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Original Article

Activation of STING signaling accelerates skin wound healing

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ABSTRACT

Background: The process of repair after skin injury is precisely regulated by a variety of mediators such as cytokines and chemokines. Recent reports demonstrated that cytoplasmic DNA-sensor cyclic GMP-AMP synthase (cGAS) activates the stimulator of interferon genes (STING) via production of cyclic GMP-AMP (cGAMP) and subsequently induces inflammatory cytokines, including type I interferon (IFN).
Objective: We examined whether activation of the STING pathway by cGAMP affects the process of skin wound repair.
Methods: The skin wound repair model was established using wild-type (WT) mice. Two full-thickness skin biopsies were taken from the right and left subscapular regions. One site was treated with ointment containing cGAMP, and the other was treated with a control ointment. Changes in wound size over time were calculated using photography.
Results: Treatment with cGAMP significantly accelerated skin wound healing up to day 6. Biochemical analyses showed that topical treatment with cGAMP on wound sites promoted STING signaling pathway and enhanced the expression of IFN-β, CXCL10 and CCL2 in the wound sites treated with cGAMP markedly compared with the control. The scratch assay also revealed that cGAMP treatment accelerated wound closure in mouse embryonic fibroblasts. The acceleration of skin wound repair by cGAMP in WT mouse was impaired by administration of anti-IFNR antibody and anti-CXCR3 antibody respectively.
Conclusion: These results revealed that topical treatment with cGAMP accelerates skin wound healing by inducing type I IFN and CXCL10/CXCR3. Topical administration of cGAMP might contribute to new effective treatments for accelerating skin wound healing.

1. Introduction

Wound healing is a process of repair after skin injury. The complex signaling network involves growth factors, cytokines and chemokines, and the healing process can be divided into three overlapping phases: inflammatory phase, proliferative phase, and tissue remodeling. These steps in physiological healing are precisely regulated by cytokines/chemokines and growth factors [1]. Recent reports indicate that the innate immune system can influence the time needed for wound healing. Wound healing was significantly accelerated by local administration of CpG (oligodeoxynucleotides) ODN, a trigger of the innate immune system, via toll-like receptor (TLR) 9 [2]. Moreover, inflammasomes are activated after skin injury, and the activation of inflammasomes also accelerates skin repair after skin injury [3]. Thus, inflammation is critical for the acceleration of skin wound healing.

The rapid infiltration of plasmacytoid dendritic cells (pDCs), which are specialized type I interferon (IFN)–producing cells, to the skin wound sites and their activation to produce type I IFN through endosomal TLR9 play an important role in inducing early inflammatory responses and re-epithelization of skin wounds [4]. Recently, certain reports suggested that innate sensing of DNA in pDCs is not limited to endosomes and might also occur via cytosolic receptors in a TLR9-independent manner [5,6]. In addition, much progress has been reported in understanding how nucleic acids are recognized by the discovery of the cytoplasmic DNA-sensor cyclic GMP-AMP synthase (cGAS) [7]. Upon DNA binding, cGAS activates the stimulator of interferon genes (STING) adaptor via the production of cyclic GMP-AMP (cGAMP) as a second messenger [8,9]. Activated STING subsequently recruits tank-binding kinase 1 (TBK1) to phosphorylate interferon regulatory factor 3 (IRF3), and IRF3 subsequently dimerizes and translocates to the nucleus where the production of type I IFNs is induced [10,11]. An increasing number of studies suggest that the cGAS-STING pathway likely plays a major role in both immune defense against several microbial pathogens and

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autoimmune diseases caused by excessive cytoplasmic DNA such as SLE [12].

Based on these observations, it is likely that an innate immune response is involved in optimal wound healing. The STING pathway is also critical to the development of innate immune response. However, it is unknown whether the activation of STING pathway is involved in skin wound healing. In the current study, we examined the effect of STING ligands such as cGAMP on the process of skin wound repair using the skin wound repair model.

2. Materials and methods

2.1. Mice

C57BL/6 J wild-type (WT) mice (age 7–9 weeks, male) were obtained from Japan SLC Inc. (Shizuoka, Japan). All procedures were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the guidelines for the care and use of animals established by the Animal Care and Use Committee of Gifu University (Gifu, Japan)

2.2. Reagents

cGAMP (cyclic [G(3′,5′)pA(3′,5′)p]) was obtained from InvivoGen (San Diego, CA). In selected studies, cGAMP (2 μg/wound) was added to 100 μl of white petrolatum. The formulation was heated to 60 °C and quickly mixed to emulsify the components. Monoclonal antibodies (Ab) specific for murine interferon (IFN)-receptor (500 μg/mouse) and CXCR3 (300 μg/mouse) were purchased from Bio X cell (West Lebanon, NH). These antibodies were intraperitoneally administered on day 0 immediately before creation of skin wounds. Amlexanox (1 mg/mouse), purchased from Invivo Gen (San Diego, CA), was administered by daily oral gavage from 2 days before wound creation for 3 days.

2.3. Murine in vivo wound repair model

The wound repair model of Devalaraja et al. was used [13]. Mice were anesthetized, and the skin on the back was cleaned, shaved, and sterilized with betadine solution followed by 70 % ethanol. A 6-mm full-thickness (including the panniculus carnosus) excisional biopsy was taken using a biopsy punch (Kai Industries Co., Gifu, Japan) from the right- and left-upper paravertebral region of each animal. In certain experiments, the biopsy sites were coated with 100 μl of white petrolatum including 2 μl of cGAMP (5 μg/μl) and 3 μl of PBS, or 5 μl of PBS as control, on day 0 and 2. Mice were wrapped with a tight-fitting bandage to protect the biopsy sites. Wounds were checked and photographed every other day. Changes in wound contraction over time were calculated using ImageJ software (version 1.37; NIH, Bethesda, MD). The surface area of the wound defects was expressed as a percentage of the closure relative to the initial surface of each wound. Each treatment was tested and the results averaged in a minimum of 4 independent animals/group.

