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Microphthalmia-associated transcription factor as the molecular target of cadmium toxicity in human melanocytes

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ABSTRACT

Dietary intake of cadmium is inevitable, causing age-related increase in cadmium accumulation in many organs, including hair, choroid and retinal pigment epithelium (RPE). Cadmium has been implicated in the pathogenesis of hearing loss and macular degeneration. The functions of cochlea and retina are maintained by melanocytes and RPE, respectively, and the differentiation of these pigment cells is regulated by microphthalmia-associated transcription factor (MITF). In the present study, we explored the potential toxicity of cadmium in the cochlea and retina by using cultured human melanocytes and human RPE cell lines. MITF consists of multiple isoforms, including melanocyte-specific MITF-M and widely expressed MITF-H. Levels of MITF-M protein and its mRNA in human epidermal melanocytes and HMV-II melanoma cells were decreased significantly by cadmium. In parallel with the MITF reduction, mRNA levels of tyrosinase, the key enzyme of melanin biosynthesis that is regulated by MITF-M, were also decreased. In RPE cells, however, the levels of total MITF protein, constituting mainly MITF-H, were not decreased by cadmium. We thus identify MITF-M as the molecular target of cadmium toxicity in melanocytes, thereby accounting for the increased risk of disability from melanocyte malfunction, such as hearing and vision loss among people with elevated cadmium exposure.

1. Introduction

Mammalian pigment cells include the melanocyte of the neural crest origin and the retinal pigment epithelium (RPE), derived from the optic cup of the developing brain. Microphthalmia-associated transcription factor (Mitf) is a key regulator for development of the melanocyte and the RPE in mice [1–3]. The human *MITF* gene contains multiple promoters and consecutive first exons, thereby generating distinct isoforms, such as a melanocyte-lineage specific isoform MITF-M and widely expressed MITF-H [4–6]. A loss-of-function mutation in the *MITF* gene is associated with a dominantly inherited auditory-pigmentary disorder, Waardenburg syndrome type 2, characterized by sensorineural hearing loss and depigmentation of the skin and/or iris [7]. Thus, the amount of functional MITF is critical for maintaining the functions of melanocytes in humans. Likewise, Mitf-M, the mouse counterpart of human MITF-M, is essential for the development of follicular and choroidal melanocytes [3,8] and cochlear melanocytes [9,10]. The blood vessel-enriched choroid is located adjacent to the RPE that constitutes the blood-retinal barrier that protects the photoreceptors against blood-born toxicants [11]. On the other hand, cochlear melanocytes are responsible for the production of endolymph that is essential for sensing sound.

Cadmium, an environmental toxicant, has been implicated in the pathogenesis of various chronic diseases [12,13], including age-related macular degeneration (AMD) [14–16] and hearing loss [17,18]. Diet and cigarette smoking are the main sources of cadmium in the general population [19]; the estimated amount of cadmium intake ranges from 10 to 30 μg/day in developed countries. Because of inevitable cadmium intake via diet and a lack of active mechanism for its elimination, levels of cadmium accumulation rise with age in various tissues, including RPE and choroid [20,21]. Higher levels of cadmium are found in the RPE and the

Abbreviations: AMD, age-related macular degeneration; HO, heme oxygenase; MITF, microphthalmia-associated transcription factor; NHEMs, normal human epidermal melanocytes; RPE, retinal pigment epithelium.

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choroid from subjects with AMD, compared with those without AMD [15]. Likewise, cadmium is detected in human scalp hair [22]. We have therefore hypothesized that the age-related increases in cadmium accumulation may cause functional impairment of RPE and melanocytes of the choroid, hair follicles, and cochlea.

In the present study, we analyzed the effects of cadmium exposure on the expression of MITF isoforms in human pigment cells with differing MITF isoform expression profiles. We, therefore, used epidermal melanocytes and melanoma cells, which predominantly express MITF-M [23,24] together with the RPE cell lines, ARPE-19 and D407, both of which predominantly expressed MITF-H [5,25] and have been used by us to explore the biochemical consequences of cadmium exposure [26,27]. This is the first report of MITF isoform-specific effect of cadmium, which could explain the increased risk of hearing and vision disability among people with elevated cadmium exposure.

2. Materials and methods

2.1. Human epidermal melanocytes and cell lines

Normal human epidermal melanocytes (NHEMs) were obtained from KURABO (Osaka, Japan) and cultured in Medium 154S (KURABO) containing human melanocyte growth supplement and 0.5% fetal bovine serum (FBS). NHEMs used were established from the abdominal skin of a 22-year-old Caucasian female (Strain number: 01179). Every two days, the medium was changed to maintain the growth of NHEMs. HMV-II human melanoma cells [28] were obtained from RIKEN Bio-Resource Center, Japan and were cultured in Ham's F-12 medium supplemented with 10% FBS (Gibco, Invitrogen, Mexico). Human RPE cell lines, ARPE-19 [29] and D407 [30], were cultured in 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM) and Ham's F-12 and DMEM, respectively [26,27,31].

