Cellular Engineering: Bioengineering of the skin

Pigmented human skin equivalent—as a model of the mechanisms of control of cell–cell and cell–matrix interactions

K. Nakazawa[1] M. Kalassy[2] F. Sahuc[1] C. Collombel[1] O. Damour[1,3]

[1] Laboratory of Skin Substitutes, Edouard Herriot Hospital, Bat. 5, Federation of Biochemistry, 5, place d'Arsonval, 69437 Lyon, France
[2] International Agency for Research on Cancer, 150, Cours Albert Thomas, Lyon, France
[3] IBCP, CNRS, UPR412, Lyon, France

Abstract—*The melanin pigment system in human skin is extraordinarly well developed and assures the photoprotection of the skin against harmful solar radiation. Specific cell–cell interactions between one melanocytes and keratinocytes play a fundamental role in the regulation of melanogenesis and melanin pigementation, the two key elements of this system, giving rise to the concept of a structural, functional collaborative 'epidermal melanin unit,' Early experiments strongly suggested that melanocyte growth and differentiation are regulated by paracrine factors from keratinocytes and other skin cells. In addition, co-culture studies with keratinocytes has shown that the extracellular matrix acts as a local environmental signal for dendrite formation and melanogenesis. Attempts to reconstruct pigmented human skin in vitro have made great progress over the last decade. The behavior of cells in these pigmented human skin equivalents closely resembles that in vivo, and the cells can still respond to appropriate extrinsic regulatory stimuli such as ultraviolet radiation. Keratinocytes and fibroblasts have been shown to be active partners in the regulation of melanocyte distribution, viability and other differentiation functions, presumably by direct contact and the effects of various soluble paracrine factors. By reproducing cell–cell and cell–matrix interactions, these culture systems provide a promising experimental model for investigating regulation of the skin pigmentary system and the role of photoprotection against harmful solar radiation.*

Keywords—*Melanocytes, Skin equivalent, UVB*

Med. Biol. Eng. Comput., 1998, **36**, 813–820

1 Introduction

THE HUMAN skin pigementary system, consisting of one melanocyte and about 36 keratinocytes in the epidermis, is considered to be a structural and functional collaborative unit, known as an 'epidermal melanin unit,' The best-known functions of this system are (i) production of melanin in melanosomes (melanogenesis) and (ii) transfer of melanosomes into surrounding keratinocytes through dendritic processes (melanin pigmentation). This ensures a primary physical defence against harmful solar radiation (FITZPATRICK *et al.*, 1983). Epidemiological studies have shown that constitutive melanin protects against solar radiation by absorbing and scattering it. Thus, highly pigmented races are less susceptible to skin cancers induced by ultraviolet (UV) radiation than lightly pigmented races (EPSTEIN, 1989), and DNA from lightly pigmented melanocytes contained significantly more cyclobutane pyrimidine dimers than DNA from heavily pigmented melanocytes after UVB irradiation (BARKER *et al.*, 1995). DNA damage (ELLER *et al.*, 1994) and its excision repair (ELLER *et al.*, 1996; GILCHREST *et al.*, 1993) is an important initial signal in the pigmentation responses to UV radiation, although the mechanism by which these melanosomes are subsequently transferred to keratinocytes is unknown.

Correspondence should be addressed to Dr O. Damour
First received 4 November 1997 and in final form 26 January 1998

2 Cell–cell and cell–matrix interactions among melanocytes, keratinocytes and fibroblasts

Since the first selective cultivation normal human melanocytes (EISINGER and MARKO, 1982), many attempts have been made to produce a culture medium that will support melanocyte proliferation (HALABAN *et al.*, 1988; HERLYN *et al.*, 1988; PITTELKOW and SHIPLEY, 1989; WILKINS *et al.*, 1985). The resulting improvements in cell culture conditions have greatly advanced our understanding of melanocyte biology (YAAR and GILCHREST, 1991), including the identification of growth factors and the observation of direct activation of melanocytes by UV irradiation (FRIEDMAN and GILCHREST, 1987).

Under hyperproliferative conditions, however, the specific differentiation functions of melanocytes are often altered or lost. The morphology of melanocytes *in vivo*, which is characterised by dendrite formation among surrounding keratinocytes, has great importance for the transfer of melanosomes into keratinocytes. However, in monolayer cultures, without keratinocytes, *in vitro*, the morphology of melanocytes largely depends on the culture conditions. The ability of

melanocytes to form dendrites frequently decreases after several passages, and the cells become bipolar with little cytoplasm (EISINGER and MARKO, 1982; HALABAN *et al.*, 1988; HERLYN *et al.*, 1988). In contrast, in the presence of keratinocytes, melanocytes spread their dendrites to the keratinocytes (DE LUCA *et al.*, 1988*a,b*; NAKAZAWA *et al.*, 1995; VALYI and HERLYN, 1991). It has also been shown that melanocyte proliferation can be controlled through direct cell–cell contact between the melanocyte and neighbouring keratinocytes. Generally, proliferation of normal human melanocytes in culture requires mitogens such as 12-0-tetradecanoylphorbol 13-acetate (TPA), basic fibroblast growth factor (bEGF) or cyclic AMP stimulating agents (EISINGER and MARKO, 1982; HALABANE *et al.*, 1987), and normal human melanocytes cannot grow without these mitotic factors. Melanocytes can nevertheless proliferate when in direct contact with keratinocytes (DE LUCA *et al.*, 1988*a,b*; HALABAN *et al.*, 1988; VALYI and HERLYN, 1991). The physiological ratio of melanocytes to keratinocytes *in vivo* is strictly maintained in co-culture with keratinocytes in epithelial sheets (DELUCA *et al.*, 1988*a,b*).

These results emphasise the importance of complex cell–cell interactions and, especially, the role of keratinocytes in the regulation of melanocyte functions. Many growth factors have been identified as paracrine factors from keratinocytes and fibroblasts, including bFGF (HALABAN *et al.*, 1988), nerve growth factor (YAAR *et al.*, 1991), endothelin-1 (HARA, *et al.*, 1995; YADA *et al.*, 1991; YOHN *et al.*, 1993), leukotrienes C4 and D4 (MORELLI *et al.*, 1989), prostaglandin E_2 (NORDLUND *et al.*, 1986; TOMITA *et al.*, 1987), interleukin1α and 6, vitamin D_3 (TOMITA *et al.*, 1988), tumour necrosis factor-α (SWOPE *et al.*, 1991), hepatotyce growth factor/scatter factor (HALABANE *et al.*, 1992), mast cell growth factor/stem cell factor (FUNASAKA *et al.*, 1992; SCOTT *et al.*, 1996) and α-melanoctye stimulating hormone (DE LUCA *et al.*, 1993; SCHAUER *et al.*, 1994; ABDEL-MALEK *et al.*, 1995). All of these act in the regulation of normal human melanocyte functions (Table 1).