2.4. Extraction of RNA and quantitative RT-PCR

Tissues from biopsy site was excised 0, 8, 24, 48, 96 h, and 8 days after creating wound. The wound site tissues taken from the 2–3 mm surrounding the wound edge were frozen soon after collection. Total RNA from the wound site was extracted using the ISOGEN II reagent (Nippon Gene, Tokyo, Japan), and first-strand cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Quantitative real-time RT-PCR was performed using specific primer-probe sets that amplified the IFN-β1 mRNA genes (TaqMan® Gene Expression Assays and Universal PCR Master Mix; Applied Biosystems) and QuantiTect SYBR green PCR Master Mix (Qiagen GmbH, Hilden, Germany) for IFN-γ, F4/80, CXCL10, and CCL2. Each sample was analyzed on the Light-Cycler® 480 system (Roche Diagnostic Systems, Basel, Switzerland). The expression level of each gene was normalized against that of 18S rRNA. The primer sequences used for qRT-PCR are as follows : IFN-γ-fwd TGAACGCTACACTGCATCTTGG, IFN-γ-rev CGACTCCTTTTCCGCTTCCTGAG, F4/80-fwd AAGCATCCGAGACACACACAGTCT, F4/80-rev TGACTGTACCCACATGGCTGATGA, CXCL10-fwd GACGGTCCGCTGCAACTG, CXCL10-rev GCTTCCCTATGGCCCTCATT, CCL2-fwd GTTGGCTCAGCCAGATGCA, CCL2-rev AGCCTACTCATTGGGATCATCTTG, 18S-fwd GGGAGCCTGAGAAACGGC, 18S-rev GGGAGCCTGAGAAACGGC.

2.5. Enzyme- linked immunosorbent assay (ELISA)

Wound sites taken from around 2 mm surrounding wound edge were homogenized in CelLytic MT Cell Lysis Reagent (Sigma-Aldrich, St. Louis, MO, USA) and then centrifuged. Supernatants were used for measuring IFN-β protein levels by Mouse IFN-β ELISA Kit (R&D SYSTEMS) according to the manufacturer's instructions.

2.6. Western blot analysis

Wound sites taken from around 2 mm surrounding wound edge were homogenized in CelLytic MT Cell Lysis Reagent. Protein from the lysate was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane. After being blocked with 5 % skim milk and 5 % bovine serum albumin in Tris-buffered saline-Tween at room temperature for an hour, the membrane was incubated with rabbit anti-TBK1 (Cell Signaling, D1B4/#3504, 1:1,000), rabbit anti-pTBK1 (Ser172) (Cell Signaling, D52C2/#5483, 1:1,000), rabbit anti-CXCL10 (10H11L3) (Invitogen #701225) and anti-GAPDH (Cell Signaling Technology) primary antibodies respectively for 60 min and then incubated with a peroxidase labelled anti rabbit IgG antibody (Santa Cruz Biotechnology) for 60 min at room temperature. Detection of proteins bands was performed with ECL plus (GE Healthcare UK Ltd., England).

2.7. Scratch assay

Timed pregnant WT mice were euthanized, and the embryos were harvested at embryonic day 14.5. Mouse embryonic fibroblast (MEF) cultures were prepared using standard techniques [14]. Cells were maintained in complete DMEM medium supplemented with 10 % fetal bovine serum, penicillin/streptomycin, and L-glutamine (Gibco®, Invitrogen, Life Technologies, Grand Island, NY). Cultured MEFs from mice were grown in 12-well plates. When the cells reached confluence, a scratch was made across the cell monolayer with a yellow pipette tip (approximately 0.5 mm width). cGAMP (0.1 μg/ml or 1.0 μg/ml) was immediately added into the culture medium after generation of a scratch wound. After 8 h, the culture medium was removed and the cell was immersed in 4 % paraformaldehyde to be immobilized for 30 min. After that, it was stained with Crystal violet for 1 h and three representative images of the scratched areas were photographed for each experimental condition, and changes in the non-wound closure area were measured using ImageJ software.

2.8. Statistics

Values are expressed as the means ± standard errors of the mean (SEMs). Differences in the rate of healing of the wound sites were assessed using one way repeated measures analysis of variance (ANOVA). Comparisons between the experimental groups

were analyzed with the Kruskal-Wallis test followed by Scheffe's F-test. Significance was established at $p < 0.05$.

