

TNF- α -induced Inhibition of Protein Myristoylation Via Binding Between NMT1 and Sorbs2 in Osteoblasts

忽那, 重彦

<https://hdl.handle.net/2324/7182396>

出版情報 : Kyushu University, 2023, 博士 (歯学) , 課程博士
バージョン :

権利関係 : © 2024, International Institute of Anticancer Research (Dr. George J. Delinasios),
All rights reserved



TNF- α -induced Inhibition of Protein Myristoylation Via Binding Between NMT1 and Sorbs2 in Osteoblasts

SHIGEHICO KUTSUNA, GORO SUGIYAMA, TAKUMA KOMIYAMA, HANAE KAMOHARA,
YUKIKO OHYAMA, WATARU KUMAMARU and TOMOHIRO YAMADA

Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Abstract. *Background/Aim:* Bone resolution due to tumor invasion often occurs on the surface of the jaw and is important for clinical prognosis. Although cytokines, such as TNF- α are known to impair osteoblasts, the underlying mechanism remains unclear. Protein myristoylation, a post-translational modification, plays an important role in the development of immune responses and cancerization of cells. A clear understanding of the mechanisms underlying this involvement will provide insights into molecular-targeted therapies. N-myristoyltransferase1 (NMT1), a specific enzyme involved in myristoylation, is expressed in cancer cells and in other normal cells, suggesting that changes in myristoylation may result from the regulation of NMT1 in cancer cells. *Materials and Methods:* Using newly emerging state-of-the-art techniques such as the Click-it assay, RNA interference, mass spectrometry, immunoprecipitation, immunocytochemistry, and western blotting, the expression of myristoylated proteins and the role of TNF- α stimulation on NMT1 and Sorbs2 binding were evaluated in a murine osteoblastic cell line (MC3T3-E1). *Results:* The expression of myristoylated proteins was detected; however, TNF- α stimulation resulted in their inhibition in MC3T3-E1 cells. The expression of NMT1 also increased. Immunoprecipitation and mass spectrometry identified Sorbs2 as a novel binding protein of NMT1, which upon TNF- α stimulation, inhibited myristoylation. *Conclusion:* The binding between NMT1 and Sorbs2 can regulate

myristoylation, and NMT1 can be considered as a potential target molecule for tumor invasion.

In gingival cancer, tumor invasion of the jaw is mainly characterized by bone resolution. Appropriate evaluation and determination of the margins are important factors regarding clinical prognosis. Since delays in treatment can lead to poor outcomes, a marker of invasion is required. Inflammation caused by a tumor on the bone surface results in osteoblastic impairment and osteoclast activation (1). The cytokines are a major source of inflammation, with TNF- α being a potent inflammatory cytokine released from tumor cells in response to noxious stimuli (2). These inflammatory responses suppress immune functions of osteoblasts (3). Although bone resolution in response to TNF- α -induced inflammation results from dysfunctional bone metabolism and the activation of osteoclasts, its effect on osteoblasts remains unclear.

Post-translational modifications (PTM) are chemical responses that play an important role in cellular functions (4). These processes include phosphorylation, glycosylation, ubiquitination, methylation, acetylation, and lipid synthesis. Myristoylation, an irreversible reaction that occurs ubiquitously in cells, is a type of PTM that plays an important role in immune responses and cancer (5, 6). For example, it is essential for the oncogenic activity of the Src protein, which is a major molecule in cancer (7). In addition to cancer, this lipid modification is related to immune disorders, such as human immunodeficiency virus (HIV) infection and fungus activation (8-10). Although PTM are important in mediating the immune system, detection methods for myristoylation have not been established compared to those for other PTMs for a long time. However, a unique chemical approach using binds between azide and alkyne called “click chemistry” has been recently developed (11). This method is safer compared to the traditional method using radioisotopes and therefore, helps in conducting easy research on cellular processes.

N-myristoyltransferase (NMT) is a catalytic enzyme involved in myristoylation (12). Some isoforms (NMT1 and

Correspondence to: Dr. Goro Sugiyama, Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan. Tel: +81 926426452, Fax: +81 926426392, e-mail: gs5603@dent.kyushu-u.ac.jp

Key Words: Myristoylation, NMT1, Sorbs2, osteoblast, TNF- α .



This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) 4.0 international license (<https://creativecommons.org/licenses/by-nc-nd/4.0>).

