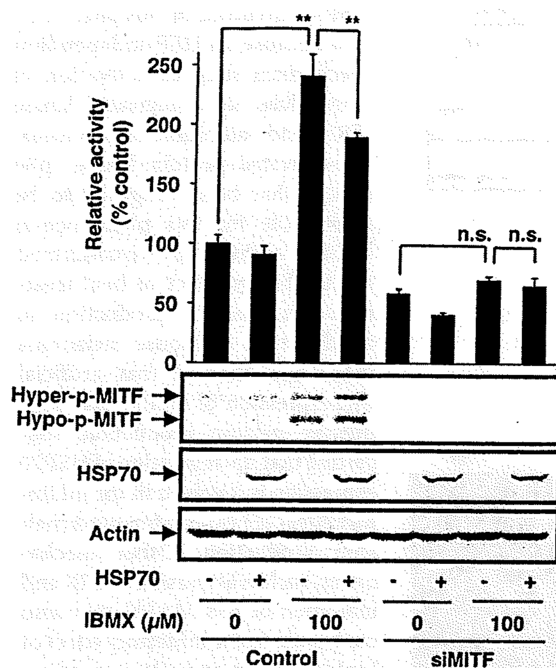

FIGURE 4. **Effect of siRNA for MITF on the IBMX-stimulated promoter activity of the tyrosinase gene.** HSP70-overexpressing B16 cells (*HSP70* +) or mock transfectant control cells (*HSP70* −) were transiently transfected with not only pRL-SV40 and pGL4-tyrosinase-luc but also with siRNA for MITF (*siMITF*) or non-silencing siRNA (*Control*). After 24 h, the cells were incubated for 24 h with or without 100 μM IBMX. Luciferase activity was measured and expressed as described in the legend of Fig. 2. Values are given as the mean ± S.D. ($n = 3$). **, $p < 0.01$; *n.s.*, not significant. Whole cell extracts were analyzed by immunoblotting as described in the legend of Fig. 3.

with MITF to modulate its effect on transcription of the tyrosinase gene. At first, we examined the physical interaction between HSP70 and MITF by a co-immunoprecipitation assay; we immunoprecipitated HSP70 and looked for the presence of MITF. Efficient precipitation of HSP70 was observed in a manner that was dependent on both the overexpression of HSP70 (Fig. 5*A*) and the specific antibody (data not shown). MITF was co-immunoprecipitated, and this co-immunoprecipitation was stimulated by overexpression of HSP70 (Fig. 5*A*). We also performed a reciprocal co-immunoprecipitation assay; we immunoprecipitated MITF and looked for the presence of HSP70. Efficient precipitation of MITF was observed in a manner that was dependent on the specific antibody (data not shown), and HSP70 was co-immunoprecipitated (Fig. 5*A*). These results suggest that HSP70 can physically interact with MITF.

We also examined the interaction using purified proteins. After incubation of purified glutathione *S*-transferase (GST)-fused MITF and HSP70, we precipitated GST-MITF and looked for the presence of HSP70. As shown in supplemental Fig. S3, precipitation of HSP70 was observed with full-length GST-MITF (GST-MITF-1) but not with GST alone, suggesting the direct interaction between MITF and HSP70. We also constructed a series of deletion mutants of GST-MITF to identify the domain of MITF responsible for its interaction with HSP70. Deletion of the N-terminal region of MITF (1–99 amino acid residues) diminished the interaction with HSP70, and the N-terminal fragment of MITF (1–99 amino acid residues) interacted with HSP70, suggesting that this region is responsible for the interaction (supplemental Fig. S3). AQ: G ZSI

We then tested the co-localization of HSP70 and MITF by immunoblotting and immunostaining assays. As shown in Fig. 5*B*, MITF was detected in the nuclear extract irrespective of whether HSP70 was overexpressed, and HSP70 was also detected in the nuclear extract from cells overexpressing HSP70 but not in extract from control cells. These observations were confirmed by an immunostaining assay; MITF localized in the nucleus irrespective of the overexpression of HSP70, and HSP70 localized in the nucleus in a manner that was dependent on its overexpression (Fig. 5*C*). As a result, co-localization of HSP70 and MITF in the nucleus was observed in HSP70-overexpressing cells (see the *Merge panel* in Fig. 5*C*).

