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Keratinocytes regulate the function of melanocytes

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ABSTRACT

Mammalian keratinocytes compose the bulk of the epithelium, undergo keratinization, and form the dead superficial layer of the skin. These superficial keratinized cells are continuously replaced by cells derived from mitotic cells in the lowest layer of the epidermis (i.e., the basal layer). Melanocytes locate in the basal layer and do not keratinize; however, they can produce melanin pigments. Melanin is accumulated in small granules called melanosomes. The melanosomes are transported to dendrites from which the melanosomes are transferred to keratinocytes. Epidermal invaginations such as keratinocytes and melanocytes extend to the dermis to form hair follicles. In addition to these two cells, dermal fibroblasts are also required for the formation of hair follicles. The homeostasis of the epidermis and hair follicle is primarily regulated by the cellular interaction between keratinocytes and melanocytes. Keratinocytes stimulate melanocyte functions such as proliferation, differentiation, melanogenesis, and dendritogenesis. Using the techniques of tissue culture, biochemistry, and molecular biology, factors that have been derived from keratinocytes are hormones, growth factors, and cytokines such as α-melanocyte-stimulating hormone, adrenocorticotrophic hormone, basic fibroblast growth factor, nerve growth factor, endothelins, granulocyte-macrophage colony-stimulating factor, stem cell factor, leukemia inhibitory factor, and hepatocyte growth factor. These keratinocyte-derived paracrine factors have a key role in regulating melanocyte function through receptor-mediated signaling pathways, followed by maintaining epidermal and hair follicular homeostasis.

Introduction

The skin is the largest organ in mammals. It covers the surface of the body and consists of three main layers: the surface epidermis, the subjacent dermis, and the subcutaneous tissue (the lowest layer). An important function of the skin is to protect an animal's body from external stimuli. The skin consists primarily of three cell types: keratinocytes, melanocytes, and fibroblasts. Keratinocytes compose the bulk of the epithelium, undergo keratinization, and form the dead superficial layer of the skin. These superficial keratinized cells continuously desquamate from the surface and are replaced by cells derived from mitotic cells in the lowest layer of the epidermis (i.e., the basal layer). The higher level cells are successively displaced by the population of new cells below them. As they move upwards, they elaborate keratin and accumulate it in the cytoplasm, and finally the cells are mostly occupied by keratin.[1]

Melanocytes, which are pigment-producing cells, are originally derived from neural crest cells in the embryonic skin. Neural crest cells migrate from the dorsal to ventral side and localize all over the body. Melanoblasts, which are a precursor of melanocytes, differentiate from the neural crest cells, proliferate, and colonize the epidermis in the embryonic stage. In the epidermis, the melanocytes locate in the basal layer and do not keratinize, but they can produce melanin pigments.[2] Mammalian hair forms from hair follicles derived from hair germ cells that begin as an epidermal invagination, and include keratinocytes, melanoblasts, and melanocytes.[2] The dermis, which consists of fibroblasts, forms a thickening beneath the epidermis and the end of the invagination surrounds the thickening. The dermal thickening develops into a dermal papilla, and the surrounding part of the invagination forms the hair bulb.[2–4] The lower half of the hair bulb is called a hair matrix, and numerous functional melanocytes are localized there.[2] Keratinocyte stem cells[5] and melanocyte stem cells[6] locate in the bulge area of hair follicles (i.e., at the site of attachment of the

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arrector pili muscle). They produce new proliferating and differentiating keratinocytes and melanocytes.

Recent studies using tissue culture, biochemistry, and molecular biology techniques demonstrate that keratinocytes regulate epidermal and hair follicular melanocyte functions such as proliferation, differentiation, melanogenesis, and dendritogenesis. This article reviews studies on the regulation of melanocyte function by keratinocytes and discusses in detail the mechanism of regulation.

Structure and function of the epidermis

The epidermis is a histologically stratified squamous epithelium and constantly requires renewal from birth to death. An important role of the epidermis is to protect the skin from many types of environmental stresses such as exposure to bacteria; viruses; chemicals; UV radiation; ionizing radiation; electromagnetic waves; and physical, thermal, and mechanical injuries (i.e., barrier function). Keratinocytes compose the bulk of the epidermis, undergo differentiation (i.e., keratinization), and form a dead superficial layer on the skin (this layer is called the "keratinized layer" or "cornified layer").[2] A function of keratinocytes is to produce keratin and filaggrin, which are involved in regulating the barrier function. The renewal of the epidermis is supported by the proliferation and differentiation of keratinocytes (i.e., epidermal homeostasis primarily depends on a balance between the proliferation and differentiation of keratinocytes).[2]