NMT2) are expressed ubiquitously in cells, especially during embryonic development, with NMT1 being the most important (13). Given its functional importance, NMT1 is not essential for the viability of mammalian cells, but is required for embryonic cell development. NMT1 is highly expressed in several cancers, including colorectal, breast, and oral cancers (14-16). Inhibition of NMT1 through an NMT inhibitor effectively inhibits myristoylation, highlighting a potential novel drug target in clinical trials (17). However, the expression and regulation of myristoylation in normal cells in response to cytokines, such as TNF- α , remain unclear. While changes in the expression of NMT1 lead to the regulation of myristoylation, information regarding molecules interacting and regulating NMT1 is still not well understood.

To this end, this study aimed to confirm whether the expression of myristoylated proteins is suppressed by TNF- α stimulation in osteoblasts, using newly emerging state-of-the-art techniques.

Materials and Methods

Antibodies and reagents. Recombinant murine TNF- α was obtained from Peprotech (Rocky Hill, NJ, USA); NMT-1 and Sorbs2 antibodies, Sorbs2 and the control siRNA-A from Santa Cruz Biotechnology (Dallas, TX, USA); PCLX-001, a specific NMT1 inhibitor, from MCE (Monmouth Junction, NJ, USA); Click-iT cell Reaction Buffer Kit, myristic acid azide, Alexa-alkyne 488, Alexa 532, and Lipofectamine RNAiMAX Reagent from Invitrogen (Carlsbad, CA, USA).

Cell culture and transfection. MC3T3-E1 cells were supplied by the RIKEN Cell Bank. For stimulation, the cells were cultured with or without TNF- α (20 ng/ μ l) as described previously (18). The cells were transfected with siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

RNA interference. The siRNA duplexes against mouse Sorbs2 and control (scrambled) siRNAs were synthesized by Eurofins Genomics (Ebersberg, Germany). The sense strands of the siRNAs were as follows: negative control, private; siSorbs2, 5'-GAAGUCUAU GCCCAUUCUAtt-3'; 5'-UAGAUUGGGCAUAGACUUCtt-3'. The cells were transfected with siRNA using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After incubation, the medium was replaced.

Click chemistry assay. Click chemistry assays were performed using the Click-iTTM cell Reaction Buffer, followed by incubation of MC3T3-E1 cells with myristic acid for 12 h. Myristoylated proteins, with or without TNF- α and PCLX-001 stimulation, were labeled using Alexa-alkyne 488 or Alexa 532, and detected using a fluorescence microscope (Bioevo BZ-9000, Keyence, Osaka, Japan). For detection of the nuclei, the cells were stained with 4',6-diamidino-2-phenylindole.

Immunocytochemistry. The immunocytochemistry assay using the anti-NMT1 antibody (1:100) in MC3T3-E1 cells was performed as previously described (18). MC3T3-E1 cells were fixed with 3.7% formaldehyde and 0.2% glutaraldehyde, blocked with 5% skim milk

in PBS, and incubated overnight with the antibody. The cells were incubated with Alexa Fluor 430-conjugated anti-rabbit IgG (1:10,000; Invitrogen, Carlsbad, CA, USA) for 90 min at 37°C. The detection was performed using a fluorescence microscope.

Western blotting. Western blotting was performed following established protocols (18). Protein lysates from cell homogenates were subjected to SDS-PAGE and transferred to nitrocellulose membranes. Immunoreactive bands were visualized using computer-assisted densitometry (MultiImagerII-Mi-600CB; Bio-Tools).

Mass spectrometry. Cell lysates from the homogenates were prepared for mass spectrometry. Equal amounts of protein (30 μ g) from each sample were electrophoresed on 7.5% SDS-PAGE and stained with Coomassie brilliant blue (Bio-Rad Laboratories). After washing with PBS, the gel was cut, and the two samples were analyzed. For protein identification using peptide mass fingerprinting (PMF), protein spots were excised, digested with trypsin (Promega, Madison, WI, USA), mixed with α -cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid (TFA), and subjected to Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) analysis (Microflex LRF 20, Bruker Daltonics). Spectra were collected from 300 shots per spectrum over the m/z range 700-4,000 and calibrated by two-point internal calibration using trypsin autodigestion peaks (m/z 842.5099, 2211.1046). A peak list was generated using Flex Analysis 3.0. The threshold used for peak-picking was as follows: 500 for minimum resolution of monoisotopic mass and 6 for S/N. The search program MASCOT, developed by Matrixscience, was used for protein identification by PMF (19). The following parameters were used for the database search: trypsin as the cleaving enzyme; a maximum of one missed cleavage; iodoacetamide (Cys) as a complete modification; oxidation (Met) as a partial modification; monoisotopic masses; and a mass tolerance of ± 0.2 Da. The PMF acceptance criterion is probability scoring.

Immunoprecipitation. Immunoprecipitation assay was performed in MC3T3-E1 cells using 2 μ g anti-NMT1 rabbit polyclonal antibody. The lysate was incubated for 1 h with protein G-Sepharose beads. The prepared samples were analyzed by western blotting as described previously (18).