3. Results

3.1. Topical treatment with cGAMP accelerated skin wound healing in WT mice

Type I IFN plays an important role for skin wound healing at the early stage. We first examined the role of type I IFN in skin wound healing using the anti-type I IFN receptor antibody (IFNR Ab). To evaluate the effect of anti-IFNR Ab on the skin wound healing process, we established the skin wound repair model using WT mice. As shown in Fig. 1A and B, administration of anti-IFNR Ab significantly decelerated skin wound healing in WT mice. We also evaluated the topical effect of cGAMP in the skin wound healing process. Two identical full-thickness skin biopsies were taken from the right and left subscapular regions of individual mice. One site was treated with ointments containing cGAMP, and the other site was treated with PBS as control ointments. The wounds were observed every other day, and changes in wound size over time were calculated using photography. Treatment with cGAMP significantly accelerated wound healing on day 2 and 4 (Fig. 1C and E). Moreover, administration of anti-IFNR Ab impaired the

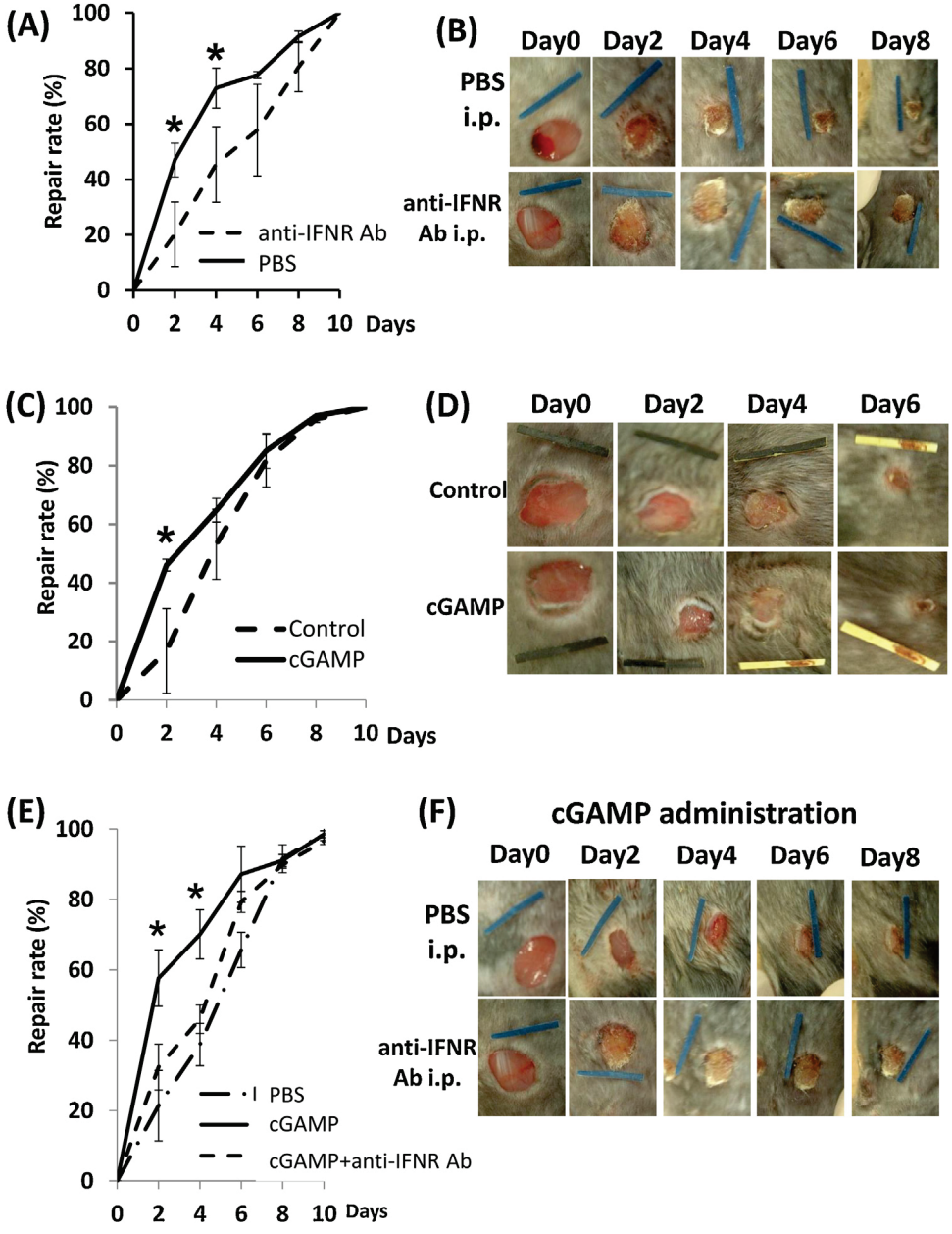

Fig. 1. Acceleration of skin wound healing via topical treatment with cGAMP.
(A, B) Multiple independent excisional biopsies (6 mm diameter) were taken from the right and left dorsum of WT mice (n = 6). Anti-IFNR Ab was administered before skin wound creation. (C, D) One biopsy site (selected at random) was treated on days 0 and 2 with PBS, and the other was treated with PBS formulated with cGAMP (n = 6). (E, F) Multiple independent excisional biopsies (6 mm diameter) were taken from the right and left dorsum of WT mice (n = 4). Anti-IFNR Ab was administered intraperitoneally just before skin wound creation. One biopsy site (selected at random) was treated on days 0 and 2 with PBS, and the other was treated with PBS formulated with cGAMP (n = 4). Each wound was recorded via digital photograph with a scale bar indicating 10 mm. The wounds were observed every other day, and changes in wound size over time were calculated using photography. The mean rate of repair was calculated based on the original wound area of each biopsy site. The percentage repair over time is shown. *Indicates statistically significant differences; $p < 0.05$.