We then examined the effect of HSP70 overexpression on the specific binding of MITF to the promoter of the tyrosinase gene by chromatin immunoprecipitation assay. As shown in Fig. 6, F6 DNA fragments containing the promoter of tyrosinase gene were precipitated with antibody against MITF more efficiently than control DNA fragments, suggesting that MITF specifically binds to the promoter of the tyrosinase gene in cells. We also found that this binding was stimulated by treatment of cells with IBMX, and HSP70 overexpression significantly inhibited this binding (Fig. 6). Results in Fig. 6 also suggest that HSP70 does not bind to the promoter of the tyrosinase gene so apparently in cells.

Finally, we examined the effect of HSP70 on the transcription of the tyrosinase gene in nuclear extract. DNA fragments containing the promoter region of the tyrosinase or *grp78* (control) were incubated with nuclear extract to promote the transcription, and transcripts were detected by autoradiography after separation on polyacrylamide gel electrophoresis. The band with the expected size was detected depending on the template DNA and nuclear extract (data not shown), showing that the AQ: H band corresponds to the transcript of tyrosinase or *grp78*. The intensity of bands corresponding to the transcript of tyrosinase but not that of *grp78* increased by treatment of cells with IBMX (Fig. 7*A*). As shown in Fig. 7*A*, the intensity F7 of band corresponding to the transcript of tyrosinase was lower with extracts prepared from HSP70-overexpressing cells treated with IBMX than those from control cells treated with IBMX. Such effect was not observed for the band corresponding to the transcript of *grp78* (Fig. 7*A*). Furthermore, the addition of purified HSP70 to extract prepared from control cells decrease the intensity of band corresponding to the transcript of tyrosinase but not of *grp78* (Fig. 7*B*). These results suggest that HSP70 directly suppresses the transcription of the tyrosinase.

Effect of Expression of HSP70 on UVB-induced Melanin Production in Vivo—Finally, we tested the *in vivo* relevance of our *in vitro* results using transgenic mice expressing HSP70. The transgenic mice and wild-type mice were exposed to UVB irradiation for 8 days, and the melanin content was estimated by Fontana-Masson staining of sections or by a narrow-band simple reflectance meter (Mexameter). For this we used the tail skin because murine tail skin resembles human skin, as epidermal melanocytes are present and UV-dependent melanin production has been observed (52). We confirmed the overexpression of HSP70 in the skin of the transgenic mice by an

