Original Article

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Opsin 3-mediated regulation of blue light-induced β-hexosaminidase release from mast cells

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SUMMARY: The human body is constantly exposed to light from the environment, and intense light is a source of skin inflammation. Although cellular responses to high-energy ultraviolet light have long been reported, the photoresponsive mechanism occurring after skin exposure to visible light remains unclear. This study focused on mast cells involved in inflammation and examined the expression of photoreceptors and their effects on degranulation in mast cells. Photoreceptors expressed in two mast cell cultures (P-815 and RBL-2H3) were examined by RT-PCR and western blotting to demonstrate that OPN3 was expressed in RBL-2H3 cells. Next, the effect of visible light exposure on degranulation was evaluated by measuring β-hexosaminidase activity in the culture medium. The results show that β-hexosaminidase release was most strongly induced at wavelengths of ~460 nm, which corresponds to the absorption peak of OPN3. In addition, suppression of OPN3 expression by siRNA reduced β-hexosaminidase release at 460 nm. These results suggest that OPN3 expressed in mast cells mediates degranulation in skin inflammation that occurs upon exposure to intense light.

Keywords: blue light, opsin 3, mast cell, degranulation, photoresponse

1. Introduction

Photosensitivity is a condition that causes itching, redness, and rashes on the skin under exposure to sunlight, which may be manifested by ultraviolet (*1*) or visible light (*2,3*). Although cellular responses to high-energy ultraviolet light have long been reported, the photoresponsive mechanism occurring after skin exposure to visible light remains unclear.

The induction of inflammation in the skin following exposure to visible light occurs through the secretion of inflammatory cytokines. In keratinocytes, the activation of opsins promotes the secretion of inflammatory cytokines such as IL-3 and IL-6 (*4,5*), which is known to induce inflammation. Additionally, blue light is absorbed by intracellular chromophores, such as those found in mitochondria, leading to the generation of reactive oxygen species (ROS) (*6*). The ROS produced then induce the secretion of inflammatory cytokines (TNF-α, IL-1β, *etc.* (*5-7*)), thereby promoting inflammatory responses. These mechanisms are considered to be one of the causes of skin inflammation induced by visible light.

Mast cells are distributed in many tissues, and together with basophils constitute the main source of histamine (*8*). The mast cell surface expresses FcεRI, a receptor for IgE antibodies, which causes increased intracellular calcium ion concentrations, then degranulation and the release of histamine and other chemical transmitters from the granules to the extracellular environment (*9*). Histamine causes vasodilation, increased vascular permeability, and contraction of smooth muscle, leading to a variety of immediate allergic symptoms (*10*).

In recent years, the expression of photoreceptors in skin has been clarified and reported to show various responses to exposure to visible light and ultraviolet radiation (*11-14*). However, the effects of visible light on mast cells have not been reported so far. In this study, we therefore focused on opsin as a photoreceptor and explored its expression in mast cells. We also examined the effects of light exposure on degranulation, focusing on the induction of allergies, in which mast cells play a major role.

2. Materials and Methods

2.1. Cell culture

Cells of the mouse mast cell line P-815 (*15*) and rat basophilic leukemia and mast cell line RBL-2H3

(*16,17*), which are widely employed as models for immunologically induced mast cell degranulation, were supplied by Health Science Research Resources Bank (Osaka, Japan). Cells were cultured in DMEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS, Moretate Biotech, Bulimba, Australia) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

2.2. Expression of opsins in mast cell lines

The expression of opsins was assessed using reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from the P-815 cells and RBL-2H3 cells using TriPure Isolation Reagent (Roche Life Science, Indianapolis, IN, USA), and genomic DNA was removed with DNase I. cDNA was synthesized using ReverTra Ace (Toyobo, Osaka, Japan) according to manufacturer instructions. The primers used are listed in Table 1. PCR amplification was performed using Quick Taq® HS DyeMix (Toyobo) following the manufacturer's instructions. The following PCR cycles were employed: initial denaturation at 95°C for 5 min, followed by 30 to 40 cycles (depending on the primer) at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. PCR products were electrophoresed on ethidium bromide-containing 2% agarose gels and visualized under UV illumination.

For quantification of OPN3 expression, quantitative PCR was performed using a StepOne system (Applied Biosystems, Foster City, CA, USA) with THUNDERBIRD® Next SYBR™ qPCR Mix (Toyobo). The primers used are listed in Table 1. Specificity of PCR products was verified by melt curve. The Ct values for the samples were normalized to that of β-actin, and the relative expression was calculated using the comparative Ct method.