2.2. Chemicals and antibodies

Cadmium chloride ($CdCl_2 2H_2O$) was from Sigma–Aldrich (St. Louis, MO). Mouse monoclonal anti-MITF antibody (MS-771-P1 clone C5) and mouse monoclonal anti-α-tubulin antibody were from NeoMarkers (Fremont, CA). Mouse monoclonal anti-tyrosinase antibody (clone T311) was purchased from Santa Cruz (Dallas, TX, USA). Rabbit polyclonal anti-heme oxygenase 1 (HO-1) antibody (ADI-SPA-896) and anti-heme oxygenase 2 (HO-2) antibody (SPA-897) were from Enzo Life science (Farmindale, NY) and from StressGen Biotechnologies (Victoria, BC, Canada), respectively. Mouse monoclonal IgG1 anti-β-actin antibody (A-5441 clone AC-19) was from Sigma–Aldrich (St. Louis, MO). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit immunoglobulin (NA9340) and HRP-conjugated sheep anti-mouse immunoglobulin (NA931) were from GE Healthcare (Buckinghamshire, UK).

2.3. Cadmium exposure experiments

The culture medium of NHEMs (about 80% confluent) was changed with the fresh medium and incubated for 8 h, and a same volume of vehicle solution or cadmium stock solution was added to the medium (0 time). NHEMs were then incubated in the absence or presence of cadmium (1, 2.5 or 5 μM) for up to 24 h. Note that higher concentrations of cadmium (⩾10 μM) killed NHEMs. HMV-II melanoma cells or RPE cells were grown to 70–80% confluent, before addition of cadmium stock solution or vehicle solution. Final Cd^{2+} concentrations in culture medium were between 1 and 40 μM. Cells were maintained in culture under 5% CO_2 for various times and subjected to preparation of RNA or whole cell lysates.

2.4. Western blot analysis

Collected cells were immediately dissolved in ice-cold lysis buffer, containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS and 0.1% protease inhibitor cocktail (Sigma). Each cell-lysate sample (10–30 μg protein per lane) was loaded for separation, using SDS–PAGE with 10% or 12% gel concentration (AE-6500 ATTO, Japan). Following electrophoresis, proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Immobilon™-P, Millipore Corporation), as detailed previously [31]. Blotted membranes were incubated at room temperature for 2 h with each primary antibody at a specified dilution shown in parentheses: MITF (1:1000), tyrosinase (1:1000), β-catenin (1:5000), HO-1 (1:1000), HO-2 (1:1000), β-actin (1:5000), and α-tubulin (1:5000). Blotted membranes were then incubated with the secondary antibody, HRP-conjugated donkey anti-rabbit immunoglobulin at a 1:5000 dilution for β-catenin, HO-1 and HO-2 proteins. For detecting MITF, tyrosinase, β-actin, and α-tubulin, membranes were incubated with HRP-conjugated sheep anti-mouse immunoglobulin (1:1000–5000). Immunoreactive proteins were detected, according to the manufacturer's protocol (Immobilon™ Western, Millipore).

2.5. Real-time RT-PCR

RNA was subjected to cDNA synthesis with PrimeScript RT reagent Kit (TAKARA, Japan). RNA, prepared from NHEMs, was purified prior to RT-PCR using OneStep™ PCR Inhibitor Removal Kit (Zymo Research, Alameda, CA), because of contaminated melanin. The real-time RT-PCR was performed in triplicate, using SYBR Premix Ex Taq II (TAKARA, Japan) and a real-time PCR system ABI7500 (Applied Systems, CA). The conditions of the real-time PCR were initial enzyme activation at 95 °C for 30 s, followed by 40 cycles of reaction profiles: denaturation at 95 °C for 5 s, annealing at 55 °C for 5 s, and extension at 60 °C for 60 s for MITF-H, -D, and -M; denaturation at 95 °C for 5 s, annealing and extension at 60 °C for 60 s for total MITF, tyrosinase, HO-1, HO-2, and 18S rRNA; and denaturation at 95 °C for 5 s, annealing and extension at 62 °C for 60 s for MITF-A. The PCR for MITF isoform mRNAs was performed, based on the reported procedures [32]. The locations of primers for MITF isoforms are schematically shown in Fig. 1A. For normalization, the cDNA for 18S rRNA was also amplified. The primer sets used are shown below: tyrosinase, Fp 5′-TTGGCAGATTGTCTGTAGCC-3′ and Rp 5′-AGGCATTGTGCATGCTGCTT-3′; 18S rRNA, Fp 5′-GATATGCTCATGTGGTGTTG-3′ and Rp 5′-AATC TTCTTCAGTCGCTCCA-3′. HO-1, Fp 5′-GGGGGCCAGGTGCTCAAA AAGATT-3′ and Rp 5′-GGAGCCTGGGAGCGGGTGTTGAGTG-3′; and HO-2, Fp 5′-AGCACACGACCGGGCAGAAAACAC-3′ and Rp 5′-CA AAGGGGCAAAGGCTGGATGGTC-3′.