Results

Effect of TNF- α on the expression of myristoylated proteins in MC3T3-E1 cells. We examined whether myristoylated proteins were expressed in MC3T3-E1 cells using a click chemistry assay. Compared to the control, myristoylated proteins were detected around the nucleus (Figure 1A). To confirm the involvement of myristoylation and NMT1, we assessed the effect of PCLX-001, a specific NMT1 inhibitor. Upon treatment, a dose-dependent decrease in the levels of myristoylated proteins was observed (Figure 1B). To investigate cellular behavior under inflammatory conditions, the effect of TNF- α as an inflammatory cytokine, was examined, and the expression of myristoylated proteins was found to be decreased and some of them translocated to the membrane (Figure 1C). The expression of myristoylated proteins was induced by NMT1 in MC3T3-E1 cells, and our

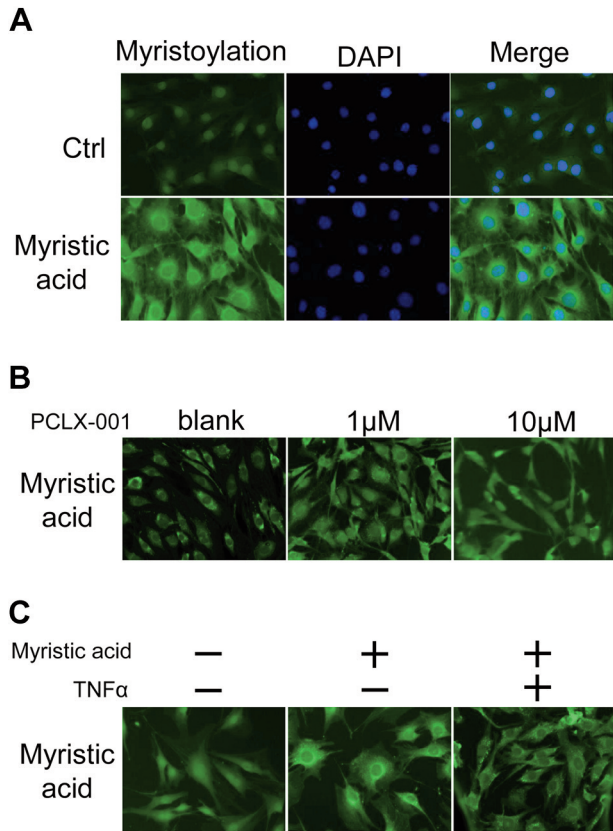


Figure 1. Effect of $\text{TNF-}\alpha$ on expression of myristoylated proteins in MC3T3-E1 cells. (A) Myristoylated proteins as determined by click chemistry assay in MC3T3-E1 cells treated with myristic acid with azide (10 μM) for 12 h. For click chemistry, the cells were incubated with Alexa-alkyne 488, fixed with 3.7% formaldehyde and 0.2% glutaraldehyde, and then blocked with 1% BSA in PBS for detection. The sample without myristic acid is indicated as a control. (B) Click chemistry analysis of myristoylated proteins in MC3T3-E1 cells treated with or without PCLX-001. Blank is indicated as a control. (C) Click chemistry analysis of myristoylated proteins in MC3T3-E1 cells treated with or without $\text{TNF-}\alpha$.

results suggested that $\text{TNF-}\alpha$ is associated with the inhibition and translocation of myristoylated proteins to the membrane.

Effect of $\text{TNF-}\alpha$ stimulation on the expression of NMT1 in MC3T3-E1 cells. To confirm that NMT1 is a specific enzyme for myristoylation, we examined the expression of NMT1 and the effect of $\text{TNF-}\alpha$ on MC3T3-E1 cells using immunocytochemistry. We found that NMT1 was expressed in MC3T3-E1 cells and upon $\text{TNF-}\alpha$ stimulation, its expression increased (Figure 2A). Furthermore, western blotting confirmed that the expression increased in response to $\text{TNF-}\alpha$ stimulation in a time-dependent manner (Figure 2B).

