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Dual abnormal effects of mutant MITF encoded by *Mi*^{wh} allele on mouse mast cells: decreased but recognizable transactivation and inhibition of transactivation☆

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Abstract

MITF is a basic helix-loop-helix leucine zipper-type transcription factor and is important for development of mast cells. MITF encoded by *Mi*^{wh} allele (*Mi*^{wh}-MITF) was mutated at a single amino acid of basic domain, and possessed a deficient but apparent DNA-binding ability. Here, we characterized the unique effects of *Mi*^{wh}-MITF on the expression of mast cell-related genes. The expression level of mouse mast cell protease (mMCP)-4, -5, and -6 genes in *Mi*^{wh}/*Mi*^{wh} cultured mast cells (CMCs) was intermediate between levels of normal (+/+) CMCs and *tg*/*tg* CMCs, which did not express any MITFs. *Mi*^{wh}-MITF appeared to show the positive transactivation effect through the remaining DNA-binding ability. On the other hand, the expression level of tryptophan hydroxylase gene was lower in *Mi*^{wh}/*Mi*^{wh} CMCs than in *tg*/*tg* CMCs, suggesting the inhibitory effect of *Mi*^{wh}-MITF on the transactivation. *Mi*^{wh}-MITF possessed dual abnormal effects on transactivation of mast cell-related genes.

Keywords: MITF; Mast cell; Mutant mice; Transcription factor

The *mi* locus of mice encodes MITF that is a basic helix-loop-helix leucine zipper-type transcription factor [1,2]. MITF plays an important role in development of mast cells [3,4]. Many mutants have been reported at the *mi* locus [5,6]. Among them, *tg*/*tg* mice are useful as a standard for evaluating the function of other mutant MITFs, since *tg* is considered to be a null mutant allele [1,7–10]. Mast cells showed the more severely impaired phenotype in *mi*/*mi* and *Mi*^{or}/*Mi*^{or} mice than in *tg*/*tg* mice, suggesting that MITF encoded by *mi* and *Mi*^{or} alleles (*mi*- and *Mi*^{or}-MITF, respectively) possessed inhibitory effects [7,8]. Mast cells of *mi*^{ew}/*mi*^{ew} and *mi*^{ce}/*mi*^{ce} mice showed a comparable phenotype to that of *tg*/*tg* mast cells, suggesting that the MITFs encoded by *Mi*^{ew} and *mi*^{ce} alleles (*mi*^{ew}- and *mi*^{ce}-MITF, respectively) had no detectable effects [9,10]. The *mi*- and *Mi*^{or}-MITFs were mutated at a single amino acid in the basic domain [11], whereas *mi*^{ew}-MITF lacked most of the basic domain and *mi*^{ce}-MITF lacked the zipper domain [11].

MITF encoded by the *Mi*^{wh} allele (*Mi*^{wh}-MITF) was mutated at a single amino acid of the basic domain as in the cases of *mi*- and *Mi*^{or}-MITFs [11], but the effect of *Mi*^{wh}-MITF on phenotype of mast cells was different from that of other previously examined mutant MITFs [8]. No DNA-binding ability was observed in the other mutant MITFs [8–13], whereas a deficient but apparently recognizable DNA-binding ability was detected in *Mi*^{wh}-MITF [8]. In the present study, we investigated the unique effect of *Mi*^{wh}-MITF on mouse mast cells. The *Mi*^{wh}-MITF showed dual effects, decreased but recognizable transactivation effect on some genes and an apparent inhibitory effect on transactivation of another gene.

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Materials and methods

Mice and cells. All mice used were in the C57BL/6 genetic background. The *mi*/+, *mi*/*mi*, Mi^{wh}/+, Mi^{wh}/Mi^{wh}, and *tg*/*tg* mice were described previously [7,8]. Female *mi*/+ or Mi^{wh}/+ mice and male *tg*/*tg* mice were mated and the resulting *mi*/*tg* or Mi^{wh}/tg mice were selected by their white coat color. Cultured mast cells (CMCs) were maintained in α-minimal essential medium (α-MEM, ICN Biomedicals, Costa Mesa, CA) supplemented with 10% fetal calf serum (FCS, Nippon Biosupp Center, Tokyo, Japan) and 10% pokeweed mitogen-stimulated spleen cell conditioned medium as mentioned before [7]. The MST cells, provided by Dr. J. D. Esko (University of California, San Diego) [14], were maintained in RPMI1640 (Sigma, St. Louis, MO) supplemented with 10% FCS.

