Melanogenesis:Experimental Models

Hee-Young Park*, Ph.D., and Jin Lee

Department of Dermatology Boston University School of Medicine
Boston, Massachusetts 02118

Melanogenesis, or synthesis of melanin has been a focus of intense investigation by pigment cell biologists during the past few decades. Melanogenesis provides pigment in skin, thus serving as a unique, if not only, physiological defense against sun-induced injuries, including photocarcinogenesis. Moreover, skin color plays a major role in visual esthetics of an individual. Therefore, unwanted hyper- or hypo-pigmentation, especially on facial skin, could cause significant psychological stress.

Epidermal melanocytes, derived from neural crest cells, are mainly responsible for melanin in skin[1]. In human skin, nearly all normal pigmentation is due to melanin and with the exception of hemoglobin, it is one of the only endogenously synthesized pigments in man[1]. Melanin has numerous functions in mammals, including increasing the optical efficiency of the eye, producing color patterns in various organs, including hair or skin, serving as camouflage, heat exchange, sexual recognition and protection from sunlight[1,2]. The incidences of malignant melanoma have been increasing dramatically in western countries, at least by a factor of 15 over the past 60 years, and this has caused an intense interest in understanding melanogenesis[3-5]. More than 40,000 new cases of malignant melanoma have been diagnosed in 1997[6], and it is one of the most common cancers in young adults[7]. The research in the area of melanogenesis has exploded during the last ten years because model systems to study molecular mechanisms regulating melanogenesis have become available. This review examines currently available in vitro and in vivo model systems to study melanogenesis.

(Ann Dermatol 15(2) 45~51, 2003).

Key Words : Pigmentation, Melanocytes, Melanoma cells, Model systems

I. *IN VITRO* SYSTEMS

1. Human melanocytes

The concept of culturing human primary melanocytes was first introduced in 1957 by Hu and colleagues[8] who obtained a short-term culture of normal human melanocytes in vitro. Achieving homogenous cultures of human melanocytes was initially proven to be very difficult due to the overgrowth of the cultures by either keratinocytes and/or fibroblasts[9-11]. Further compounding the difficulty in obtaining pure human melanocyte culture was that the number of melanocytes are few in the normal epidermis where there is one melanocyte for every ~35 keratinocytes[12], readily available normal human skins were scarce and the proliferation rate of melanocytes was intrinsically slow[9,10].

Eisinger and Marko successfully cultured normal human melanocytes in 1982[9,10] by employing using 12-0-tetradeconoylphorbol-13-acetate(TPA) (10ng/ml), cholera toxin and 5% fetal bovine serum in culture media. Epidermal cell suspensions were obtained from facial skin or neonatal fore-

Received January 4, 2002
Accepted for publication May 10, 2002
Reprint request to : Hee-Young Park, Ph.D., Boston University School of Medicine Department of Dermatology 609 Albany Street Boston, MA 02118
Tel. 617-638-5527
E-mail. hypark@bu.edu

skins, and the foreskins were subsequently shown to be a good source to culture melanocytes because foreskins are readily available and their melanocytes have a higher proliferation rate attributed to the age of the donor[9,10]. The use of TPA and cholera toxin together was shown to extend the life span of melanocytes in culture, allowing for successful serial passages and large quantities of melanocytes. Moreover, TPA was shown to be a potent inducer for keratinocyte differentiation[13,14] and slows the growth of fibroblasts[15], thus, eliminating these cells from the melanocyte culture.

Subsequently, a second approach for culturing homogenous human melanocytes was reported by Tsuji and Karasek[16]. These investigators were able to culture melanocytes from both newborn and adult skins and maintain in culture medium consisting of 10% calf serum, cholera toxin, and isobutyl methyxanthine (IBMX) for up to 6 weeks. Rather than employing TPA, this system exposed epidermal cells to 5-fluorouracil to remove keratinocytes and fibroblasts[16].

The advantage of the system containing TPA is that TPA allows multiple passage of melanocytes[17], thus allowing large quantities of cells for experiments. However, the disadvantage is that TPA is a potent regulator of protein kinase C (PKC), which later proves to be critical for melanogenesis[18,19].