Effect of $\text{TNF-}\alpha$ stimulation upon NMT1 and Sorbs2 binding in MC3T3-E1 cells. Treatment with $\text{TNF-}\alpha$ resulted in a

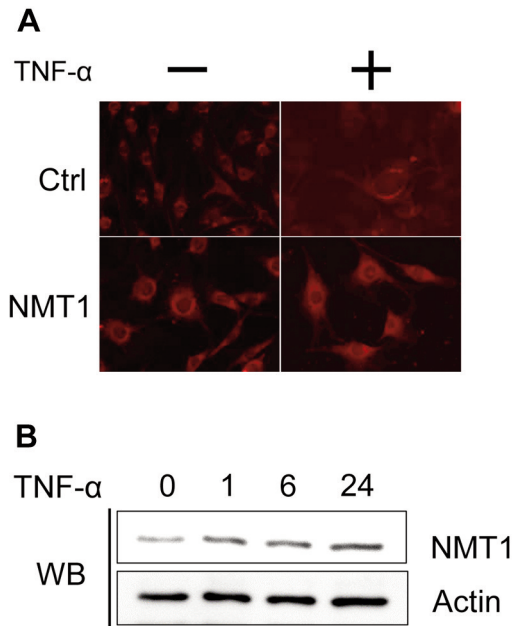


Figure 2. Expression of NMT1 in MC3T3-E1 cells in response to $\text{TNF-}\alpha$ stimulation. (A) Immunocytochemistry analysis of NMT1 in MC3T3-E1 cells treated with or without $\text{TNF-}\alpha$, and fixed with 3.7% formaldehyde and 0.2% glutaraldehyde, blocked with 1% BSA in PBS, and incubated with the NMT1 antibody (1:250). (B) NMT1 was detected using anti-NMT1 antibody by western blotting analysis.

significant increase in the expression of NMT1; however, this change was not likely associated with the suppression of myristoylated proteins. Thus, we hypothesized that might be an interaction between regulatory molecules and NMT1. To elucidate this interaction, we investigated the binding of NMT1 to other proteins using immunoprecipitation with an anti-NMT1 antibody. Compared with the control, two additional protein bands were observed in the $\text{TNF-}\alpha$ stimulated sample after staining with Coomassie brilliant blue (Figure 3A). Mass spectrometry was performed using the sample isolated from the gel (Figure 3B). Although the data revealed binding of several proteins, Sorbs2 was identified as the most likely binding target. We performed immunoprecipitation and detected Sorbs2 using western blotting (Figure 3C). The results imply that NMT1 binds to Sorbs2 upon $\text{TNF-}\alpha$ stimulation in MC3T3-E1 cells. These results indicate the possibility that Sorbs2 plays an important role in the suppression of myristoylation.

The expression and effect of Sorbs2 on myristoylation in MC3T3-E1 cells. To examine the expression and localization of Sorbs2 in MC3T3-E1 cells, immunocytochemistry was performed using an anti-Sorbs2 antibody. The expression pattern was clearly spindle-like (Figure 4A). Additionally, suppression of Sorbs2 using siRNA rescued myristoylation

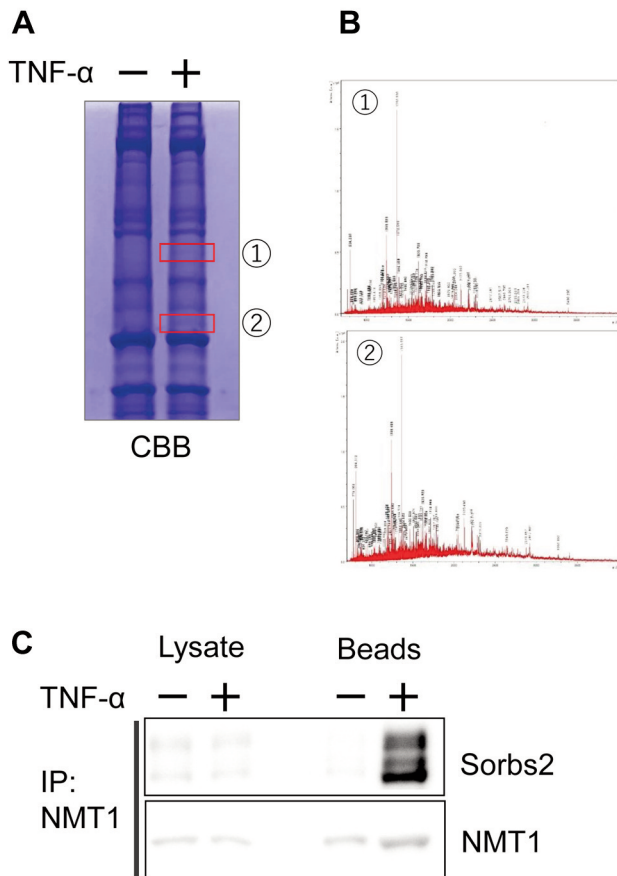


Figure 3. Effect of TNF- α stimulation on the binding between NMT1 and Sorbs2 in MC3T3-E1 cells. (A) An equal amount of protein lysate from cells treated with or without TNF- α homogenate was electrophoresed and stained using Coomassie brilliant blue. The bands indicated by red boxes were identified as specific interacting proteins. (B) Mass spectrometry spectra are shown in 1 and 2. The analysis was performed using PMF and the search program MASCOT. Based on the figures, both binding proteins are identified as Sorbs2, including isoforms. (C) Protein extracts from cells treated with or without TNF- α were subjected to immunoprecipitation using an anti-NMT1 antibody immobilized by Protein G Sepharose beads.

from TNF- α induced suppression (Figure 4B). These data suggest that NMT1 and Sorbs2 are involved in TNF- α stimulation *via* binding, which, in turn, is important for the regulation of myristoylation in MC3T3-E1 cells.