Staining and counting of mast cells. Three weeks after birth, mice were killed by decapitation after ether anesthesia. Pieces of dorsal skin were removed, smoothed onto a piece of the filter paper to keep them flat, fixed in Carnoy's solution, and embedded in paraffin. Sections of skin pieces were stained with alcian blue or berberine sulfate. The staining and counting methods have been described previously [15].

Northern blot analysis. Total RNAs (20 μg) isolated with the litium chloride–urea method [16] were used for Northern blot. The fragments of tryptophan hydroxylase (TPH) [17], c-*kit* receptor tyrosine kinase (KIT) [18], mouse mast cell protease (mMCP)-4 [19], mMCP-5 [20], and mMCP-6 [21] cDNAs were labeled with [α-^{32}P]dCTP (DuPont/NEN Research Products, Boston, MA; 10 mCi/mL) by random oligonucleotide priming. After hybridization at 42 °C, blots were washed to a final stringency of 0.2× SSC (1× SSC is 150 mmol/L NaCl and 15 mmol/L trisodium citrate, pH 7.4) and subjected to autoradiography.

Concentration of serotonin. The concentration of serotonin was measured using high-performance liquid chromatography with electrochemical detection as described before [8]. The concentration of serotonin per 1.0×10^6 cells was calculated.

Construction of reporter and expression plasmids. The reporter vector (pSPLuc) was provided by Dr. K. Nakajima (Osaka City University Medical School, Osaka, Japan) [22]. To construct reporter plasmids, a DNA fragment containing a promoter region of the TPH gene (−160 to +22, +1 shows the transcription initiation site) [17] was obtained with PCR and subcloned into pSPLuc. The reporter plasmid containing the deleted promoter region of TPH gene (−126 to +22) and that containing the mutated promoter region were also constructed by PCR. The effector plasmids were constructed by introducing the fragment of whole coding region of +-, *mi*-, and Mi^{wh}-MITF cDNAs into the pEF-BOS expression vector provided by Dr. S. Nagata (Osaka University Medical School, Osaka, Japan) [23]. The sequence of all constructs was verified with ABI 3100 sequencer (Applied BioSystems, Foster City, CA) in both directions.

Transient cotransfection assay. Five μg reporter, 0.5 or 1 μg an effector plasmid, and 1 μg expression vector containing β-galactosidase gene were cotransfected into MST cells by electroporation. In some experiments, the transfection was carried out without an effector plasmid. The expression vector containing β-galactosidase gene was used as an internal control. The cells were harvested 48 h after the transfection and the soluble extracts were assayed for luciferase activity and for β-galactosidase activity as described previously [9]. The normalized value by the β-galactosidase activity was expressed as the relative luciferase activity.

Results and discussion

We have reported that the number of skin mast cells decreased in *mi*/*mi* and *tg*/*tg* mice but not in Mi^{wh}/Mi^{wh} mice [8]. As expected, the number of skin mast cells in *mi*/*tg* mice was comparable to the value of *mi*/*mi* and *tg*/*tg* mice (Table 1). The number of skin mast cells in Mi^{wh}/tg mice was intermediate between the values of *tg*/*tg* and Mi^{wh}/Mi^{wh} mice (Table 1), indicating that the Mi^{wh}-MITF possessed a positive effect on the number of skin mast cells in a gene dose-dependent manner.

