Biochemical and Biophysical Research Communications 443 (2014) 980–986

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

miR-142-3p enhances FcεRI-mediated degranulation in mast cells

Yoji Yamada, Kyoko Kosaka [1], Tatsuya Miyazawa, Kazumi Kurata-Miura, Tetsuo Yoshida *

Biologics Research Laboratories, Kyowa Hakko Kirin Co., Ltd., 3-6-6, Asahi-machi, Machida-shi, Tokyo 194-8533, Japan

ARTICLE INFO

Article history:
Received 30 November 2013
Available online 19 December 2013

Keywords:
Mast cell
microRNA
Degranulation
miR-142-3p

ABSTRACT

Mast cells are immune cells derived from hematopoietic progenitors. When they are activated by stimuli, they immediately release granule-associated mediators, leading to allergic inflammation. Several factors controlling mediator release have been identified; however, little is known whether microRNAs (miRNAs) are involved in this process. miRNAs are a small class of non-coding RNAs that negatively regulate gene expression. In this study, we investigated the relationship between miRNAs and degranulation in LAD2 cells, a human mast cell line. We demonstrated that silencing of Dicer, a key enzyme of miRNA biogenesis, attenuates degranulation, indicating that miRNAs are involved in mast cell degranulation. We furthermore discovered that the overexpression of miR-142-3p enhances FcεRI-mediated degranulation and that miR-142-3p rescues the reduction of degranulation by silencing Dicer. Similar effects were observed in bone marrow-derived mast cells obtained miR-142-3p-deficient mice. Our studies suggest that miR-142-3p is a potential therapeutic target in pathological conditions caused by mast cells, such as mastocytosis and allergies.

1. Introduction

Mast cells are immune cells derived from hematopoietic progenitors in the bone marrow that mature in peripheral tissues. Mast cells are characterized by secretory granules containing active mediators, such as histamine, proteases and cytokines. When they are activated by stimuli, they immediately release granule-associated mediators that induce allergic inflammation [1,2]. The major mechanism underlying the stimulation of mast cells is the interaction between antigens and IgE bound to its high-affinity receptor, FcεRI, on the cell surface. Receptor aggregation initiates a signaling cascade leading to the release of mediators, a process referred to as degranulation. On the other hand, mast cells can also be stimulated by a large number of chemical molecules known as basic secretagogues that elicit degranulation through an IgE/FcεRI-independent mechanism [1].

Recent studies suggest that microRNAs (miRNAs), a family of 21–25 nucleotide small RNAs, regulate gene expression via translational repression and/or mRNA degradation by binding to the 3′ untranslated regions (3′ UTRs) of their target mRNAs [3]. They are involved in important biological events, such as proliferation, apoptosis, differentiation and stem cell maintenance. The inappropriate expression of miRNAs is associated with cancer and a variety of other pathologies [4,5].

While some miRNAs have already been shown to be important for differentiation and proliferation of mast cells [6,7], little is known regarding the role of miRNAs in mast cell degranulation. In this study, we therefore investigated whether miRNAs are involved in the degranulation of LAD2 cells, a human mast cell line. We observed that the degranulation activity in LAD2 cells transfected with Dicer siRNA is decreased, thus suggesting that miRNAs are involved in mast cell degranulation. Specifically, we found that miR-142-3p enhances FcεRI-mediated degranulation in LAD2 cells. A similar function was observed in the bone marrow-derived mast cells obtained miR-142-3p-deficient mice. Overall, our studies provide new insight into the role of miRNAs regulating mast cell degranulation, indicating that miR-142-3p is a potential therapeutic target in pathological conditions caused by mast cells, such as mastocytosis and allergies.

2. Materials and methods

2.1. Cell culture and transfection

LAD2 cells [8] were obtained from the National Institutes of Health. The cells were cultured in Stem Pro-34 SFM (Life Technologies, Carlsbad, CA) containing Stem-Pro supplement, 2 mM L-glutamine, 100 U/ml penicillin, 50 μg/ml streptomycin and 100 ng/ml SCF.

Transfection of oligonucleotides into LAD2 cells was performed using a Gene Silencer (Genlantis, San Diego, CA). Pre-miR miRNA

* Corresponding author. Fax: +81 42 726 8330.
E-mail address: tetsuo.yoshida@kyowa-kirin.co.jp (T. Yoshida).
[1] Present address: Division of Infectious Disease Control, Center for Infectious Disease, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe-shi, Hyogo 650-0017, Japan.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.12.078

Precursors (Life Technologies) were used as synthetic miRNA mimics. Synthesized fully 2′-O-methylated RNA oligonucleotide, whose sequence was complementary to the mature miRNA sequence, was used as miRNA antisense. Anti-miR miRNA Inhibitors (Life Technologies) were used as synthetic miRNA inhibitors. siRNAs were purchased from Life Technologies and Dharmacon (Chicago, IL). The target sequences of the siRNAs were listed in the Supplemental methods.