A third approach to culture a homogenous culture of human melanocyte cultures was described by Gilchrest and colleagues[20]. A selective cultivation of human melanocytes from both newborn and adult epidermis was achieved in the absence of TPA and with minimal serum supplementation in growth factor defined M199 media[20]. The removal of phorbol esters from melanocyte culture media paved a way to the findings that PKC is intimately involved in melanogenesis, specifically activation of tyrosinase[18,19].This culture method capitalized on dependency of initial growth of melanocytes to the presence of differentiating keratinocytes. The media was supplemented with epidermal growth factor, basic fibroblast growth factor, transferrin, triiodothyronine, holera toxin, insulin and bovine pituitary extract, which acts as a potent growth factor for melanocytes[21]. 2% fetal bovine serum was also used transiently, during the time of initial inoculation and at the time of each passage, however serum was excluded from the medium otherwise. In this system Gilchrest *et al.* showed that the proliferation rate of melanocytes were inversely related to the age of the donor[22], and that cells could be maintained up to the third passage[20]. Subsequent to the first passage, the same media was used except that Ca^{++} was eliminated in order to minimize the growth of fibroblasts[23].

Further modifications to M199-based culture medium has been achieved by removing cholera toxin[24]. Cholera toxin is a non-physiologic substance which constitutively activates cAMP-dependent pathway, known to mediate melanogenesis[25-27].

The culturing of human melanocytes from roof of suction blisters in adult Asians has also provided a source of pure melanocytes. Epidermal roofs of blisters did not contain any fibroblasts and keratinocytes do not attach to cultures dishes in presence of TPA, able to obtain pure melanocytes cultures[28].

Culture of adult human melanocytes can also be obtained from vitiligo donors using MCDB-153 media which included basic fibroblast growth factor, crude bovine pituitary extract, insulin, transferring, hydrocortisone, 5% FCS, α-tocopherol (antioxidant), calcium (200uM), and TPA (4-8nM)[29]. In this system, cholera toxin and IBMX, exogenous catalase (20ug/ml) are needed during isolation and primary seeding, but are eliminated once cells are attached.

2. Human melanoma cells

As an alternative to primary human melanocytes, both primary and established cell lines of human melanoma are subsequently used to study melanogenesis. Primary melanoma cells are initially cultured from fresh surgical specimens of primary cutaneous malignant melanoma. For example, the radical growth phase and vertical growth phase from a patient with melanoma could be excised, washed with calcium- and magnesium-free phosphate buffered saline and incubated in 0.25% trypsin for 37°C for 1hr in order to dissociate cells from the extracellular matrix. Then they are placed into the appropriate medium ranging from defined media to generic DME containing 10% fetal bovine serum or calf serum[30]. Recurrent primary melanoma and metastatic melanoma could also be established via explant cultures[30]. These primary melanoma cells could be kept indefinitely in culture,

thus, becoming an established cell line. The advantage of the established cell line is that it provides an unlimited supply of cells, but the disadvantage is that the characters and properties of the cells change as the passage number increases.

The characterization of a human melanoma cell line has been difficult to achieve because a large degree of heterogenicity exists among the cell lines. Byers *et al.* studied cell migration and actin organization in cultured human primary, recurrent and metastatic melanomas. Results displayed heterogenicity in cell shape, actin organization and cell migration between various lines, as well as within the same line[30]. Cell lines established from lymph node metastasis had higher migration rates than those derived from visceral metastasis. Moreover, the ability of cells to organize actin into stress fibers directly correlated with significantly higher random migration rates and lacked colony formation[30].