Most mast cells in the skin of +/+ and *tg*/*tg* mice were stained with berberine sulfate that binds heparin, but only few mast cells were berberine sulfate-positive in the skin of *mi*/*mi* mice (Table 1) [24]. This showed that the *mi*-MITF possessed an inhibitory effect on the synthesis of heparin [24]. The proportion of berberine sulfate-positive cells in the skin of *mi*/*tg* mice was intermediate between values of *tg*/*tg* and *mi*/*mi* mice, indicating that the inhibitory effect of *mi*-MITF on the synthesis of heparin was gene dose-dependent (Table 1). The proportion of berberine sulfate-positive cells in Mi^{wh}/tg and Mi^{wh}/Mi^{wh} mice was comparable to the value of *tg*/*tg* mice. In contrast to *mi*-MITF, Mi^{wh}-MITF did not appear to possess the inhibitory effect on the heparin synthesis.

Next, we compared the expression level of various genes among *tg*/*tg*, *mi*/*tg*, and *mi*/*mi* CMCs and among *tg*/*tg*, Mi^{wh}/tg, and Mi^{wh}/Mi^{wh} CMCs by Northern blot. As reported previously, the expression level of TPH gene reduced slightly in *tg*/*tg* CMCs and severely in *mi*/*mi* CMCs [7]. The TPH expression level of *mi*/*tg* CMCs was intermediate between levels of *tg*/*tg* and *mi*/*mi* CMCs, indicating that the *mi*-MITF showed an inhibitory effect on the expression of TPH gene in a gene dose-dependent manner (Fig. 1A). The TPH expression level of Mi^{wh}/tg CMCs was also intermediate between levels of *tg*/*tg* and Mi^{wh}/Mi^{wh} CMCs (Fig. 1A), indicating that the inhibitory effect of Mi^{wh}-MITF on the TPH expression was gene dose-dependent as well.

The expression of KIT gene was slightly impaired in *tg*/*tg* CMCs, moderately in *mi*/*tg* CMCs, and severely in *mi*/*mi* CMCs (Fig. 1A). The *mi*-MITF showed the gene dose-dependent inhibitory effect also on the expression of KIT gene. The effect of Mi^{wh}-MITF on the KIT expression was different from that of *mi*-MITF; the expression of KIT was not impaired in Mi^{wh}/tg and

Table 1
Number of mast cells and proportion of berberine sulfate-positive to alcian blue-positive cells in the skin of mice

Genotype of mice	Number of alcian blue$^+$ cells (per cm skin)[a]	Proportion of berberine sulfate$^+$ to alcian blue$^+$ cells (%)[a]
+/+	226 ± 17[b]	92 ± 3
Mi^{wh}/Mi^{wh}	209 ± 2[b]	86 ± 4
Mi^{wh}/tg	156 ± 11[b,c]	84 ± 6
tg/*tg*	89 ± 8[c]	81 ± 4
mi/*tg*	82 ± 10[c]	58 ± 5[b,c]
mi/*mi*	88 ± 9[c]	3 ± 1[b,c]

[a] Mean ± SE of 10 mice.
[b] $p < 0.05$ when compared with the value of *tg*/*tg* mice by *t* test.
[c] $p < 0.05$ when compared with the value of +/+ mice by *t* test.