UV radiation has long been known to be the major stimulus for the skin pigmentary system *in vivo* (PATHAK *et al.*, 1965; QUEVEDO *et al.*, 1965; JIMBOW *et al.*, 1975; ROSEN *et al.*, 1987) and *in vitro* (FRIEDMANN and GILCHREST, 1987). Melanocytes are directly stimulated by UV irradiation to increase the length of their dendrites and the number of melanosomes. Melanocytes are indirectly stimulated by the release of some of the paracrine factors listed above, such as bFGF (HALABAN *et al.*, 1988), nerve growth factor (YAAR *et al.*, 1991), endothelin-1 (IMOKAWA *et al.*, 1992) and α-melanocyte stimulating hormone (CHAKRABORTY *et al.*, 1995), after UV irradiation.

Functional differentiation of melanocytes is also affected by molecules of extracellular matrix (ECM) which act as local environmental signals (CILCHREST *et al.*, 1985; MCCLENIC *et al.*, 1989; SCOTT *et al.*, 1992; SCOTT and BUSACCO, 1997). The structural components of dermal ECM produced by embroyonic tissues can induce melanocyte differentiation from neural crest cells (DERBY, 1982). The intact ECM produced by bovine corneal endothelial cells has been shown to affect tyrosinase activity and melanin production as well as melanocyte proliferation (RANSON *et al.*, 1988).

Table 1 Action of different soluble factors on normal human melanocyte functions

Proliferation	bFGF, ET-1, LT-C4, LT-D4, IL-1a, IL-6, TNF-α, MGF(SCF), α-MSH and HGF
Migration	LT-C4 and TNF-α
Melanogenesis	IL-1α, IL-6, TNF-a, ET-1, LT-B4 and α-MSH
Dendrite formation	PGE_2, LT-C4, ET-1, NGF and VD3

Strong effects of ECM deposited by keratinocytes on melanocyte dendrite formation, adhesion and proliferation and additional physiological and environmental stimuli have been reported (NAKAZAWA *et al.*, 1995). Similarly, it has been reported that the ECM proteins derived from normal human fibroblasts or dermal papilla cells of hair follicles strongly induce tyrosinase activity (BUFFEY *et al.*, 1994). The effects of fibroblast ECM have been observed most clearly when myocytes were cultures under poor conditions without mitogens (HEDLY *et al.*, 1996, 1997).

The difference in the proliferation rate in monolayer cultures and *in vivo* should also be noted. To stimulate conditions *in vivo*, the three-dimensional pigmented human skin equivalent began to be used in 1986, and several potentially useful pigmented skin equivalents (PSEs) have been developed in the last decade (Table 2).

3 Pigmented human skin equivalent models

The first PSE was reported by TOPOL *et al.* (1986). The model was constructed by plating human neonatal foreskin melanocytes onto a dermal equivalent before overgrowth with keratinocytes and culturing in Earle's modified Eagle's medium with 20 ng/ml epidermal growth factor (EGF) and 10^{-10} M of cholera toxin for five days. The cells were then lifted onto grids for 12 days. The dermal equivalent arises through the action of fibroblasts which compact matrix proteins into a tissue (BELL *et al.*, 1979, 1981). Keratinocytes migrated out of 2 mm punch biopsies of human neonatal foreskin embedded in the dermal equivalent and covered it within 14 days (COULOMB *et al.*, 1986). Using the same dermal equivalent, BERTAUX *et al.* (1988) reported the outgrowth of melanocytes from a foreskin punch biopsy without the addition of keratinocytes. Recently, foreskin melanocytes and keratinocytes were seeded at a ratio of 1:10 onto the same dermal equivalent and cultured in Dulbecco's modified Eagle's medium (DMEM) with relatively high concentrations of bFGF (10 ng/ml), EGF (20 ng/ml) and cholera toxin (10^{-9} M) (ARCHAMBAULT *et al.*, 1995). SCOTT and HAAKE (1991; HAAKE and SCOTT, 1991) combined cultured foetal or neonatal keratinocytes with cultured melanocytes (7:3 ratio) on the same dermal equivalent. The PSE was maintained in DMEM for 6–10 days and then lifted onto the air-liquid interface and cultured for 1–2 weeks.

The second PSE model was established on a fibroblast-coated nylon filtration mesh, which serves as the dermal equivalent (NAUGHTON *et al.*, 1989). Keratinocytes were plated, after three days, onto melanocytes that had previously been seeded onto the firbroblast-coated mesh, when cultured in PRMI 1640 with no further supplement.

In the third model, PSEs were prepared by seeding mixtures of adult human keratinocytes and melanocytes in various ratios onto deepidermised dermis, originally designed by FREEDMAN *et al.* (1976) and further developed by PRUNIÉRAS *et al.* (1983), in which the fibroblasts are devitalised (ASSELINEAU and PRUNIÉRAS, 1984). The dermal substrate does not contain living fibroblasts, but it is presumed to provide sufficient degradation products of the matrix and still contain enough soluble factors (FUSENIG, 1992). The cells were cultured in DMEM/Ham's F12 (3:1) for three days and then lifted onto the air-liquid interface and maintained for 11 days (TODD *et al.*, 1993).

Franchi reported the use of the same PSE but with a different culture medium (Rheinwald-Green's modified medium) with 1.8 mmol/litre, $CaCl_2$, EGF and bFGF. More recently, BESSOU *et al.* (1996) used this model and developed autologus epidermal reconstructs with keratinocytes and mel-

Table 2 Pigmented human skin equivalent models

		Dermal equivalents	Epidermis	Studies
1986	Topol *et al.*	Collagen Gel + FB	Punch Biopsy + MC (foreskin)	UVB Melanosome transfer Dopa-reaction
1988	Naughton *et al.*	Nylon Mesh + FB	KC + MC	Cytotoxicity asay
1988	Bertaux *et al.*	Collagen Gel + FB	Punch Biopsy (foreskin)	UVB, UVA + 8-MOP Melanosome transfer Dopa-reaction
1991	Haake and Scott	Collagen Gel + FB	KC + MC (foreskin)	Melanoma-melanocyte-associated antigens Melanosome
1991	Scott and Haake	Collagen Gel + FB	KC + MC (foreskin, 7:3)	Melanocyte density HMB-45
1993	Todd *et al.*	Deepidermized Dermis	KC + MC (adult skin, 5-25:1)	UVB Dopa-reaction, TRP-1
1994	Franchi *et al.*	Deepidermized Dermis	KC + MC (adult skin, 5-10:1)	UVB S-100, T4-tyrosinase Dopa-reaction Melanosome
1995	Archambault *et al.*	Collagen Gel + FB	KC + MC (foreskin, 10:1)	UVB Melanin synthesis Viability
1996	Bessou *et al.*	Deepidermized Dermis	KC + MC (adult skin, 20:1)	UVB Phototypes I–IV Dopa-reaction Melanosome
1997	Nakazawa *et al.*	Dermal Substrate + FB	KC + MC (mixed skin, 10:1) in epithelial sheet	UVB Melanocyte number TRP-1, Ki67 Epidermal proliferation

anocytes from healthy donors of skin phototypes I to VI. The melanocytes and keratinocytes were seeded onto the de-epidermised dermis in a 1:20 ratio, and grown in MCDB 153–Iscove's modified Dulbecco's medium (3:1) with 45 μg/ml bovine pituitary extract for 15 days at the air-liquid interface.

