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MELANOCYTE MITOSIS IN UVB-IRRADIATED MOUSE SKIN

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Abstract. Little is known about the mitotic activity of the epidermal melanocyte population. Vincristine sulphate has now been used in vivo to study the frequency of mitosis of epidermal melanocytes in the ear skin of C57Bl mice. It is shown that there is continuous formation of new melanocytes in unstimulated skin due to mitosis of pigment-producing epidermal melanocytes. The increase in this mitotic rate following daily UVB irradiation suggests that this is a major mechanism responsible for the UV-induced increase in the epidermal melanocyte population.

Key words: Melanocyte; Mitosis; UVB irradiation; Vincristine sulphate

Ultraviolet (UVB) irradiation induces marked alterations in the epidermal pigmentary system. Following irradiation there is an increase in tyrosinase activity in single pigment cells, giving an increased production of melanin. In addition, repeated UVB irradiation induces an increase in the number of epidermal melanocytes (9, 13, 14). Since dividing melanocytes have only rarely been observed in vivo (6, 8, 13, 14) it has been suggested that this increase is due to activation of pre-existing amelanotic epidermal melanocytes, which become DOPA-positive and visible through stimulation of a resting tyrosinase system (13, 14). Migration of dermal melanocytes into the epidermis is another mechanism which has been thought to account for the increase in the melanocyte population (13, 14). Using cumulative labelling with ^{3}H-methylthymidine Rosdahl and Szabó recently showed that there is a turnover of melanocytes in the unstimulated epidermis of the mouse ear and that the UVB-induced increase in the melanocyte population is the result of cell division (11, 12).

In the present study, vincristine sulphate (VCR), which arrests dividing cells in metaphase, has been used to determine whether the new melanocytes are formed by division of melanin-producing melanocytes or by mitosis of postulated tyrosinase negative precursor cells (13, 14).

MATERIALS AND METHODS

Eleven-week-old male mice of a pigmented strain (C57Bl/6J, Jackson Laboratory, Maine) were used. This mouse strain has epidermal melanocytes in less hairy areas such as the tail, soles and ears (5). Six animals had the dorsal aspect of their right ear irradiated with 0.1 Joule/cm^2 daily at 9.30 a.m. as described in a previous study (12). The light source was a fluorescent sun lamp (Westinghouse FS, wave length spectrum 290–350 nm). The left ear was folded backwards, covered and kept as a control. The irradiation was repeated for 6 days, at which time there is a peak melanocyte proliferation rate under the experimental conditions used (12). On the seventh day at 8.30 a.m. the animals were injected intraperitoneally with vincristine sulphate (2 mg/kg body weight) and were sacrificed by cervical dislocation 3.5 hours later (1, 2, 3, 4, 18). The skin on the dorsal side of the ear was mechanically separated from the cartilage and incubated for 30 min in 37°C buffered L-3,4-dihydroxyphenylalanine (DOPA). After fixation and dehydration, the skin was embedded in paraffin. Four μm sections were cut and stained with hematoxylin-eosin. The relative melanocyte population density was estimated by counting the number of melanocytes per unit length of the skin in the section. To avoid double counts only every 5th section was counted. Three animals, irradiated as above, but not injected with VCR were subjected to the same procedure.

RESULTS

Macroscopical and microscopical changes induced in the mouse ear skin by 6 days of UVB irradiation were consistent with earlier findings (11, 12). In the present animals there was a 5–7-fold increase in the number of epidermal melanocytes in the irradiated ear as compared with corresponding fields from the control ear (Table I). These counts are only approximate due to the uneven distribution of epidermal melanocytes in the mouse ear. However, it indicates that the UVB irradiation induced an increase in the number of melanocytes comparable to that found in the previous study.