2.2. Degranulation assay of LAD2 cells

LAD2 cells were sensitized with 0.3 μg/ml human myeloma IgE (Athens Research & Technology, Athens, GA) for 24 h. For cell activation, the cells were stimulated with 10 or 15 μg/ml anti-human IgE antibody (Dakocytomation, Carpinteria, CA) or 0.5 μg/ml compound 48/80, a mixture of polymers derived from N-methyl-p-methoxy-phenylethylamine (Sigma–Aldrich, St. Louis, MO) for 20 min in Tyrode's buffer (126.1 mM NaCl, 4.0 mM KCl, 1.0 mM $CaCl_2$, 0.6 mM $MgCl_2$, 0.6 mM KH_2PO_4, 10 mM HEPES, 5.6 mM D-glucose, 0.1% Bovine Serum Albumin (pH 7.4)). The enzymatic activity of β-hexosaminidase in the supernatants was measured with 4-nitrophenyl N-acetyl-β-D-glucosaminide (Sigma–Aldrich) in 0.1 M sodium citrate (pH 4.5). The absorbance was read at 405 nm using ARVOsx (Perkin Elmer, Waltham, MA). Following calculation of the enzymatic activity, 1% Triton X-100 was added and the absorbance was measured again. The degranulation activity was calculated as the percentage by dividing the absorbance of the supernatant by that of the detergent-solubilized cells. Triplicate wells were used for each condition.

2.3. Intracellular Ca^{2+} measurement

IgE-sensitized LAD2 cells were labeled with the 1 μM of fluorescent Ca^{2+} indicator, Fluo 4-AM (Dojindo, Japan). The cells were suspended in Ca^{2+} assay buffer (20 mM HEPES, 115 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 13.8 mM D-glucose, 2.5 mM Probenecid (Sigma–Aldrich), 0.2% BSA, pH 7.4). For the measurement of Ca^{2+} mobilization, the Fluo-4 fluorescence intensity in each well was monitored with FDSS6000 (Hamamatsu Photonics, Japan). The intensity was monitored every second and plotted as the ratio to the resting calcium level.

2.4. Luciferase miRNA target reporter assay

The MREs (miR-142-3p recognition element) of human and murine LPP mRNAs were inserted between the XhoI–NotI restriction sites of the psiCHECK-2 vector (Promega), immediately 3′ downstream from the Renilla luciferase gene. The wild-type and mutant sequences in MREs are described in Fig. 3A.

HepG2 cells (ATCC) were co-transfected with 500 ng of reporter vector and 20 nM of miR-142-3p mimic or miRNA mimic negative control (Ambion) using Lipofectamine 2000 (Life Technologies). Firefly and Renilla luciferase activities were measured consecutively using the Dual-Glo Luciferase Assay System (Promega) and ARVOsx (Perkin Elmer) 24 h after transfection. All the experiments were done in triplicate.

2.5. Generation of miR-142-3p-deficient mice

A vector was constructed to replace the endogenous pre-miR-142 site with a PGK-neo cassette using homologous recombination in ES cells. The 5′ and 3′ sequences flanking the endogenous 87-bp pre-miR-142 site were amplified via PCR from a murine genome, generating 2.0-kb and 6.0-kb fragments, respectively. The sequence of the endogenous 87-bp was as follows: 5′-GACAGTGCAGTCACCCATAAAGTAGAAAGCACTACTAACAGCACTGGAGGGTGTAGTGTTTCCTACTTTATGGATGAGTGTACTGTG-3′.

These homology arms were cloned into a vector incorporating both a neomycin resistance gene for positive selection, and a diphtheria toxin (DTA) gene for negative selection. The targeting vector was transfected into ES cells. Positive clones were selected following culture in medium containing G418 antibiotics, and screened by means of PCR amplification. The successfully recombined ES cells were injected into blastocysts, and chimeric mice were bred with C57BL/6 mice to obtain germline transmission of the mutant allele.

All experiments were approved by the Animal Care and Use Committee of Kyowa Hakko Kirin and the handling of the mice and the experimental procedures were in accordance with the requirements of the Committee.

2.6. Generation of murine mast cells and degranulation assay

Cultured murine mast cells were generated from the femoral bone marrow cells of mice according to the method of Supajatura [9]. Following 4–6 weeks of culture, the cells were analyzed using a degranulation assay.

The primary cultured murine mast cells were sensitized with 1 ng/ml murine monoclonal anti-trinitrophenyl (TNP) IgE antibodies (BD Pharmingen, Franklin Lakes, NJ) in the culture medium for 24 h. Following sensitization, the cells were washed and suspended in Tyrode's buffer. The cells were stimulated with 1 ng/ml of TNP derivatives of bovine serum albumin (LSL Co., Japan) for 20 min. The degranulation activity was calculated by measuring the enzymatic activity of β-hexosaminidase in the supernatants.

3. Results

3.1. Effects of silencing Dicer on mast cell degranulation

To elucidate whether degranulation in mast cells is regulated by miRNAs, we measured the degranulation activity of the LAD2 cells after silencing of the Dicer expression by siRNA transfection. Dicer is a key enzyme of the miRNA biogenesis; therefore, silencing Dicer is expected to lead to the global repression of miRNAs [3,10].