A porous dermal substrate made of collagen types I/III, was first developed by YANNAS and BURKE (1980) and modified by COLLOMBEL *et al.* (1987) with a chitosan cross-linking process. Because of the presence of pores of about 100 μm in diameter, fibroblasts migrate and colonise the dermal substrate and further synthesise a new ECM, which forms a dense, non-contractile dermal equivalent (BERTHOD *et al.*, 1993). First, adult skin melanocytes and keratinocytes were seeded at a ratio 1:10 on a foreskin fibroblast feeder layer in DMEM/MCDB 153 (1:1) with cholera toxin (10^{-10} M), TPA (4 nmol/litre), bFGF (0.25 ng/ml) and bovin pituitary extract (25 μg/ml), forming an epithelial sheet. The dermal equivalent was epidermised by transfer of this epithelial sheet and the PSE was then lifted onto the air-liquid interface and cultured in DMEM with bFGF (0.5 ng/ml), EGF (10 ng/ml) and cholera toxin (10^{-10} M) for two weeks (NAKAZAWA *et al.*, 1997).

By mimicking the normal epidermal environment, with growth of the cells at the air-liquid interface, the models allow the cells to grow, differentiate and finally form a skin tissue-like structure with multilayered differentiated epithelium and a well-organised extracellular matrix in the dermal region (Fig. 1).

Fig. 1 *Immunostaining of TRP-1 using MEL-5 anti-melanosomal protein gp75 of human skin (A) and pigmented skin equivalent (B) using a collagen GAG-Chitosane sponge as cell culture support. Melanocytes appear in black on basal layer*

4 Responses of PSEs to UV radiation: melanogenesis and melanin pigmentation

Exposure of the skin to UV radiation results in a deeper skin colour. Immediate darkening occurs within few seconds, delayed pigment formation or tanning (melanin formation) begins on the second or third day, and melanin migrates upwards in the epidermis several days later (SNELL, 1963). Tyrosinase and TRP-1 are the best-characterised melanogenic proteins associated with melanin synthesis. Regulation of the expression of TRP-1 and tyrosinase is independent (VIJAYASARADHI *et al.*, 1995) but they can interact (BOISSY *et al.*, 1996; ZHAO *et al.*, 1996), although the functional consequence

of this interaction is not yet fully understood. The activity of tyrosinase can be determined by measuring L-dopa and L-tryosine, which are two key substrates of tyrosinase (SLOMINSKI *et al.*, 1988). The morphological features of melanocytes are particularly important in melanin pigmentation: a brown colour is imparted to the skin by melanosomes within keratinocytes and not melanocytes loaded with melanosomes (FITZPATRICK *et al.*, 1983).

In guinea-pigs exposed to UV irradiation (230 nm, infra-red, 5 min/day for two weeks) melanocytes had enlarged perikarya and an increased number of enlarged dendrites were filled with melanin granules (SNELL, 1963). The morphological changes after UVB irradiation are most marked at day 5 or 7, but still persist at day 10 or 14 (JIMBOW *et al.*, 1975). The delayed response consistent with UVB-induced pigmentation is visible only after three days. The numbers of tyrosinase- and TRP-1-positive melanocytes increase significantly (from 4.0 ± 0.6 to 51 ± 9 and from 48 ± 10 to 130 ± 3 per 500 basal keratinocytes in normal human skin, respectively), and the intensity of cellular staining also increased after UVB irradiation (0.5 minimal erythema dose per day for five days) (TOBIN *et al.*, 1994).

Exposure of the PSE established on collagen gel to UVB radiation (4×10^4 and 8×10^4 erg/cm^2/day) for 14 days stimulated and enhanced the pigment transfer from melanocytes to keratinocytes. Melanosomes, identified by their pigment and by dopa oxidase staining, were dispersed throughout the keratinocyte cytoplasm (TOPOL *et al.*, 1986). BERTAUX *et al.* (1988) reported that treatment of the PSE with 8-MOP + UVA (320–400 nm, 500 mJ/cm^2) or UVB (313 nm, 200 mJ/cm^2) did not modify the surface colour of the epidermis, but the dopa-positive melanocytes were more numerous and the dendritic melanocytes were denser than in controls.

Although the PSE established on deepidermised dermis became more heavily pigmented affer UV irradiation (total UVB, 4760 J/m^2 over three days), the quantity and distribution of melanin at the light microscopic level appeared to be unchanged. However, the numbers of dendrites and of melanocytes increased, as did their staining with dopa and TRP-1 (TODD *et al.*, 1993). ARCHAMBAULT *et al.* (1995) reported that melanocytes in UV-irradiated PSE (solar simulator, 285 ± 5 nm, 2.5–10 mJ/cm^2), developed many more dendrites than sham-irradiated cells. More melanin was consistently produced in melanocytes in the PSE than in those in monolayer culture, and the most melanin was found in melanocytes in the PSE containing both keratinocytes and fibroblasts, strongly suggesting an influence of neighbouring keratinocytes and fibroblasts. BESSOU *et al.* (1996) has shown that epidermal reconstructs with keratinocytes and melanocytes obtained from different skin phototypes (I to IV) reproduce the initial phototype, with few modifications, and that the intensity of melanin transfer correlates with the situation *in vivo* after five consecutive UVB irradiations (312 nm, 150 mJ/cm^2). After exposure of the non-contractile PSE to UVB (312 nm, 100 mJ/cm^2/day) for seven consecutive days, the intensity of TRP-1 staining was increased (NAKAZAWA *et al.*, 1997).

In these PSEs, the functional activity of the melanin pigmentary system, melanogenesis (melanin production) and melanin pigmentation (melanosome transfer to keratinocytes) are maintained in a manner comparable to that *in vivo*. The results thus confirm the validity of the models for further use.

5 Response of PSEs to UV radiation: melanocyte proliferation

Skin colour can become darker with or without an increase in the number of melanocytes.

In a guinea-pig irradiated with UV (230 nm, infra-red, 5 min/day for two weeks), the total number of melanocytes (estimated by dopa reaction) was greatly increased (SNELL, 1963). In contrast, in mouse skin exposed repeatedly to UVB radiation (290–340 nm, 0.1 J/cm^2 per day for 16 days), increased incorporation of ^{3}H-methylthymidine into epidermal melanocytes was found, indicating enhanced melanocyte proliferation (ROSDAHL and SZABO, 1978). In irradiated human skin (304 nm, 80–200 mJ/cm^2 and 365 nm, 50–150 J/cm^2), the ratio of dopa-positive melanocytes to keratinocytes increased from 1:12–1:18 to 1:5–1:7 (ROSEN *et al.*, 1987). An increase in the number of dopa-positive melanocytes was also observed in shielded human skin after UVB irradiation (270–380 nm, three times/week for 17 days) (STIERNER *et al.*, 1989).