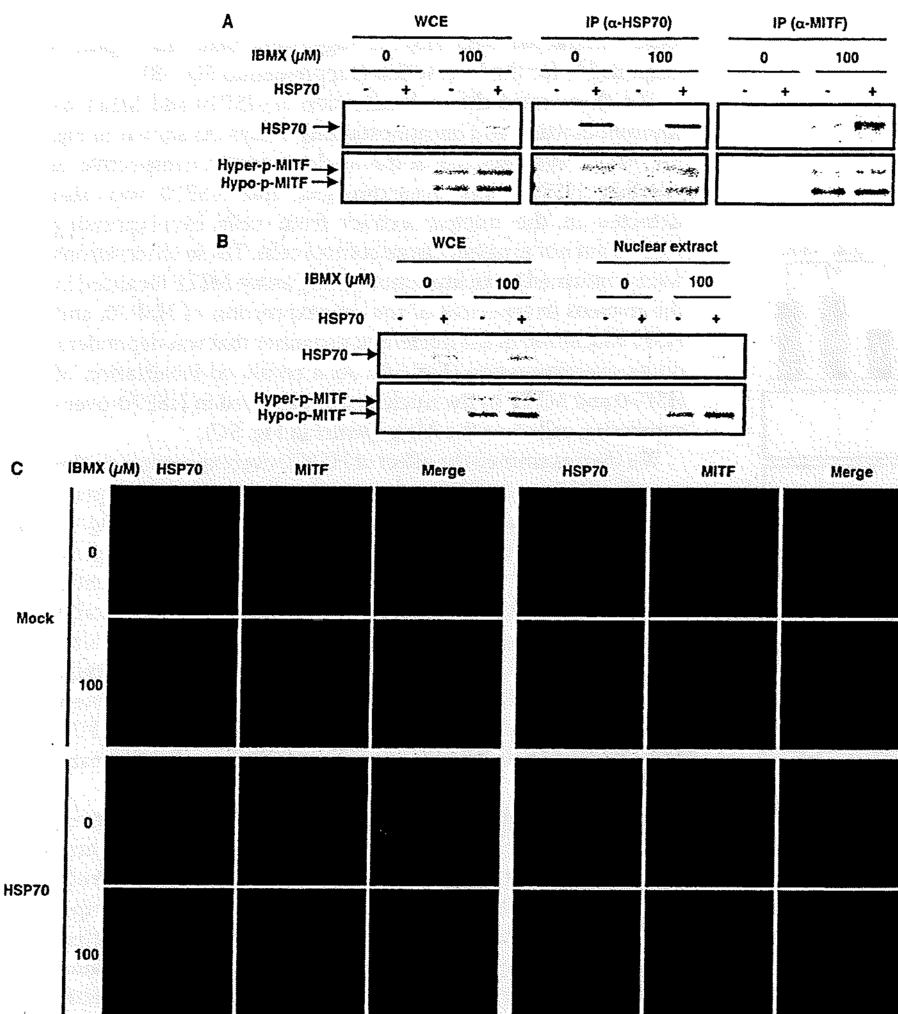

FIGURE 5. **Physical interaction between MITF and HSP70 and their intracellular co-localization.** HSP70-overexpressing B16 cells (*HSP70* +) or mock transfectant control cells (*HSP70* −) were incubated for 3 h with or without 100 μM IBMX (*A–C*), and whole cell extracts (*WCE*) were prepared (*A* and *B*). WCE were immunoprecipitated with antibodies against HSP70 (*IP (α-HSP70)*) or those against MITF (*IP (α-MITF)*) (*A*), or nuclear extracts were prepared from whole cell extracts (*B*). Each fraction was analyzed by immunoblotting as described in the legend of Fig. 1 (*A* and *B*). After fixation, samples were incubated with antibody against HSP70 or MITF. After incubation with the respective secondary antibody, cells were inspected using fluorescence microscopy. The *left three panels* are the magnified image of the *right three panels*. *Scale bar*, 20 μm (*C*).

COLOR

immunoblotting assay (data not shown). As shown in Fig. 8*A*, an increase in melanin staining at the basal layer of the epidermis (the dermal/epidermal border) was observed in the wild-type mice after UVB irradiation, but this increase was not so obvious in sections prepared from the transgenic mice expressing HSP70. Measurement of melanin content by a Mexameter also showed that the melanin content was lower in the UVB-treated transgenic mice expressing HSP70 than in the wild-type controls (Fig. 8*B*). We also found that heat treatment of tail skin caused overexpression of HSP70 and a lower level of melanin content after irradiation with UVB (supplemental Fig. S4).

DISCUSSION

It has been reported that heat treatment of mouse melanoma cells (Mel-Ab) suppresses melanin production; however, it is not clear whether up-regulation of expression of HSPs is involved in this phenomenon because an HSP-independent mechanism such as activation of extracellular signal-regulated kinase (ERK) and inhibition of p38 mitogen-activated protein kinase (p38 MAPK) has been proposed to be responsible for this phenomenon (35, 36). In this study we confirmed the inhibitory effect of heat treatment on melanin production in another type of mouse melanoma (B16) and showed that artificial overexpression of HSP70 also suppresses melanin production, suggesting that up-regulation of HSP70 expression is involved in the inhibitory effect of heat treatment on melanin production. Other mechanisms, such as activation of ERK and inhibition of p38 MAPK, may also contribute to the inhibitory effect of heat treatment on melanin production (35, 36). Furthermore, a decrease in the level of MITF after heat treatment (supplemental Fig. S2, *E* and *F*) should be involved in the inhibitory effect of heat treatment on melanin production.

ZS1

Because HSPs are known to affect the intracellular traffic of vesicles, it is possible that expression of HSP70 affects the intracellular traffic of melanosomes, resulting in alterations to the amount of melanin in the culture medium. However, as well as heat treatment, artificial overexpression of HSP70 decreased the amount of melanin not only in the culture medium but also in cell extracts. This suggests that the decrease in the amount of melanin in the culture medium cannot be simply explained by the alteration of intracellular traffic of melanosomes and that synthesis of melanin by melanosomes is suppressed by overexpression of HSP70. In fact, we found that the activity and expression of tyrosinase (a rate-limiting enzyme in the synthesis of melanin) are suppressed in cells overexpressing HSP70. It is well known that the activity of tyrosinase is regulated mainly at the level of transcription, and we found by real-time RT-PCR analysis and luciferase reporter assay that the transcriptional activity of the tyrosinase gene is suppressed in cells overexpressing HSP70. Furthermore, in cells transfected with siRNA specific for MITF (a key transcription factor regulating the transcription of the tyrosinase gene), overexpression of HSP70 did not affect the promoter activity of the tyrosinase gene, suggesting that MITF plays an important role in HSP70-dependent regulation of the transcription of the tyrosinase gene.