2.3. β-Hexosaminidase assay

Cells were prepared in DMEM with 10% FBS at 5 × 10^5 cells/mL and cultured in 96-well microplates at 200 μL per well. After 24 h incubation, DMEM with 10% FBS was removed, 100 μL of DMEM with 1% FBS was added to each well, and the cells were irradiated with light for 15 min. The irradiation was performed using the Okazaki Large Spectrograph with wavelengths of 350 nm, 450 nm, 550 nm, 650 nm and 750 nm. Each wavelength was regulated at 1.7-2.2 W/m^2. After irradiation, the supernatants were collected for measuring β-hexosaminidase activity. The supernatant of cells not exposed to light was used as a negative control, while total β-hexosaminidase extracted using 100 μL of Triton X-100 was used as a positive control. A moiety of 10 μL per prepared sample was transferred to a 96-well microplate, and 100 μL of 2 mM p–nitrophenyl–*N*–acetyl–β–*D*–glucosaminide solution was added and then incubated at 37°C for 30 min. After incubation, the amount of paranitrophenol produced by the reaction was determined based on absorbance at a wavelength of 410 nm.

2.4. Western blotting analysis

Samples (20 μg) of the cell lysates were separated on a 12% (w/v) polyacrylamide gel (*18*). Proteins were blotted onto nitrocellulose membranes (Protran BA85; GE Healthcare, Chicago, IL, USA) in a semi-dry blotting system (NA-1513; Nihon Eidoh Co., Tokyo, Japan) (*19*). Nitrocellulose membranes were blocked with 2% (w/v) skim milk in Tris-buffered saline. Blocked membranes were incubated with rabbit anti-opsin 3 antibody (1:3,000; Genetex, Irvine, CA, USA), mouse anti-GAPDH antibody (1:10,000; Fujifilm Wako Pure Chemical, Osaka, Japan). This was followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) antibody (1:10,000; Seracare, Camarillo, CA, USA) or horseradish peroxidase-conjugated goat anti-mouse IgG antibody (1:3,000; Seracare). The blots were subsequently developed using the ImmunoStar LD chemiluminescent reagent

Table 1. Primers for RT-PCR

Target	Sense primer	Antisense primer
rat OPN1-SW	TCTTCACAGTCTTCATCGCCAG	CCAGGTATAGTGCTCGCTTC
rat OPN1-MW/LW	AGCAGAGACCATTATTGCCAGC	GTCCATACAGCAGCCCAGAC
rat OPN2	CACCTCACTGCATGGCTACTT	ATGGGGATGGTGAAGTGGAC
rat OPN3	GTCTGGGCGATCTGCTGGTA	ATGCCAAAGAGTAGAGCCAGAT
rat OPN4	TGGAACAGCACTCAGAACATC	AAAGACAGCCCCACAGAAGG
rat OPN5	AAGCCTGATTACCATGACTGC	TGGCAATGATCTTCGCGTATG
rat β-actin	AACCCTAAGGCCAACCGTGAAAAG	CGACCAGAGGCATACAGGGACAAC
mouse OPN1-SW	TGGGCTCTGTAGCAGGTCTA	CAGGACCACCATCAGTGCAT
mouse OPN1-MW/LW	AACTTGGCAGTTGCTGACCT	AGGCCTGTGATTCCACACAAT
mouse OPN2	GGGCCCCAATTTTTATGTGCC	ACAGGGCGATTTCACCTCCAA
mouse OPN3	CATTACCACCCTCACTGTGCT	GATCTTCAACACAGCGAAGCATT
mouse OPN4	GTTCTGAGAGTGAAGTGGGCT	AAGCTTCCAGGCTTGTGACAT
mouse OPN5	AACCACACTGCCCTACCTCA	TCTCTTCAGCCAGACCCCATA
mouse β-actin	TGACAGGATGCAGAAGGAGA	CATCTGCTGGAAGGTGGACA

(Fujifilm Wako Pure Chemical) and a LuminoGraph chemiluminescent imaging system (Atto Corporation, Tokyo, Japan).

2.5. RNAi-induced opsin 3 knockdown assay

INTERFERin (Polyplus-transfection, Illkirch, France) was used to transfect rat opsin 3 siRNA (AGCAAUGGGCUAUGACACC(dTdT); Bioneer Corporation, Daejeon, Republic of Korea) into RBL-2H3 cells. At 48 h after transfection, opsin 3 expression was measured through quantitative RT-PCR analysis.