3. Results and discussion

3.1. Cadmium decreases the expression level of MITF-M in human epidermal melanocytes

The *MITF* gene encodes distinct isoforms that differ in the amino-termini, such as MITF-M, MITF-A and MITF-H (Fig. 1A) [4,5,23]. To explore the cadmium toxicity in melanocytes, we analyzed the effect of cadmium exposure on the expression levels of MITF-M and tyrosinase proteins in NHEMs, in which MITF-M mRNA constitutes about 80% of total MITF mRNAs, as judged by S1-nuclease protection assay [24]. Tyrosinase is the rate-limiting enzyme for melanin biosynthesis and its gene expression is regulated by MITF-M [33]. By Western blot analysis we detected only MITF-M protein in NHEMs, based on its molecular mass (Fig. 1B).

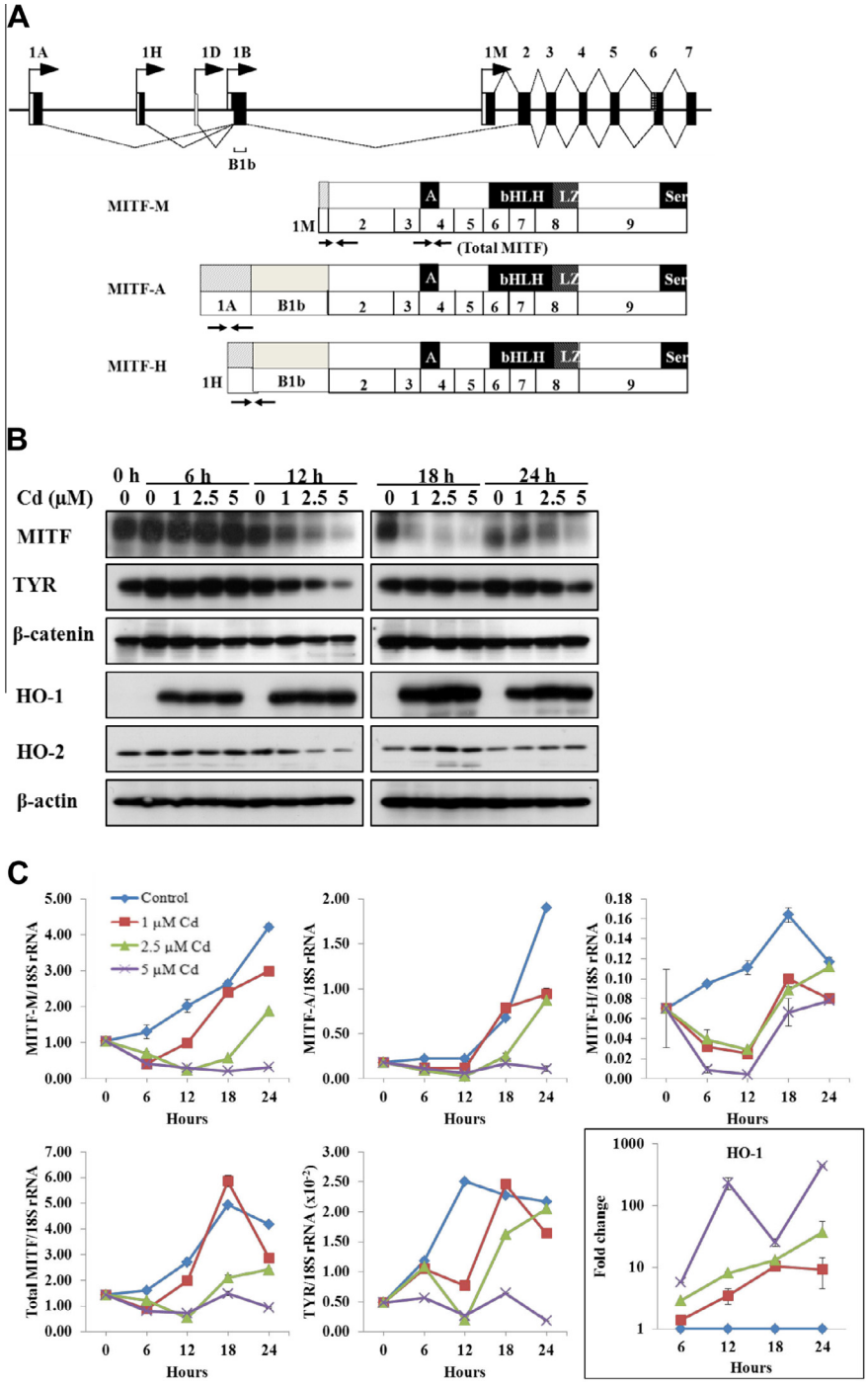

Fig. 1. Decreased expression of MITF-M in human epidermal melanocytes after cadmium exposure. (A) Schematic representation of the MITF gene. Open boxes and closed boxes indicate the 5′- and 3′-untranslated exons and the protein-coding exons, respectively. The promoter region upstream from exon 1 M is the melanocyte-specific promoter [4]. Exon 1 M encodes the amino-terminus of MITF-M. Exon B1b, coding for domain B1b, is used as a second exon for generation of MITF-A and MITF-H mRNAs [5]. Representative MITF isoforms are shown beneath the gene. Small arrows indicate primer sets that were used to amplify the isoform-specific regions. The primer set beneath exon 3 and exon 4 were used for total MITF isoform mRNAs. (A) Activation domain; bHLH-LZ, basic helix-loop-helix leucine zipper structure; and Ser, Ser-rich domain. (B) Western blot analysis of MITF-M and tyrosinase (TYR) proteins. NHEMs were treated with vehicle or cadmium at indicated concentrations for indicated hours. Other proteins are shown as positive or negative control. (C) Real-time RT-PCR analysis of MITF isoform and tyrosinase mRNAs, showing the time-dependent changes in their relative expression levels. Each symbol represents the mean of three normalized values. The data for HO-1 mRNA, shown as inset, represent the fold change that was normalized with the value of NHEMs without cadmium exposure, and are shown as Log scale, because of large magnitudes of induction.

Treatment with cadmium (1, 2.5 or 5 μM) caused a decrease in the expression levels of MITF-M and tyrosinase proteins in NHEMs after 12 h in a dose-dependent manner (Fig. 1B). In particular, exposure to 5 μM cadmium resulted in the long-lasting decrease in the MITF-M and tyrosinase proteins, while both protein levels tended to increase after 24-h exposure to the lower cadmium levels tested (1 or 2.5 μM). In contrast, the expression level of HO-1 protein was increased by 6 h after treatment with cadmium even at 1 μM, while the expression level of HO-2 protein remained unchanged. HO-1 and HO-2 are the rate-limiting enzymes for