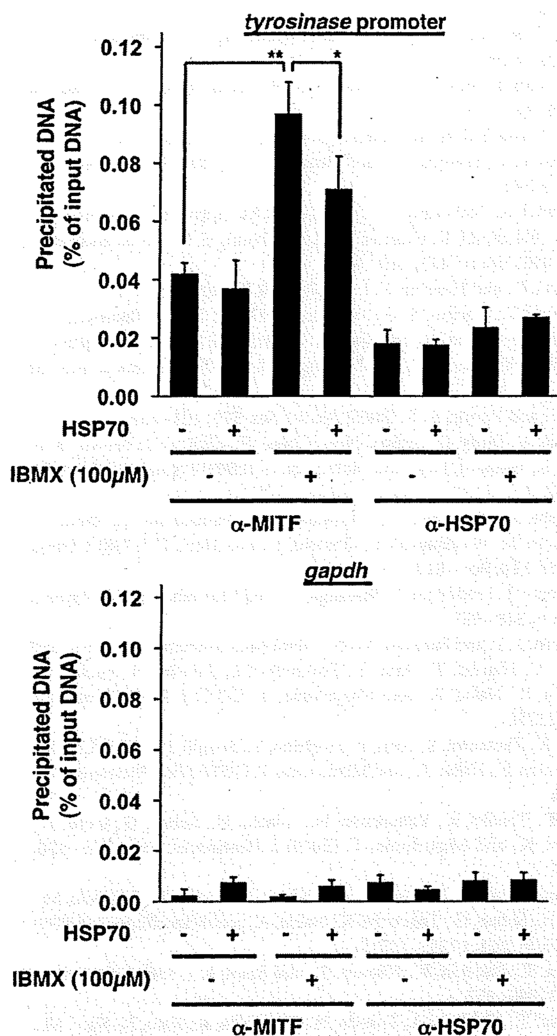

FIGURE 6. **The binding of MITF to the promoter of the tyrosinase gene in cells.** HSP70-overexpressing B16 cells (*HSP70* +) and mock transfectant control cells (*HSP70* −) were incubated for 24 h with or without 100 μM IBMX. Whole cell extracts were immunoprecipitated with an antibody against MITF or HSP70. DNA fractions were prepared from the immunoprecipitated samples, and whole cell extracts and subjected to real time RT-PCR with specific primer sets for the tyrosinase promoter (*upper panel*) and *gapdh* gene (*lower panel*). Values are expressed relative to the input DNA (whole cell extracts) and are given as mean ± S.D. ($n = 3$). **, $p < 0.01$; *, $p < 0.05$.

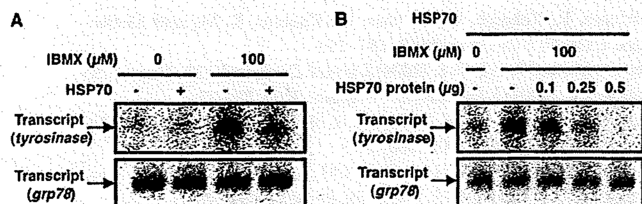

FIGURE 7. **Effect of HSP70 on the transcription of the tyrosinase gene in nuclear extract.** HSP70-overepressing B16 cells (*HSP70* +) or mock transfectant control cells (*HSP70* −) were incubated for 3 h with or without 100 μM IBMX. Nuclear extracts were prepared and incubated with DNA fragments containing the tyrosinase or *grp78* promoter region in the presence (*B*) or absence (*A*) of indicated amounts of purified HSP70 (*B*). Isolated RNAs were electrophoresed on a 15% polyacrylamide gel and autoradiographed.

The pathway of activation of adenylate cyclase followed by an increase in the intracellular cAMP level and activation of protein kinase A/cAMP response element-binding protein has been proposed to play an important role in the UV-induced expression of tyrosinase mainly through induction of MITF expression (7). In this study we have confirmed that IBMX up-regulates the expression of MITF. However, surprisingly, overexpression of HSP70 did not affect this up-regulation. Thus, we propose an alternative mechanism for the inhibitory effect of HSP70 on expression of the tyrosinase gene; HSP70 directly binds to MITF in the nucleus and inhibits its specific binding to the promoter of the tyrosinase gene. This is based on the following results; HSP70 can physically interact with MITF (co-immunoprecipitation assay), overexpressed HSP70 co-localized with MITF in the nucleus (co-immunostaining assay), overexpression of HSP70 inhibited the specific binding of MITF to the promoter of the tyrosinase gene (chromatin immunoprecipitation assay), and HSP70 inhibited the transcription of the tyrosinase gene in nuclear extract.