BERTAUX *et al.* (1988) reported that only melanocytes obtained from donors under two years of age could grow *in vitro* in the PSE established on collagen gel, even after stimulation with 8-MOP + UVA (320–400 nm, 500 mJ/cm^2) or UVB (313 nm, 200 mJ/cm^2) five times every two days. Another report showed that the viability of cells was decreased after seven daily UV irradiations (solar simulator, 285 ± 5 nm, 2.5–10 mJ/cm^2), whereas melanin production was increased (ARCHAMBAULT *et al.*, 1995). In the PSE model established on de-epidermised dermis, the number of melanocytes was not increased after UVB irradiation (TODD *et al.*, 1993). BESSOU *et al.* (1996) showed, however, an increase in the number of dopa-positive melanocytes in various reconstructed phototypes after five consecutive UVB irradiations (312 nm, 150 mJ/cm^2), except for phototype I involving a mixed medium with MCDB-153 and DMEM, and suggested augmentation of the mitotic activity of melanocytes. In the non-contractile PSE, the mean melanocyte to basal keratinocyte ratio was 3.14–3.47:40, and this ratio was not changed after UVB treatment (NAKAZAWA *et al.*, 1997).

Melanocyte survival in PSEs seems to be strongly affected by the seeding ratio and culture conditions. Moreover, individual and regional anatomical differences may exist in the responses of the skin pigmentary system to UV radiation (SNELL, 1963; QUEVEDO *et al.*, 1965). In human skin, the UV-induced increase in the number of melanocytes may be due to activation of 'silent melanocytes' (STIERNER *et al.*, 1989), 'dormant melanocytes' (PATHAK *et al.*, 1965; TOBIN *et al.*, 1994) or 'initially dopa-negative melanocytes' (ROSEN *et al.*, 1987) as the number of melanocytes was usually estimated by tyrosinase activity in the dopa reaction.

MISHIMA and WILDEN (1967) proposed the use of a 'combined dopa-premelanin reaction' and electron microscopy to distinguish enzymatically inactive (with no or minimal tyrosinase) and structurally inactive (with few premelanosomes) melanocyte populations from active (melanosome and tyrosinase synthesising) melanocytes. The formation of premelanosomes is independent of tyrosinase synthesis and of the deposition of melanin, athough these process are normally coordinated in the cytoplasm of the melanocyte. With this method, it was shown that the melanocyote population of the skin is consistently larger than that revealed by the dopa reaction, showing that enzymatically inactive melanocytes exist in significant numbers in normal skin. Therefore, UV irradiation may act by both increased tyrosinase synthesis and induced processes of premelanosome formation.

PATHAK *et al.* (1965) suggested that an increase in the number of dopa-positive melanocytes is not needed to increase the degree of pigmentation in the skin: melanocytes may have greater functional activity, such as a larger cell body and longer dendrites loading pigment granules, to increase pigmentation. Furthermore, it has been suggested that the increased number of melanocytes in normal skin leads to neoplastic changes in some individuals (FITZPATRICK *et al.*,

1983). The equilibrium between proliferation and differentiation is precarious; it can be studied by combined analysis of both using distinct methods.

Melanocyte migration is also stimulated by UV irradiation. After stimulation with UVB or 8-MOP + UVA, melanocytes grew out of a biopsy sample (BERTAUX *et al.*, 1988). Melanocyte migration from the centre to the periphery of the keratinocyte layer was also stimulated by UVB irradiation (ARCHAMBAULT *et al.*, 1995). These responses of melanocytes to UV radiation must also be taken into account.

6 Other responses of PSEs to UV radiation

Histochemical analysis of UV-irrradiated human skin demonstrates a distinctive pattern of damage, including the appearance of 'sunburn cells' with characteristic eosinophilic cytoplasm and pyknotic nuclei (DANIELS *et al.*, 1961). The cumulative effects of repeated exposures to UVB and UVA (solar simulated radiation, 290–400 nm and 320–420 nm, 28 times daily) are the presence of epidermal hyperplasia and stratum corneum thickening (LAVKER *et al.*, 1995). ROSEN *et al.* (1987) showed that sunburn cells appeared in human skin one day after exposure to 304 nm irradiation, but not on day 7 or day 14 or at any time after exposure to 365 nm. Another important effect of UV radiation on the skin is the alteration of the underlying dermis known as solar elastosis, which is characterised by decomposition of collagen fibres. Pigmented individuals show this change to a much lesser degree than red-headed people (FITZPATRICK *et al.*, 1983).

These responses have not been well documented in PSEs. ARCHAMBAULT *et al.* (1995) reported that cells in the upper layer had the appearance of sunburn cells, but there was no evidence of cellular necrosis in the other PSEs (BERTAUX *et al.*, 1988). In the non-contractile PSE, although there was no signficant change in the epidermal organisation, a dense stratum corneum was noted (NAKAZAWA *et al.*, 1997).

Thickening of the stratum corneum is the other potent protective factor after UV irradiation. This reaction allows longer retention of free melanin and prevents loss of melanin from the surface of skin (FITZPATRICK and SZABO, 1959; SNELL, 1963). The two factors therefore probably play a combined protective role.

7 Functional activity of PSEs

The stratus of keratinocyte differentiation, which can be modified by culture conditions, appears to affect various melanocyte functions. In addition, the mitotic activity of keratinocytes in the long-term is extremely important for studies of the pathogenesis and carcinogenesis of the skin *in vitro* (YUSPA and DLUGOSZ, 1991). In neonatal PSE, the mean melanocyte to basal keratinocyte ratio determined by HMB-45 staining was 1:31.2, and the melanocytes were individually distributed on the basal epidermal layer. The radio in foetal skin equivalent was 1:3.3, and the melanocytes were grouped both basally and suprabasally. Nevertheless, both models parallel the situation *in vivo* (HAAKE and SCOTT, 1991). Because mitoses are generally restricted to the basal layer in the normal neonatal epidermis, whereas there is proliferative activity throughout the foetal epidermis, the distribution of melanocytes is probably related to compartmentalisation of proliferating keratinocytes within the epidermis. When cultured PSE to athymic mice, however, differentiated epithelium was better able to support melanisation of the skin than undifferentiated epithelium (BOYCE *et al.*, 1993).

Autoradiographic analysis after incorporation of radioactive thymidine or immunohistochemical analysis with specific antibody after BrdU-labelling are usually used to identify proliferating cells in the S phase in tissues. Alternatively, immunohistochemical identification of cycling cells by Ki67 is suited for comaprative studies as it does not require prelabelling or radioactivity.