Discussion

Myristoylation is important for adapting to the extracellular environment and maintaining homeostasis (20). Therefore, its functional abnormalities can lead to serious diseases, such as infection and cancer. NMT1 is highly expressed in cancer cells and plays an important role in the regulation of myristoylation. Recent reports have suggested that

myristoylation is involved in the suppression of fungal activity and HIV-related immune dysfunction. Fungal plasma membrane and pH sensing are involved in the microenvironment (21). In this study, Sorbs2 was identified as a novel protein that binds to NMT1, providing insight into the mechanism of membrane trafficking in the immune system. Considering its important role in the generation of immune responses and cancer, a deeper understanding of its regulatory mechanism will aid in the development of novel targeted therapies. Although the effect of myristoylation on inflammatory stimulation in osteoblasts has not yet been investigated, it is thought to be important in bone immunology during inflammation or tumor invasion. While this PTM is an interesting aspect of immune and cancer activation, its molecular mechanism in osteoblasts remains unclear. In this study, using a new approach based on click chemistry, which is useful for myristoylation, we showed that myristoylated proteins are expressed in osteoblasts. Furthermore, alterations in their expression in response to TNF- α stimulation, suggests that the immune system is influenced by inflammatory stimulation. Despite extensive research on bone resolution resulting from the invasive growth of oral cancer, effective preventive or long-term treatment strategies have not yet been proposed (22). Recent reports have revealed that osteoclast activation related to tumor invasion results from an inflammatory response induced by cytokines (23). However, little is known about the mechanisms underlying osteoblast inhibition. In addition to myristoylated proteins, the expression of NMT1, a catalytic enzyme of myristoylation, has been confirmed in osteoblasts. Contrary to the suppression of myristoylated proteins, NMT1 expression was found to increase upon TNF- α stimulation. These finding led us to hypothesize that this interaction could modulate the function of NMT1. NMT1 is known to be expressed not only in cancer cells but also in eukaryotic cells, such as T cells, and to play an important role in immune suppression during HIV infection in host cells (24). In *Candida albicans*, NMT1 plays an important role in immune function, rendering it a potential target for therapeutic interventions (25). Similarly, the expression of NMT1 induced by TNF- α suggested a model in which NMT1 plays a central role in suppressing the function of osteoblasts.

Sorbs2 functions as an adaptor protein that coordinates the operation of multiple regulatory mechanisms that converge upon the actin cytoskeleton (26). In addition, its expression has been confirmed in the cardiac muscle (27), where knockout of Sorbs2 in cardiomyocytes led to dilated cardiomyopathy in mice (28). In some cases, cardiac disease is associated with immune dysfunction, such as ease of infection or diabetes metabolism, which may explain the expression of Sorbs2 in the heart. In contrast, Sorbs2 also plays an important role as a tumor suppressor in pancreatic