3. Murine melanocytes

In 1987, subsequent to culturing human melanocytes, the first culture of murine melanocytes was obtained31. The primary obstacle to obtaining primary murine melanocytes cultures included finding an ideal source for melanocytes, identifying the necessary mitogens and growth factors required and how to overcome the growth advantage of fibroblasts[31]. *Tamura et al.* described a technique which pure cultures of murine melanocytes could be obtained by using dorsal dermis specimens from newborn mice. Dermal specimens were incubated in trypsin and Eagle's minimal essential medium without calcium and magnesium at 37°C for approximately 2-4 hours, or 4°C overnight[31]. Depending on the coloration of the mouse, specimens were split from the epidermis in different manners. For example, darker strains were separated using forceps whereas those from lightly pigmented strains were shaken vigorously via Vortex mixer. The tissues were placed into a flask and incubated at 37°C in humidified air with 5% CO_2. Tissue debris was removed by suction and medium was changed after supplemented with geneticin. When pure colonies of melanocytes were obtained, medium was modified by replacing newborn calf serum with 20% fetal calf serum. Normal murine melanocytes in culture appeared to require growth factors and mitogens similar to those of human melanocytes[9,10,32]. Since the initial protocol was established, minor modifications have been made such as initial plating on a feeder layer of mitomycin-treated immortal murine keratinocytes (ie. XB2) which after third passage, feeder cells are omitted, and melanocytes are grown in RPMI-1640 medium with 10% fetal calf serum, TPA and cholera toxin[33,34].

Another hallmark in the culturing of murine melanocytes was the discovery of TM10, the first reported immortal murine melanocyte line reported by Sato[35]. Initially cells were grown in the same supplements as used by Eisinger and Marko, however, they later lost their dependence to cholera toxin and TPA[36]. The disadvantages of this line is that they are aneuploid and their tumorigenicity was not tested[36].

4. Melanin-A

In 1987, the first known line of non-tumorigenic immortalized mouse melanocytes was discovered by Bennet *et al.*[36]. Melan-a, an immortal line of pigmented melanocytes, was derived from normal epidermal melanoblasts of embryos of inbred C57BL mice. Melan-a cells are named so in order to designate the genotype *a/a* (non-agouti or black) mice. Proliferation of cells was accomplished by creating an environment with a low extracellular pH in the presence of a tumor promoter (TPA or teleocidin) in culture. Melan-a cells were of great significance as they provided a parallel non-tumorigenic line for which melanoma could be studied concurrently[32].

5. Murine melanoma cells (B16, S91, GR1)

Initial experimental studies of pigment cells were performed in murine melanoma models, specifically melanoma cell of GR1, B16 and Cloudman S91 cells[25,26]. These cells are popular models because they require simple media such as DME plus 10% calf serum and display robust response to agents elevating the intracellular level of cAMP[37-42]. α-MSH or agents elevating the intracellular level of cAMP will induce tyrosinase mRNA protein and activity in these cells up to 10 fold[25,26,27,37-42].

II. *IN VIVO* MODELS

1. Human Subjects

For many years recruited human subjects were used to test various compounds on their skin color.

In 1955, it was observed that when an alkali-treated extract from hog pituitary glands injected intramuscularly into humans, generalized darkening, similar to that seen in adrenocortical insufficiency was noted[26,43]. It was later discovered that the extract contained α- and β-melanocyte-stimulating hormone[26,43]. Two other cases of synthetic and pig (melanocyte-stimulating hormone (α-MSH) causing darkening of human skin were documented in 1961 and in 1962, respectively[44]. Another example of pigmentation induced by α-MSH was documented in 1991 in a randomized, placebo-controlled, double-blind clinical trial[45]. Twenty-eight patients received ten subcutaneous injections of either saline or purified NDP (NleD-Phe), a synthetic analogue of α-MSH, over a course of twelve days. Clinically visible tanning, especially on the face and neck, was noted on subjects receiving NDP. The extremities and sun-exposed areas were also seen to darken, however, no darkening was noted on the trunk and buttocks. Mild side effects including flushing and mild gastrointestinal discomfort typically lasting less than one hour after injections[45]. Using Melanotan-II, a more potent synthetic MSH analog, similar results were obtained with 0.01 mg/kg body weight injections and 0.005 mg/kg dose escalation over 10 injections[46].

Skin biopsies of subjects injected with 0.16 mg/kg [Nle^4, D-Phe^7]- α-MSH for 10 days showed a 1.5-fold increase of eumelanin in forehead skin, and 2-fold increase in forearm skin, via quantitative HPLC analysis[47]. However, side effects of [Nle^4, D-Phe^7]- α-MSH injections included erythematous flushing, yawning, lethargy, nausea and spontaneous penile erections[46-48]. Given these adverse reactions, unevenness of tanning and difficulty of administration, MSH analogs have thus had limited use as potential tanning agents[48].