Mammalian melanocytes locate in the basal layer; they do not keratinize but produce melanin pigments.[7] Melanin is produced from L-tyrosine (L-Tyr) with the aid of enzymatic reactions by tyrosinase (Tyr), Tyr-related protein (Tyrp)-1, and Tyrp2.[8] Most epidermal melanocytes migrate to the hair follicles and colonize hair matrix melanocytes in hairy general body (i.e., trunk) skin in animals. In the glabrous skin of the ear, nose, foot, and tail in animals and in human skin (except for the scalp, underarm, and pubic skin), numerous differentiated melanocytes are present in the epidermis—even in adults.[2,9] However, in the epidermis of hairy skin in mice, epidermal melanocytes exist only during the early weeks after birth.[2] In human skin, the epidermal melanin unit, which comprise keratinocytes and melanocytes, has a key role in regulating pigmentation and homeostasis of the epidermis.[9]

Structure and function of the hair follicle

Hair follicular melanocytes derived from epidermal melanocytes are highly dendritic and colonize the hair matrix. Hair matrix melanocytes secrete mature melanosomes (i.e., melanin-containing organelles) to surrounding keratinocytes.[2] Keratinocytes develop the hair cortex and medulla in which melanosomes are incorporated. In mammals, the process of morphogenesis of the hair follicle is cyclic[10] and is called the hair cycle (i.e., growth cycle).[3] The hair cycle consists of three phases: resting (i.e., telogen phase), growth (i.e., anagen phase), and regression (i.e., catagen phase). Anagen hair follicles produce hair shafts formed by fully keratinized cells and pigmented melanocytes. This deposition of mature melanosomes continues during the entire anagen phase (approximately 17 days). This phase is divided into six subphases (i.e., anagen I–VI). When cell proliferation of hair matrix keratinocytes ceases (i.e., catagen), the melanocytes also cease producing melanosomes and no further cells enter the hair shafts. This phase is followed by the telogen phase. In mice, hair growth is synchronized and proceeds in waves all over the body. This growth pattern does not occur in humans.

Hair matrix melanocytes differ from epidermal melanocytes in that hair matrix melanocytes are larger than epidermal melanocytes, possess longer dendrites, and interact with fewer keratinocytes.[2] A functional melanin unit between melanocytes and keratinocytes is established in mature hair follicles. Melanocytes locate in the basal layer of the hair matrix close to the dermal papillae and rest on the glassy membrane (i.e., the basement membrane).[2] Pigmentation is strictly coupled to the growth phase of the hair cycle (i.e., anagen III–VI).[3] Towards the end of the anagen phase, identifiable melanocytes decrease in number and the melanocytes lose their dendrites, shrink, become less pigmented, and disappear in the catagen phase.[3] Keratinocytes and melanocytes die and finally hair follicles move upwards to form a rudimentary hair germ near a sebaceous gland.[6] In the telogen phase, keratinocyte stem cells and melanocyte stem cells continue to reside in the bulge area.[6]

Regulation of melanocyte function

The main function of melanocytes is to produce melanin.[2,7,8] Melanin absorbs UV waves to prevent DNA damage to the keratinocytes. The quality and quantity of melanin present in an animal's body is determined by the differentiated state of melanocytes, melanocyte number, degree of melanogenesis, and dendricity, and by environmental factors such as the surrounding tissue environment, blood supply, UV radiation, and ionizing radiation.[2,7,8] Melanin synthesis in melanocytes is primarily controlled by Tyr, Tyrp1, and Tyrp2.[7,8,11] Tyrosinase initiates melanin synthesis by catalyzing the oxidation of L-Tyr to dopaquinone.[12] Tyrosinase-related protein-1 possesses 5,6-dihydroxyindole-2-carboxylic acid (DHICA) oxidase activity.[13] By contrast, Tyrp2 possesses dopachrome tautomerase activity,[14] which converts dopachrome to DHICA.[15] Melanocytes produce two types of melanin: brown-black eumelanin and red-yellow pheomelanin.[7,16] Differences exist in their molecular size and general properties, although these melanins arise from a common metabolic pathway in which dopaquinone is a key intermediate.[16]

