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UV guided dendritic growth patterns and the networking of melanocytes

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Abstract. Whole skin organ cultures of vitiliginous skin show that the marginal melanocytes are highly sensitive to a pulse of UV exposure (210–380 nm) during the G_2 phase of the cell cycle, as seen by prominent dendricity. Melanocytes are highly dendritic in the epidermis overlying rapidly growing tumors, as well as within proliferative lesions such as basal cell carcinomas and aggressive seborrheic keratosis. In the organ cultures the dendrites extend towards the source of UV, i.e. the surface, while the main body lies along the basement membrane. The epidermal melanocytes overlying tumors lie almost vertically, dendrites aligned towards the underlying tumor on one side and the surface on the other. Within tumors dendritic elongation is guided by mitotic and PCNA positive (S-phase) tumor cells, which are a source of ultraweak UV emissions in the range of 210–330 nm. These observations indicate that ultraweak biophoton emissions from neighbouring cells can simulate environmental cues and contribute to the plasticity of networks such as the melanocytes or the visual pathways.
Key words. Dendrites; directional growth; UV exposure; ultraweak biophoton emission; plasticity; networking.

Melanocytes have a bimodal morphology, varying from dendritic to non-dendritic[1]. The highly dendritic melanocytes are seen in a variety of lesions and also in a proportion of vitiligo cases[2,3]. Melanocytes are very sensitive to UV, showing prominent dendricity on exposure[4,5]. The melanocytes form a UV-sensitive neuron-like network in the skin. A prominent network of highly dendritic melanocytes is seen in some proliferative lesions. Since melanocyte dendricity is an expression of UV-sensitivity in G_2-phase, this study investigates the orientation and growth direction of the dendritic processes of the melanocytes and the importance of ultraweak ultraviolet biophoton emissions by proliferating cells.

Materials and methods

Several proliferative lesions containing highly dendritic melanocytes have been used in the study. These include: 2 pigmented basal cell carcinoma (BCCa), 2 seborrheic keratosis (one of which is rapidly growing with local infiltration), the surface epidermis overlying malignant tumors with a high proliferative index (including melanomas), and marginal melanocytes in vitiligo exposure to UV in G_2-phase.

UV exposure. Whole skin organ culture was done using biopsies from 11 cases of vitiligo. The tissues were transported in MEM medium. These were cut under sterile conditions into 3 pieces each, 2 mm in width including the marginal zone between the pigmented and vitiliginous areas. One piece was fixed in cold formol-glutaraldehyde, the rest were incubated at 37 °C in MEM containing 200 µg/ml of adriamycin. One of these pieces was incubated in the dark while the other was exposed to a 120 s pulse of UV after 2 h of incubation. Both pieces were harvested at 5 h.

Specifications of UV tube: 15 W, emitting 210–380 nm, with a photometric reading of 12.9 mV at a distance of 30 cm (site of specimen) so that the resultant energy supplied to each melanocyte is 9.71×10^{-5} J (451 J/cm^2).

Histology and immunohistochemistry. The tissues were fixed overnight in cold buffered formol-glutaraldehyde and 5 µm thick serial sections were cut at −25 °C on a Lipshaw Cryostat. Histochemical and immunohistochemical characterization was done with the following stains: HE, catecholoxidase using dopa as substrate, and mAbs PCNA (DAKO). Camera lucida (CL) diagrams were drawn from dopa-stained sections as well as those stained with PCNA to study the orientation of the dendrites. Only CL diagrams are shown since the PCNA-positive nuclei are overlapped by the dendrites which are at a different focus in the frozen sections. On CL the dendrite can be followed at different foci to show the true relationship.

Results

Vitiligo. The melanocytes show a prominent increase in dendricity 3 h after exposure to a pulse of 120 s UV when in G_2-phase, while they are non-dendritic after dark incubation. All melanocytes lie on the basement membrane and the dendrites extend into the upper layers of the epidermis towards the source of UV, through the interstices between the intercellular bridges. Growth cones, as in neurites, are evident (fig. 1).

Epidermal melanocytes overlying tumors (basal cell carcinoma and melanomas). The melanocytes are highly dendritic, the cell bodies arranged vertically within the basement membrane (fig. 2). Dendrites have growth cones which extend towards basal cells showing nuclear PCNA positivity, as well as towards the underlying tumor growth and upwards towards the surface. This