Very few studies have been performed on this aspect of PSEs. In the skin equivalent on collagen gel, the percentage of nuclei of basal cells labelled with BrdU decreased rapidly from 48% on day 6 to 5.6% on day 12 (TSUNENAGA *et al.*, 1994). In another experiment, keratinocytes cultured with serum-free medium in the modified collagen gel model had a thymic labelling index of 11.9% on day 14 (2–4% in normal human epidermis), suggesting a hyperproliferative phenotype (CHEN *et al.*, 1995). In the non-contractile PSE, $18.1 \pm 7.4\%$ of cells within the basal layer were stained with Ki67 by day 14, indicating mitotic activity very similar to that of basal cells in normal human epidermis ($17.6 \pm 6.6\%$) (NAKAZAWA *et al.*, 1997). It has been reported that 1–3% of all epidermal cells were Ki67-positives (SOINI *et al.*, 1994). KNAGGS *et al.* (1994) showed an increase in Ki67-positive cells ($25.3 \pm 6.8\%$) in epidermis surrounding inflamed lesions from acne patients ($5.3 \pm 3.4\%$ in basal cells in normal epidermis). The percentage of Ki67-positive proliferating cells was higher than that observed by radioactive thymidine or BrdU labelling, consistent with the presence of Ki67 nuclear antigen throughout the cell cycle (GARDES *et al.*, 1984).

Further study of the optimal culture conditions for proliferation of keratinocycles at an appropriate rate and for differentiation adequate to maintain epidermal integrity in the PSE will be indispensable for long-term experiments.

8 Conclusion

These results suggest that melanocytes in the PSEs can function in a manner similar to that *in vivo*, including the expression of a number of melanoma/melanocyte-associated antigens in the collagen gel model (HAAKE and SCOTT, 1991). The studies confirm the idea that isolated, recultivated melanocytes grow and regain certain specific differentiation functions.

Each model has some drawbacks, such as high contractile rate in the collagen gel model (TSUNENAGA *et al.*, 1994), relatively low contractile rate for cross-linked dermal substrates (BERTHOD *et al.*, 1993; BOYCE *et al.*, 1993), and the complete absence of dermal fibroblasts in the de-epidermised dermis model. The contractile rate in collagen gel was much improved in a recently modified model, however, and hyperproliferation was reported (CHEN *et al.*, 1995). The presence of both living fibroblasts and keratinocytes has been shown to be necessary for optimal melanocyte function, such as survival and melanin synthesis after UV irradiation (ARCHAMBAUT *et al.*, 1995), while the number and distribution of melanocytes were closely regulated by keratinocytes (SCOTT and HAAKE, 1991), even in the absence of dermis (DE LUCA *et al.*, 1988). Although de-epidermised dermis is one of the best dermal substrates, with a preserved, well organised basal lamina for epidermal differentiation, the presence of biologically active fibroblasts and dermal components might be important for studying the regulatory mechanisms of the skin pigment system.

9 Perspectives and future applications

Even though the models have some drawbacks, these PSE models are potentially useful, as they provide conditions in which cell–cell and cell–matrix interactions can be maintained

under conditions resembling those in tissue homeotaxis *in vivo*. BESSOU *et al.* (1997) have used their system to investigate reagents that can affect melanogenesis, such as isobutylmethylxanthine, 1-oleoyl–2-acetyl snglycerol and kojic acid, either by adding them to the culture medium or by applying them topically at the air-liquid interface. The various PSE models could be used not only for studying the molecular mechanisms that regulate normal melanocyte proliferation and differentiation, but also for investigating pathogenesis in the skin, including the progression of malignant melanoma, other disease developments (possibly using genetically altered skin cells) and therapeutic evaluation of new drugs.

Acknowledgments—This work was supported by Direction de la Recherche et de la Technologie, Centre National de la Recherche Scientifique and Hospices Civils de Lyon, France.

References

ABDEL-MALEK, Z., SWOPE, V. B., SUZUKI. I., ACKALI, C., HARRIGER, M. D., BOYCE, S. T., URABE, K., and HEARING, V. J. (1995): 'Mitogenic and melanogenic stimulation of normal human melanocytes by melanotropic peptides,' *Proc. Natl. Acad. Sci. USA*, **92**, pp. 1789–1793

ARCHAMBAULT, M., YAAR, M., and GILCHREST, B. A. (1995) 'Keratinocytes and fibroblasts in a human skin equivalent model enhance melanocyte survival and melanin synthesis after ultraviolet irradiation,' *J. Invest. Dermatol.*, **104**, pp. 859–867

ASSELINEAU, D., and PRUNIÉRAS, M. (1984): 'Reconstruction of "simplified" skin: control of fabrication,' *Br. J. Dermatol.*, **111**, pp. 219–222

BARKER, D., DIXON, K., MEDRANO, E. E., SMALARA, D., IM, S., MITCHELL, D., BABCOCK, G., and ABDEL-MALEK, Z. A. (1995): 'Comparison of the responses of human melanocytes with different melanin contents to UVB irradiation,' *Cancer Res.*, **55**, pp. 4041–4046

BELL, E., EHRLICH, H. P., BUTTLE, D. J., and NAKATSUJI, T. (1981): 'Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness,' *Science*, **211**, pp. 1052–1054

BELL, E., IVARSSON, B., and MERRILL, C. (1979): 'Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*,' *Proc. Natl. Acad. Sci. USA*, **76**, pp. 1274–1278

BERTAUX, B., MORLIERE, P., MORENO, G., COURTALON, A., MASSE, J. M., and DUBERTRET, L. (1988): 'Growth of melanocytes in a skin equivalent model *in vitro*,' *Br. J. Dermatol.*, **119**, pp. 503–512

BERTHOD, F., HAYEK, D., DAMOUR, O., and COLLOMBEL C. (1993): 'Collagen synthesis by fibroblasts cultured within a collagen sponge,' *Biomaterials*, **14**, pp. 749–754

BESSOU, S., PAIN, C., and TAIEB, A. (1997): 'Use of human skin reconstructs in the study of pigment modifiers,' *Arch. Dermat.*, **133**, pp. 331–336

BESSOU, S., SURLEVE BAZEILLE, J. E., PAIN, C., DONATIEN, P., and TAIEB, A. (1996): '*Ex vivo* study of skin phototypes,' *J. Invest. Dermatol.*, **107**, pp. 684–688

BOISSY, R. E., ZHAO, H., OETTING, W. S., AUSTIN, L. M., WILDERNBERG, S. C., BOISSY, Y. L., ZHAO, Y,. STURM, R. A., HEARING, V. J., KING, R. A., and NORDLUND, J. J. (1996): 'Mutation in and lack of expression of tyrosine-related protein-1 (TRP-1) in melanocytes from an individual with brown oculocutaneous albinism: a new subtype of albinism classified as "OCA3'," *Am. J. Hum. Genet.*, **58**, pp. 1145–1156

BOYCE, S. T., MEDRANO, E. E., ABDEL, M. Z., SUPP, A. P., DODICK, J. M., NORDLUND, J. J., and WARDEN, G. D. (1993): 'Pigmentation and inhibition of wound contraction by cultured skin substitutes with adult melanocytes after transplantation to athymic mice,' *J. Invest. Dermatol.*, **100**, pp. 360–365