Melanin synthesis occurs in specialized organelles called melanosomes.[17] Melanosome maturation is categorized into four stages: stages I and II include unmelanized immature premelanosomes, whereas stages III and IV contain melanized melanosomes.[18] In animals, coat colors are determined by the quantity and properties of melanosomes transferred to neighboring keratinocytes from hair matrix melanocytes. Melanosomes are produced in various sizes, numbers, and densities. They are passed on to the hair shaft where the final distribution patterns of the pigment are determined. This distribution determines the coat coloring of animals.[19] Eumelanin-containing melanosomes (i.e., eumelanosomes) are elliptical in morphology with longitudinal depositions of pigments in intraluminal fibrils.[20] By contrast, pheomelanin-containing melanosomes (i.e., pheomelanosomes) are spherical with granular depositions of pigments within multivesicular bodies. Agouti (*A*/—) melanocytes contain pheomelanosomes in the pheomelanin-producing stage of the anagen phase and in yellow melanocytes (lethal yellow, A^y/—; recessive yellow, $Mc1r^e$/$Mc1r^e$ or *e*/*e*).[21] Thus, the differences in melanin synthesis correspond to differences in melanosome morphology. Numerous genetic and epigenetic factors regulate melanin synthesis in melanocytes.[2,19] Among these factors, the coat color genes are the most important.[19] In mice, more than 300 genes are involved in melanocyte-proliferation and differentiation; approximately one-half of the genes have been cloned and their functions have been clarified.[22] Furthermore, epigenetic factors from the surrounding tissue environment, especially keratinocytes, are also important for regulating melanin synthesis.

Mechanism of the regulation of melanocyte function by keratinocytes

To understand the mechanism of the regulation of melanocyte function by keratinocytes, analysis by a co-culture system of melanocytes and keratinocytes is useful. For the co-culture of melanocytes and keratinocytes, pure culture of melanocytes and keratinocytes should be prepared first. Serum-free primary culture system of melanoblasts/melanocytes and keratinocytes is preferable because unidentified factors present in serum can be eliminated. Serum-free chemically defined culture media to optimize and maintain mouse melanoblasts and melanocytes have been developed by using suspensions of epidermal cells from newborn C57BL/10JHir (B10) mice.[23] Mouse epidermal melanoblasts can be preferentially cultured using melanoblast-defined medium (MDM), which consists of Ham's F-10 medium supplemented with insulin (Ins), bovine serum albumin, ethanolamine, phosphoethanolamine, and sodium selenite. After 14 days, nearly all keratinocytes that exist predominantly in the early stage of the primary culture die and a pure culture of melanoblasts can be obtained. Pure culture of differentiated melanocytes can be obtained using melanocyte-differentiation medium (MDMM), which consists of MDM supplemented with α-melanocyte-stimulating hormone (MSH). α-Melanocyte-stimulating hormone induces the differentiation of melanocytes. Proliferation of melanocytes can be elicited by melanocyte-proliferation medium (MDMD), which consists of MDM supplemented with dibutyryl adenosine 3′,5′-cyclic monophosphate (DBcAMP).[23] Pure populations of numerous melanoblasts (approximately 90%) and melanocytes (approximately 10%) can be obtained using melanoblast-proliferation medium (MDMDF), which consists of MDMD supplemented with basic fibroblast growth factor (bFGF).[23] Differentiation and proliferation of melanocytes can be induced by DBcAMP. By contrast, bFGF can induce the proliferation of melanoblasts in the presence of DBcAMP. Table 1[23] summarizes these culture systems.

By contrast, a pure culture of primary mouse keratinocytes can be obtained by culturing epidermal cell suspensions from B10 mice using a keratinocyte-defined medium (KDM) that consists of calcium (Ca^{2+})-free minimal essential medium (MEM) supplemented with MEM-nonessential amino acids, Ins, bovine serum albumin, ethanolamine, phosphoethanolamine, sodium selenite, epidermal growth factor, hydrocortisone, dexamethasone, and 0.03mM calcium chloride ($CaCl_2$).[23] At 14 days, the primary keratinocytes can be trypsinized and seeded into pure melanoblasts or melanocytes obtained using MDM, MDMM, MDMD, or MDMDF.[23] Proliferation-stimulating, differentiation-stimulating, melanogenesis-stimulating, and dendritogenesis-stimulating factors derived from keratinocytes have been studied using this co-culture system.[22] Endothelin (ET)-1, ET-2, ET-3, leukemia inhibitory factor (LIF), stem cell factor (SCF), hepatocyte growth factor (HGF), and granulocyte-macrophage colony-stimulating factor (GMCSF) are keratinocyte-derived factors (Table 2).[22] Other co-culture systems using mouse melan-a cells and SP-1 keratinocytes have been reported.[24] The SP-1 keratinocytes stimulate the proliferation, differentiation, melanogenesis, and dendritogenesis of melan-a cells. However, interleukin (IL)-1α inhibits the proliferation of mouse epidermal melanoblasts cultured with MDMDF, irrespective of the presence or absence of keratinocytes, and IL-1α inhibits the proliferation of melanocytes cultured with MDMD only in the presence of keratinocytes. By contrast, IL-1α stimulates the differentiation of melanocytes, Tyr activity, melanogenesis, and dendritogenesis, irrespective of the presence or absence of keratinocytes.[25] Thus, undefined factors derived from keratinocytes seem to be involved in regulating the proliferation of melanocytes in cooperation with IL-1α.