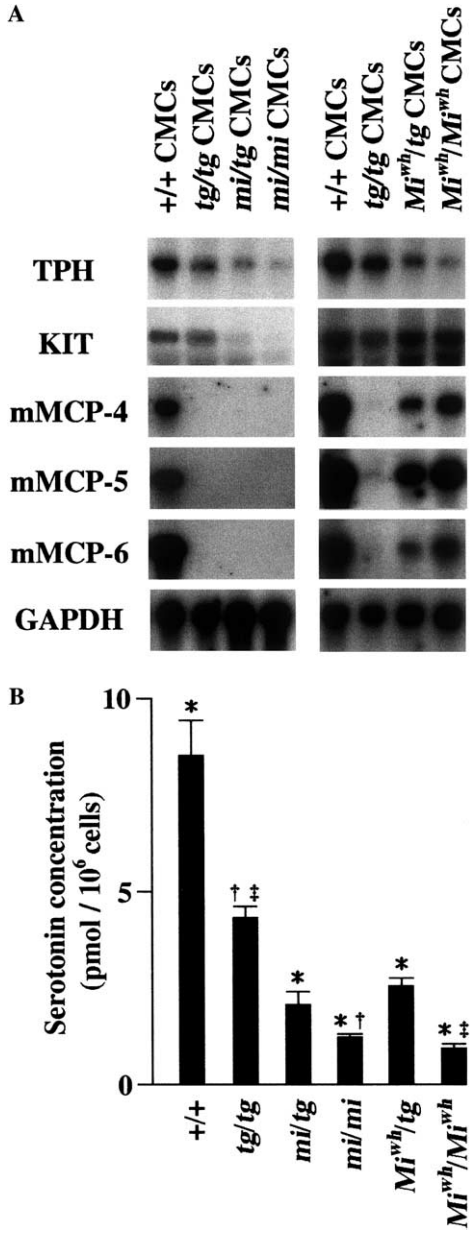

Fig. 1. Phenotype of CMCs derived from +/+, *tg/tg*, *mi/tg*, *mi/mi*, Mi^{wh}/tg, and Mi^{wh}/Mi^{wh} mice. (A) Expression of various genes was examined by Northern blot. The blot was hybridized with ^{32}P-labeled cDNA probe of TPH, KIT, mMCP-4, mMCP-5, mMCP-6, and GAPDH. Three independent experiments were done and comparable results were obtained. A representative experiment is shown. (B) Serotonin concentration was determined in CMCs derived from mice of each genotype. The bars represent the mean ± standard error (SE) of three independent experiments. $^{*}p < 0.05$ by *t* test when compared with the value of *tg/tg* CMCs. $^{\dagger}p < 0.05$ by *t*-test when compared with the value of *mi/tg* CMCs among the values of $tg/tg, mi/tg$ and mi/mi CMCs. $^{\ddagger}p < 0.05$ by *t* test when compared with the value of Mi^{wh}/tg CMCs among the values of tg/tg, Mi^{wh}/tg, and Mi^{wh}/Mi^{wh} CMCs.

Mi^{wh}/Mi^{wh} CMCs (Fig. 1A). The Mi^{wh}-MITF possessed a positive effect on the expression of KIT gene. A single gene dose of +-MITF or Mi^{wh}-MITF appeared sufficient for the expression of KIT gene.

The expression of mMCP-4, -5, and -6 genes was hardly detectable in *tg/tg* CMCs as described previously [7, Fig. 1A]. This indicated that the presence of normal MITF was essential for the expression of mMCP-4, -5, and -6 genes [7,25–27]. The *mi*-MITF did not show such a positive effect on the expression of mMCP-4, -5, and -6 genes [25–27]. Therefore, the *mi/tg* and *mi/mi* CMCs did not express these genes as in the case of *tg/tg* CMCs (Fig. 1A). In contrast, Mi^{wh}/Mi^{wh} CMCs showed only slightly impaired levels of mMCP-4, -5, and -6 gene expression (Fig. 1A). The expression level of these genes in Mi^{wh}/tg CMCs was intermediate between levels of *tg/tg* and Mi^{wh}/Mi^{wh} CMCs (Fig. 1A). Mi^{wh}-MITF showed a slightly impaired but apparently positive effect on the expression of mMCP-4, -5, and -6 genes in a gene dose-dependent manner.

Mi^{wh}-MITF showed a positive effect on the expression of KIT, mMCP-4, -5, and -6 genes but an inhibitory effect on the expression of TPH gene. To confirm the inhibitory effect of Mi^{wh}-MITF on the TPH gene expression, we analyzed the serotonin content of CMCs, since TPH is the rate-limiting enzyme of serotonin synthesis [28]. The serotonin content paralleled to the expression level of TPH gene in CMCs; $+/+ > tg/tg > mi/tg \sim Mi^{wh}/tg > mi/mi \sim Mi^{wh}/Mi^{wh}$ (Fig. 1B). Mi^{wh}-MITF showed an inhibitory effect on serotonin production in a gene dose-dependent manner, as in the case of *mi*-MITF.