We selected LAD2 cells as human mature mast cells. LAD2 cells were originally established from a patient with mast cell sarcoma-leukemia and closely resemble the primary cultures of $CD34^+$-derived human mast cells [8]. For example, LAD2 cells express FcεRI and c-Kit without mutations and are dependent on the cytokine stem cell factor (SCF) and can be stimulated to degranulation in an FcεRI-dependent manner. LAD2 cells were sensitized with IgE, and were stimulated by anti-human IgE antibodies leading to the crosslinking of FcεRI. The release of β-hexosaminidase was used as a measure of the extent of degranulation.

Two different siRNAs against human Dicer were transfected into LAD2 cells, and the degranulation activities were measured. Compared to that observed in the cells transfected with control siRNA (Lamin A/C siRNA) and cells without siRNA (mock), the transfection of Dicer siRNA reduced the degranulation activity (Fig. 1A). This result indicates that the global repression of miRNAs reduced the degranulation activity in the LAD2 cells, suggesting that miRNAs are involved in mast cell degranulation.

3.2. Identification of miRNA regulating degranulation in LAD2 cells

We next investigated the specific miRNAs related to degranulation in LAD2 cells. We constructed a small RNA library of LAD2 cells and found that miR-142-3p was the most abundant clone, with an overall abundance of the 24% in LAD2 cells (Supplemental

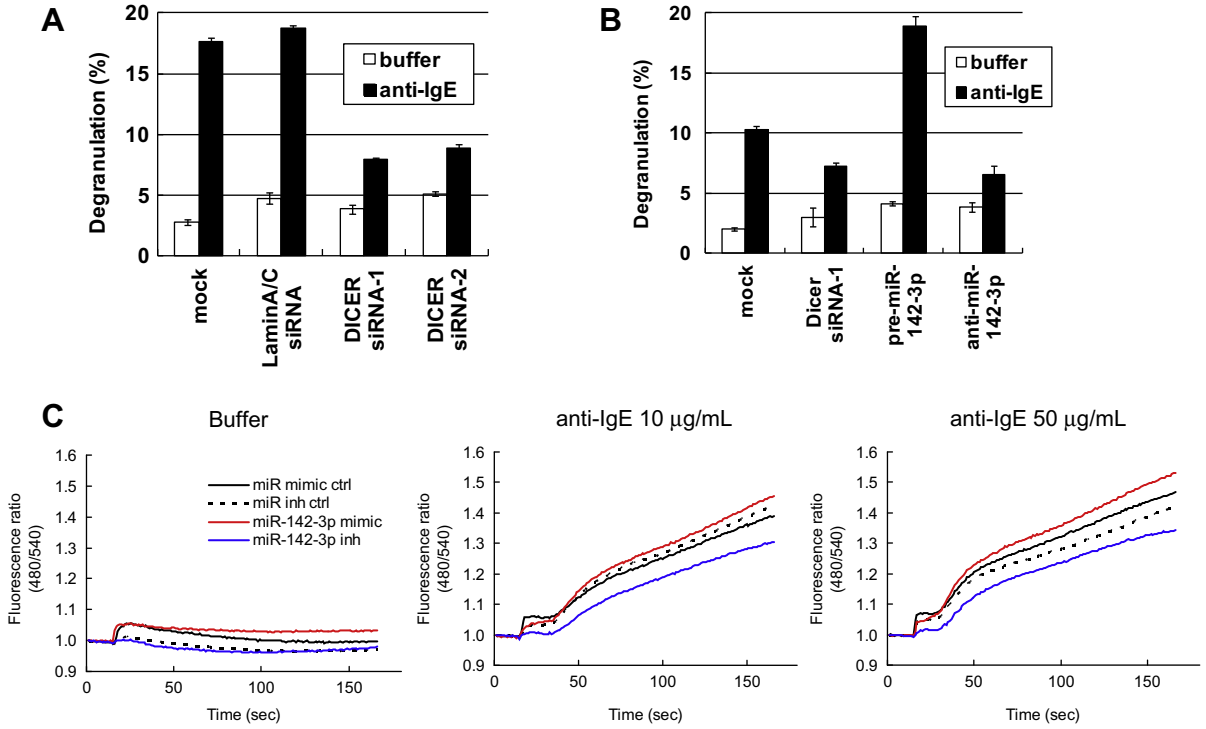

Fig. 1. miR-142-3p regulate degranulation in LAD2 cells. (A) Silencing of Dicer attenuates degranulation in LAD2 cells. Seven days after transfection of siRNA (30 nM), the degranulation activity was measured with or without anti-IgE (15 μg/ml) stimulation. The data represent the average ± SD of three experiments. (B) LAD2 cells were transfected with 30 nM of miR-142-3p mimic, antisense and Dicer-siRNA. Three days after transfection, the degranulation activity was measured with or without anti-IgE (10 μg/ml) stimulation. (C) The degree of Ca^{2+} mobilization was measured 3 days after transfection of miRNA mimics or inhibitors. IgE-sensitized LAD2 cells were preloaded with Fluo 4-AM and then stimulated with Ca^{2+} assay buffer or the indicated concentrations of anti-IgE at 15 s. The relative fluorescence intensity was plotted.