Studies using *in vivo* systems demonstrate that α-MSH is produced in the skin of rats and gerbils.[26] In guinea pigs, SCF stimulates the proliferation and pigmentation of melanocytes in UVB-irradiated skin.[27] α-Melanocyte-stimulating hormone is produced in the skin of adult hairless mice.[26] The SP-1 keratinocytes also produce and release α-MSH and stimulate the melanogenesis of murine melan-a cells.[28] However, the gene for proopiomelanocortin was not expressed in cultured keratinocytes of newborn mice or expressed in the epidermis and dermis (which were separated by trypsin) of pre- and postnatal mice. This suggests that keratinocytes in pre- and neonatal mouse skin do not produce nor release α-MSH/adrenocorticotrophic hormone (ACTH).[29] In mice, the major source of α-MSH and/or ACTH may be derived from the pituitary through the blood stream, but not from epidermal keratinocytes—at least in the pre- and postnatal stages. Studies using transgenic mice demonstrate that SCF[30] and HGF[31] are keratinocyte-derived mitogens towards melanocytes.

Human epidermal melanoblasts and melanocytes can be cultured with MDMDF and MDMD with a slight modification.[32] Supplementing MDMDF with transferrin and ET-1 (i.e., MDMDFα) can serially grow and passage human melanoblasts (approximately 90%) and melanocytes (approximately 10%), and MDMD supplemented with transferrin, L-Tyr, ET-1, and SCF (i.e., MDMDα) can

Table 1 Culture media used for the co-culture of mammalian melanoblasts/melanocytes and keratinocytes.

Species	Culture media	Cells	Effects of keratinocytes on melanoblasts/melanocytes				Refs
			Proliferation	Differentiation	Melanogenesis	Dendritogenesis	
Mouse	MDM	Primary Mb + Primary K	↑	—	—	—	23
	MDMM	Primary M + Primary K	→	↑	↑	↑	23
	MDMD	Primary M + Primary K	↑	↑	↑	↑	23
	MDMDF	Primary Mb/M + Primary K	↑	—	—	—	23
	SKM	Melan-a (M) + SP-1 (K)	↑	↑	↑	↑	24
Human	MDMDFα	Human Mb + Human K	↑	—	—	—	32
	MDMDα	Human M + Human K	↑	↑	↑	↑	32
	KGM	Human M + Human K	—	—	—	↑	35
	M2	Human M + Human K	↑	↑	↑	↑	36
	M2:KM = 1:2	Human M + HaCaT	—	—	—	↑	44

"→" = no change; "↑" = stimulated; KGM (keratinocyte growth medium) = MCDB153 medium + ethanolamine (EA) + phosphoethanolamine (PEA) + hydrocortisone + insulin (Ins) + epidermal growth factor + bovine pituitary extract + low calcium; KM (keratinocyte medium) = Dulbecco's modified Eagle medium (DMEM) + glucose + L-glutamine + pyridoxine + 10% fetal bovine serum (FBS); M = melanocyte; M2 (melanocyte culture medium) = DMEM:F12 medium (1:1) + fibroblast growth factor (bFGF); Mb = melanoblast; MDM (melanoblast-defined medium) = F-10 medium + insulin + bovine serum albumin + EA + PEA + sodium selenite; MDMD (melanocyte-proliferation medium) = MDM + dibutyryl adenosine 3′:5′-cyclic monophosphate; MDMDα = MDMD + transferrin (Tf) + L-tyrosine + endothelin (ET)-1 + stem cell factor (SCF); MDMDF (melanoblast-proliferation medium) = MDMD + bFGF; MDMDFα = MDMDF + Tf + ET-1; MDMM (melanocyte-differentiation medium) = MDM + α-melanocyte-stimulating hormone; SKM (standard keratinocyte medium) = minimal essential medium (MEM) + 8% chelex-treated FBS + low calcium.