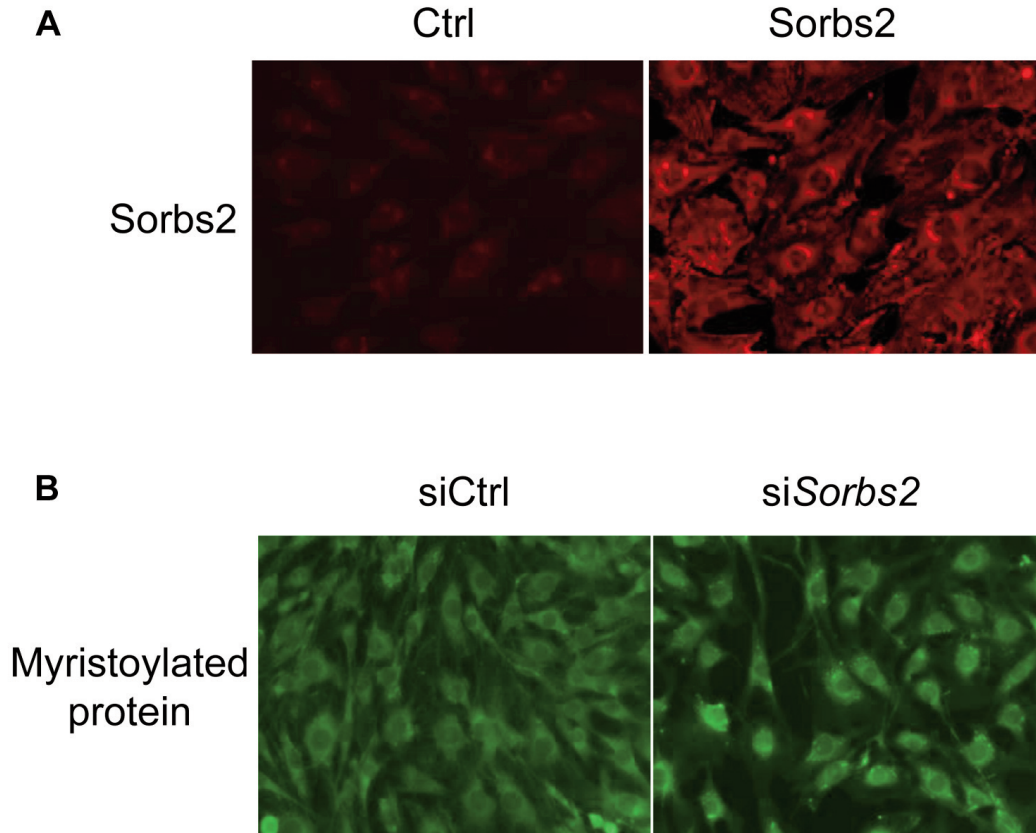


Figure 4. The expression and effect of Sorbs2 on myristoylation in MC3T3-E1 cells (A) The expression of Sorbs2 was confirmed using immunocytochemistry with an anti-Sorbs2 antibody. (B) Immunocytochemistry-based analysis of myristoylated proteins in MC3T3-E1 cells transfected with siSorbs2 or negative control siRNA with TNF- α .

cancer (29). Thus, Sorbs2 plays a role in connecting cells that are essential for immunosuppression in cancer. However, the bone commonality remains unclear. In this study, we demonstrated its expression in MC3T3-E1 cells, thereby suggesting that binding to NMT1 is important for inhibition of myristoylation in response to TNF- α stimulation. These findings indicate the potential of NMT1 as a target molecule for tumor invasion and for understanding its regulation *via* the interaction between NMT1 and Sorbs2. The immune system is normally composed of cell-to-cell networking, and myristoylation further establishes this aspect because of its relation to cell adhesion.

Conclusion

Taken together, our findings suggest that dysfunction in membrane trafficking *via* the interaction between Sorbs2 and myristoylation by NMT1 is involved in immune regulation. We showed a schematic representation of the regulatory mechanisms underlying protein myristoylation *via* the binding interaction between NMT1 and Sorbs2 in MC3T3-

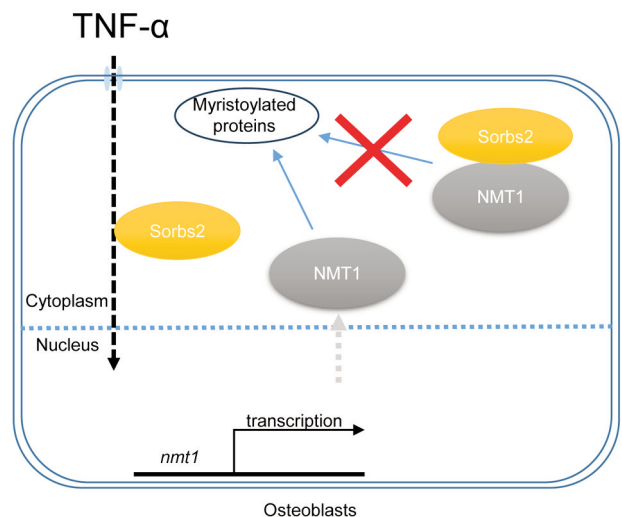


Figure 5. Schematic representation of the proposed mechanism. TNF- α stimulation promotes NMT1 expression and binding to Sorbs2. This binding between NMT1 and Sorbs2 suppresses protein myristoylation, suggesting that Sorbs2 can act as a suppressor protein during its interaction with NMT1.

E1 cells (Figure 5). As a result, bone resolution may also decrease in the surface osteoblasts, as supported by our study findings using osteoblast cell lines. However, additional investigations are necessary to confirm whether experiments conducted using cultured cells are consistent with *in vivo* experiments, thereby establishing translational relevance.