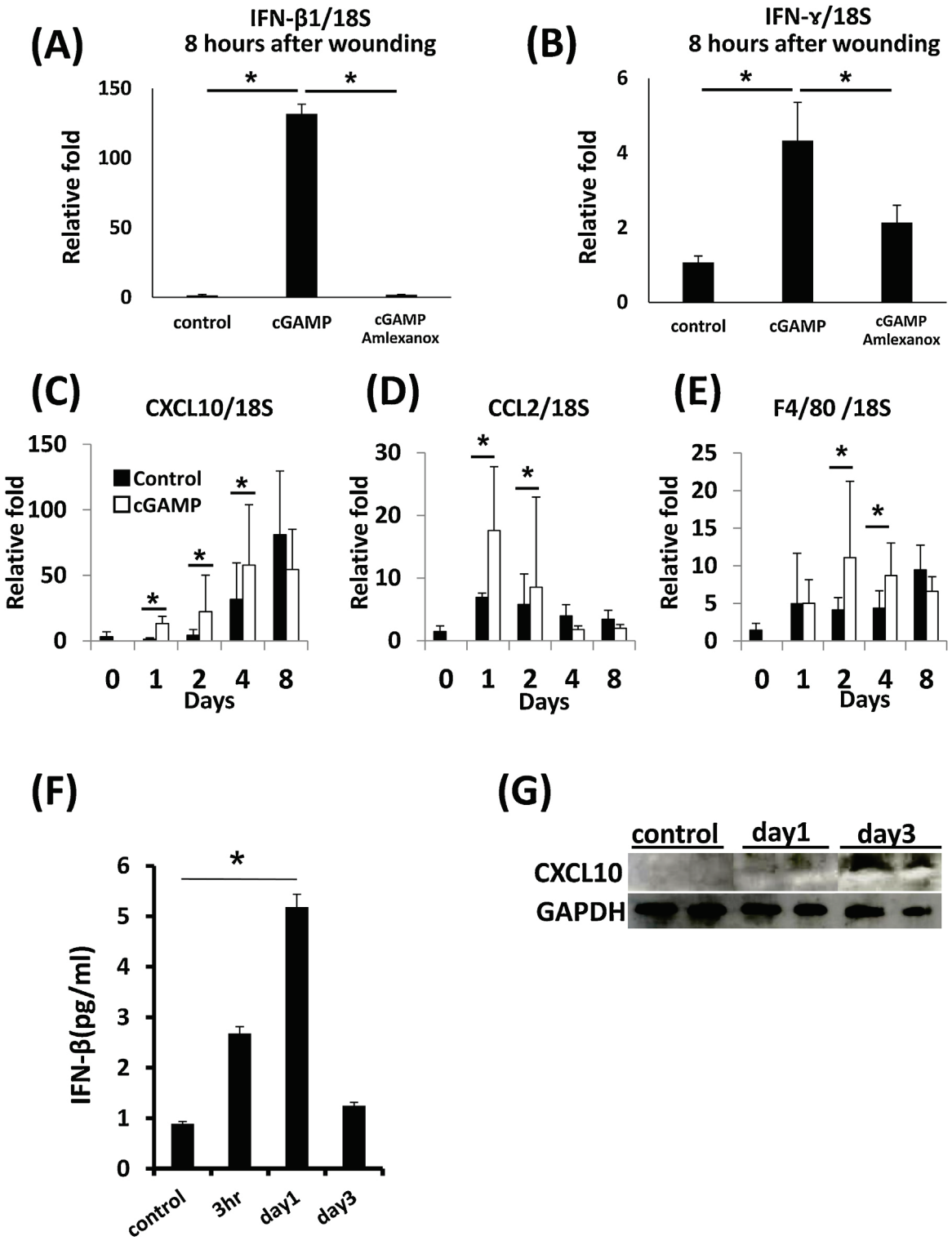

Fig. 2. Topical administration of cGAMP at the wound site up-regulates the production of IFNs and chemokines.
(A–E) Multiple independent excisional biopsies (6 mm diameter) were taken from the right and left dorsum of WT mice. WT mice were assigned to three groups; topical treatment with PBS as control, topical treatment with cGAMP and administration with Amlexanox as well as topical treatment with cGAMP. Amlexanox was administered orally just before skin wound creation. mRNA expression of IFN-β1 (A) and IFN-γ (B) 8 h after creation of the wound. mRNA expression of CXCL10 (C), CCL2 (D), and F4/80 (E) in the wound sites was measured using quantitative RT-PCR after wound creation on days 0, 1, 2, 4 and 8 (n = 3 mice with 2 wound sites in each groups). Each value is the mean ± SE for three mice. The results were normalized to the expression of 18S rRNA. (F) The concentration of IFN- β at the wound site treated with cGAMP in WT mice was measured by ELISA (n = 3 mice with 2 wound sites in each time points). (G) Expression of CXCL10 protein at the wound site treated with cGAMP were examined using western blot analysis. Each data point and error bar represent the mean and SE, respectively, of data from triplicate samples. *Indicates statistically significant differences; $p < 0.05$