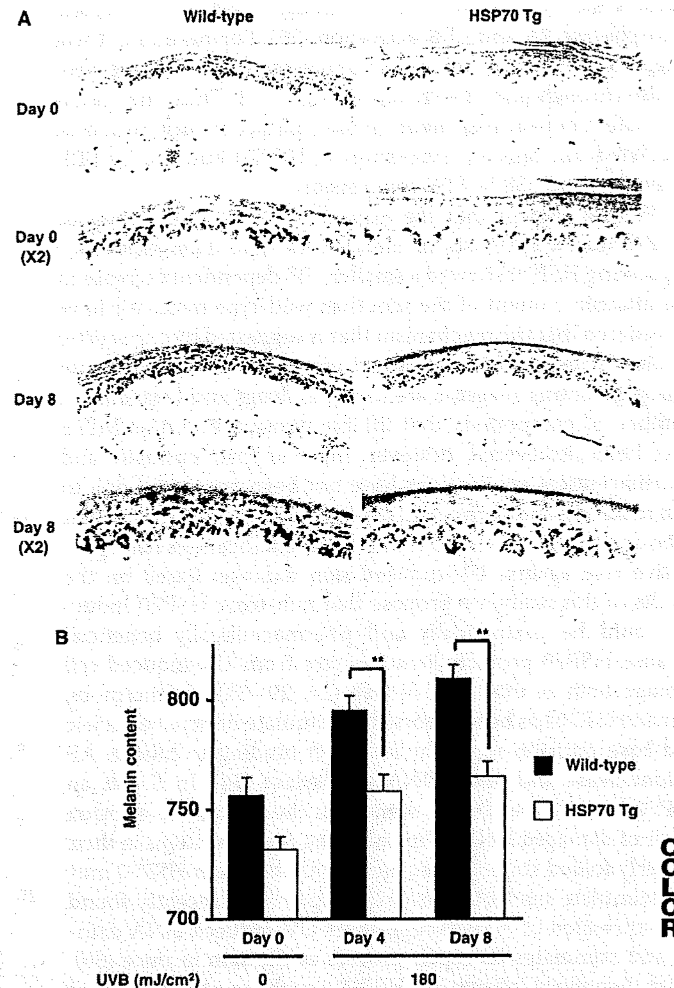

FIGURE 8. **Effect of overexpression of HSP70 on UVB-induced melanin production in the skin *in vivo*.** Transgenic mice expressing HSP70 (*HSP70 Tg*) and wild-type mice (C57/BL6) were irradiated with 180 mJ/cm² UVB once per 2 days for 8 days (totally 4 times) (*A* and *B*). Sections were prepared from the tail skin and subjected to Fontana-Masson staining (*A*). The amount of melanin in the tail skin was measured as described under "Experimental Procedures." Values are given as the mean ± S.E. ($n = 17$). **, $p < 0.01$ (*B*).

On the other hand, it has been reported that heat treatment suppresses the promoter activity of the *mitf* gene and sup-

presses the expression of MITF through inhibition of protein phosphatase 2A and ERK activation (36). Furthermore, it was suggested that heat treatment suppresses the activity of tyrosinase through p38 MAPK suppression (35). Thus, the inhibitory effect of heat treatment on melanin production would be mediated not only by expression of HSP70 but also by ERK activation and p38 MAPK suppression.