Table S1). Several miRNAs were selected based on their expression abundance and specificity in LAD2 cells, and were overexpressed by transfection of miRNA mimics into the LAD2 cells. Then, FcεRI-induced degranulation activity was assessed. We found that LAD2 cells transfected with miR-142-3p was significantly increased (Fig. 1B and Supplemental Fig. S1). Conversely, we also introduced miR-142-3p antisense into LAD2 cells to inhibit endogenous miR-142-3p. As shown in Fig. 1B, miR-142-3p antisense decreased the degranulation activity.

It has been shown that the activation of mast cells triggered by FcεRI crosslinking leads to calcium influx [11]. Therefore, we introduced miR-142-3p mimic or inhibitor into LAD2 cells, and Ca^{2+} mobilization was measured. Among the control RNA-transfected LAD2 cells, no intracellular Ca^{2+} increases were observed in the cells without stimulation; however, the 10 and 50 μg/ml concentrations of anti-human IgE antibodies induced a Ca^{2+} response (Fig. 1C). The miR-142-3p-transfected LAD2 cells exhibited an elevated level of intracellular Ca^{2+} following stimulation with human-IgE antibodies. On the other hand, the miR-142-3p inhibitor decreased the Ca^{2+} levels from the control RNA.

These data showed that the level of Ca^{2+} mobilization was in proportion to the level of miR-142-3p, comparable with the phenomena observed in association with β-hexosaminidase release. These results suggest that miR-142-3p originally controls the FcεRI-mediated degranulation activity to enhance.

3.3. miR-142-3p rescues the reduction of degranulation by silencing Dicer

As we found that miR-142-3p is highly expressed in LAD2 cells, we speculated that the reduction of degranulation activity achieved by silencing Dicer mRNA was primarily attributed to the reduction of endogenous miR-142-3p. Therefore, we investigated whether the overexpression of miR-142-3p rescues the reduction of the degranulation activity in Dicer siRNA-transfected LAD2 cells, in which most miRNAs were repressed.

When Dicer siRNA was introduced into the LAD2 cells, miR-142-3p was reduced as Dicer mRNA was reduced (Fig. 2A and B). Meanwhile in the LAD2 cells transfected with both Dicer siRNA and miR-142-3p mimic, the Dicer mRNA was reduced, while miR-142-3p was increased (Fig. 2A and B).

We assessed the FcεRI-induced degranulation activity under these conditions. In the LAD2 cells transfected with Dicer siRNA and miR-142-3p mimic, the degranulation activity was increased compared to that observed in the LAD2 cells transfected with Dicer siRNA and control miRNA mimic. The level of degranulation activity in the LAD2 cells transfected with Dicer siRNA and miR-142-3p mimic was similar to that observed in the LAD2 cells without transfection (Fig. 2C). These results suggest that the cause of the reduction of degranulation activity following stimulation with anti-human IgE antibodies in Dicer siRNA-transfected LAD2 cells was the reduction of the miR-142-3p expression level triggered by the reduction of Dicer mRNA and that the increase in the amount of miR-142-3p rescued the degranulation activity.

3.4. Role of miR-142-3p in degranulation following stimulation with compound 48/80

Mast cells are activated not only by IgE/FcεRI stimulation, but also by FcεRI-independent stimulation. For example, compound 48/80, a basic secretagogue, induces degranulation independently of FcεRI [1,12]. Therefore, we investigated the degranulation activity in LAD2 cells following stimulation with compound 48/80, not human anti-IgE antibodies.

When Dicer siRNA was introduced into the LAD2 cells, the compound 48/80-induced degranulation activity was decreased