2.6. Statistical analysis

Statistical analyses were performed using Student's *t*-tests implemented in SPSS Statistics (version 29.0.2.0; IBM, Chicago, Il, USA). A p-value < 0.05 was considered indicative of statistical significance.

3. Results

3.1. Expression of opsins in mast cells

We analyzed the expression of opsin in the mouse mast cell line P-815 and rat basophilic leukemia cell line RBL-2H3 using RT-PCR (Figure 1A). OPN2, OPN3 and OPN4 mRNA were expressed in P-815 cells. Expression of OPN1-MW/LW, OPN2 and OPN3 was observed in RBL-2H3 cells. OPN1-MW/LW and OPN2 in RBL-2H3 cells demonstrated two different PCR products. The expression of OPN3 mRNA in RBL-2H3 cells was confirmed by Western blotting (Figure 1B). A band of approximately 40 kDa was detected in RBL-2H3 cells.

3.2. Effect of light exposure on β-hexosaminidase release

We evaluated whether light exposure induces degranulation from mast cells, using β-hexosaminidase release as an indicator (Figure 2A). The results show that exposure to wavelengths between 350 and 550 nm increased β-hexosaminidase release. In contrast, no change in β-hexosaminidase release was observed with exposure to wavelengths longer than 650 nm. Next, to examine in more detail the wavelengths to which RBL-2H3 cells respond, we measured β-hexosaminidase released from RBL-2H3 cells when exposed to various wavelengths at 20 nm intervals (Figure 2B). The results show that wavelengths in the ultraviolet region at 340 nm and around 460 nm induced the release of β-hexosaminidase.

3.3. Downregulation of OPN3 expression by siRNA and its effect on β-hexosaminidase release

We examined the release of β-hexosaminidase after light exposure in RBL-2H3 cells in which OPN3 expression was suppressed by siRNA. siRNA knockdown of OPN3 in RBL-2H3 cells reduced OPN3 expression to approximately 28% of the control level (Figure 3A). Western blotting further revealed that OPN3 expression

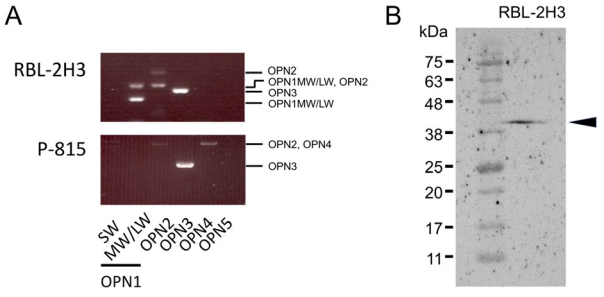

Figure 1. Expression of opsins in murine mast cell line P-815 and rat mast cell line RBL-2H3. RT-PCR analysis of opsin mRNA expression in RBL-2H3 cells and P-815 cells (**A**). Western blotting for OPN3 using RBL-2H3 cells (**B**).

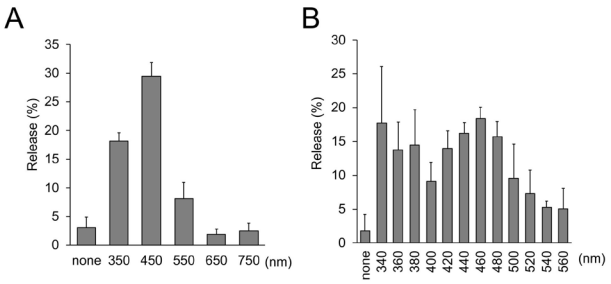

Figure 2. Wavelength dependency of degranulation in RBL-2H3. The degranulation from mast cells was evaluated with β-hexosaminidase release as an indicator. Monochromatic light was separated using the Okazaki Large Spectrograph, and cellular responses were evaluated at intervals of 100 nm (**A**) or 20 nm (**B**).