BUFFEY, J. A., MESSENGER, A. G., TAYLOR, M., ASHCROFT, A. T. T., WESTGATE, G. E., and MACNEIL, S. (1994): 'Extracellular matrix derived from hair and skin fibroblasts stimulates human skin melanocyte tyrosinase activity,' *Br. J. Dermatol.*, **131**, pp. 1–7

CHAKRABORTY, A., SLOMINSKI, A., ERMAK, G., HWANG, J., and PAWELEK, J. (1995): 'Ultraviolet B and melanocyte-stimulating hormone (MSH) stimulate mRNA production for alphaMSH receptor and proopiomelanocortin-derived peptides in mouse melanoma cells and transformed kerationcytes,' *J. Invest. Dermatol.*, **105**, pp. 655–659

CHAKRABORTY, A. K., FUNASAKA, Y., SLOMINSKI, A., ERMAK, G., HWANG, J., PAWELEK, J. M., and ICHIHASHI, M. (1996): 'Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B,' *Biochim. Biophs. Acta.*, **1313**, pp. 130–138

CHEN, C. J., LAVKER, R. M., RODECK, U., RISSE, B., and JENSEN, P. J. (1995): 'Use of a serum-free epidermal culture medel to show deleterious effect of epidermal growth factor on morphogeneis and differentiation,' *J. Invest. Dermatol.*, **104**, pp. 107–112

COLLOMBEL, C., DAMOUR, O., GAGNIEU, C., POINSIGNON, F., ECHINARD, C., and MARICHY, J. (1987): 'Peau artificielle et son procédé de fabrication,' Brévet d'invention fransi n°87-08752, 15 June 1987, Brévet d'invention europién n°88-420194.8, 14 June 1988

COULOMB, B., SAIAG, P., BELL, E., BREITBURD, F., LEBRETON, C., HESLAN, M., and DUBERTRET, L. (1986): 'A new method for studying epidermalization *in vitro*,' *Br. J. Dermatol.*, **114**, pp. 91–101

DANIELS, F., BROPHY, D., and LODITZ, W. C. (1961): 'Histological responses of human skin following ultraviolet irradiation,' *J. Invest. Dermatol.*, **37**, pp. 351–357

DELUCA, M., D'ANNA, F,. BONDANZA, S., FRANZI, A. T., and CANCEDDA, R. (1988*a*): 'Human epithelial cells induce human melanocyte growth *in vitro* but only skin keratinocytes regulate its proper differentiation in the absence of dermis,' *J. Cell. Biol.*, **107**, pp. 1919–1926

DELUCA, M., FRANZI, A. T., D'ANNA, F., ZICCA, A., ALBANESE, E., BONDANZA, S., and CANCEDDA, R. (1988*b*): Coculture of human keratinocytes and melanocytes: differentiated melanocytes are physiologically ortganized in the basal layer of the cultured epithelium,' *Eur. J. Cell. Biol.*, **46**, pp. 176–180

DELUCA, M., SIEGRIST, W., BONDANZA, S., MATHOR, M., CANCEDDA, R., and EBERLE, A. N. (1993): 'α-melanocyte stimulating hormon (α-MSH) stimulates normal melanocyte growth by binding to high affinity receptors,' *J. Cell. Sci.*, **105**, pp. 1079–1084

DERBY, M. A. (1982): 'Environmental factors affecting neural crest differentiation: melanocyte differentiation by crest cells exposed to cell-free (deoxycholate-exracted) dermal mesenchyme matrix,' *Cell Tissue Res.*, **225**, pp. 379–386

EISINGER, M., and MARKO, O. (1982): 'Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin,' *Proc. Natl. Acad. Sci. USA*, **79**, pp. 2018–2022

ELLER, M. S., OSTROM, K., and GILCHREST, B. A. (1996): 'DNA damage enhances melanogenesis,' *Proc. Natl. Acad. Sci. USA*, **93**, pp. 1087–1092

ELLER, M. S., YAAR, M., and GILCHREST, B. A. (1994): 'DNA damage and melanogenesis, *Nature*, **372**, pp. 413–414

EPSTEIN, J. H. (1989): 'Photocarcinogenesis, skin cancer and aging' *In* KLIGMAN, B. A. K., and KLIGMAN, A. M. (Eds).: 'Aging and the skin' (Raven Press, New York), pp. 307–349.

FITZPATRICK, T. B., and SZABO, G. (1959): 'The melanocyte cytology and cytochemistry,' *J. Invest. Dermatol.*, **32**, pp. 197–209

FITZPATRICK, T. B., SZABO, S., and WICK, M. M. (1983), 'Biochemistry and physiology of melanin pigmentation;' *in* GOLDSMITH L. A. (Ed.) 'Biochemistry and physiology of the skin. Vol. 2 (Oxford University Press), pp. 687–712

FREEMAN, A. E., IGEL, H. J., HARRMAN, B. J., and KLEINFIELD, K. L. (1976): 'Growth and characterization of human skin epithelial cell culture,' *In Vitro*, **12**, pp. 352–362

FRIEDMAN, P. S., and GILCHREST, B. A. (1987) 'Ultraviolet radiation directly induces pigment production by cultured human melanocytes,' *J. Cell. Physiol.*, **133**, pp. 88–94

FUNASAKA, Y., BOULTON, T., and COBB, M. (1992): 'c-Kit-kinase induces a cascade of protein tyrosine phosphorylation in normal human melanocytes in responses to mast cell growth factor and simulates mitogen-activated protein kinase but is down-regulated by melanosomes,' *Mol. Biol. Cell*, **3**, pp. 197–209

FUSENIG, N. E. (1992): 'Cell interaction and epithelial differentiation' *in* FRESHNEY, R. I. (Ed.): 'Culture of epithelial cells' (Wiley-Liss, New York), pp. 25–57

GARDES, J., LEMKE, H., BAISCH, H., WACKER, H.-H., SCHWAB, U., and STEIN, H. (1984): 'Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67,' *J. Immunol.*, **133**, pp. 1710–1715

GILCHREST, B. A., ALBERT, L. S., KARASSIK, R. L., and YAAR, M. (1985): 'Substrate influences human epidermal melanocyte attachment and spreading *in vitro*, *In Vitro Cell. Dev. Biol.*, **21**, pp. 114–120

GILCHREST, B. A., ZHAI, S., ELLER, M. S., YAROSH, D. B., and YAAR, M. (1993): 'Treatment of human melanocytes and S91 melanoma cell with the DNA repaire enzyme T4 endonuclease V enhances melanogenesis after ultraviolet irradiation,' *J. Invest. Dermatol.*, **101**, pp. 666–672

HAAKE, A. R., and SCOTT, G. A. (1991): 'Physiologic distribution and differentiation of melanocytes in human fetal and neonatal skin equivalents,' *J. Invest. Dermatol.*, **96**, pp. 71–77

HALABAN, R., LANGDON, R., BIRCHALL, N., CUONO. C., BAIRD, A., SCOTT, G., MOELLMANN, G., and MCGUIRE, J. (1988) 'Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes,' *J. Cell. Biol.*, **107**, pp. 1611–1619