The use of MSH analogs in topical creams has also been pursued in a cosmetic avenue. Melasyn100 ® is an aloe vera and all-plant derived synthetic melanin which is commercially available for use as a tanning agent. In comparison to other commercial self-tanning agents containing dihydroxyacetone (DHA), it absorbs both UV and visible light, exhibits free radical scavenger activity and achieves a skin spectrum similar to a natural tan, rather than the yellow-orange spectrum that is seen by DHA induced self-tanning[49]. This compound is only one of many that are being tested for achieving tanning which mimics the protective effects of tanning seen at the physiologic level.

2. Murine mutant strains with abnormalities in pigmentation

With advances in genomic research, unique murine strains with certain genes altered were created. 'Knockout' mice, which derived via transgenic, targeted mutagenesis and 'gene switch' mice, or conditional mice have been used more frequently in research, and has allowed us to study human disease processes more thoroughly[50]. These murine models range from developmental defects resulting in a large array of coat color phenotypes to melanoma metastasis model[50,51]. An example of such a mouse model includes the tetracycline-inducible system, which can be used as a model to study human melanoma. In this system, the *Tet* operon allows for an inducible gene over-expression in mice, and the *rtTA*, tetracycline-regulated transactivators which are linked to the tyrosinase gene promoter-enhancer elements (*Tyr-rtTA*), allow for temporally restricted and tissue-specific gene induction/suppression by doxycycline administration[50].

In addition to genetically altered mice affecting the coat color, many mice strains with genetic defects associated with their coat color have been identified[51]. These mice with genetic defects provided identification of numerous proteins critical for melanogenesis[51], biogenesis of melanosomes[51], and transport of melanosomes[51].

3. Guinea pigs

As both guinea pigs and humans have active melanocytes that are located in the basal layer of the interfollicular epidermis[52], guinea pigs serve as excellent pigmentation model for studying the effects of targeted compounds on human skin[53-56]. Guinea pigs also respond similar to humans after exposure to UVA, UVB, UVC irradiation[55], PUVA[55], topical MSH plus UVB[54], and injections of MSH or estrogen[57,58]. Hyperpigmentation has been observed to be induced by UVB in a histamine mediated manner, as topical applications of a H2 antagonist was shown to decrease pigmentation[59]. Strains of pigmented guinea pigs may include American Shorthair X Abyssian guinea pigs[60] and A-1 and Hartley strain[59].

A hairless pigmented guinea pig model devel-

oped by Bolognia et al.[52], provide a hairless animal with a pigmentary system that mimics the human one. Hairless albino guinea pigs on an outbred Hartley background were mated with red-haired guinea pigs, and subsequently red-haired heterozygotes were then mated with each other or with hairless albino guinea pigs, resulting in F2 progeny having hairless pigmented guinea pigs[52]. This model system provides the advantage of having a greater cutaneous surface area, similar to humans of active interfollicular epidermal melanocytes[52]. Moreover, UV irradiation of hairless pigmented guinea pigs also results in increased cutaneous pigmentation in an even distribution[52]. Also, the hairless nature of the guinea pig also minimizes undesired artifacts during experimentation, such as irritation due to hair removal[52].

4. Yucatan pigs

The Yucatan hairless minipig has been known to be the closest animal model to humans. Yucatan minipigs are hairless and are a breed of swine with baseline pigmentation varying from light brown to dark brown skin. The skin morphology, pattern of pigment distribution resembles human skin[53]. Consequently, studies have been performed on these models in order to assess laser injuries at specific wavelengths, in order to assess damage from energy absorption by skin pigmentation (melanin) and a more highly pigmented system Yucatan minipig[53]. Additional studies to test for hyper and hypopigmentation, and depigmentation of skin and hair via intraperitoneal injection have been pursued[53,61].

SUMMARY

These available models allowed the advancement in pigment research during the last decade. However, each model, whether *in vivo or in vitro*, has advantages and disadvantages. The continued revision and modification of each model to better reflect human skin would be important as we move forward with our pigment research.