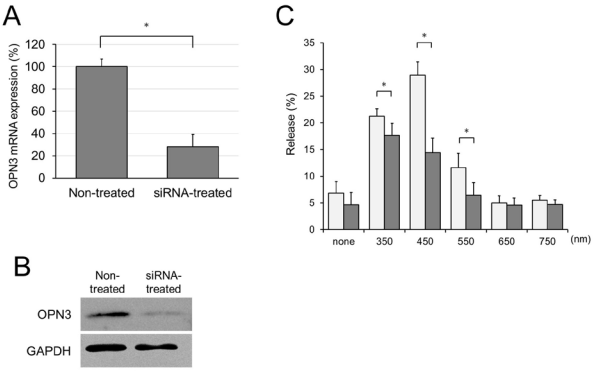

Figure 3. Effect of blue light on degranulation in mast cells with OPN3 knockdown. Evaluation of the OPN3 knockdown efficiency in RBL-2H3 cells following siRNA treatment by quantitative RT-PCR (**A**) and western blotting (**B**). Quantitative RT-PCR results are presented as mean ± SD (n = 3). Statistical significance was determined by Student's *t*-tests compared to non-treated control cells (p < 0.05). Comparison of degranulation in response to blue light exposure between non-treated control cells and OPN3 knockdown (OPN3-KD) cells (**C**). Open bars represent the non-treated control cells, while filled bars indicate OPN3-KD cells. Data are shown as mean ± SD (n = 8). *p < 0.05, determined by Student's *t*-tests compared to non-treated control cells.

was reduced in cells treated with OPN3 siRNA (Figure 3B). Next, the light response of RBL-2H3 cells with OPN3 expression reduced by siRNA was examined based on β-hexosaminidase release. As shown in Figure 3C, β-hexosaminidase release was significantly suppressed upon stimulation with light at wavelengths of 350 nm, 450 nm and 550 nm. Among these, the most pronounced suppression (approximately 49.8%) was observed at 450 nm, which is close to the peak response wavelength of OPN3, while the release observed at 350 nm was less pronounced.

4. Discussion

In this study, we explored opsin expressed in non-visual cells using RBL-2H3 cells and P-815 cells as mast cell models and found that OPN3 is commonly expressed. Several groups have previously reported that opsin receptors are expressed in multiple cells in skin, a non-visual tissue (*11,12,20*). It has been shown that 11-*cis*-retinal is produced by all-*trans*-retinal in response to light and metabolism of retinal, which is required for opsin activity, and it has been reported that OPN expressed in skin is functional (*21*). The presence of a photoreceptor cycle in skin has suggests that opsins expressed on mast cells would also be functional.

To determine the wavelengths to which mast cells respond, spectrograms were used to evaluate β-hexosaminidase release as an indicator of spectral light response. β-hexosaminidase release was maximal at ~460 nm (*22*). Since the strongest response of the photoreceptor OPN3 was observed at this wavelength, the release of β-hexosaminidase is considered to be mediated by OPN3.

OPN3 has been reported to increase intracellular calcium ion concentrations. Increased intracellular calcium ion concentrations have been shown to trigger the release of intracellular secretory granules in various cells (*23,24*). Based on this observation, it was assumed that the activation of OPN3 is responsible for the elevated β-hexosaminidase release recorded at the 460 nm wavelength. Accordingly, when OPN3 expression was suppressed with siRNA, the release of β-hexosaminidase at ~450 nm was reduced. These findings indicate that exposure of mast cells to blue light increases the release of β-hexosaminidase *via* expressed OPN3.

Short wavelengths of light penetrate less deeply into the tissues of biological organisms than do long wavelengths. With a transmittance of ~30%, only a small proportion of blue light is able to reach the dermal layer, where mast cells are located (*25*). The observed phenomena may therefore constitute the response mechanism of the organism when blue light reaches the dermal layer due to exposure to intense light or damage to the epidermal layer. In general, the response to blue light varies between different skin cells. In keratinocytes, exposure to blue light decreases their migration ability (*21*). In melanocytes, melanin production is increased (*26*). In fibroblasts, the expression of matrix metalloproteinase, a collagen-degrading enzyme, is increased resulting in decreased skin elasticity (*27*).

Our results demonstrate that OPN3 is expressed in mast cells and that blue light induces mast cell degranulation *via* OPN3. The enhancement of degranulation by blue light may be considered to be one of the defensive mechanisms involved in skin homeostasis.