HALABANE, R., GHOSH, S., and BAIRD, A. (1987): 'bFGF is the putative natural growth factor for human melanocytes,' *In Vitro Cell. Dev. Biol.*, **23**, pp. 47–52

HALABANE, R., RUBIN, J. S., FUNASAKA, Y., COBB, M., BOULTON, T., FALETTO, D., ROSEN, E., CHAN, A., YOKO, K., WHITE, W., COOK, C., and MOELLMAN, G. (1992): 'Met and hepatocyte growth factor/scatter factor signal transduction in normal melanocytes and melanoma cells,' *Oncogene*, **7**, pp. 2195–2206

HARA, M., YAAR, M., and GILCHREST, B. A. (1995): 'Endothelin-1 of keratinocyte origin is a mediator of a melanocyte dendricity,' *J. Invest. Dermatol.*, **105**, pp. 744–748

HEDLEY, S., GAWKRODGER, D. J., WEETMAN, A. P., and MACNEIL, S. (1996): 'Investigation of the influence of extracellular matrix proteins on normal human melanocyte tyrosinase activity,' *Br. J. Dermatol.*, **135**, pp. 888–897

HEDLY, S., WAGNER, M., BIELBY, S., SMITH-THOMAS, L., GAWKRODGER, D. J., and S., M. N. (1997): 'The influence of extracellular matrix proteins on cutaneous and uveal melanocytes,' *Pigment Cell. Res*, **10**, pp. 54–59

HERLYN, M., MANCIANTI, M., JAMBROSIC, J., BOLEN, J. B., and KOPROWSKI, H. (1988): 'Regulatory factors that determine growth and phenotype of normal human melanocytes,' *Exp. Cell. Res.*, **179**, pp. 322–331

IMOKAWA, G., YADA, Y., and MIYAGISHI, M. (1992): 'Endothelins secreted from keratinocytes are intrinsic mitogens for human melanocytes,' *J. Biol. Chem.*, **267**, pp. 24657–24680

JIMBOW, K., SZABO, G., PATHAK, M. A., and FITZPATRICK, T. B. (1975) 'Ultrastructural changes in human melanocytes after ultraviolet irradiation, *in* PATHAK, M. A. (Ed.): 'Sunlight and man: normal and abnormal photobiologic responses' (University of Tokyo Press), pp. 195–215

KNAGGS, H. E., HOLLAND, D. B., MORRIS, C., WOOD, E. J., and CUNLIFFE, W. J. (1994): 'Quantification of cellular proliferation in acne using the monoclonal antibody Ki-67,' *J. Invest. Dermatol.*, **102**, pp. 89–92

LAVKER, R. M., GERBERICK, G. F., VERES, D., IRWIN, C. J., and KAIDBEY, K. H. (1995): 'Cumulative effects from repeated exposures to suberythermal doses of UVB and UVA in human skin,' *J. Am. Acad. Dermatol.*, **32**, pp. 53–62

MCCLENIC, B. K., MITRA, R. S., RISER, B. L., NICKOLOFF, B. J., DIXIT, V. M., and VARANI, J. (1989) 'Production and utilization of extracellular matrix components by human melanocytes,' *Exp. Cell. Res.*, **180**, pp. 314–25

MISHIMA, Y., and WIDLEN, S. (1967): 'Enzymatically active and inactive melanocyte populations and ultraviolet irradiation: combined dopa-premelanin reactions and electron microscopy,' *J. Invest. Dermatol.*, **49**, 273–281

MORELLI, G. J., YOHN, J. J., LYONS, M. B., MURPHY, R. C., and NORRIS, D. A. (1989): Leukotrienes C4 and D4 as potent mitogens for cultured human neonatal melanocytes,' *J. Invest. Dermatol.*, **93**, pp. 719–722

NAKAZAWA, K., NAKAZAWA, H., COLLOMBEL, C., and DAMOUR, O. (1995): 'Keratinocyte extracellular matrix-mediated regulation of normal human melanocyte functions,' *Pigment Cell Res.*, **8**, 10–18

NAKAZAWA, K., NAKAZAWA, H., SAUC, F., LEPAVEC, A., COLLOMBEL, C., and DAMOUR, O. (1997): 'Pigmented human skin equivalent—mew method of reconstitution by grafting an epithelial sheet onto a non-contractile dermal equivalent,' *Pigment Cell Res.*

NAUGHTON, G. K., JACOB, L., and NAUGHTON, B. A. (1989): 'A physiological skin model for in vitro toxicity studies. In A. M. GOLDBERG (Ed.) '*In vitro* toxicology new directions. Vol. 7' (Mary Ann Liebert Inc., New York), pp. 183–189

NORDLUND, J. J., COLLINS, C. E., and RHEINS, L. A. (1986): 'Prostaglandin E2 and D2 but not MSH stimulate the proliferation of pigment cells in the pinnal epidermis of the DBA/2 mouse,' *J. Invest. Dermatol.*, **86**, pp. 433–437

PATHAK, M. A,. SINESI, S. J., and SZABO, G. (1965): 'The effect of a single dose of ultraviolet radiation on epidermal melanocytes,' *J. Invest. Dermatol.*, **45**, pp. 520–528

PITTELKOW, M. R., and SHIPLEY, G. D. (1989): 'Serum-free culture of normal human melanocytes: growth kinetics and growth factor requirements,' *J. Cell. Physiol.*, **140**, pp. 565–576

PRUNIÉRAS, M., RÉGNIER, M., and WOODLEY, D. (1983): 'Methods for cultivation of keratinocytes with an air-liquid interface,' *J. Invest. Dermatol.*, **81**, pp. 28s–33s

QUEVEDO, W. C., SZABO, G., VIRKS, J., and SINESI, S. J. (1965): 'Melanocypte populations in UV-irradiated human skin,' *J. Invest. Dermatol.*, **45**, pp. 295–298

RANSON, M., POSEN, S., and MASON, R. S. (1988): 'Extracellular matrix modulates the function of human melanocytes but not melanoma cells,' *J. Cell. Physiol.*, **136**, pp. 281–288

ROSDAHL, I. K., and SZABO, G. (1978): 'Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin,' *J. Invest. Dermatol.*, **70**, pp. 143–148

ROSEN, C. F., SEKI, Y., FARINELLI, W. STERN, R. S., FITZPATRICK, T. B., PATHAK, M. A., and GANGE, R. W. (1987): 'A comparison of the melanocyte response to narrow band UVA and UVB exposure *in vivo*,' *J. Invest. Dermatol.*, **88**, pp. 774–779

SCHAUER, E., TRAUTINGER, F., KOCK, A., SCHWARTZ, A., BHARDWAJ, R., SIMON, M., ANSEL, J. C., SCHWARZ, T., and LUGER, T. A. (1994): 'Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes,' *J. Clin. Invest.*, **93**, 2258–2262

SCOTT, G., LIANG, H., and LUTHRA, D. (1996): 'Stem cell factor regulates the melanocyte cytoskeleton,' *Pigment Cell Res.*, **9**, pp. 134–141

