performed cytokine profiling to examine the processes that resulted from the absence of SIRT1 in order to fill the knowledge gaps between phenotype and functioning of the SIRT1 protein, as well as to further understand the relationship between SIRT1 and neutrophils. The basal levels of most cytokines were increased in SIRT1 knockout neutrophils, in the absence of stimulation of the neutrophils. We could not observe cytokine levels of stimulated conditions. It may be difficult to correlate the results of previous studies with our cytokine array results, as previous studies were performed under the stimulated conditions. Further experiments are necessary to confirm how SIRT1 regulates neutrophils from a basal status to inflammatory status.

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ABSTRACT (IN KOREAN)

호중구 이동현상에서의 SIRT1 기능 규명

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강경이

SIRT1은 sirtuin의 약자이며 SIRT1 gene에 의해 인코딩 되어있다. 포유동물에서 SIRT1은 탈 아세틸 화 역할과 p53 단백질을 비활성화 하는 것으로 잘 알려져 있다. 또한, 노화는 만성적이고 아주 낮은 단계의 염증반응으로 분류되는데, 이때 중요하게 작용하는 NF-kappa B는 SIRT1에 의해 조절되는 전사인자로서, 염증반응의 주 요소이다. SIRT1은 NF-kappa B의 소단위인 RelA/p65의 리신 310 잔기를 탈 아세틸 화 하여 NF-kappa B를 억제시킨다. 이렇게 SIRT1이 다양한 과정에 관여하고 있지만, 몇몇 조절 기작은

아직 알려진 바가 적다.

이 연구에서, 본 그룹은 기존에 알려진 바가 적던 호중구에서의 SIRT1 역할을 규명하였다. 식세포작용이나 호중구의 운동성을 SIRT1 유무에 따라 분석하였고, SIRT1 결여시 나타나는 표현형을 확인하였다.

SIRT1이 결여된 호중구와 야생형 호중구의 식세포작용을 비교 하였을 때, SIRT1이 결여된 호중구에서 식세포작용이 감소되었고, 빠른 면역반응시 중요한 호중구의 이동 능력도 야생형과 비교하였을 때 감소되어 있는 것을 볼 수 있었다. 또한 Ionomycin을 통해 호중구내 칼슘양을 측정 하였을 때, 칼슘양이 감소되어 있음을 확인하였다.

SIRT1이 결여된 호중구에서 면역반응에 중요한 역할을 하는 요소들이 감소된 양상을 보였고, 이러한 결과는 SIRT1이 선천면역에서 주요 역할을 하고있는 호중구를 조절함으로써, 면역조절에 중요한 역할을 할 수 있음을 암시한다.

핵심되는 말: 호중구, SIRT1, 백혈구, 식세포 작용, 세포이동