REFERENCES

1. Prota, G. Melanins and Melanogenesis, Academic Press, Inc. Boston, 1992
2. Prota G, D'Ischia M, Napolitano A. The chemistry of melanins and related metabolites. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne J-P. The Pigmentary System: Physiology and Pathophysiology. New York: Oxford University Press; 1998, pp 307-322.
3. Gilchrest BA, Eller MS, Geller AC, Yaar M. The Pathogenesis of Melanoma Induced by Ultraviolet Radiation. NEJM 340:1341-1348, 1999.
4. Glass AG, Hoover RN. The emerging epidemic of melanoma and Squamous cell skin cancer. JAMA 262:2097-2100, 1989.
5. Koh HK, Geller AC, Miller DR, Davis BB, Lew RA. Skin cancer: prevention and control. In: Greenwald P, Kramer BS, Weed DL, Marcel Dekker, eds. Cancer prevention and control. New York. 1995, pp 611-640.
6. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1997. CA Cancer J Clin 47: 5-27, 1997.
7. Kosary CL, Ries LAG, Miller BA, Hankey BF, Harras A, Edwards BK, eds. SEER cancer statistics review, 1973-1992: tables and graphs. Bethesda, Md.: National Cancer Institute, 1996. (NIH publication no. 96-2789).
8. Hu F, Staricco RJ, Pinkus H, Fosnauch RP. Human melanocytes in tissue culture. J Invest Dermatol 28: 15-32, 1957.
9. Eisinger E, Marko O. Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin. Proc Natl Acad Sci USA. 79:2018-2022, 1982.
10. Eisinger E, Marko O, Ogata S-L, Old LJ. Growth regulation of human melanocytes. Science 229:984-986, 1984.
11. Klaus SN. Prospects for growing normal human melanocytes in vitro. Methods Cell Biol 21 A:277-288. 1980.
12. Frenk E, Schellhorn JP. Morphology of the epidermal melanin unit. Dermatologica 139: 271-277, 1969.
13. Garmyn M, Yaar M, Boileau N, Backendorf C, Gilchrest BA. Effect of aging and habitual sun exposure on the genetic response of cultured human keratinocytes to solar-simulated irradiation. J Invest Dermatol 99:743-748, 1992.
14. Yaar M, Gilani A, DiBenedetto P, Harkness DD, Gilchrest BA. Gene modulation accompanying differentiation of normal versus malignant keratinocytes. Exp Cell Res 206:235-243, 1993.
15. Park HY, Campisi, J. Posttranslational control of cyclic AMP-dependent protein kinase by phorbol ester in normal but not in chemically transformed