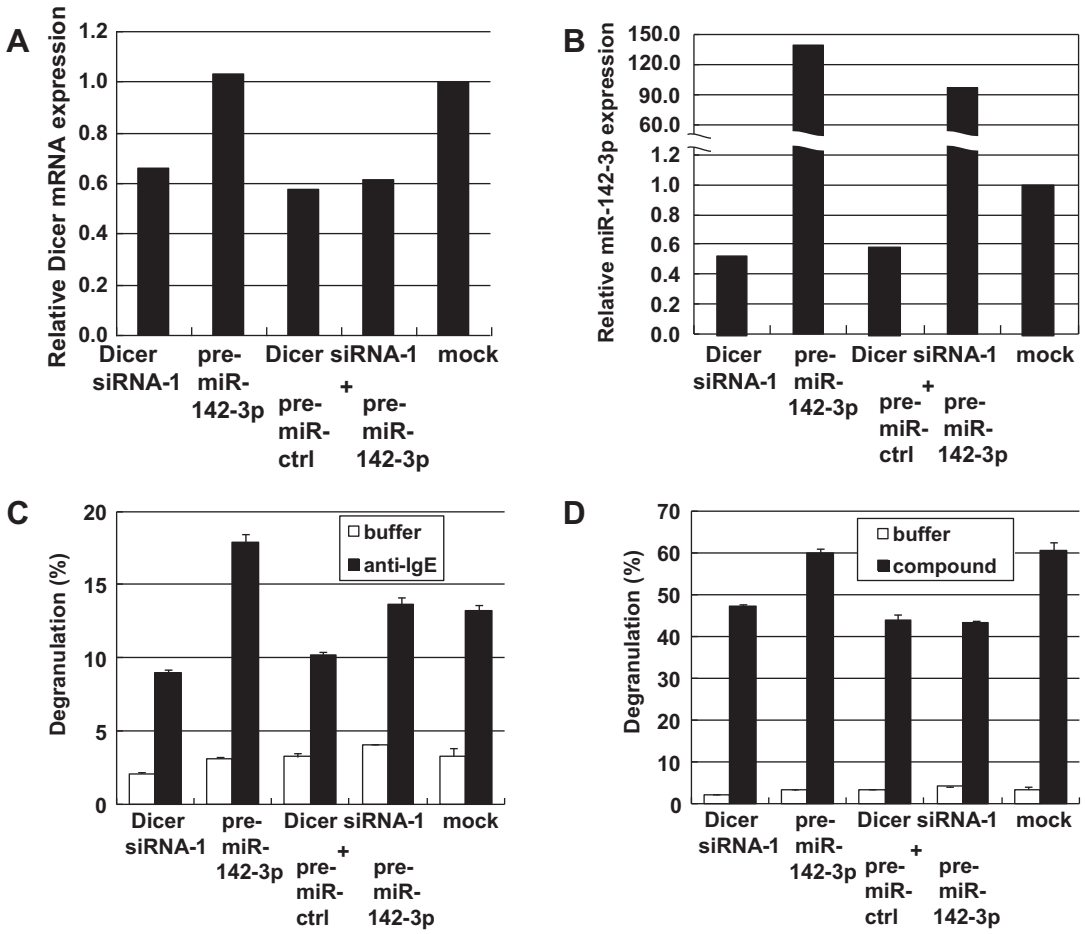

Fig. 2. miR-142-3p rescues the reduction of the degranulation activity by silencing Dicer. Dicer siRNA (30 nM), miR-142-3p mimic (30 nM), Dicer siRNA (30 nM) + miRNA mimic ctrl (30 nM) and Dicer siRNA (30 nM) + miR-142-3p mimic (30 nM) were transfected into LAD2 cells. (A, B) Two days after transfection, the expression levels of Dicer mRNA (A) and hsa-miR-142-3p (B) were measured using qRT-PCR. (C, D) Two days after transfection, the cells were sensitized with IgE, and the following day, the degranulation activity was measured following anti-IgE (10 μg/ml) (C) or compound 48/80 (0.5 μg/ml) (D) stimulation. The data represent the average ± SD of three experiments.

compared with that observed in the control (Fig. 2D), consistent with the reaction noted following anti-human IgE antibody stimulation. On the other hand, in the LAD2 cells transfected with miR-142-3p mimic, the degranulation activity did not change compared with that observed in the control (Fig. 2D).

Moreover, we found that the degranulation activity following stimulation with compound 48/80 in the LAD2 cells transfected with both Dicer siRNA and miR-142-3p mimic was decreased, as in the LAD2 cells transfected with Dicer siRNA only (Fig. 2D). Namely, miR-142-3p did not rescue the reduction of degranulation activity that occurred following stimulation with compound 48/80 in the LAD2 cells silenced Dicer mRNA, unlike that observed following stimulation with anti-human IgE antibodies. These results suggest that miR-142-3p is not independent of pathways triggered by compound 48/80 and that it is involved in FcεRI-dependent pathways.

3.5. miR-142-3p target prediction

In general, one miRNA regulates multiple mRNA expressions, which results in the formation of a complex regulatory network. We predicted several candidate mRNAs of miR-142-3p primarily based on pairing between the miRNA and assessed the effects of candidate mRNAs in the FcεRI-induced degranulation activities. Among the siRNAs of the target candidate mRNAs, silencing of LPP, WASL and c-cbl (CBL) mRNA enhanced the degranulation activity following stimulation with anti-human IgE antibodies (Supplemental Fig. S2A). These siRNAs were obviously confirmed to be potential to silence target mRNAs (Fig. S3). Supplemental Fig. S2B and C shows the putative miR-142-3p recognition elements (MREs) in their 3′ UTRs. The degranulation activity silenced by these mRNAs was similar to that of the overexpression of miR-142-3p; therefore, we speculate that these mRNAs may be parts of target genes of miR-142-3p.

Among these target genes, we constructed a luciferase reporter plasmid that inserted miR-142-3p MRE, as well as MRE with a mutated seed region, in the human and murine LPP mRNA at the downstream of Renilla luciferase gene, and tested the luciferase activity. As shown in Fig. 3, we observed a marked reduction in luciferase activities in cells co-transfection of miR-142-3p and the reporter vector compared with co-transfection of the negative control miRNA and the reporter vector. In contrast, no change in the mutant reporter vector was observed in the presence of miR-142-3p. These results indicated that LPP would be a direct target of miR-142-3p.