SCOTT, G., RYAN, D. H., and MCCARTHY, J. B. (1992): 'Molecular mechanisms of human melanocyte attachment to fibronectin,' *J. Invest. Dermatol.*, **99**, pp. 787–794

SCOTT, G. A., and HAAKE, A. R. (1991): 'Keratinocytes regulate melanocyte number in human fetal and neontal skin equivalents,' *J. Invest. Dermatol.*, **97**, pp. 776–781

SCOTT, G. C. L., and BUSACCO, A. (1997): 'Fibronectin suppresses apoptosis in normal human melanocytes through an integrin-dependent mechanism,' *J. Invest. Dermatol.*, **108**, pp. 147–153 (1997)

SLOMINSKI, A., MOELLMANN, G., KUKLINSKA, E., BOMIRSKI, A., and PAWELEK, J. (1988): 'Positive regulation of melanin pigmentation by two key substrates of the melanogenic pathway, L-tyrosine and L-dopa,' *J. Cell. Sci.*, **89**, pp. 287–296

SNELL, R. S. (1963): 'The effect of ultraviolet irradiation on melanogenesis,' *J. Invest. Dermatol.*, **40**, pp. 127–132

SOINI, Y, KAMEL, D., PAAKKO, P., LEHTO, V. P., OIKARINEN, A., and VAHAKANGAS, K. (1994): 'Aberrant accumulation of p53 associates with Ki67 and mitotic count in benign skin legions,' *Br. J. Dermatol.*, **131**, pp. 514–520

STIERNER, U., ROSDAHL, I., AUGUSTSSON, A., and KAGEDAL, B. (1989): 'UVB irradiation induces melanocyte increase in both exposed and shielded human skin,' *J. Invest. Dermatol.*, **92**, pp. 561–564

SWOPE, V. B., ABDEL-MALEK, Z., KASSEM, L. M., and NORDLUND, J. J. (1991): 'Interleukins 1 alpha and 6 and tumor necrosis factor-alpha are paracrine inhibitors of human melantocyte proliferation and melanogenesis,' *J. Invest. Dermatol.*, **96**, pp. 180–185

TOBIN, D., QUINN, A. G., ITO, S., and THODY, A. J. (1994): 'The presence of tyrosinase and related proteins in human epidermis and their relationship to melanin type,' *Pigment Cell Res.*, **7**, pp. 204–209
TODD, C., HEWITT, S. D., HEMPENAAR, J., NOZ, K., THODY, A. J., and PONEC, M. (1993): 'Co-culture of human melanocytes and keratinocytes in a skin equivalent model: effect of ultraviolet radiation,' *Arch. Dermatol. Res.*, **285**, pp. 455–459
TOMITA, Y, IWAMOTO, M., MASUDA, T., and TAGAMI, H. (1987): 'Stimulatory effect of prostaglandin E2 on the configuration of normal human melanocytes *in vitro*' *J. Invest. Dermatol.*, **89**, pp. 299–301
TOMITA, Y., TORINUKI, W., and TAGMI, H. (1988): 'Stimulation of human melanocytes by Vitamin D3 possibly mediates skin pigmentation after sun exposure, *J. Invest. Dermatol.*, **90**, pp. 882–884
TOPOL, B. M., HAIMES, H. B., DUBERTRET, L., and BELL, E. (1986): 'Transfer of melansomes in a skin equivalent model *in vitro*,' *J. Invest. Dermatol.*, **87**, pp. 642–647
TSUNENAGA, M., KOHNO, Y., HORII, I., YASUMOTO, S., HUH, N. H., TACKIKAWA, T., YOSHIKI, S., and KUROKI, T. (1994): 'Growth and differentiation properties of normal and transformed human keratinocytes in organotypic culture,' *Jpn. J. Cancer Res.*, **85**, pp. 238–244
VALYI,. N. I., and HERLYN, M. (1991) 'Regulation of growth and phenotype of normal human melanocytes in culture, *Cancer Treat. Res.*, **54**, pp. 85–101
VIJAYASARADHI, S., DOSKOCH, P. M., WOLCHOK, J., and HOUGHTON, A. N. (1995): 'Melanocyte differentiation marker gp75, the brown locus protein, can be regulated independently of tyrosinase and pigmentation,' *J. Invest. Dermatol.*, **105**, pp. 113–119
WILKINS, L., GILCHREST, B. A., SZABO, G., WEINSTEIN, R., and MACIAG, T. (1985): 'The stimulation of normal human melanocyte proliferation *in vitro* by melanocyte growth factor from bovine brain,' *J. Cell Physiol.*, **122**, pp. 350–61
YAAR, M., and GILCHREST, B. A. (1991): 'Human melanocyte growth and differentiation: a decade of new data,' *J. Invest. Dermatol.*, **97**, pp. 611–617
YAAR, M., GROSSMAN, K., ELLER, M., and GILCHREST, B. A. (1991): 'Evidence for nerve growth factior-mediated paracrine effects in human epidermis,' *J. Cell Biol.*, **115**, pp. 821–828
YADA, Y., HIGUCHI, K., and IMOKAWA, G. (1991): 'Effects of endothelins on signal transduction and proliferation in human melanocytes,' *J. Biol. Chem.*, **266**, pp. 18352–18357
YANNAS, I. V., and BURKE, J. F. (1980): 'Design of the artificial skin I. Basic design principals,' *J. Biomed. Mat. Res.*, **14**, pp. 65–81
YOHN, J. J., MORELLI, J. G., WALCHAK, S. J., RUNDELL, K. B., NORRIS, D. A., and ZAMORA, M. R. (1993): 'Cultured human keratinocytes synthesize and secrete endothelin-1,' *J. Invest. Dermatol.*, **100**, pp. 23–26
YUSPA, S. H., and DLUGOSZ, A. A. (1991): 'Cutaneous carcinogenesis: natural and experimental' *in* GOLDSMITH, L. A. (Ed.): 'Physiology biochemistry and molecular biology of the skin' (Oxford University Press), pp. 1365–1402
ZHAO, H., ELING, D. J., MEDRANO, E. E., and BOISSY, R. E. (1996): 'Retroviral infection with human tyrosinase-related protein-1 (TRP-1) cDNA upregulates tyrosinase activity and melanin synthesis in a TRP-1-deficient melanoma cell line,' *J. Invest. Dermatol.*, **106**, pp. 744–752

Author's biography

ODILE DAMOUR was born in Noyon, France, in 1949. She received her diploma in pharmacy in 1973 and in immunotoxicology-immunopharmacology in 1982. From 1975 to 1980 she studied for certificates in haematology, biochemistry, general immunology, medical pathology, general bacteriology and virology. She obtained her PhD in pharmaceutical sciences in 1985 from Lyon. She received her certificate in cellular biology in 1992. She is supervisor of the Laboratoire des Substituts Cutanés in the department of Pr. C. Collombel at the Hôpital Edonard Herriot.