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References

1. Cavinato M, Jansen-Dürr P. Molecular mechanisms of UVB-induced senescence of dermal fibroblasts and its relevance for photoaging of the human skin. Exp Gerontol. 2017; 94:78-82.
2. Sklar LR, Almutawa F, Lim HW, Hamzavi I. Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: A review. Photochem Photobiol Sci. 2013; 12:54-64.
3. Ezekwe N, Maghfour J, Kohli I. Visible Light and the Skin. Photochem Photobiol. 2022; 98:1264-1269.
4. Lago JC, Ganzerla MD, Dias ALA, Savietto JP. The influence of blue light exposure on reconstructed 3-dimensional skin model: Molecular changes and gene expression profile. JID Innov. 2024; 4:100252.
5. Liu L, Ma J, Chen W, Zhang J, Zuo W, Wang M, Li J. UVB-induced HaCat cell damage and Myricaria Paniculata's molecular effects. Sci Rep. 2025; 15:10909.
6. Nakashima Y, Ohta S, Wolf AM. Blue light-induced oxidative stress in live skin. Free Radic Biol Med. 2017; 108:300-310.
7. Kumar D, Ahmed M, Andrabi NI, Singh CP, Saroch D, Bharitkar YP, Kour G, Madishetti S, Bhagat A, Shukla SK, Ahmed Z. Anti-inflammatory and anti-oxidant potential of Dispiro-indanedione hybrid of parthenin *via* regulating Nrf2 and NF-κB/MAPK pathways. Eur J Pharmacol. 2025; 177547.
8. Dileepan KN, Raveendran VV, Sharma R, Abraham H, Barua R, Singh V, Sharma R, Sharma M. Mast cell-mediated immune regulation in health and disease. Front Med (Lausanne). 2023; 10:1213320.
9. Macglashan D, Jr. IgE and Fc{epsilon}RI regulation. Ann N Y Acad Sci. 2005; 1050:73-88.
10. Church MK. Allergy, histamine and antihistamines. Handb

Exp Pharmacol. 2017; 241:321-331.
11. Tsutsumi M, Ikeyama K, Denda S, Nakanishi J, Fuziwara S, Aoki H, Denda M. Expressions of rod and cone photoreceptor-like proteins in human epidermis. Exp Dermatol. 2009; 18:567-570.
12. Haltaufderhyde K, Ozdeslik RN, Wicks NL, Najera JA, Oancea E. Opsin expression in human epidermal skin. Photochem Photobiol. 2015; 91:117-123.
13. Castellano-Pellicena I, Uzunbajakava NE, Mignon C, Raafs B, Botchkarev VA, Thornton MJ. Does blue light restore human epidermal barrier function *via* activation of Opsin during cutaneous wound healing? Lasers Surg Med. 2019; 51:370-382.
14. Wicks NL, Chan JW, Najera JA, Ciriello JM, Oancea E. UVA phototransduction drives early melanin synthesis in human melanocytes. Curr Biol. 2011; 21:1906-1911.
15. Schellenberg H, Domen Joz R. P-815 mast cell tumor in suspension as a substrate for the testing of carcinostatic substances. Med Exp Int J Exp Med. 1962; 7:137-143.
16. Morita Y, Siraganian RP. Inhibition of IgE-mediated histamine release from rat basophilic leukemia cells and rat mast cells by inhibitors of transmethylation. J Immunol. 1981; 127:1339-1344.
17. Hemmerich S, Pecht I. Isolation and purification of an Fc epsilon receptor activated ion channel from the rat mast cell line RBL-2H3. Biochemistry. 1988; 27:7488-7498.
18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227:680-685.
19. Burnette WN. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate--polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem. 1981; 112:195-203.
20. Suh S, Choi EH, Atanaskova Mesinkovska N. The expression of opsins in the human skin and its implications for photobiomodulation: A Systematic Review. Photodermatol Photoimmunol Photomed. 2020; 36:329-338.
21. Yamamoto H, Okada M, Sawaguchi Y, Yamada T. Expression of opsin and visual cycle-related enzymes in fetal rat skin keratinocytes and cellular response to blue light. Biochem Biophys Rep. 2024; 39:101789.
22. Sugihara T, Nagata T, Mason B, Koyanagi M, Terakita A. Absorption characteristics of vertebrate non-visual Opsin, Opn3. PLoS One. 2016; 11:e0161215.
23. Bertram R, Sherman A, Satin LS. Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion. Am J Physiol Endocrinol Metab. 2007; 293:E890-900.
24. Stojilkovic SS. Ca^{2+}-regulated exocytosis and SNARE function. Trends Endocrinol Metab. 2005; 16:81-83.
25. Akihiro K, Eisaku K, Tomohisa Y, Shunki Y, Shuichi K, Shuichi M. Ultra long-range visible light propagation of human skin. Nihon Gazo Gakkaishi. 2017; 56:466-472.
26. Regazzetti C, Sormani L, Debayle D, Bernerd F, Tulic MK, De Donatis GM, Chignon-Sicard B, Rocchi S, Passeron T. Melanocytes sense blue light and regulate pigmentation through Opsin-3. J Invest Dermatol. 2018; 138:171-178.
27. Moriwaki S. Light-emitting diodes as a new medical tool in aesthetic dermatology. Photomed Photobiol. 2018; 39:1-5.

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