heme catabolism, with distinct expression profiles [34]. The induction of HO-1 expression is the consequence of transcriptional activation of the HO-1 gene by cadmium [35]. In this context, the concentration of aqueous humor cadmium in patients with AMD was reported to be about 1 μmol/L (1 μM) [16]. Importantly, there were no noticeable changes in the expression levels of β-catenin and β-actin proteins over the 24-h treatment with cadmium. β-Catenin may also function as a coactivator for MITF in melanocytes [36]. Moreover, the expression level of α-tubulin protein, another loading control, was not noticeably changed (data not shown).

We next measured the relative expression levels of MITF isoform and tyrosinase mRNAs in NHEMs using the quantitative real-time RT-PCR (Fig. 1C). The data at the 0 time indicate that MITF-M mRNA represents a major species of total MITF mRNAs in NHEMs, consistent with our report [24]. The relative abundance of MITF-A and MITF-H mRNAs is about 17.3% and 6.7% of MITF-M mRNA, respectively. Unexpectedly, under the basal conditions (no cadmium), the expression levels of MITF isoform mRNAs were increased during the 24-h incubation (Fig. 1C), although such increases did not noticeably influence their protein levels (Fig. 1B). Likewise, the expression level of tyrosinase mRNA was increased to the maximum level at 12 h and remained at the high levels by 24 h. Treatment with cadmium decreased the expression levels of MITF isoform and tyrosinase mRNAs in a dose-dependent manner (Fig. 1C). However, the levels of MITF-M, MITF-A, MITF-H and tyrosinase mRNAs tended to increase after 12-h treatment with 1 or 2.5 μM cadmium. In particular, the level of MITF-H mRNA was increased even with 5 μM cadmium. In contrast, the expression level of HO-1 mRNA was remarkably increased during treatment with cadmium (Inset in Fig. 1C), while the level of HO-2 mRNA remained unchanged (data not shown). In summary, cadmium exposure decreased the expression levels of MITF-M and MITF-A mRNAs and MITF-M and tyrosinase proteins in NHEMs.

3.2. Cadmium decreases the expression level of MITF-M in human melanoma cells

To further explore the implication of the cadmium-mediated reduction of MITF-M protein in NHEMs, we repeated the similar experiments using HMV-II melanoma cells, in which MITF-M mRNA constitutes about 75% of total MITF isoform mRNAs [23]. In contrast to NHEMs, high-level cadmium (40 μM) did not significantly decrease the viability of HMV-II melanoma cells (data not shown), which may be due to the fact that HMV-II melanoma cells were cultured in the medium containing 10% FBS, unlike NHEMs cultured in the special medium containing 0.5% FBS.