3.6. Degranulation activity in primary mast cells derived from miR-142-3p-deficient mice

We further investigated whether miR-142-3p has the same function other than LAD2 cells. To examine the effects on the degranulation activity of miR-142-3p in murine mast cells, we

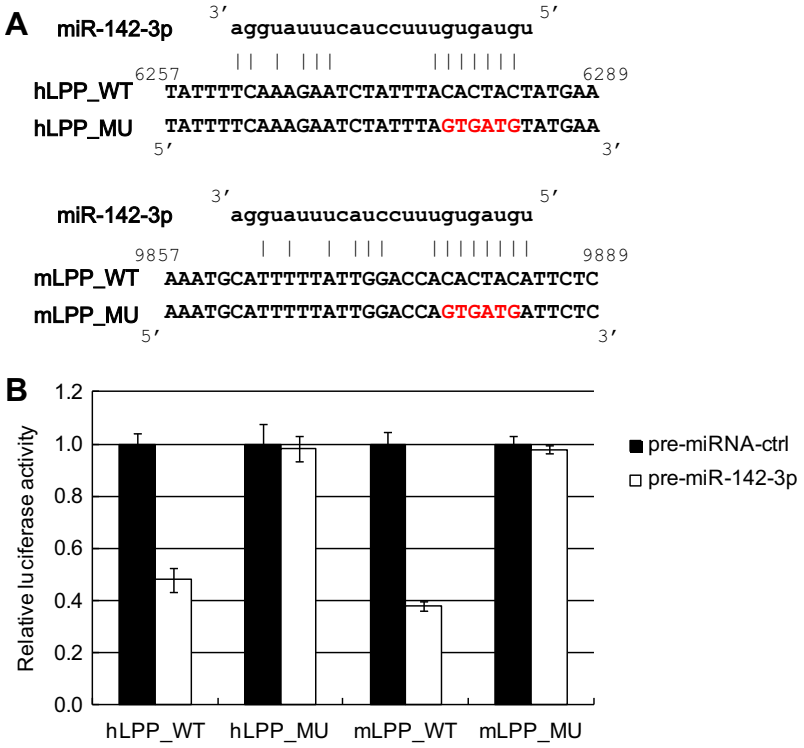

Fig. 3. miR-142-3p targets LPP. (A) miR-142-3p response element sequences in the human or murine LPP inserted reporter vectors were shown as schematic representation. Red letters denote point mutations that disrupt base pairing. (B) pre-miR-142-3p or pre-miRNA control were co-transfected into HepG2 cells with luciferase reporters containing the wild-type (WT) or mutant (MU). Renilla/Firefly luciferase activity is expressed as relative values against the negative control. Average ± SD (n = 3) are shown.

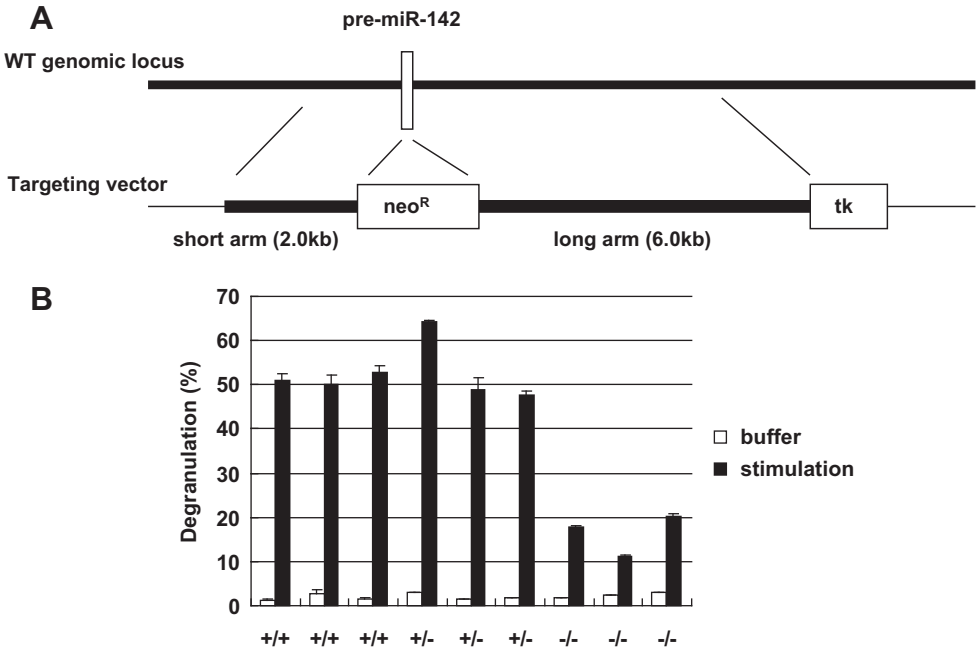

Fig. 4. Analysis of miR-142-3p-deficient mice. (A) Schematic representation of the homologous recombination approach used to delete the pre-miR-142 genomic sequence. (B) The degranulation activity of mast cells derived from the bone marrow of miR-142-3p-deficient mice. +/+ indicates wild-type mice, +/− indicates heterozygous mice and −/− indicates homozygous mice.

generated miR-142-3p-deficient mice. Based on the information in miRBase (http://www.mirbase.org), the murine miR-142-3p locus is located on the 11 chromosome and the mature sequence is identical to that of human miR-142-3p; thus, we deleted the endogenous pre-miR-142 site. The targeting was performed by introducing a neomycin resistance gene into murine embryonic stem cells (Fig. 4A). Targeted embryonic stem (ES) cell clones were used to generate chimeric mice, which transmitted the mutant

allele through the germline, yielding heterozygous mice. Breeding of these mice yielded homozygous for the miR-142-3p deletion, which were viable.