The effect of *mi*-MITF and Mi^{wh}-MITF on the expression of TPH gene was examined by reporter assay. First, the region that mediated the transactivation of TPH promoter was determined. We constructed the reporter plasmid containing the promoter region of TPH gene (−160 to +22). The reporter plasmid, in which the promoter region was deleted, was also constructed (−126 to +22). Each reporter plasmid was transfected to MST mastocytoma cells that expressed TPH mRNA (data not shown). When the reporter plasmid starting from nt −126 was transfected, the reporter activity decreased one-sixth that of the reporter plasmid starting from nt −160 (Fig. 2A). The promoter region between nt −160 and −126 contained a GC-rich region (28 of 35 nucleotides were G or C). We constructed the reporter plasmid mutated at the GC-rich region (CCCGCCCC from nt −147 to −140 was changed to AAATAAAA). The mutation at the GC-rich region reduced the promoter activity to the level of the promoter starting from nt −126 (Fig. 2A), indicating that the GC-rich region partly mediated the transactivation of TPH gene.

The effect of +-MITF, *mi*-MITF, and Mi^{wh}-MITF on the transactivation of TPH gene was examined. The reporter plasmid starting from nt −160 was cotransfected with the expression plasmid containing +-MITF, *mi*-MITF or Mi^{wh}-MITF cDNA. The cotransfection of +-MITF cDNA did not affect the reporter activity, whereas the cotransfection of *mi*-MITF or Mi^{wh}-MITF

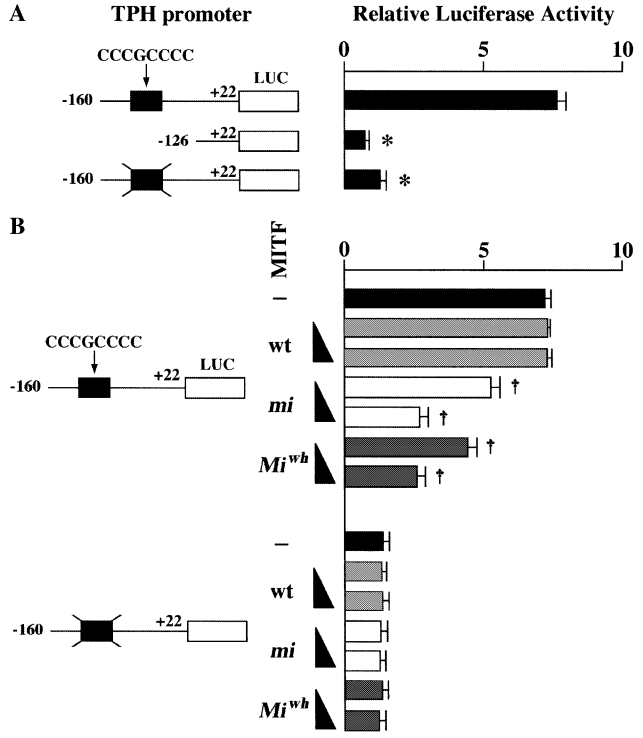

Fig. 2. Effect of +-MITF, *mi*-MITF or Mi^{wh}-MITF on the promoter activity of TPH gene. (A) Various reporter plasmids were transfected into MST cells, in which TPH gene was expressed. The solid square shows the CCCGCCCC motif located nt −147 to −140. (B) Various reporter and effector plasmids were cotransfected. The bars represent the mean ± SE of three independent experiments. $^{*}p < 0.05$ by *t* test when compared with the value obtained with the reporter plasmid starting from nt −160. $^{\dagger}p < 0.05$ by *t* test when compared with the control, in which expression vector containing no insert was cotransfected.

cDNA reduced the reporter activity in a dose-dependent manner (Fig. 2B). Mi^{wh}-MITF as well as *mi*-MITF appeared to inhibit the expression of TPH gene through the GC-rich region.