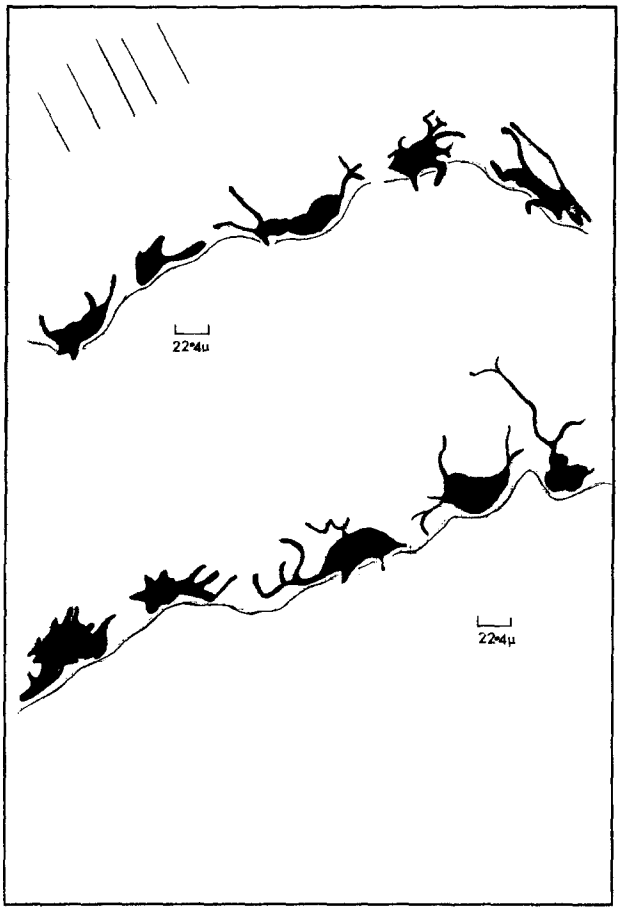

Figure 1. Marginal zone melanocyte in vitiligo showing dendrites extending towards the external source of UV [dopa × 200,000 – Camera lucida (CL)].

Figure 2. Vertically oriented melanocytes within the epidermis overlying a rapidly proliferating melanoma [PCNA × 200,000 – CL]: ⏲, ● PCNA + ve S-phase nucleus.

orientation is lost in the epidermis at some distance from the tumor growth.

Seborrheic keratosis. In an aggressive seborrheic keratosis with local infiltration, many of the proliferative keratinocytes show nuclear PCNA positivity. In addition, mitotic activity is high. On dopa-staining the melanocytes are highly dendritic (fig. 3). Only a few melanocytes show nuclear PCNA positivity. In comparison, the melanocytes within a classical seborrheic keratosis taken from the same patient (fig. 4) are non-dendritic, and the basaloid keratinocytes are negative for PCNA.

Pigmented basal cell carcinoma. The proliferating basal cells show prominent nuclear PCNA positivity as well as numerous mitoses. The melanocytes are amelanotic but are highly dendritic on dopa-staining, the nuclei being mostly negative for PCNA (figs. 5, 6).

Orientation of dendritic processes. On PCNA staining the highly dendritic melanocytes show cytoplasmic positivity. This fact has been utilized to study the orientation of dendrites.

In the UV-exposed whole skin organ culture the dendrites extend through the intercellular spaces towards the surface in the direction of UV exposure (fig. 1). The epidermal melanocytes overlying tumors show a vertical orientation, with dendrites extending towards the surface and the underlying tumor (fig. 2).

In the proliferative lesions, i.e. the aggressive seborrheic keratosis and the basal cell carcinomas, the melanocyte dendrites extend towards cells showing PCNA-positive nuclei and mitotic figures (figs 3, 6). The extension of the dendrites follows a path marked by the PCNA-positive cells and mitotic figures.

Discussion

As seen in earlier studies the melanocytes are highly sensitive to UV exposure. It is observed that G_2 phase dendricity is a function of melanocyte UV sensitivity. They become highly dendritic and show neuron-like differentiation[4]. It has also been observed that there is a correlation between the cell size, dendricity and the amount of photic input[5].

In the present study, it was shown that melanocytes become highly dendritic within proliferative lesions or within the epidermis overlying tumors as they do when exposed to a pulse of UV.

Figure 3. Highly dendritic melanocytes forming a network within an aggressive seborrheic keratosis [PCNA × 2000,000 – CL] ①, ● PCNA + ve S-phase nucleus.