We also suggest that the expression of HSP70 suppresses UVB-induced synthesis of melanin *in vivo*; transgenic mice expressing HSP70 showed a smaller UV-dependent increase in the melanin content of the skin than wild-type mice. We have considered that the mechanism that is suggested by our *in vitro* studies is responsible for this *in vivo* phenomenon. Because hypopigmenting reagents are useful as drugs and cosmetics, a number of compounds that inhibit tyrosinase and/or MITF have been discovered; however, most of their cosmetic and pharmaceutical applications have not been successful due to skin irritation and permanent depigmentation (53). This seems to be due to the fact that UV-induced melanogenesis has a protective role against UV-induced skin damage. Based on the results of this study, we propose that non-toxic HSP70-inducers could be cosmetically and pharmaceutically beneficial because HSP70 protects keratinocytes from UV-induced cell damage both *in vitro* and *in vivo* (24, 29–33). Furthermore, human HSP70 has been reported to stimulate deoxyribonucleic acid base excision repair by its direct binding to human AP endonuclease and uracil DNA glycosylase (54). In *E. coli*, an HSP70 homologue (DnaK) stimulates the nucleotide excision repair of damaged DNA by maintaining repair proteins in their properly folded state (55), suggesting that human HSP70 may also stimulate nucleotide excision repair. We recently found that expression of HSP70 suppresses UV-induced DNA damage and stimulates its repair process at the skin in mice (56). Other beneficial effects of HSP70, such as stimulation of wound-healing at the skin and anti-aging activity have also been suggested (57–60). Furthermore, because recent reports suggest that MITF is an oncogene and its activation is involved in the progression of melanoma (61), the inhibitory effect of HSP70 on MITF would be beneficial for the prevention of UV-induced melanoma. We have already screened for non-toxic HSP70 inducers from natural products and found that some of their HSP-inducing activities were more potent than geranylgeranylacetone (62). We hope to develop some of these as whitening cosmetics or drugs for melanin-related diseases.

Acknowledgments—We thank Drs. C. E. Angelidis and G. N. Pagoulatos (University of Ioannina) or Dr. M. Funaba M (Kyoto University) for generously providing transgenic mice expressing HSP70 or a plasmids, respectively.