3T3 cells. Cancer Res 50:7145-7152, 1990.
16. Tsuji T, Karasek M. A procedure for the isolation of primary cultures of melanocytes from newborn and adult human skin. J Invest Dermatol 81: 179-180, 1983.
17. Yaar M, Gilchrest BA. Human melanocyte growth and differentiation: A decade of new data and speculation. J Invest Dermatol 97:611-617, 1991.
18. Park HY, Perez JM, Laursen R. and Gilchrest BA. Protein kinase C-(activates tyrosinase by phosphorylating serine residues in its cytoplasmic domain. J Biol Chem 274:16470-16478, 1999.
19. Park HY, Russakovsky V, Ohno S, and Gilchrest BA. The beta isoform of protein kinase C stimulates human melanogenesis by activating tyrosinase in pigment cells. J Biol Chem 268:11742-11749, 1993.
20. Gilchrest BA, Vrabel MA, Flynn BS, Szabo G. Selective Cultivation of Human Melanocytes from Newborn and Adult Epidermis. J Invest Dermatol 83:370-376, 1984.
21. Wilkins LM, Gilchrest BA, Maciag T, Szabo G, Connell L: Growth of enriched human melanocyte cultures, Growth of Cells in Hormonally-Defined Media. Edited by GH Sate, AB Pardee, DA Sirbasker. Cold Spring Harbor Conferences on Cell Proliferation. 9: 929-936, 1982.
22. Gilchrest BA Ageing of differentiated cells in kin. In "Ageing and the Skin" (AK Balin, AM Kligman, eds.) Raven Press, New York, 1985, pp-77-83.
23. Naeyaert JM, Eller M, Gordon PR, Park HY, Gilchrest BA. Pigment content of cultured human melanocytes does not correlate with tyrosinase message level. Br J Dermatol 125:297-303, 1991.
24. Park HY, Murphy-Smith M, Wu HH, Gilchrest BA. Induction of protein kinase C-(and tyrosinase mRNA by cAMP-dependent pathway in cultured human melanocytes. J Invest Dermatol 117: 716, 2001.
25. Silagi S. Control of pigment production in mouse melanoma cells in vitro. Evocation and maintenance. J Cell Biol 43:263-74, 1969.
26. Lerner AB. Shizume K, Banding I. Mechanism of endocrine control of melanin pigmentation. J Clin Endocrinol Metab 14: 1463-1490, 1954.
27. Park HY, Russakovsky V, Ao Y, Fernandez E, Gilchrest BA. Alpha-melanocyte stimulating hormone-induced pigmentation is blocked by depletion of protein kinase C. Exp Cell Res 227:70-79, 1996.
28. Tomita Y, Yamamoto H, Sato C, Takeuchi T, Tagami H. Efficient culturing of human melanocytes from suction blisters. Tohoku J Exp Med 147(2):219-220, 1985
29. Medrano EE, Nordlund JJ. Successful culture of adult human melanocytes obtained from normal and vitiligo donors. J Invest Dermatol 95: 441-444, 1990.
30. Byers HR, Etoh T, Doherty JR, Sober AJ, Mihm MC Jr. Cell Migration and actin organization in cultured human primary, recurrent cutaneous and metastatic melanoma. Am J Pathol 139: 423-435, 1991.
31. Tamura A, Halaban R, Moellmann G, Cowan JM, Lerner MR, Lerner AB. Normal murine melanocytes in culture. In Vitro Cell Dev Biol 23:519-522, 1987.
32. Halaban R, Ghosh S, Baird A. bFGF is the putative natural growth factor for human melanocytes. In Vitro Cell Dev Biol. 23:47-52, 1987.
33. Sviderskaya EV, Bennett DC, Ho L, Bailin T, Lee ST, Spritz RA. Complementation of hypopigmentation in p-mutant (pink-eyed dilution) mouse melanocytes by normal human PcDNA, and defective complementation by OCA2 mutant sequences. J Invest Dermatol 108: 30-34, 1997.
34. Sviderskaya EV, Hill SP, Evans-Whipp TJ, Chin L, Orlow SJ, Easty DJ, Ching Cheong S, Beach D, DePinho RA, Bennett DC. P16Ink4a in melanocyte senescent and differentiation. J National Cancer Institute. 94: 2002.
35. Sato C, Ito S, Takeuchi T. Establishment of a mouse melanocyte clone which synthesizes both eumelanin and pheomelanin. Cell Struct Funct 10: 421-425, 1985.
36. Bennett DC, Cooper PJ, Hart IR. A line of non-tumorigenic mouse melanocytes, syngeneic with the B16 melanoma and requiring a tumour promoter for growth. Int J Cancer. 39:414-418, 1987.
37. Martinez-Liarte JH, Solano F, Garcia-Borron JC, Jara JR, Lozano JA. α-MSH and other melanogenic activators mediate opposite effects on tyrosinase and dopachrome tautomerase in B16/F10 mouse melanoma cells. J Invest Dermatol. 99:435-439, 1992.
38. Wong G, Pawelek J: Control of phenotypic expression of cultured melanoma cells by melanocyte stimulating hormone. Nature (New Biol) 241:213-215, 1973.
39. Fuller BB, Lunsford JB, Iman DS. Alpha-melanocyte-stimulating hormone regulation of ty-