Most mutant MITFs possessed only the inhibitory effect (*mi*- and Mi^{or}-MITFs) [7,8] or lacked any detectable effects (Mi^{ew}- and Mi^{ce}-MITFs) [9,10]. On the other hand, Mi^{wh}-MITF possessed both positive and negative effects; the positive effect on the number of skin mast cells and the expression levels of KIT, mMCP-4, -5, and -6 genes, and the negative effect on the expression of TPH gene. The magnitude of effects would be parallel to the amount of Mi^{wh}-MITF. In fact, the gene dose-dependency was observed in both the positive effect of Mi^{wh}-MITF on the expression of mMCP-4, -5, and -6 genes and the inhibitory effect of Mi^{wh}-MITF on the expression of TPH gene. The Mi^{wh}-MITF possessed dual effects on the transactivation of mast cell-related genes.

The +- and Mi^{wh}-MITFs possessing DNA-binding ability showed the positive effect, whereas the *mi*-, Mi^{or}-, Mi^{ew}- and Mi^{ce}-MITFs lacking DNA-binding ability did not show it. The DNA-binding ability appeared to be necessary for the positive effect. The affinity to DNA paralleled to the magnitude of positive effect. The expression level of mMCP-4, -5, and -6 genes reduced in Mi^{wh}/Mi^{wh} CMCs when compared to the level of +/+ CMCs. The reduction may be due to the deficient DNA-binding ability of Mi^{wh}-MITF [8]. Huang et al. [29] demonstrated that MyoD, a member of bHLH proteins, showed a moderately impaired transactivation potential when its DNA-binding ability was reduced.

The inhibitory effect was observed in either mutant MITFs possessing DNA-binding ability or mutant MITFs lacking it. The DNA-binding ability did not appear necessary for the inhibitory effect. The *mi*-, Mi^{or}- and Mi^{wh}-MITFs showing the inhibitory effect were mutated at a single amino acid of the basic domain, whereas the Mi^{ew}- and Mi^{ce}-MITFs lacking the inhibitory effect showed a large deletion. The large deletion may make the protein–protein interaction impossible. Such protein–protein interactions would be prerequisite for the inhibition of other transcription factors. MITFs possessing a single amino acid mutation may interact with other proteins, whereas MITFs possessing a large deletion may lack the ability of interaction. In fact, we found that MAZR, which interacted with +-MITF, bound *mi*-MITF but not Mi^{ce}-MITF that lacked the zipper domain [30]. The Mi^{wh}-MITF, as well as *mi*-MITF, appeared to have the inhibitory effect on the transactivation ability of an unidentified transcription factor that binds the GC-rich region of the TPH promoter.

Taken together, the Mi^{wh}-MITF possessed dual effects on the phenotype of mast cells. A decreased but recognizable positive effect of Mi^{wh}-MITF may be due to the residual DNA-binding ability and an inhibitory effect may be due to its ability to interact with other transcription factors.

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References

[1] C.A. Hodgkinson, K.J. Moore, A. Nakayama, E. Steingrimsson, N.G. Copeland, N.A. Jenkins, H. Arnheiter, Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein, Cell 74 (1993) 395–404.

[2] M.J. Hughes, J.B. Lingrel, J.M. Krakowsky, K.P. Anderson, A helix-loop-helix transcription factor-like gene is located at the *mi* locus, J. Biol. Chem. 268 (1993) 20687–20690.

[3] Y. Kitamura, E. Morii, T. Jippo, A. Ito, *mi* transcription factor as a regulator of mast cell differentiation, Int. J. Hematol. 71 (2000) 197–202.