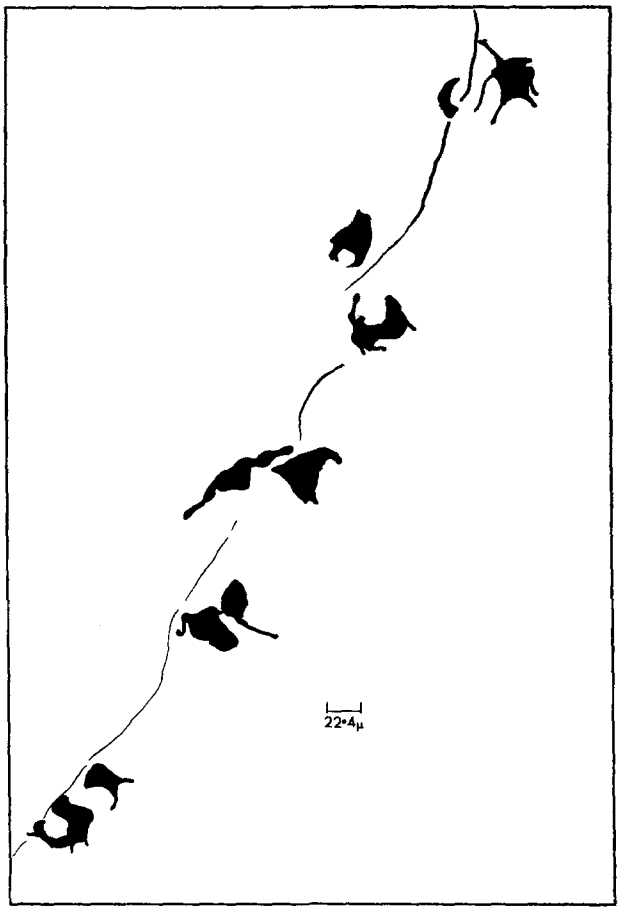

Figure 4. Non-dendritic melanocytes in a conventional seborrheic keratosis in the same patient [dopa × 200,000 – CL].

Since the melanocyte can respond to UV exposure with dendritic outgrowth this study provides circumstantial evidence that they are sensitive to the ultraweak UV emissions from the cells in S/G_2 and mitotic phase as identified by PCNA positivity. The melanocytes are themselves in the S/G_2 phase, as seen by their cytoplasmic positivity[7].

The study of biophoton emissions[8] is an emerging field, although many reports have been in existence since the beginning of the century. Substantial work has been done in the field by Russian and European workers although several controversies exist.

Many studies have indicated the emission of 'mitogenetic irradiation' from proliferating cells of several species[9–13]. Earlier work by Gurwitsch[9] showed that there is an emission of UV during the mitotic phase in several species of bacteria. Recent work has confirmed this by sensitive assessment of the photoemission, using photomultiplier tubes[10]. Gurwitsch[9] claimed that dividing cells emit very weak UV light which can itself stimulate division in other cells if incident upon them at the proper stage of their cycle. Konev and his group[10] indicated that this emission was in the 210–330 nm range during the exponential growth phase in synchronised cultures of several bacterial and yeast species. Visible emission (450–620 nm) can be detected in the stationary phase. The spectral emission of UV in the growth phase is similar across the genera and phyla. This indicates that DNA is the source of this UV and acts as a 'photon store'[12]. It is interesting that the DNA absorption spectrum is also in the same range of 210–330[13].

From the present study, it appears that the plasticity of the melanocytes and their dendritic activity, specifically the directional growth, is dependent on a source of UV emission within the 210–380 nm range and a chemical milieu arresting them in the S/G_2 phase. Both external UV emission, as in sunlight or experimental exposure, as well as biophoton UV emission by mitotic cells, mould the path followed by the extending dendritic process.

This finding is of interest since melanocyte dendricity is associated with neuron-like appearance and noradrenalin secretion[1,4]. Several aspects of neuronal plasticity during embryogenesis and in adults is akin to this. Embryological maturation of visual pathways depend on photic inputs so that the final connectivity is estab-

Figure 5. Highly dendritic melanocytes forming intricate patterns within the sheets of basal cell carcinoma [dopa × 200,000 – CL].

Figure 6. Melanocytes dendrites are extended and moulded in relation to S-phase and mitotic cells as brought out on PCNA staining. [PCNA × 200,000 – CL] ①, ●: PCNA + ve S-phase nucleus: ⊛ Mitotic figure.

lished on exposure to light/UV[15]. Since mitotic activity is high and regulated in the CNS during embryogenesis, axonal and dendritic extensions may depend on the patterns of the cell cycles and mitoses in the surrounding cells, as observed in this study.

Similarly, neuronal plasticity is well known in adult auditory/vocal pathways in birds where there is a seasonal variation in the dendricity of neurons with a change in bird-song[16,17]. The addition of new cells is attributed to androgen secretions. As observed earlier[5] variations in the length of UV exposure bring about changes in dendricity of melanocytes similar to seasonal changes. Complicated hormonal fluctuations associated with coat-colour changes are found in nature[18]. Thus, melanocytic and selected neuronal plasticity are dependent on environmental cues such as day length and UV exposure. The intricate moulding of the neuronal and melanocytic morphology might be regulated by selective biophoton emissions from adjacent cell groups which are in different phases of the cell cycles. Since the emissions vary with the phase of the cell cycle the local milieu can fine tune the chemical as well as the photic components, leading to responses by sensitive cell groups.

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