Western blot analysis showed that the expression level of MITF-M protein was decreased in HMV-II melanoma cells after 6-h exposure to cadmium (10, 20 or 40 μM), while the induction of HO-1 expression was apparent (Fig. 2A). In contrast, there were no noticeable changes in the expression levels of HO-2, β-catenin

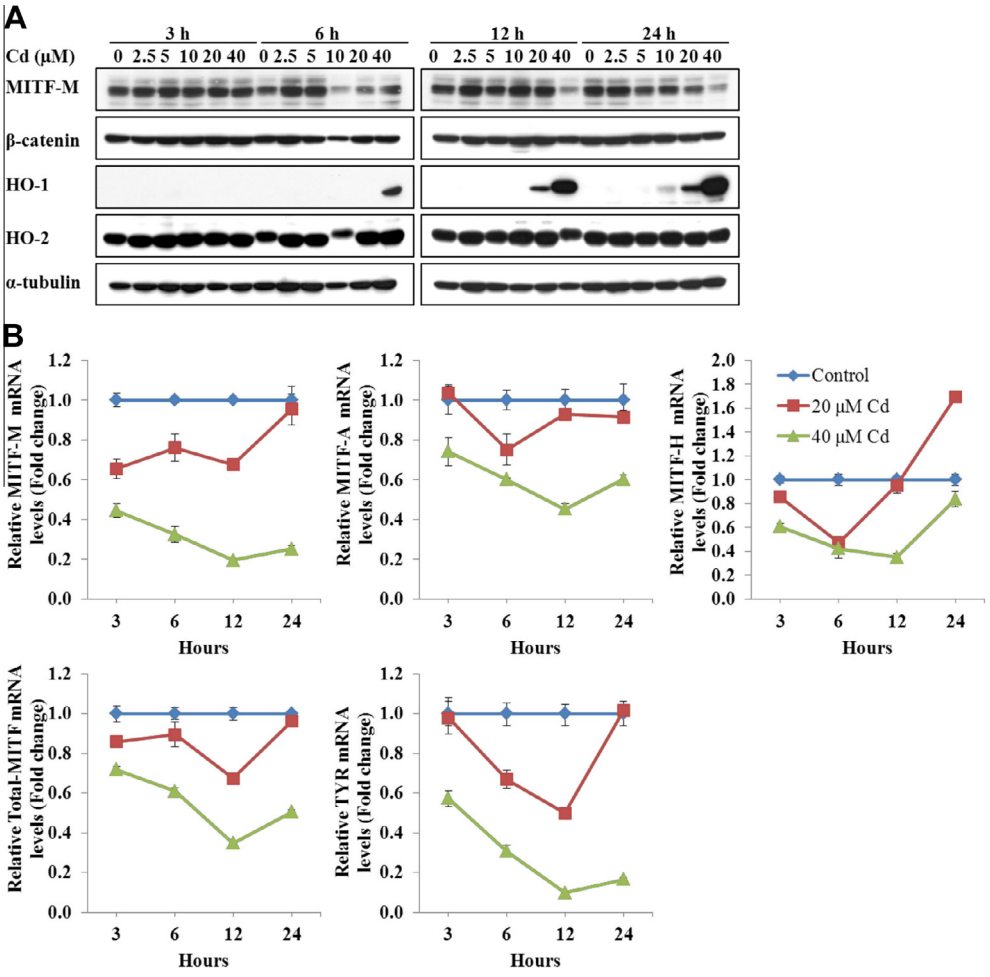

Fig. 2. Decreased expression of MITF-M in human melanoma cells after cadmium exposure. (A) Western blot analysis of MITF-M. HMV-II melanoma cells were treated with vehicle or cadmium at the indicated concentrations and hours. (B) Real-time RT-PCR analysis of MITF isoform and tyrosinase mRNAs. The data represent the fold change in the relative expression level, compared to the level in HMV-II cells exposed to vehicle solution. The data shown are one representative of two independent experiments.

and α-tubulin proteins during the 24-h treatment with cadmium. We were unable to identify the tyrosinase protein with certainty due to the presence of multiple bands around the molecular mass of 60 kDa in melanoma cell extracts (data not shown). The real-time RT-PCR analysis showed that the treatment with 40 μM cadmium reduced the expression levels of MITF-M and tyrosinase mRNAs in a time-dependent manner (Fig. 2B). In this analysis, the data are presented as fold change, because the levels of MITF and tyrosinase mRNAs were only marginally varied in vehicle-treated HMV-II cells. Importantly, the levels of MITF-A and MITF-H mRNAs were similar and about 60-fold lower than the level of MITF-M mRNA. The treatment with 40 μM cadmium decreased the expression levels of MITF-A and MITF-H mRNAs, but the level of MITF-H mRNA returned to the control level by 24 h even with 40 μM cadmium, suggesting that MITF-H is less sensitive to cadmium toxicity. In contrast, the expression level of HO-1 mRNA was increased by more than 50 times and that of HO-2 mRNA remained unchanged (data not shown), as reported previously [26,27]. Thus, cadmium exposure decreased the levels of MITF-M mRNA and protein in HMV-II melanoma cells. Moreover, similar to the NHEMs, cadmium-mediated decrease of MITF-M expression was associated with the decreased tyrosinase mRNA expression. Taken together, we propose that MITF-M is the molecular target of cadmium toxicity in melanocyte-lineage cells.