Table 2 Keratinocyte-derived factors involved in regulating the function of mammalian melanocytes.

Species	K-factors[a]	Experimental system	Proliferation	Differentiation	Melanogenesis	Dendritogenesis	Refs
Mouse	ET-1	Serum-free co-culture, Ab	↑	↑	↑	↑	22
Mouse	ET-2	Serum-free co-culture, Ab	↑	↑	↑	↑	22
Mouse	ET-3	Serum-free co-culture, Ab	↑	↑	↑	↑	22
Mouse	HGF	Serum-free co-culture, Ab, TG	↑	→	↑	↑	22,30
Mouse	GMCSF	Serum-free co-culture, Ab, ELISA	↑	↑	↑	↑	22
Mouse	LIF	Serum-free co-culture, Ab	↑	↑	↑	↑	22
Mouse	SCF	Serum-free co-culture, Ab, TG	↑	↑	↑	↑	22,29
Mouse	IL-1α	Serum-free co-culture	↓	↑	↑	↑	25
Mouse	α-MSH	*In vivo*, RIA	—	—	—	—	26
Rat	α-MSH	*In vivo*, RIA	—	—	—	—	26
Gerbil	α-MSH	*In vivo*, RIA	—	—	—	—	26
Guinea pig	SCF	*In vivo*, IHC	↑	—	↑	—	27
Human	α-MSH	*In vivo*, RIA, RT-PCR, LC-MS	—	—	↑	↑	26,37–40
Human	SCF	*In vivo*, co-culture, ELISA	↑	↑	↑	↑	27,32
Human	HGF	co-culture	↑	↑	↑	↑	32
Human	GMCSF	Culture, ELISA	↑	↑	↑	↑	32,42
Human	ET-1	Culture, Northern	↑	↑	↑	↑	32,42
Human	bFGF	Co-culture, Northern, Ab	↑	—	—	↑	45
Human	ACTH	*In vivo*, RIA, RT-PCR, LC-MS	—	—	↑	↑	38–40
Human	NGF	Culture, PCR	—	—	—	↑	41
Human	PGE_2, $PGF_{2\alpha}$	Culture, ELISA	—	—	—	↑	43
Human	IL-6, IL-8	Culture	—	—	—	↑	44

"↓" = inhibition of the proliferation of melanoblast and/or melanocytes; "↑" = stimulation of the proliferation of melanoblasts and melanocytes or stimulation of the differentiation, melanogenesis, and dendritogenesis of melanocytes; "→" = no effects on melanocyte function; Ab = antibody is added to the culture system; ACTH = adrenocorticotropic hormone; bFGF = basic fibroblast growth factor; ELISA = enzyme-linked immunosorbent assay; ET = endothelin; GMCSF = granulocyte-macrophage colony-stimulating factor; HGF = hepatocyte growth factor; IHC = immunohistochemistry; IL = interleukin; LC-MS = liquid chromatography-mass spectrometry; LIF = leukemia inhibitory factor; α-MSH = melanocyte-stimulating hormone; NGF = nerve growth factor, Northern = Northern blot analysis; PGE_2 = prostaglandin E_2; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; RIA = radioimmunoassay; RT-PCR = reverse transcription polymerase chain reaction; SCF = stem cell factor; TG = analysis using transgenic mice.

[a] Studies using *in vivo* and *in vitro* systems suggest the presence of keratinocyte (K)-derived factors.

serially grow and passage differentiated melanocytes.[32] Human epidermal melanoblasts cultured with MDMDFα can differentiate pigment-producing melanocytes when the culture medium is changed to MDMDα.[32] By contrast, human epidermal keratinocytes fail to be cultured by KDM.[32] Pure culture of human keratinocytes can be obtained by culturing epidermal cell suspensions of neonatal foreskin with KG2 medium, which consists of MCDB153 medium supplemented with bovine pituitary extract, Ins, epidermal growth factor, and hydrocortisone.[33] Keratinocytes can be trypsinized and seeded into pure melanoblasts and melanocytes cultured with MDMDFα. Human epidermal melanoblasts can be stimulated to proliferate when they are cultured with MDMDFα in the presence of keratinocytes.[32] Furthermore, human epidermal melanoblasts can be stimulated to differentiate to functional melanocytes (i.e., fully pigmented and dendritic cells) when they are cultured with MDMDα in the presence of keratinocytes.[32] Thus, human epidermal keratinocytes appear to stimulate the proliferation, differentiation, melanogenesis, and dendritogenesis of epidermal melanocytes. Other culture systems have been reported in which human melanoblasts used melanocyte growth medium (MGM),[34] which consisted of MCDB153 + 8% chelated fetal bovine serum (FBS) + 2% FBS + L-glutamine + cholera toxin + SCF + ET-3 + bFGF and human melanocytes using M2 medium[35,36] (Table 1). Thus, human keratinocytes can stimulate the proliferation and differentiation of melanocytes.