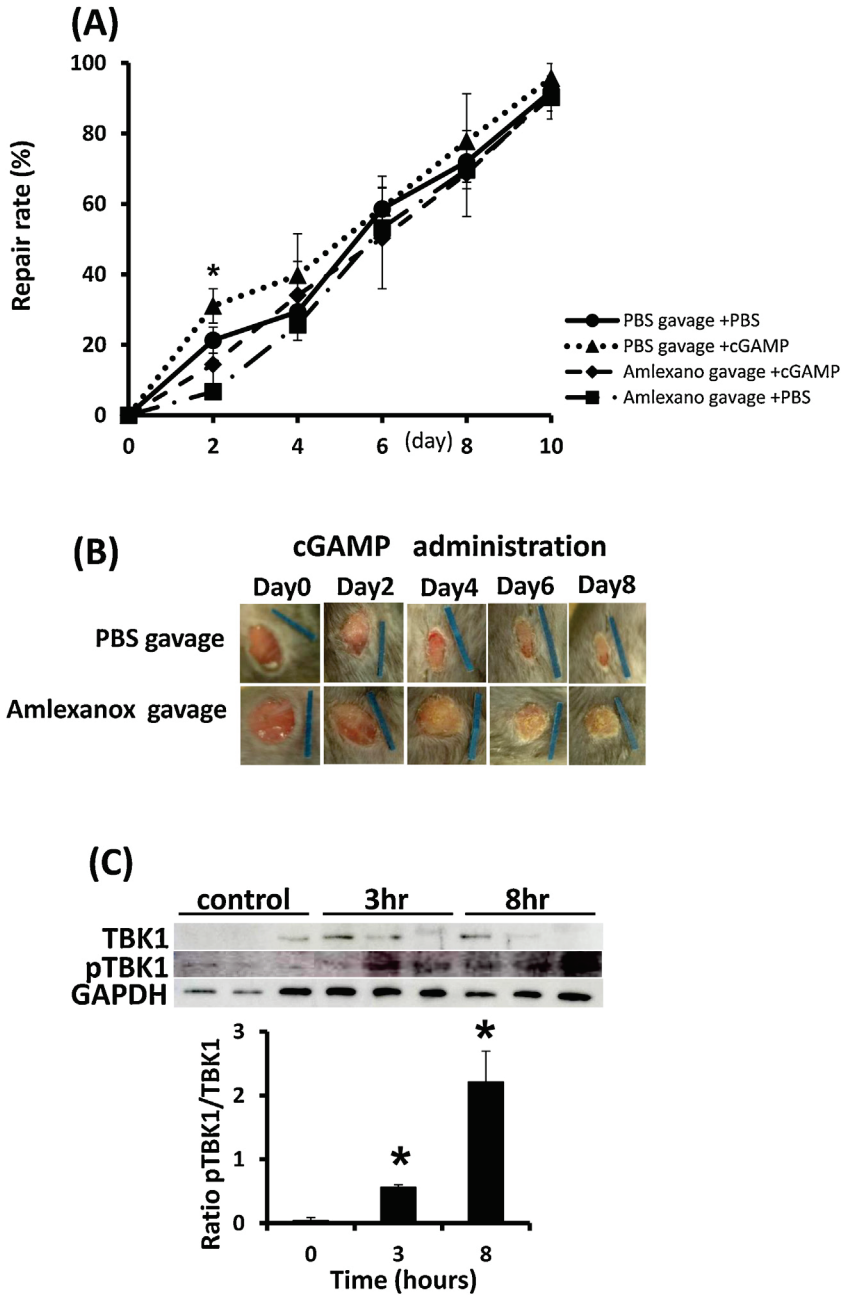

Fig. 3. Inhibition of TBK1 impaired the acceleration of skin wound healing by cGAMP.
(A, B) Multiple independent excisional biopsies (6 mm diameter) were taken from the right and left dorsum of WT mice. We compared the rate of wound closure among 4 groups; PBS gavage + PBS administration, PBS gavage + cGAMP administration, Amlexanox gavage + cGAMP administration, and Amlexanox gavage + PBS administration (n = 3 mice with 2 wound sites in each groups). Amlexanox was administered by daily oral gavage from 2 days before wound creation for 3 days. Each wound was recorded via digital photograph with a blue bar indicating 10 mm (B). The wounds were observed every other day, and changes in wound size over time were calculated using photography. The mean rate of repair was calculated based on the original wound area of each biopsy site. The percentage repair over time is shown. *Indicates statistically significant differences; $p < 0.05$. (C) Expression of TBK1 and pTBK1 protein at the wound site treated with cGAMP were examined using western blot analysis. The TBK1 phosphorylation level was evaluated as the ratio of pTBK1 and TBK1 chemiluminescent signals. Each data point and error bar represent the mean and SE, respectively, of data from triplicate samples. *Indicates statistically significant differences; $p < 0.05$

acceleration of skin wound healing by cGAMP (Fig. 1E and F). These results indicated that topical administration of cGAMP accelerated skin wound healing in WT mice via inducing type I IFNs.

3.2. Topical administration of cGAMP at the wound site up-regulates the production of IFNs and chemokines

A previous report demonstrated that administration of cGAMP enhanced the expression of type I IFN and IFN-related genes [15]. Therefore, we measured the mRNA expression of IFNs and chemokines at the wound site after treatment with cGAMP. mRNA expression of IFN-β1 and IFN-γ at wound sites treated with cGAMP significantly increased compared with that at wound sites without cGAMP at 8 h after creation of the wound (Fig. 2A and B). Moreover, the up-regulation of IFN-β1 and IFN-γ expression was impaired by amlexianox, a specific inhibitor of TBK1 [16]. We also examined IFN-β protein level at the wound site treated with cGAMP in WT mice using ELISA. IFN-β expression was significantly increased by the administration with cGAMP, as shown in Fig. 2F.