REFERENCES

1. Rabe, J. H., Mamelak, A. J., McElgunn, P. J., Morison, W. L., and Sauder, D. N. (2006) *J. Am. Acad. Dermatol.* 55, 1-19
2. Svobodova, A., Walterova, D., and Vostalova, J. (2006) *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech. Repub.* 150, 25–38
3. McMillan, T. J., Leatherman, E., Ridley, A., Shorrocks, J., Tobi, S. E., and Whiteside, J. R. (2008) *J. Pharm. Pharmacol.* 60, 969-976
4. Shorrocks, J., Paul, N. D., and McMillan, T. J. (2008) *J. Invest Dermatol.* 128, 685-693
5. Douki, T., Reynaud-Angelin, A., Cadet, J., and Sage, E. (2003) *Biochemistry* 42, 9221–9226
6. Matsumura, Y., and Ananthaswamy, H. N. (2004) *Toxicol. Appl. Pharmacol.* 195, 298-308
7. Buscà, R., and Ballotti, R. (2000) *Pigment Cell Res.* 13, 60-69
8. Yamaguchi, Y., Brenner, M., and Hearing, V. J. (2007) *J. Biol. Chem.* 282, 27557–27561
9. D'Orazio, J. A., Nobuhisa, T., Cui, R., Arya, M., Spry, M., Wakamatsu, K., Igras, V., Kunisada, T., Granter, S. R., Nishimura, E. K., Ito, S., and Fisher, D. E. (2006) *Nature* 443, 340-344
10. Costin, G. E., and Hearing, V. J. (2007) *FASEB J.* 21, 976–994
11. Miyamura, Y., Coelho, S. G., Wolber, R., Miller, S. A., Wakamatsu, K., Zmudzka, B. Z., Ito, S., Smuda, C., Passeron, T., Choi, W., Batzer, J., Yamaguchi, Y., Beer, J. Z., and Hearing, V. J. (2007) *Pigment Cell Res.* 20, 2-13
12. Agar, N., and Young, A. R. (2005) *Mutat. Res.* 571, 121-132
13. Sturm, R. A., Duffy, D. L., Box, N. F., Chen, W., Smit, D. J., Brown, D. L., Stow, J. L., Leonard, J. H., and Martin, N. G. (2003) *Pigment Cell Res.* 16, 266–272
14. Kobayashi, N., Nakagawa, A., Muramatsu, T., Yamashina, Y., Shirai, T., Hashimoto, M. W., Ishigaki, Y., Ohnishi, T., and Mori, T. (1998) *J. Invest. Dermatol.* 110, 806–810
15. Bustamante, J., Bredeston, L., Malanga, G., and Mordoh, J. (1993) *Pigment Cell Res.* 6, 348-353
16. Morimoto, R. I., and Santoro, M. G. (1998) *Nat. Biotechnol.* 16, 833–838
17. Tanaka, K., Namba, T., Arai, Y., Fujimoto, M., Adachi, H., Sobue, G., Takeuchi, K., Nakai, A., and Mizushima, T. (2007) *J. Biol. Chem.* 282, 23240–23252
18. Tanaka, K., Tsutsumi, S., Arai, Y., Hoshino, T., Suzuki, K., Takaki, E., Ito, T., Takeuchi, K., Nakai, A., and Mizushima, T. (2007) *Mol. Pharmacol.* 71, 985–993
19. Asano, T., Tanaka, K., Yamakawa, N., Adachi, H., Sobue, G., Goto, H., Takeuchi, K., and Mizushima, T. (2009) *J. Pharmacol. Exp. Ther.* 330, 458–467
20. Suemasu, S., Tanaka, K., Namba, T., Ishihara, T., Katsu, T., Fujimoto, M., Adachi, H., Sobue, G., Takeuchi, K., Nakai, A., and Mizushima, T. (2009) *J. Biol. Chem.* 284, 19705–19715
21. Hirakawa, T., Rokutan, K., Nikawa, T., and Kishi, K. (1996) *Gastroenterology* 111, 345–357
22. Ohkawara, T., Nishihira, J., Takeda, H., Miyashita, K., Kato, K., Kato, M., Sugiyama, T., and Asaka, M. (2005) *Scand. J. Gastroenterol.* 40, 1049–1057
23. Ohkawara, T., Nishihira, J., Takeda, H., Katsurada, T., Kato, K., Yoshiki, T., Sugiyama, T., and Asaka, M. (2006) *Int. J. Mol. Med.* 17, 229-234
24. Wilson, N., McArdle, A., Guerin, D., Tasker, H., Wareing, P., Foster, C. S., Jackson, M. J., and Rhodes, L. E. (2000) *J. Cutan. Pathol.* 27, 176-182
25. Morris, S. D. (2002) *Clin. Exp. Dermatol.* 27, 220–224
26. Jonak, C., Klosner, G., and Trautinger, F. (2006) *Int. J. Cosmet. Sci.* 28, 233-241
27. Trautinger, F., Kokesch, C., Klosner, G., Knobler, R. M., and Kindas-Mügge, I. (1999) *Exp. Dermatol.* 8, 187-192
28. Allanson, M., and Reeve, V. E. (2004) *J. Invest. Dermatol.* 122, 1030-1036
29. Trautinger, F. (2001) *J. Photochem. Photobiol. B* 63, 70–77
30. Trautinger, F., Kindås-Mügge, I., Barlan, B., Neuner, P., and Knobler, R. M. (1995) *J. Invest. Dermatol.* 105, 160-162
31. Park, K. C., Kim, D. S., Choi, H. O., Kim, K. H., Chung, J. H., Eun, H. C., Lee, J. S., and Seo, J. S. (2000) *Arch. Dermatol. Res.* 292, 482–487
32. Maytin, E. V., Wimberly, J. M., and Kane, K. S. (1994) *J. Invest. Dermatol.* 103, 547-553
33. Kwon, S. B., Young, C., Kim, D. S., Choi, H. O., Kim, K. H., Chung, J. H., Eun, H. C., Park, K. C., Oh, C. K., and Seo, J. S. (2002) *J. Dermatol. Sci.* 28, 144–151
34. Trautinger, F., Knobler, R. M., Hönigsmann, H., Mayr, W., and Kindås-Mügge, I. (1996) *J. Invest. Dermatol.* 107, 442–443
35. Kim, D. S., Park, S. H., Kwon, S. B., Na, J. I., Huh, C. H., and Park, K. C. (2007) *Arch. Pharm. Res.* 30, 581-586
36. Kim, D. S., Park, S. H., Kwon, S. B., Youn, S. W., Park, E. S., and Park, K. C.