rosinase in Cloudman S-91 mouse melanoma cell cultures. J Biol Chem 262, 4024-4033, 1987.
40. Halaban R, Pomerantz SH, Marshall S, Lerner AB. Tyrosinase activity and abundance in Cloudman melanoma cells. Arch Biochem Biophys 230:383-387, 1984.
41. Hoganson GE, Ledwitz-Rigby F, Davidson RL, Fuller BB. Regulation of tyrosinase mRNA levels in mouse melanoma cell clones by melanocyte-stimulating hormone and cyclic AMP. Somat Cell Mol Genet 15:255-263, 1989.
42. Rungta D, Corn TD, Fuller BB. Regulation of tyrosinase mRNA in mouse melanoma cells by alpha-melanocyte-stimulating hormone. J Invest Dermatol 107:689-93, 1996.
43. Pears JS, Jung RT, Bartlett W, Browning MCK, Kenicer K, Thody AJ. A case of skin hyperpigmentation due to α -MSH hypersecretion. Br J Dermatol 126: 286-289, 1992.
44 Lerner AB, McGuire JS. Effect of alpha- and beta-melanocyte stimulating hormones on the skin color of man. Nature 189: 176-179, 1961.
45. Levine N, Sheftel SN, Eytan T, et al. Induction of skin tanning by subcutaneous administration of a potent synthetic melanotropin. JAMA 266: 2730-2736, 1991.
46. Dorr RT, Lines R, Levine N, Brooks C, Xing L, Hruby V, Alberts DA. Evaluation of Melanotan-II, a superpotent cyclic melanotropic peptide in a pilot phase-I clinical study. Life Sci 58: 1777-1784, 1996.
47. Dorr RT, Dvorakova K, Brooks C, Lines R, Levine N, Schram K, Miketova P, Hruby V, Alberts DA. Increased eumelanin expression and tanning is induced by a superpotent melanotropin [Nle4, D-Phe7]- α -MSH in humans. Photochem Photobiol 72: 526-532, 2000.
48. Brown DA. Skin Pigmentation Enhancers. J. Photochem Photobiol 63:148-161, 2001.
49. Pawelek JM. Approaches to increasing skin melanin with MSH analogs and synthetic melanins. Pigment Cell Res 14:155-160, 2001.
50. Nakamura M, Tobin DJ, Richards-Smith B, Sundberg JP, Paus R. Mutant laboratory mice with abnormalities in pigmentation: annoted tables. J Dermatol Sci 28: 1-33, 2002.
51. Lamoreux ML. The inbred mouse in pigmentation research: significance of a congenic developmental system. Pigment Cell Res 13:421-30, 2000.
52. Bolognia JL, Murrary MS, Pawelek JM: Hairless pigmented guinea pigs: a new model for the study of mammalian pigmentation. Pigment Cell Res 3:150-156, 1990.
53. Nair X, Tramposch KM. The Yucatan miniature swine as an in vivo model for screening skin depigmentation. J Dermatol Sci 2: 428-33, 1991.
54. Imokawa G, Kawai M: Differential hypermelanosis induced by allergic contact dermatitis. J Invest Dermatol 89:540-546, 1987.
55. Imokawa G, Kawai M, Mishima Y, Motegi I: Differential analysis of experimental hypermelanosis induced by UVB, PUVA, and allergic contact dermatitis using a brownish guinea pig model. Arch Dermatol Res 278:352-362, 1986.
56. Whitman GB, Leach EE, DeLeo VA, Fleiss JL: Comparative study of erythema response to UVA radiation in guinea pig skin. Photochem Photobiol 42:399-403, 1985.
57. Bolognia J, Murray M, Pawelek J. UVB-induced melanogenesis may be mediated through the MSH-receptor system. J Invest Dermatol 92:651-656, 1989.
58. Snell RS, Bischitz PG. The effect of large doses of estrogen and progesterone in melanin pigmentation. J Invest Dermatol 35:73-82,
59. Yoshida M, Hirotsu S, Nakahara M, Uchiwa H, Tomita Y. Histamine is involved in ultraviolet B-induced pigmentation of guinea pig skin. J Invest Dermatol. 118:255-260, 2002.
60. Allan AE, Archambault M, Messana E, Gilchrest BA. Topically Applied Diacylglycerols Increase Pigmentation in Guinea Pig Skin. J Invest Dermatol. 105:687-692, 1995.
61. Nair X, Parab P, Suhr L, Tramposch KM. Combination of 4-hydroxyanisole and all-trans retinoic acid produces synergistic skin depigmentation in swine. J Invest Dermatol 101: 145-149, 1993.