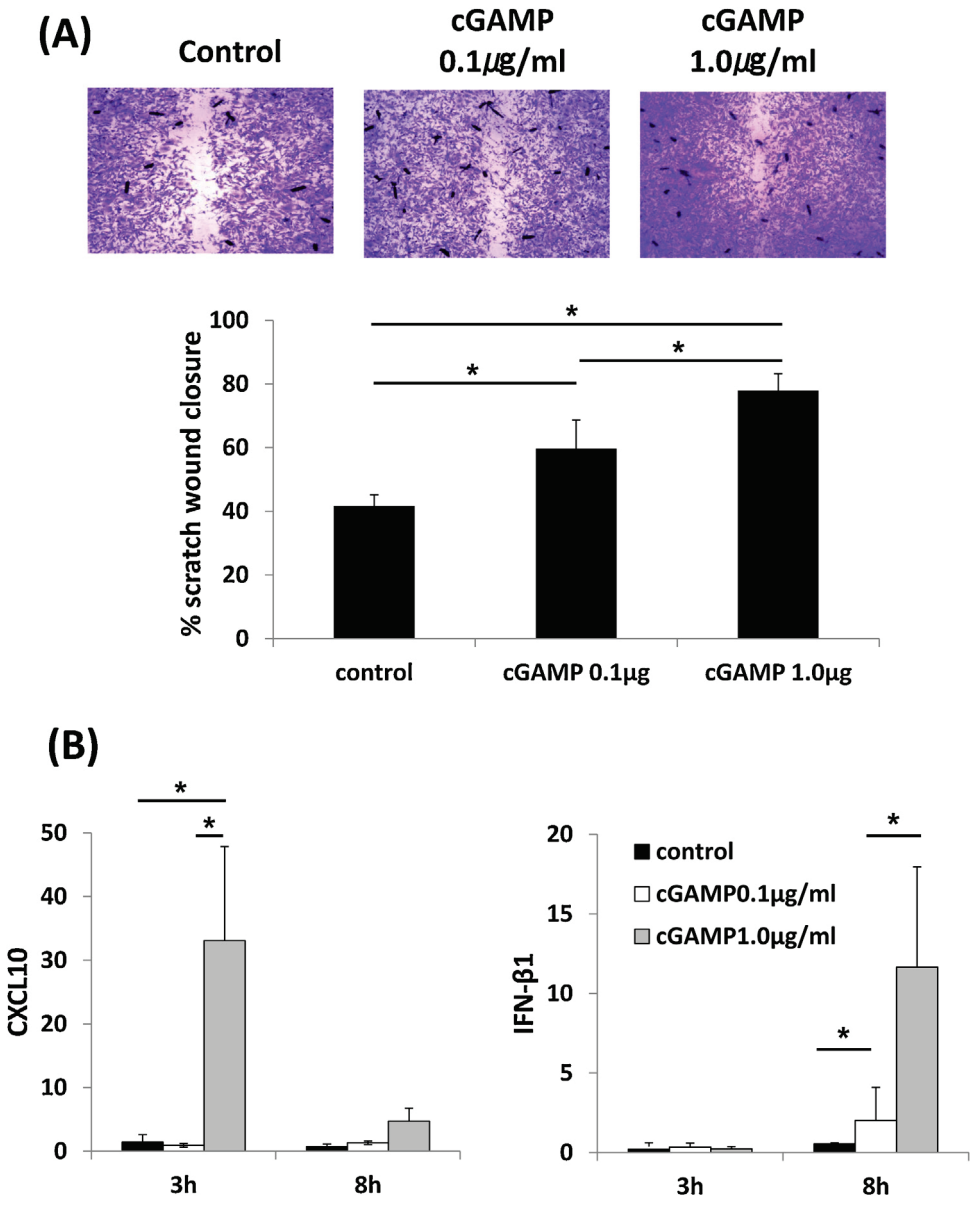

Fig. 4. Administration with cGAMP accelerated the scratch wound closure in MEFs.
Cultured MEFs from WT mice were grown on 12-well plates. When the cells reached confluence, the monolayer was injured by scratching across the plate with a yellow pipette tip (approximately 0.5 mm in width). (A) Digital photographic images were taken 8 h after creating the scratch wound. The repair rate of scratch wounds was determined for each treatment. Each data point and error bar represent the mean and SEM, respectively, of data from triplicate samples. (B) The relative expression levels of IFN-β1 and CXCL10 mRNA in MEFs from WT mice 3 and 8 h after administration with cGAMP were measured by quantitative real-time RT-PCR. The results were normalized to the expression of GAPDH mRNA. *Indicates statistically significant differences; $p < 0.05$

As shown in Fig. 2C and D, mRNA expression of CXCL10 and CCL2 in the wound sites treated with cGAMP markedly increased compared with those in the wound sites treated with PBS. We also examined CXCL10 protein level using western blot analysis. The expression of CXCL10 with cGAMP treatment significantly increased 3 days after creating skin wound (Fig. 2G). Moreover, the expression of F4/80 mRNA level was also significantly enhanced in the wound sites after the treatment with cGAMP (Fig. 2E). These results indicated that the administration with cGAMP promoted several chemokines such as CXCL10 and CCL2 and recruited macrophages in the wound site.

3.3. cGAMP promotes production of type I IFN through the STING-TBK1-IRF3 axis

To further investigate wherther administration of cGAMP at the wound site promotes production of type I IFN via activation of the STING pathway, we administered amlexianox by daily oral gavage to WT mice 2 days before creation of full-thickness wound sites. Skin wound healing rate of WT mice treated with cGAMP was impaired by the administration with amlexanox (Fig. 3A and B). Furthermore, oral administration of amlexanox significantly decreased mRNA expression of IFN-β1 and IFN-γ at wound sites treated with cGAMP 8 h after creation of the wound (Fig. 2A and B).