3.3. Cadmium does not decrease the expression levels of total MITF proteins in RPE cell lines

We next analyzed the effect of cadmium exposure on the expression of MITF protein in ARPE-19 and D407 RPE cells, both of which mainly express MITF-H mRNA [5,32]. In fact, under the PCR conditions employed, the expression level of MITF-H mRNA was higher than the level of MITF-A mRNA by about 3-fold in both ARPE-19 and D407 cells, and the expression levels of MITF-D and MITF-M mRNAs were more than 100-fold lower than the level of MITF-A mRNA (data not shown). Western blot analysis showed that the expression levels of MITF isoform proteins that represent MITF-H and MITF-A varied within narrow ranges in ARPE-19 and D407 cells during the 24-h treatment with cadmium at 10 or 20 μM, compared to the levels in vehicle-treated cells (Fig. 3A). In contrast, cadmium remarkably increased the expression level of HO-1 protein at 6 h in D407 cells, whereas the induction of HO-1 was apparent only after 24 h in ARPE-19 cells. Such a time lag suggests that ARPE-19 cells may be more resistant to cadmium toxicity. Indeed, increased sensitivity to cadmium toxicity has been noted in ARPE-19 cells, when they were exposed to cadmium under low-serum medium conditions [37]. Importantly, cadmium did not noticeably change the levels of HO-2 and β-catenin proteins.

The real-time RT-PCR analysis showed that the expression levels of MITF-H and total MITF mRNAs were maintained within narrow ranges in ARPE-19 and D407 cells after the 24-h treatment with cadmium (Fig. 3B). Cadmium decreased the expression level of MITF-A mRNA in D407 cells, as observed in melanocytes and melanoma cells, but not in ARPE-19 cells. Such a difference also supports that ARPE-19 cells may be more resistant to cadmium toxicity. By contrast, cadmium increased the expression level of HO-1 mRNA in both RPE cell lines after 24-h cadmium exposure (data not shown), as reported previously [26,27].

In summary, cadmium exposure did not decrease the expression level of total MITF proteins in RPE cells, which is different from the reduced expression of MITF-M seen in melanocytes and melanoma cells. MITF isoform multiplicity has been evolved to enable RPE to maintain total MITF protein levels, most likely MITF-H. This may provide RPE survival advantage because they are at increased risk for cadmium toxicity due to cumulative lifetime exposure [15,16,21].

3.4. Implications

We have shown that cadmium causes a decrease in MITF-M expression in melanocytes and melanoma cells. Age-related increase in accumulation of cadmium may lead to the dysfunction or the loss of melanocytes located in the hair follicles, stria vascularis and choroid, thereby contributing to the onset of graying, hearing loss, and/or vision disability. Notably, each of these manifestations commonly seen in elderly might have been overlooked as "normal" outcomes of aging.

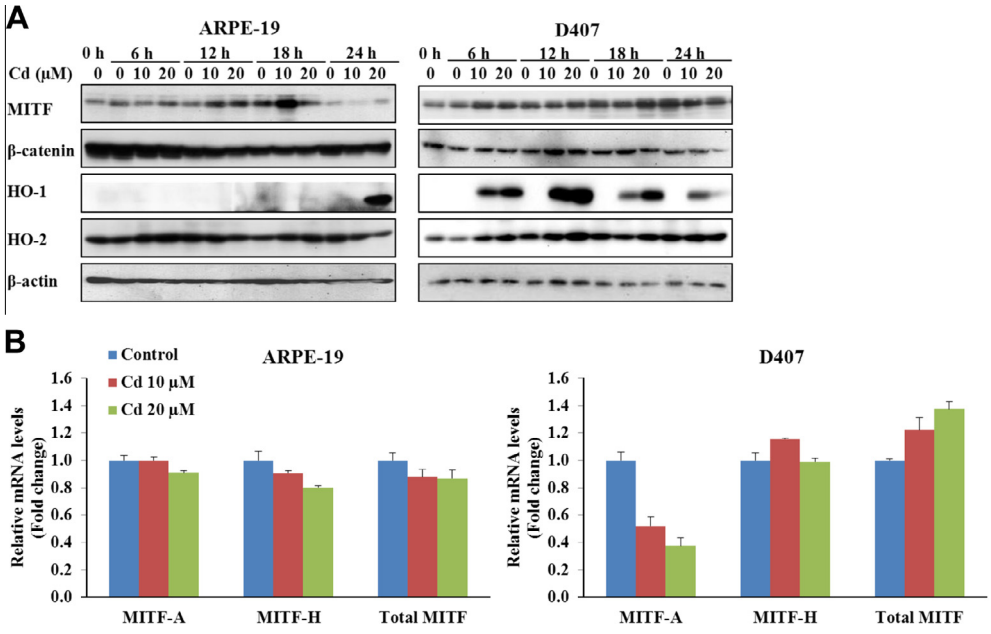

Fig. 3. Effects of cadmium on the expression of MITF in human RPE cells. (A) Western blot analysis of MITF isoform. ARPE-19 or D407 cells were treated with vehicle or cadmium for the indicated hours. (B) Real-time PCR analysis of MITF-H and MITF-A mRNAs. The data are expressed as fold change, compared to the relative expression level in RPE cells exposed to vehicle solution. The data shown are one representative of two independent experiments.