Conflicts of Interest

The Authors have no potential conflicts of interest to disclose in relation to this study.

Authors' Contributions

Conceptualization, S.K. and G.S.; Methodology, S.K.; Software, S.K.; Validation, S.K., T.K. and H.K.; Formal Analysis, W.K.; Investigation, Y.O.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, G.S.; Visualization, G.S.; Supervision, T.Y.; Project Administration, S.K.; Funding Acquisition, G.S.

Acknowledgements

This study was supported by JSPS KAKENHI Grant number JP23K09314. The Authors would like to thank Editage (www.editage.jp) for the English language editing.

References

- Epsley S, Tadros S, Farid A, Kargilis D, Mehta S, Rajapakse CS: The effect of inflammation on bone. *Front Physiol* 11: 511799, 2021. DOI: 10.3389/fphys.2020.511799
- Kitaura H, Marahleh A, Ohori F, Noguchi T, Nara Y, Pramusa A, Kinjo R, Ma J, Kanou K, Mizoguchi I: Role of the interaction of tumor necrosis factor- α and tumor necrosis factor receptors 1 and 2 in bone-related cells. *Int J Mol Sci* 23(3): 1481, 2022. DOI: 10.3390/ijms23031481
- Zhao B: Intrinsic restriction of TNF-mediated inflammatory osteoclastogenesis and bone resorption. *Front Endocrinol (Lausanne)* 11: 583561, 2020. DOI: 10.3389/fendo.2020.583561
- Lee JM, Hammarén HM, Savitski MM, Baek SH: Control of protein stability by post-translational modifications. *Nat Commun* 14(1): 201, 2023. DOI: 10.1038/s41467-023-35795-8
- Yuan M, Song ZH, Ying MD, Zhu H, He QJ, Yang B, Cao J: N-myristoylation: from cell biology to translational medicine. *Acta Pharmacol Sin* 41(8): 1005-1015, 2020. DOI: 10.1038/s41401-020-0388-4
- Fhu CW, Ali A: Protein lipidation by palmitoylation and myristoylation in cancer. *Front Cell Dev Biol* 9: 673647, 2021. DOI: 10.3389/fcell.2021.673647
- Bagnato G, Leopizzi M, Urciuoli E, Peruzzi B: Nuclear functions of the tyrosine kinase Src. *Int J Mol Sci* 21(8): 2675, 2020. DOI: 10.3390/ijms21082675
- Retanal C, Ball B, Geddes-McAlister J: Post-translational modifications drive success and failure of fungal-host interactions. *J Fungi (Basel)* 7(2): 124, 2021. DOI: 10.3390/jof7020124
- Jha V, Rustagi K, Gharat K, Sonawane N, Rathod M, Patel R, Devkar S, Dhamapurkar V, Kaur N: Human immunodeficiency virus type 1: Role of proteins in the context of viral life cycle. *J Adv Biotechnol Exp Ther* 5(2): 307, 2022. DOI: 10.5455/jabet.2022.d117
- Li H, Dou J, Ding L, Spearman P: Myristoylation is required for human immunodeficiency virus type 1 Gag-Gag multimerization in mammalian cells. *J Virol* 81(23): 12899-12910, 2007. DOI: 10.1128/JVI.01280-07
- Giglione C, Meinel T: Mapping the myristoylome through a complete understanding of protein myristoylation biochemistry. *Prog Lipid Res* 85: 101139, 2022. DOI: 10.1016/j.plipres.2021.101139
- Price HP, Menon MR, Panethymitaki C, Goulding D, McKean PG, Smith DF: Myristoyl-CoA:protein N-myristoyltransferase, an essential enzyme and potential drug target in kinetoplastid parasites. *J Biol Chem* 278(9): 7206-7214, 2003. DOI: 10.1074/jbc.M211391200
- Kumar S, Singh B, Dimmock JR, Sharma RK: N-myristoyltransferase in the leukocytic development processes. *Cell Tissue Res* 345(2): 203-211, 2011. DOI: 10.1007/s00441-011-1202-x
- Selvakumar P, Lakshmikuttyamma A, Shrivastav A, Das SB, Dimmock JR, Sharma RK: Potential role of N-myristoyltransferase in cancer. *Prog Lipid Res* 46(1): 1-36, 2007. DOI: 10.1016/j.plipres.2006.05.002
- Mackey JR, Lai J, Chauhan U, Beauchamp E, Dong WF, Glubrecht D, Sim YW, Ghosh S, Bigras G, Lai R, Berthiaume LG: N-myristoyltransferase proteins in breast cancer: prognostic relevance and validation as a new drug target. *Breast Cancer Res Treat* 186(1): 79-87, 2021. DOI: 10.1007/s10549-020-06037-y
- Shrivastav A, Sharma AR, Bajaj G, Charavaryamath C, Ezzat W, Spafford P, Hickman RG, Singh B, Copete MA, Sharma RK: Elevated N-myristoyltransferase activity and expression in oral squamous cell carcinoma. *Oncol Rep* 18(1): 93-97, 2007.
- Gamma J, Iyer A, Yap M, Zak Z, Vincent K, Ekstrom C, Beauchamp E, Postovit L, Mackey JR, Pemmaraju N, Borthakur G, Brandwein J, Berthiaume LG: Validation of *NMT1* and *NMT2* as novel drug targets in adult acute myeloid leukemia: Rationale for N-myristoyltransferase inhibition with Pclx-001 for clinical trials. *Blood* 138(Suppl 1): 4344-4344, 2021. DOI: 10.1182/blood-2021-154000
- Suzuki A, Sugiyama G, Ohyama Y, Kumamaru W, Yamada T, Mori Y: Regulation of NF- κ B signalling through the PR55 β -RelA interaction in osteoblasts. *In Vivo* 34(2): 601-608, 2020. DOI: 10.21873/in vivo.11813
- Brosch M, Yu L, Hubbard T, Choudhary J: Accurate and sensitive peptide identification with Mascot Percolator. *J Proteome Res* 8(6): 3176-3181, 2009. DOI: 10.1021/pr800982s
- Su B, Wang A, Shan X: The role of N-myristoylation in homeostasis of brassinosteroid signaling kinase 1. *Planta* 255(4): 73, 2022. DOI: 10.1007/s00425-022-03861-y
- Brown HE, Ost KS, Esher SK, Pianalto KM, Saelens JW, Guan Z, Andrew Alspaugh J: Identifying a novel connection between the fungal plasma membrane and pH-sensing. *Mol Microbiol* 109(4): 474-493, 2018. DOI: 10.1111/mmi.13998
- Coleman R, Body JJ, Aapro M, Hadji P, Herrstedt J, ESMO Guidelines Working Group: Bone health in cancer patients: ESMO Clinical Practice Guidelines. *Ann Oncol* 25(suppl 3): iii124-iii137, 2014. DOI: 10.1093/annonc/mdu103
- Newman H, Shih YV, Varghese S: Resolution of inflammation in bone regeneration: From understandings to therapeutic applications. *Biomaterials* 277: 121114, 2021. DOI: 10.1016/j.biomaterials.2021.121114