Next, we measured protein level of TBK1 and TBK1 phosphorylate (pTBK1) using western blot to confirm that cGAMP induces type I IFN through TBK1 and IRF3 activation. pTBK1/TBK1 ratio was significantly increased 8 h after creating the wound site treated with cGAMP in WT mice (Fig. 3C). These results indicated that activation of STING by topical administration with cGAMP induced the production of IFNs via activation of TBK1 and accelerated skin wound healing in WT mice.

3.4. Addition of cGAMP accelerated the scratch wound closure

To evaluate the effect of cGAMP on wound healing in vitro, we prepared MEFs from WT mice and conducted scratch wound assays using these cells. As shown in Fig. 4A, the rates of scratch wound closure in the medium with added cGAMP significantly increased compared with those in the medium without cGAMP. Moreover, the wound closure rate increased in a concentration-dependent manner. We evaluated the expression of type I IFN and CXCL10 induced by the administration of cGAMP in vitro using MEFs. The CXCL10 mRNA expression level markedly increased 3 h after the administration of cGAMP. IFN-β1 mRNA expression was also enhanced by stimulation with cGAMP (Fig. 4B). These results indicated that cGAMP also induced type I IFN and chemokines such as CXCL10, which are involved in promotion of wound healing in vitro.

3.5. Inhibition of CXCR3 signaling impaired the acceleration of skin wound healing by cGAMP

CXCL10 appears in the dermis, is produced by endothelial cells of the neovasculature, and binds in common to the ubiquitous CXCR3 chemokine receptor [17]. A previous report showed that in keratinocytes, signaling through the CXCR3 receptor promotes wound healing by accelerating re-epithelialization and promoting matrix maturation [18]. To examine whether the CXCL10/CXCR3 axis is involved in the acceleration of skin wound healing by administration with cGAMP, we evaluated the effect of anti-CXCR3 antibody on the wound repair rate after administration of cGAMP. Immediately before creation of full-thickness wound sites, we intraperitoneally injected anti-CXCR3 antibody into the WT mice. Although topical treatment with cGAMP promoted skin wound healing rate as shown in Fig. 1, the acceleration of skin wound repair was impaired by administration of the anti-CXCR3 antibody (Fig. 5). These results indicated that CXCL10/CXCR3 signaling was critical in the acceleration of wound healing induced by cGAMP treatment.

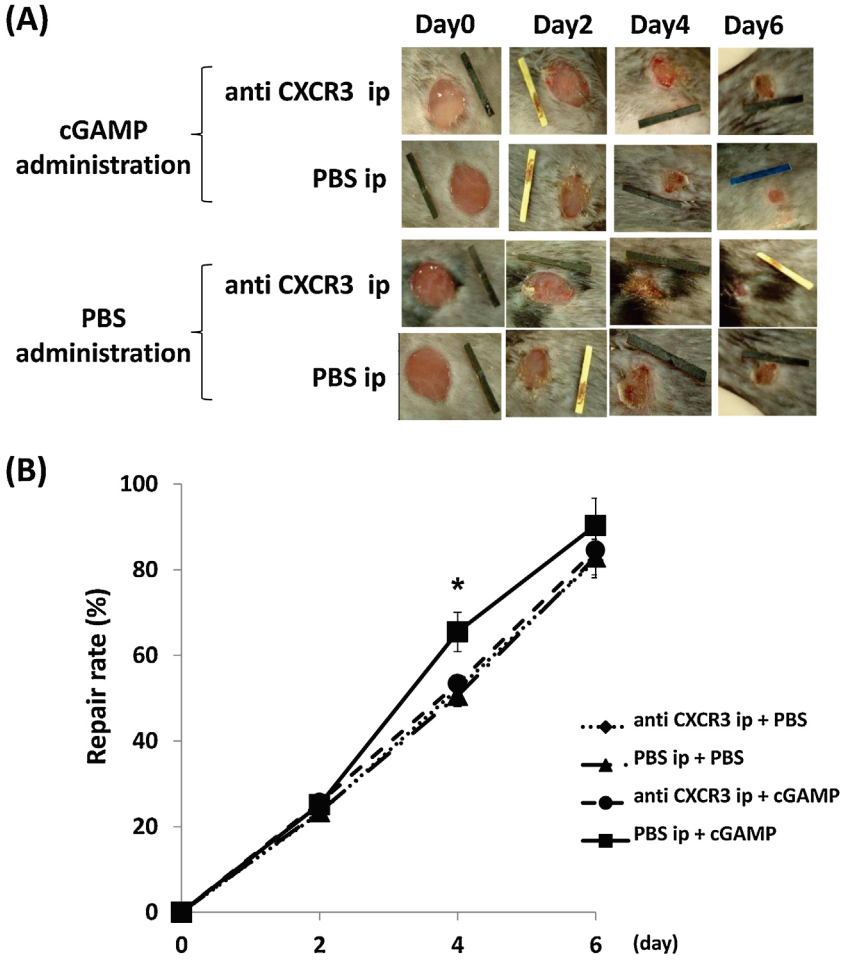

Fig. 5. Treatment with anti-CXCR3 antibody decreased the rate of wound closure in WT mice treated with cGAMP.
Multiple independent excisional biopsies (6 mm diameter) were taken from the right and left dorsum of WT mice (n = 4 in each groups). Anti-CXCR3 antibody or PBS was intraperitoneally administered to WT just before wound creation. We treated wound sites on days 0 and 2 with PBS or PBS formulated with cGAMP. We compared the rate of wound closure among four groups; PBS ip + PBS, PBS ip + cGAMP, anti-CXCR3 ip + PBS, and anti-CXCR3 ip + cGAMP. (A) The wounds were observed every other day, and changes in wound size over time were calculated using photography. (B) The mean rate of repair was calculated based on the original wound area of each biopsy site. The percentage repair over time is shown. The acceleration of skin wound repair treated with cGAMP was impaired by administration of the anti-CXCR3 antibody at day 4 significantly. The bar in all photographs indicates 10 mm. *Indicates statistically significant differences; $p < 0.05$