We next isolated bone marrow cells from homozygous, heterozygous and wild-type mice, and developed murine mast cells in vitro. Then, the β-hexosaminidase released from these mast cells upon TNP and anti-TNP IgE stimulation was assessed. While the activity of the mast cells derived from the heterozygous mice was at the same level as that observed in the wild-type mast cells, the activity of the mast cells derived from the homozygous mice was decreased by more than half compared with that observed in the wild-type mice (Fig. 4B). These results indicate that the loss of miR-142-3p reduces the degranulation activity upon IgE stimulation in mice, suggesting that murine miR-142-3p has the same function as human miR-142-3p in enhancing degranulation in an IgE/FcεRI-dependent manner.

4. Discussion

Although numerous studies have revealed that miRNAs are involved in important biological events, little is known regarding the role of miRNAs in mast cell degranulation. In this study, we showed that the total repression of endogenous miRNAs by silencing Dicer attenuates the FcεRI-mediated degranulation activity, suggesting that miRNAs intrinsically control the degranulation activity. Among miRNAs, endogeneously expressed in LAD2 cells, we found that miR-142-3p regulates the degranulation activities. miR-142-3p has been previously shown to be expressed in hematopoietic tissue [13], which is consistent with our observation that miR-142-3p is highly expressed in LAD2 cells, a type of immune cells. By focusing on the relationship between miRNAs and degranulation in mast cells, we provide new insight into the role of miR-142-3p.

On the other hand, the effects of miR-142-3p on the degranulation activity following stimulation with compound 48/80 were different from those observed following stimulation with anti-human IgE antibodies. The results indicate that miR-142-3p enhances the degranulation activity via an FcεRI-dependent pathway, not an FcεRI-independent pathway. Because compound 48/80 also induces Ca^{2+} mobilization before degranulation [14,15], miR-142-3p may function in the signal cascade that occurs between FcεRI aggregation and Ca^{2+} mobilization. The fact that LAD2 cells transfected with Dicer siRNA reduced the degranulation activity following stimulation with compound 48/80 shows that other miRNAs, not miR-142-3p, control the degranulation via FcεRI-independent pathway.

We generated miR-142-3p-deficient mice in order to elucidate the role of miR-142-3p in other than LAD2 cells, a human mast cell line. Mice homozygous for the miR-142-3p deletion were born and viable, suggesting that miR-142-3p does not have any influence on lethality, although it is involved in the differentiation of the hematopoietic lineage. While several miRNA deficient mice have been reported to date, most have not been shown to be lethal or have a severe phenotype, and some phenotypes have appeared only upon the application of certain internal or external stresses [16–18]. miR-142-3p-deficient mice exhibit the phenotype only under stimulation, as shown in other miRNA knockout mice, characteristic of the function of miRNAs.

We herein revealed that the degranulation activity in mast cells derived from miR-142-3p-deficient mice is decreased, suggesting that the function of miR-142-3p in mast cells may be the same in humans and mice. Although we cannot exclude the possibility that miR-142-3p-deficient murine cells exhibit impaired differentiation into mature mast cells rather than degranulation, the deficiency is likely to affect degranulation based on the observation in LAD2 cells. Meanwhile, degranulation was not completely suppressed even though the expression of miR-142-3p was completely suppressed. This is because miR-142-3p is not related to the initiation of degranulation, or there is an alternative mechanism to compensate for the loss of miR-142-3p. The degranulation activity was not affected in the mast cells derived from heterozygous miR-142-3p-deficient mice, indicating that more than half the level of inhibition of endogenous miR-142-3p is required to attenuate degranulation.

We did not examine the degranulation activity in murine mast cells following stimulation with compound 48/80. This is because murine mast cells derived from bone marrow cannot be activated via FcεRI-independent pathways, such as stimulation with compound 48/80 [1,19]. However, the fact remains that miR-142-3p enhances the degranulation activity in an FcεRI-dependent manner.

Because the deleted sequence of pre-miR-142 contains both miR-142-3p and miR-142-5p, the phenotype of miR-142-3p-deficient mice may also be affected by the loss of miR-142-5p. However, based on the fact that miR-142-5p was expressed at a lower level than miR-142-3p in the LAD2 cells (Supplemental Table S1) and in bone marrow-derived murine mast cells as reported [6], we speculate that the influence of miR-142-5p may be small.

miRNAs can act through regulating multiple target genes negatively. Among these potential target genes, we demonstrated that LPP would be one of the direct target genes for miR-142-3p. LPP regulates actin filament assembly by binding to α-actinin and vasodilator-stimulated phosphoprotein (VASP), which are involved in actin polymerization [20]. Previous studies have shown that the actin cytoskeleton regulates the association between crosslinked IgE–FcεRI and raft components [21] and that the inhibition of actin polymerization increases degranulation in mast cells [22]. Therefore, we speculate that LPP is associated with mast cell degranulation by organizing actin filament. It has been shown that individual miRNAs can directly downregulate hundreds of genes and that comprehensive downregulation determines the characteristics of miRNAs [23]. We herein proposed LPP as potential target genes of miR-142-3p, however, the degranulation in the LAD2 cells silenced by these genes was not as attenuated as that observed in the miR-142-3p-transfected LAD2 cells. Consequently, it is believed that this gene is only part of target genes and that miR-142-3p can regulate other target genes to enhance the degranulation activity.