[4] Y. Kitamura, E. Morii, H. Ogihara, T. Jippo, A. Ito, Mutant mice: a useful tool for studying the development of mast cells, Int. Arch. Allergy. Immunol. 124 (2001) 16–19.
[5] M.C. Green, Catalog of mutant genes and polymorphic loci, in: M.F. Lyon, A.G. Searle (Eds.), Mice Genetic Variants and Strains of the Laboratory Mouse, G. Fischer Verlag, Stuttgart, 1981, pp. 158–161.
[6] W. Silvers, The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction, Springer-Verlag, New York, 1979.
[7] A. Ito, E. Morii, D.K. Kim, T.R. Kataoka, T. Jippo, K. Maeyama, H. Nojima, Y. Kitamura, Inhibitory effect of the transcription factor encoded by the *mi* mutant allele in cultured mast cells of mice, Blood 93 (1999) 1189–1196.
[8] D.K. Kim, E. Morii, H. Ogihara, Y.M. Lee, T. Jippo, S. Adachi, K. Maeyama, H.M. Kim, Y. Kitamura, Different effect of various mutant MITF encoded by *mi*, Mi^{or}, or Mi^{wh} allele on phenotype of murine mast cells, Blood 93 (1999) 4179–4186.
[9] E. Morii, H. Ogihara, K. Oboki, T.R. Kataoka, K. Maeyama, D.E. Fisher, M.L. Lamoreux, Y. Kitamura, Effect of a large deletion of the basic domain of *mi* transcription factor on differentiation of mast cells, Blood 98 (2001) 2577–2579.
[10] E. Morii, H. Ogihara, D.K. Kim, A. Ito, K. Oboki, Y.M. Lee, T. Jippo, S. Nomura, K. Maeyama, M.L. Lamoreux, Y. Kitamura, Importance of leucine zipper domain of *mi* transcription factor (MITF) for differentiation of mast cells demonstrated using mi^{ce}/mi^{ce} mutant mice of which MITF lacks the zipper domain, Blood 97 (2001) 2038–2044.
[11] E. Steingrimsson, K.J. Moore, M.L. Lamoreux, A.R. Ferre-D'Amare, S.K. Burley, D.C. Zimring, L.C. Skow, C.A. Hodgkinson, H. Arnheiter, N.G. Copeland, N.A. Jenkins, Molecular basis of mouse microphthalmia (*mi*) mutations helps explain their developmental and phenotypic consequences, Nat. Genet. 8 (1994) 256–263.
[12] E. Morii, K. Takebayashi, H. Motohashi, M. Yamamoto, S. Nomura, Y. Kitamura, Loss of DNA-binding ability of the transcription factor encoded by the mutant *mi* locus, Biochem. Biophys. Res. Commun. 205 (1994) 1299–1304.
[13] T.J. Hemesath, E. Streingrimsson, G. McGill, M.J. Hansen, J. Vaught, C.A. Hodgkinson, H. Arnhheiter, N.G. Copeland, N.A. Jenkins, D.E. Fisher, Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family, Genes Dev. 8 (1994) 2770–2780.
[14] R.I. Montgomery, K. Lidholt, N.W. Flay, J. Liang, B. Vertel, U. Lindahl, J.D. Esko, Stable heparin-producing cell lines derived from the Furth murine mastocytoma, Proc. Natl. Acad. Sci. USA 89 (1992) 11327–11331.
[15] T. Kasugai, K. Oguri, T. Jippo-Kanemoto, M. Morimoto, A. Yamatodani, K. Yoshida, Y. Ebi, K. Isozaki, H. Tei, T. Tsujimura, S. Nomura, M. Okayama, Y. Kitamura, Deficient differentiation of mast cells in the skin of *mi/mi* mice. Usefulness of in situ hybridization for evaluation of mast cell phenotype, Am. J. Pathol. 143 (1993) 1337–1347.
[16] C. Auffray, F. Rougeon, Purification of mouse immunoglobulin heavy- chain messenger RNAs from total myeloma tumor RNA, Eur. J. Biochem. 107 (1980) 303–314.
[17] A. Ito, E. Morii, K. Maeyama, T. Jippo, D.K. Kim, Y.M. Lee, H. Ogihara, K. Hashimoto, Y. Kitamura, H. Nojima, Systematic method to obtain novel genes that are regulated by *mi* transcription factor: impaired expression of granzyme B and tryptophan hydroxylase in *mi/mi* cultured mast cells, Blood 91 (1998) 3210–3221.