- 24 Takamune N, Gota K, Misumi S, Tanaka K, Okinaka S, Shoji S: HIV-1 production is specifically associated with human NMT1 long form in human NMT isozymes. *Microbes Infect* 10(2): 143-150, 2008. DOI: 10.1016/j.micinf.2007.10.015
- 25 Galvin BD, Li Z, Villemaine E, Poole CB, Chapman MS, Pollastri MP, Wyatt PG, Carlow CK: A target repurposing approach identifies N-myristoyltransferase as a new candidate drug target in filarial nematodes. *PLoS Negl Trop Dis* 8(9): e3145, 2014. DOI: 10.1371/journal.pntd.0003145
- 26 Cestra G, Toomre D, Chang S, De Camilli P: The Abl/Arg substrate ArgBP2/nArgBP2 coordinates the function of multiple regulatory mechanisms converging on the actin cytoskeleton. *Proc Natl Acad Sci USA* 102(5): 1731-1736, 2005. DOI: 10.1073/pnas.0409376102
- 27 Liang F, Wang B, Geng J, You G, Fa J, Zhang M, Sun H, Chen H, Fu Q, Zhang X, Zhang Z: SORBS2 is a genetic factor contributing to cardiac malformation of 4q deletion syndrome patients. *Elife* 10: e67481, 2021. DOI: 10.7554/eLife.67481
- 28 McLendon JM, Zhang X, Matasic DS, Kumar M, Koval OM, Grumbach IM, Sadayappan S, London B, Boudreau RL: Knockout of sorbin and SH3 domain containing 2 (Sorbs2) in cardiomyocytes leads to dilated cardiomyopathy in mice. *J Am Heart Assoc* 11(13): e025687, 2022. DOI: 10.1161/JAHA.122.025687
- 29 Taieb D, Roignot J, André F, Garcia S, Masson B, Pierres A, Iovanna JL, Soubeyran P: ArgBP2-dependent signaling regulates pancreatic cell migration, adhesion, and tumorigenicity. *Cancer Res* 68(12): 4588-4596, 2008. DOI: 10.1158/0008-5472.CAN-08-0958

Received October 4, 2023

Revised November 6, 2023

Accepted November 7, 2023