(2005) *Cell. Signal.* **17,** 1023–1031
37. Fujimoto, M., Takaki, E., Hayashi, T., Kitaura, Y., Tanaka, Y., Inouye, S., and Nakai, A. (2005) *J. Biol. Chem.* **280,** 34908–34916
38. Hoshino, T., Tsutsumi, S., Tomisato, W., Hwang, H. J., Tsuchiya, T., and Mizushima, T. (2003) *J. Biol. Chem.* **278,** 12752–12758
39. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
40. Kim, K. S., Kim, J. A., Eom, S. Y., Lee, S. H., Min, K. R., and Kim, Y. (2006) *Pigment Cell Res.* **19,** 90–98
41. Lei, T. C., Virador, V. M., Vieira, W. D., and Hearing, V. J. (2002) *Anal. Biochem.* **305,** 260–268
42. Yang, J. Y., Koo, J. H., Song, Y. G., Kwon, K. B., Lee, J. H., Sohn, H. S., Park, B. H., Jhee, E. C., and Park, J. W. (2006) *Acta Pharmacol. Sin.* **27,** 1467–1473
43. Mima, S., Tsutsumi, S., Ushijima, H., Takeda, M., Fukuda, I., Yokomizo, K., Suzuki, K., Sano, K., Nakanishi, T., Tomisato, W., Tsuchiya, T., and Mizushima, T. (2005) *Cancer Res.* **65,** 1868–1876
44. Murakami, M., Iwata, Y., and Funaba, M. (2007) *Mol. Cell Biochem.* **303,** 251–257
45. Namba, T., Homan, T., Nishimura, T., Mima, S., Hoshino, T., and Mizushima, T. (2009) *J. Biol. Chem.* **284,** 4158–4167
46. Hoshino, T., Nakaya, T., Araki, W., Suzuki, K., Suzuki, T., and Mizushima, T. (2007) *Biochem. J.* **402,** 581–589
47. Takehara, M., Makise, M., Takenaka, H., Asano, T., and Mizushima, T. (2008) *Biochem. J.* **413,** 535–543
48. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucleic Acids Res.* **11,** 1475–1489
49. Bancroft, J. D., and Stevens, A. (1982) *Theory and Practice of Histological Techniques*, 2nd Ed., Churchill Livingstone, New York
50. Levy, C., Khaled, M., and Fisher, D. E. (2006) *Trends Mol. Med.* **12,** 406–414
51. Park, H. Y., Wu, C., Yonemoto, L., Murphy-Smith, M., Wu, H., Stachur, C. M., and Gilchrest, B. A. (2006) *Biochem. J.* **395,** 571–578
52. Cui, R., Widlund, H. R., Feige, E., Lin, J. Y., Wilensky, D. L., Igras, V. E., D'Orazio, J., Fung, C. Y., Schanbacher, C. F., Granter, S. R., and Fisher, D. E. (2007) *Cell* **128,** 853–864
53. Ando, H., Kondoh, H., Ichihashi, M., and Hearing, V. J. (2007) *J. Invest. Dermatol.* **127,** 751–761
54. Bases, R. (2006) *Cell Stress Chaperones* **11,** 240–249
55. Zou, Y., Crowley, D. J., and Van Houten, B. (1998) *J. Biol. Chem.* **273,** 12887–12892
56. Matsuda, M., Hoshino, T., Yamashita, Y., Tanaka, K., Maji, D., Sato, K., Adachi, H., Sobue, G., Ihn, H., Funasaka, Y., and Mizushima, T. (2010) *J. Biol. Chem.* **285,** 5848–5858 AQ: J
57. Simon, M. M., Reikerstorfer, A., Schwarz, A., Krone, C., Luger, T. A., Jäättelä, M., and Schwarz, T. (1995) *J. Clin. Invest.* **95,** 926–933
58. Gutsmann-Conrad, A., Heydari, A. R., You, S., and Richardson, A. (1998) *Exp. Cell Res.* **241,** 404–413
59. Kimura, K., Tanaka, N., Nakamura, N., Takano, S., and Ohkuma, S. (2007) *J. Biol. Chem.* **282,** 5910–5918
60. Rattan, S. I. (1998) *Biochem. Mol. Biol. Int.* **45,** 753–759
61. Garraway, L. A., Widlund, H. R., Rubin, M. A., Getz, G., Berger, A. J., Ramaswamy, S., Beroukhim, R., Milner, D. A., Granter, S. R., Du, J., Lee, C., Wagner, S. N., Li, C., Golub, T. R., Rimm, D. L., Meyerson, M. L., Fisher, D. E., and Sellers, W. R. (2005) *Nature* **436,** 117–122
62. Yamashita, Y., Hoshino, T., Matsuda, M., Kobayashi, C., Tominaga, A., Nakamura, Y., Nakashima, K., Yokomizo, K., Ikeda, T., Mineda, K., Maji, D., Niwano, Y., and Mizushima, T. (2010) *Exp. Dermatol.*, in press AQ: K