[18] T. Tsujimura, E. Morii, M. Nozaki, K. Hashimoto, Y. Moriyama, K. Takebayashi, T. Kondo, Y. Kanakura, Y. Kitamura, Involvement of transcription factor encoded by the *mi* locus in the expression of c-*kit* receptor tyrosine kinase in cultured mast cells of mice, Blood 88 (1996) 1225–1233.
[19] W.E. Serafin, T.P. Sullivan, G.A. Conder, A. Ebrahimi, P. Marcham, S.S. Johnson, K.F. Austen, D.S. Reynolds, Cloning of the cDNA and gene for mouse mast cell protease 4. Demonstration of its late transcription in mast cell subclasses and analysis of its homology to subclass-specific neutral proteases of the mouse and rat, J. Biol. Chem. 266 (1991) 1934–1941.
[20] H.P. McNeil, K.F. Austen, L.L. Somerville, M.F. Gurish, R.L. Stevens, Molecular cloning of the mouse mast cell protease-5 gene. A novel secretory granule protease expressed early in the differentiation of serosal mast cells, J. Biol. Chem. 266 (1991) 20316–20322.
[21] D.S. Reynolds, D.S. Gurley, K.F. Austen, W.E. Serafin, Cloning of the cDNA and gene of mouse mast cell protease-6. Transcription by progenitor mast cells and mast cells of the connective tissue subclass, J. Biol. Chem. 266 (1991) 3847–3853.
[22] K. Nakajima, T. Kusafuka, T. Takeda, Y. Fujitani, K. Nakae, T. Hirano, Identification of a novel interleukin-6 response element containing an Ets- binding site and a CRE-like sites in the junB promoter, Mol. Cell. Biol. 13 (1993) 3027–3041.
[23] S. Mizushima, S. Nagata, pEF-BOS, a powerful mammalian expression vector, Nucleic Acids Res. 18 (1990) 5322.
[24] E. Morii, H. Ogihara, K. Oboki, C. Sawa, T. Sakuma, S. Nomura, J.D. Esko, H. Handa, Y. Kitamura, Inhibitory effect of MITF encoded by the mutant *mi* allele on GABP-mediated transcript expression in mouse mast cells, Blood 97 (2001) 3032–3039.
[25] T. Jippo, Y.M. Lee, Y. Katsu, K. Tsujino, E. Morii, D.K. Kim, H.M. Kim, Y. Kitamura, Deficient transcription of mouse mast cell protease 4 gene in mutant mice of *mi/mi* genotype, Blood 93 (1999) 1942–1950.
[26] E. Morii, T. Jippo, T. Tsujimura, K. Hashimoto, D.K. Kim, Y.M. Lee, H. Ogihara, K. Tsujino, H.M. Kim, Y. Kitamura, Abnormal expression of mouse mast cell protease 5 gene in cultured mast cells derived from mutant *mi/mi* mice, Blood 90 (1997) 3057–3066.
[27] E. Morii, T. Tsujimura, T. Jippo, K. Hashimoto, K. Takebayashi, K. Tsujino, S. Nomura, M. Yamamoto, Y. Kitamura, Regulation of mouse mast cell protease 6 gene expression by transcription factor encoded by the *mi* locus, Blood 88 (1996) 2488–2494.
[28] E. Jequier, W. Lovenberg, A. Sjoerdsma, Tryptophan hydroxylase inhibition: The mechanism by which *p*-chlorophenylalanine depletes rat brain serotonin, Mol. Pharmacol. 3 (1967) 274–278.
[29] J. Huang, H. Weintraub, L. Kedes, Intramolecular regulation of MyoD activation domain conformation and function, Mol. Cell. Biol. 18 (1998) 5478–5484.
[30] E. Morii, K. Oboki, T.R. Kataoka, K. Igarashi, Y. Kitamura, Interaction and cooperation of *mi* transcription factor (MITF) and myc- associated zinc-finger protein-related factor (MAZR) for transcription of mouse mast cell protease 6 gene, J. Biol. Chem. 277 (2002) 8566–8571.