In summary, we discovered that miR-142-3p enhances degranulation in an FcεRI-dependent manner in human LAD2 mast cell lines and murine primary mast cells. Our study suggests that miR-142-3p is a potential therapeutic target for the pathological conditions caused by mast cells, such as mastocytosis and allergies.

Acknowledgments

We thank Ms. K. Hayama, Ms. I. Taki, Ms. K. Takaki and Ms. M. Kamigaki for their technical assistance and Dr. H. Hasuwa for his insightful discussions. We are grateful to Dr. A. Kirshenbaum and the agency of the United States Public Health Service for providing the LAD2 cell line. This research was supported by a grant from the New Energy and Industrial Technology Development Organization (NEDO).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.078.

References

[1] D.D. Metcalfe, D. Baram, Y.A. Mekori, Mast cells, Physiol. Rev. 77 (1997) 1033–1079.
[2] J. Kalesnikoff, S.J. Galli, New developments in mast cell biology, Nat. Immunol. 9 (2008) 1215–1223.
[3] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[4] N. Bushati, S.M. Cohen, MicroRNA functions, Annu. Rev. Cell Dev. Biol. 23 (2007) 175–205.
[5] C.M. Croce, Causes and consequences of microRNA dysregulation in cancer, Nat. Rev. Genet. 10 (2009) 704–714.
[6] S. Monticelli, K.M. Ansel, C. Xiao, et al., MicroRNA profiling of the murine hematopoietic system, Genome Biol. 6 (2005) R71.
[7] R.J. Mayoral, M.E. Pipkin, M. Pachkov, et al., MicroRNA-221-222 regulate the cell cycle in mast cells, J. Immunol. 182 (2009) 433–445.
[8] A.S. Kirshenbaum, C. Akin, Y. Wu, et al., Characterization of novel stem cell factor responsive human mast cell lines LAD 1 and 2 established from a patient with mast cell sarcoma/leukemia; activation following aggregation of FcεRI or FcγRI, Leuk. Res. 27 (2003) 677–682.
[9] V. Supajatura, H. Ushio, A. Nakao, et al., Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4, J. Immunol. 167 (2001) 2250–2256.
[10] G. Hutvágner, J. McLachlan, A.E. Pasquinelli, et al., A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA, Science 293 (2001) 834–838.
[11] S. Kraft, J.P. Kinet, New developments in FcεRI regulation, function and inhibition, Nat. Rev. Immunol. 7 (2007) 365–378.
[12] X. Ferry, S. Brehin, R. Kamel, et al., G protein-dependent activation of mast cell by peptides and basic secretagogues, Peptides 23 (2002) 1507–1515.
[13] C.Z. Chen, L. Li, H.F. Lodish, et al., MicroRNAs modulate hematopoietic lineage differentiation, Science 303 (2004) 83–86.
[14] J. Senyshyn, R.A. Baumgartner, M.A. Beaven, Quercetin sensitizes RBL-2H3 cells to polybasic mast cell secretagogues through increased expression of Gi GTP-binding proteins linked to a phospholipase C signaling pathway, J. Immunol. 160 (1998) 5136–5144.
[15] S.W. Kashem, H. Subramanian, S.J. Collington, et al., G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2, Eur. J. Pharmacol. 668 (2011) 299–304.
[16] E. van Rooij, L.B. Sutherland, X. Qi, et al., Control of stress-dependent cardiac growth and gene expression by a microRNA, Science 316 (2007) 575–579.
[17] A.H. Williams, G. Valdez, V. Moresi, et al., MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice, Science 326 (2009) 1549–1554.
[18] G.X. Zheng, A. Ravi, J.M. Calabrese, et al., A latent pro-survival function for the mir-290-295 cluster in mouse embryonic stem cells, PLoS Genet. 7 (2011) e1002054.
[19] T. Ogasawara, M. Murakami, T. Suzuki-Nishimura, et al., Mouse bone marrow-derived mast cells undergo exocytosis, prostanoid generation, and cytokine expression in response to G protein-activating polybasic compounds after coculture with fibroblasts in the presence of c-kit ligand, J. Immunol. 158 (1997) 393–404.
[20] T.G. Grunewald, S.M. Pasedag, E. Butt, Cell adhesion and transcriptional activity – defining the role of the novel protooncogene LPP, Transl. Oncol. 2 (2009) 107–116.
[21] D. Holowka, E.D. Sheets, B. Baird, Interactions between FcεRI and lipid raft components are regulated by the actin cytoskeleton, J. Cell Sci. 113 (2000) 1009–1019.
[22] L. Frigeri, J.R. Apgar, The role of actin microfilaments in the down-regulation of the degranulation response in RBL-2H3 mast cells, J. Immunol. 162 (1999) 2243–2250.
[23] M. Selbach, B. Schwanhäusser, N. Thierfelder, et al., Widespread changes in protein synthesis induced by microRNAs, Nature 455 (2008) 58–63.