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References

[1] C.A. Hodgkinson, K.J. Moore, A. Nakayama, E. Steingrímsson, 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] I. Yajima, S. Sato, T. Kimura, K. Yasumoto, S. Shibahara, C.R. Goding, H. Yamamoto, An L1 element intronic insertion in the *black-eyed white* (*Mitf*$^{mi\text{-}bw}$) gene: the loss of a single Mitf isoform responsible for the pigmentary defect and inner ear deafness, Hum. Mol. Genet. 8 (1999) 1431–1441.

[4] N. Fuse, K. Yasumoto, H. Suzuki, K. Takahashi, S. Shibahara, Identification of a melanocyte-type promoter of the microphthalmia-associated transcription factor gene, Biochem. Biophys. Res. Commun. 219 (1996) 702–707.

[5] T. Udono, K. Yasumoto, K. Takeda, S. Amae, K. Watanabe, H. Saito, N. Fuse, M. Tachibana, K. Takahashi, M. Tamai, S. Shibahara, Structural organization of the human microphthalmia-associated transcription factor gene containing four alternative promoters, Biochim. Biophys. Acta 1491 (2000) 205–219.

[6] K. Takeda, N.-H. Takahashi, S. Shibahara, Neuroendocrine functions of melanocytes: beyond the skin-deep melanin maker, Tohoku J. Exp. Med. 211 (2007) 201–221.

[7] M. Tassabehji, V.E. Newton, A.P. Read, Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene, Nat. Genet. 8 (1994) 251–255.

[8] K. Takeda, H. Hozumi, K. Nakai, M. Yoshizawa, H. Satoh, H. Yamamoto, S. Shibahara, Insertion of long interspersed element-1 in the Mitf gene is associated with altered neurobehavior of the black-eyed white Mitf$^{mi\text{-}bw}$ mouse, Genes Cells 19 (2014) 126–140.

[9] K. Watanabe, K. Takeda, K. Yasumoto, T. Udono, H. Saito, K. Ikeda, T. Takasaka, K. Takahashi, T. Kobayashi, M. Tachibana, S. Shibahara, Identification of a distal enhancer for the melanocyte-specific promoter of the MITF gene, Pigment Cell Res. 15 (2012) 201–211.

[10] S. Uehara, Y. Izumi, Y. Kubo, C.C. Wang, K. Mineta, K. Ikeo, T. Gojobori, M. Tachibana, T. Kikuchi, T. Kobayashi, S. Shibahara, C. Taya, H. Yonekawa, T. Shiroishi, H. Yamamoto, Specific expression of *Gsta4* in mouse cochlear melanocytes: a novel role for hearing and melanocyte differentiation, Pigment Cell Melanoma Res. 22 (2009) 111–119.

[11] L. Bhutto, G. Lutty, Understanding age-related macular degeneration (AMD): relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex, Mol. Aspects Med. 33 (2012) 295–317.

[12] S. Satarug, S.H. Garrett, M.A. Sens, D.A. Sens, Cadmium, environmental exposure, and health outcomes, Environ. Health Perspect. 118 (2010) 182–190.

[13] S. Satarug, M.R. Moore, Emerging roles of cadmium and heme oxygenase in type-2 diabetes and cancer susceptibility, Tohoku J. Exp. Med. 228 (2012) 267–288.

[14] J.C. Erie, J.A. Good, J.A. Butz, D.O. Hodge, J.S. Pulido, Urinary cadmium and age-related macular degeneration, Am. J. Ophthalmol. 144 (2007) 414–418.

[15] N.K. Wills, N. Kalariya, V.M. Sadagopa Ramanujam, J.R. Lewis, S. Haji Abdollahi, A. Husain, F.J.G.M. van Kuijk, Human retinal cadmium accumulation as a factor in the etiology of age-related macular degeneration, Exp. Eye Res. 89 (2009) 79–87.

[16] A.G.M. Jünemann, P. Stopa, B. Michalke, A. Chaudhri, U. Reulbach, C. Huchzermeyer, U. Schlötzer-Schrehardt, F.E. Kruse, E. Zrenner, R. Rejdak, Levels of aqueous humor trace elements in patients with non-exsudative age-related macular degeneration: a case-control study, PLoS One 8 (2) (2013) e56734.

[17] J. Shargorodsky, S.G. Curhan, E. Henderson, R. Eavey, G.C. Curhan, Heavy metals exposure and hearing loss in U.S. adolescents, Arch. Otolaryngol. Head Neck Surg. 137 (2011) 1183–1189.

[18] Y.-H. Choi, H. Hu, B. Mukherjee, J. Miller, S.K. Park, Environmental cadmium and lead exposures and hearing loss in U.S. adults: the National Health and Nutrition Examination Survey, 1999 to 2004, Environ. Health Perspect. 120 (2012) 1544–1550.

[19] S. Satarug, Long-term exposure to cadmium in food and cigarette smoke, liver effects and hepatocellular carcinoma, Curr. Drug Metab. 13 (2012) 257–271.

[20] J.C. Erie, J.A. Butz, J.A. Good, E.A. Erie, M.F. Burritt, J.D. Cameron, Heavy metal concentrations in human eyes, Am. J. Ophthalmol. 139 (2005) 888–893.