Studies using *in vivo* systems have confirmed that α-MSH,[26,37–40] ACTH[38–40]/ACTH fragments,[39,40] and nerve growth factor (NGF)[41] are produced in and released from human keratinocytes and are involved in regulating melanogenesis and/or dendritogenesis of human melanocytes in primary cultures and/or serial cultures. Endothelin-1 and GMCSF are keratinocyte-derived factors that regulate the proliferation and melanogenesis/dendritogenesis of melanocytes in UVB or UVA-irradiated human skins.[42] Prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are produced and released from human keratinocytes by stimulation of the proteinase-activated receptor 2. They stimulate the dendritogenesis of cultured human epidermal melanocytes through prostaglandin EP1, prostaglandin EP3, and prostaglandin FP receptors in a cAMP-independent manner.[43] Human keratinocytes produce and release IL-6 and IL-8, which stimulate the dendritogenesis in cultured human epidermal melanocytes.[44] By contrast, bFGF reportedly regulates the proliferation of melanocytes.[45] Stem cell factor is expressed in keratinocytes and it regulates the proliferation and differentiation of human melanocytes.[27,32]

Keratinocyte stem cells form a niche for melanocyte stem cells in the hair bulge.[6] Activation of Wnt/β-catenine signaling in keratinocyte stem cells induces the expression of ET-1, which stimulates the differentiation of melanocyte stem cells.[46] By contrast, transforming growth factor (TGF)-β has a key role in maintaining melanocyte stem cells by promoting melanocyte immaturity through the downregulation of microphthalmia-associated transcription factor (Mitf) expression.[47]

Signaling pathway of keratinocyte-derived factors

Keratinocyte-derived factors such as α-MSH, ACTH, NGF, bFGF, ET-1, ET-2, ET-3, SCF, LIF, HGF, GMCSF, PGE_2, and $PGF_{2\alpha}$ appear to bind to their specific receptors (e.g., Mc1r, NGFR [$p75^{NTR}$/TrkA], FGFR-1, FGFR-2, ETBR, Kit, gp130, LIFRα, c-Met, GMCSFR, EP1, EP2, and EP3) on the membrane of melanoblasts/melanocytes. They also stimulate 1,4,5-inositol-triphosphate formation and activate protein kinase (PK) C or mitogen-activated protein kinase (MAPK) via signal transducer and activator of transcription (STAT)1, STAT3, and STAT5.[22,42,48] They also appear to act as mitogens and melanogens towards melanocytes by upregulating the proteins required for proliferation and differentiation. The signaling pathway of these keratinocyte-derived factors forms a complex network with other factors. The proliferation of mouse melanoblasts requires three signaling pathways: (1) protein kinase A (PKA) by cAMP elevators such as α-MSH and ACTH, (2) protein kinase C (PKC) by ETs, and (3) MAPK by bFGF, SCF, LIF, HGF, and GMCSF. The proliferation of melanocytes and differentiation and/or melanogenesis/

dendritogenesis of melanocytes requires two signaling pathways: (1) PKA and PKC or (2) PKA and MAPK.[22,42]

There is also increasing evidence that the proliferation and dendricity of melanocytes is controlled by Mitf, which is regulated by MAPK signaling and stress-activated kinase p38 signaling.[49,50] Therefore, it is possible that the signaling pathway of keratinocyte-derived factors through MK or p38 regulates Mitf in melanocytes and consequently controls the proliferation of mammalian epidermal melanocytes. Thus, melanocytes and keratinocytes form well-organized units in the epidermis and hair follicles through cell-to-cell interaction.

Conclusion

The proliferation, differentiation, melanogenesis, and dendritogenesis of melanocytes in the epidermis and hair follicles of mammalian skin are primarily regulated by paracrine factors derived from keratinocytes. The paracrine regulation of melanocyte function by keratinocytes appears to have a key role in regulating the homeostasis of the epidermis and hair follicles.

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