4. Discussion

In the current study, we demonstrated that topical treatment with cGAMP significantly accelerated skin wound healing. Topical treatment with cGAMP induced an earlier increase of chemokine expression after skin wound creation. The increase in expression of several chemokines induced cell migration in the skin wound site. The enhancement of chemokine expression might be involved in the acceleration of wound healing. Moreover, we found that CXCL10-CXCR3 signaling was critical for the acceleration of skin wound healing induced by cGAMP treatment.

Wound healing is a complex event that develops in three phases: inflammatory, proliferative, and remodeling. These phases depend on the interaction of cytokines, growth factors, chemokines, and chemical mediators from various cells to perform regulatory events [19,20]. The first stage of wound repair, which starts immediately after cutaneous injury, lasts up to one week and features an inflammatory reaction mediated via growth factors and cytokines/chemokines. A previous study demonstrated that type I IFN is critical for the induction of early inflammatory responses and re-epithelization of injured skin [4]. We also found that wound closure was significantly impaired by administration with anti-IFNR Ab, especially up to day 4 (Fig. 1A and B). These results indicated that type I IFN is involved in promotion of wound healing, especially at the early stage of the wound healing process.

Innate immunity has evolved as the first line of host defense against microbial infection, and it is the prerequisite for initiating the adaptive immune responses. Recognition of different pathogen-associated molecular patterns (PAMPs) is primarily mediated by specific pattern recognition receptors (PRRs), including toll-like receptors, nod-like receptors, RIG-I-like helicases and cytosolic DNA receptors [21], followed by activation of downstream signaling pathways that initiate the production and secretion of proinflammatory cytokines, chemokines, and type I IFN to fight against pathogens. Recent studies indicated that following activation by cGAMP, STING promotes phosphorylation of TBK1, which further induces phosphorylation of IRF3 and subsequently the expression of type I IFN [10]. The present study also indicated that pTBK1/TBK1 ratio in the wound site treated with cGAMP was significantly enhanced by western blot 8 h after creation of the wound (Fig. 3D). In addition, administration of amlexanox, a specific inhibitor of TBK1, decreased mRNA expression of IFN-β1 and IFN-γ significantly at the wound site increased by cGAMP (Fig. 2A and B). Therefore, we hypothesized that topical treatment with cGAMP accelerates skin wound healing because type I IFN is involved in skin wound healing and cGAMP up-regulates the production of type I IFN via the activation of STING pathway. In the current study, we demonstrated that topical treatment with cGAMP significantly increased the expression of type I IFN and accelerated wound healing, especially at day 2 (Figs. 1C and D, 2 A and F). Moreover, wound repair acceleration by cGAMP treatment was impaired by inhibition of TBK1 especially at day 2 (Fig. 3A and B). In an in vitro assay, scratch wound closure was markedly accelerated in cell monolayers cultured in medium with added cGAMP (Fig. 3). Therefore, we concluded that cGAMP had the ability to increase the rate of wound healing in vitro and in vivo, which indicated that cGAMP contributes to the wound healing process, especially in the inflammatory phase. As shown in Fig. 2C, D and G, expression of CXCL10 and CCL2 in the wound sites treated with cGAMP markedly increased day 1 and 3 after creation of the wound. We also demonstrated that CXCL10 and type I IFN are up-regulated by cGAMP in an in vitro assay (Fig. 4B).

These results indicated that type I IFN and IFN-γ induced by cGAMP consequently up-regulate CXCL10 and CCL2 at the wound sites. A recent report also indicated that type I IFN increases production of IFN-γ by promoting T helper 1 cell differentiation [22]. CXCL10, or interferon-gamma-induced protein 10 (IP-10), was initially reported to be induced by IFN-γ and secreted by various cell types including monocytes, neutrophils, endothelial cells, keratinocytes and fibroblasts [17]. CXCL10 can activate and recruit several immune cells such as T and B lymphocytes and other leukocytes by binding to its high affinity receptor CXCR3 [23]. CXCL10 expression is strongly up-regulated in many inflammatory diseases, including atherosclerosis [24]. At wound sites, CXCL10 is a crucial mediator in signaling the end of the regenerative phase by initiating the remodeling phase [25]. Stimulation of CXCR3 signaling converts fibroblasts from a migratory to a contractile state following an increase in mature dermal collagen fibers. Thus, CXCR3 signaling is crucial for dermal maturation [25]. As shown in Fig. 5, the acceleration of skin wound repair by cGAMP was impaired by the administration of anti-CXCR3 antibody, which indicated that CXCL10 induced by cGAMP promoted wound healing via activation of CXCR3.

In conclusion, we revealed that topical treatment with cGAMP accelerates skin wound healing in early stage by inducing type I IFN and that the CXCL10/CXCR3 pathway is involved in the acceleration of skin wound healing by cGAMP treatment. The topical administration of cGAMP might contribute to new effective treatments for accelerating skin wound healing.

Declaration of Competing Interest

The authors have declared no conflicting interests.

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