[21] N.K. Wills, V.M. Sadagopa Ramanujam, J. Chang, N. Kalariya, J.R. Lewis, T.-X. Weng, F.J.G.M. van Kuijk, Cadmium accumulation in the human retina: effects of age, gender, and cellular toxicity, Exp. Eye Res. 86 (2008) 41–51.

[22] H.I. Afridi, T.G. Kazi, F.N. Talpur, A. Kazi, S.S. Arain, S.A. Arain, K.D. Brahman, A.H. Panhwar, Naeemullah, M. Shezadi, J. Ali, Interaction between essential elements selenium and zinc with cadmium and mercury in samples from hypertensive patients, Biol. Trace Elem. Res. 160 (2014) 185–196.

[23] S. Amae, N. Fuse, K. Yasumoto, S. Sato, I. Yajima, H. Yamamoto, T. Udono, Y.K. Durlu, M. Tamai, K. Takahashi, S. Shibahara, Identification of a novel isoform of microphthalmia-associated transcription factor that is enriched in retinal pigment epithelium, Biochem. Biophys. Res. Commun. 247 (1998) 710–715.

[24] K. Yasumoto, S. Amae, T. Udono, N. Fuse, K. Takeda, S. Shibahara, A big gene linked to small eyes encodes multiple Mitf isoforms: many promoters make light work, Pigment Cell Res. 11 (1998) 329–336.

[25] K. Takeda, K. Yasumoto, N. Kawaguchi, T. Udono, K. Watanabe, H. Saito, K. Takahashi, M. Noda, S. Shibahara, Mitf-D, a newly identified isoform, expressed in the retinal pigment epithelium and monocyte-lineage cells affected by Mitf mutations, Biochim. Biophys. Acta 1574 (2002) 15–23.

[26] S. Satarug, R. Wisedpanichkij, K. Takeda, B. Li, K. Na-Bangchang, M.R. Moore, S. Shibahara, Prostaglandin D_2 induces heme oxygenase-1 mRNA expression through the DP2 receptor, Biochem. Biophys. Res. Commun. 377 (2008) 878–883.

[27] S. Satarug, M. Kikuchi, R. Wisedpanichkij, B. Li, K. Takeda, K. Na-Bangchang, M.R. Moore, K. Hirayama, S. Shibahara, Prevention of cadmium accumulation in retinal pigment epithelium with manganese and zinc, Exp. Eye Res. 87 (2008) 587–593.

[28] T. Nakamura, S. Seki, O. Matsubara, S. Ito, T. Kasuga, Specific incorporation of 4-S-cysteinylphenol into human melanoma cells, J. Invest. Dermatol. 90 (1988) 725–728.

[29] K.C. Dunn, A.E. Aotaki-Keen, F.R. Putkey, L.M. Hjelmeland, ARPE-19, a human retinal pigment epithelial cell line with differentiated properties, Exp. Eye Res. 62 (1996) 155–169.

[30] A.A. Davis, P.S. Bernstein, D. Bok, J. Turner, M. Nachtigal, R.C. Hunt, A human retinal pigment epithelial cell line that retains epithelial characteristics after prolonged culture, Invest. Ophthalmol. Vis. Sci. 36 (1995) 955–964.

[31] B. Li, K. Takeda, S. Yokoyama, S. Shibahara, A prolyl-hydroxylase inhibitor, ethyl-3,4-dihydroxybenzoate, induces heme oxygenase-1 expression in human cells through a mechanism independent of hypoxia-inducible factor-1α, J. Biochem. 144 (2008) 643–654.

[32] J. Maruotti, T. Thein, D.J. Zack, N. Esumi, MITF-M, a 'melanocyte-specific' isoform, is expressed in the adult retinal pigment epithelium, Pigment Cell Melanoma Res. 25 (2012) 641–644.

[33] K. Yasumoto, K. Yokoyama, K. Shibata, Y. Tomita, S. Shibahara, Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene, Mol. Cell. Biol. 14 (1994) 8058–8070.

[34] S. Shibahara, The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism, Tohoku J. Exp. Med. 200 (2003) 167–186.

[35] K. Takeda, S. Ishizawa, M. Sato, T. Yoshida, S. Shibahara, Identification of a cis-acting element that is responsible for cadmium-mediated induction of the human heme oxygenase gene, J. Biol. Chem. 269 (1994) 22858–22867.

[36] A. Schepsky, K. Bruser, G.J. Gunnarsson, J. Goodall, J.H. Hallsson, C.R. Goding, E. Steingrimsson, A. Hecht, The microphthalmia-associated transcription factor Mitf interacts with beta-catenin to determine target gene expression, Mol. Cell. Biol. 26 (2006) 8914–8927.

[37] N.M. Kalariya, N.K. Wills, K.V. Ramana, S.K. Srivastava, F.J. van Kuijk, Cadmium-induced apoptotic death of human retinal pigment epithelial cells is mediated by MAPK pathway, Exp. Eye Res. 89 (2009) 494–502.