The melanocytes were located at the basal lamina, stained brownish due to the DOPA reaction and were densely packed with melanosomes. For melanocytes in interphase it was usually possible to

Table I. *Mitotic activity in control and irradiated mouse ear skin*

Animal	Increase in the melanocyte density by a factor of	Melanocyte mitosis/total		Basal keratinocyte mitosis/total	
		Irrad. ear	Control ear	Irrad. ear	Control ear
1	5.3	61/680 (9.0%)	6/400 (1.5%)	188/2 000 (9.4%)	33/2 000 (1.7%)
2	5.0	36/650 (5.5%)	9/418 (2.3%)	139/2 000 (7.0%)	81/2 000 (4.0%)
3	6.9	55/683 (8.0%)	6/374 (1.6%)	174/2 000 (8.7%)	19/2 000 (1.0%)
4	6.4	77/702 (10.9%)	2/299 (0.7%)	197/2 000 (9.9%)	23/2 000 (1.2%)
5	5.6	46/682 (6.7%)	7/377 (1.9%)	137/2 000 (6.9%)	29/2 000 (1.5%)
6	6.4	52/530 (9.8%)	6/334 (1.8%)	172/2 000 (8.6%)	32/2 000 (1.6%)
Mean	5.9	327/3 927 (8.3%)	36/2 202 (1.6%)	1 007/12 000 (8.4%)	217/12 000 (1.8%)

identify one or more proximal dendrites even in single sections through the cell body. The structures of the melanocyte nuclei was well preserved, but it was not always possible to avoid a certain amount of shrinkage of the perikaryon, leaving a clear zone to the next keratinocytes (Fig. 1). In VCR-injected animals, several DOPA-positive, melanosome-containing cells at the basal lamina were without nuclear membrane and had free, intensely stained chromosomes in the equatorial plane. Such cells were found in both irradiated and control skin (Fig. 1*b* and *c*), and were considered to be melanocytes in mitosis (cf. Discussion). No cells in ana-telophase were found, indicating that the VCR dose was adequate to arrest all cells in metaphase during the actual time interval. Small numbers of melanocytes in all stages of mitosis including a few cases of ana-telophase were found in both ears of the experimental animals not injected with VCR (Fig. 1*d*). The dividing melanocytes had almost spherical perikaryons, which were considerably larger than the perikaryons of interphase melanocytes. No proximal dendrites were observed in sections through the nucleus of the dividing cells. This might indicate that melanocytes retract their dendrites during mitosis. To prove this point it will be necessary to reconstruct the mitotic melanocytes from serial sections, which has not so far been attempted.

The mitotic activity of the epidermal melanocyte population was estimated by determining the proportion of melanocytes in pro- and metaphase in relation to the total sample. Due to the sparse melanocyte population in the mouse ear, the total number of counted cells in each animal was rather small (Table I). There was, in each animal, a clear difference in the frequency of mitotic melanocytes between the irradiated and control ear. On the irradiated side 5.5–10.9% of the melanocytes were arrested in pro- and metaphase, while the corresponding figures for the control side were 0.7–2.3%. This difference was significant at $p<0.05$ level (Wilcoxon's test for paired differences). No attempt was made to estimate the mitotic frequency in the control animals not injected with VCR, as the number of mitotic cells was too low to allow reliable counts. For the same reason it was not worthwhile quantitating the mitotic frequency of dermal melanocytes in the VCR-injected animals.

As a comparison, the mitotic frequency of the basal keratinocytes was also estimated. The stimulated ear contained more keratinocytes in mitosis than did the control ear, the day after the UVB irradiation was discontinued (Table I). In the irradiated skin 6.9–9.9% of the basal keratinocytes were found in pro- and metaphase, while the corresponding figures on the control side were 1.0–4.0%. These figures are based on counts of 2 000 basal cells in each ear; the difference is statistically significant ($p<0.05$). Surprisingly the mitotic frequency was approximately the same for melanocytes and basal keratinocytes in both irradiated and control ear (Table I). As shown in Fig. 2 there was a clear positive correlation between the mitotic rate of melanocytes and keratinocytes in individual animals. No such correlation was found between the mitotic rate in the stimulated and the control ear from the same animal (Fig. 3).

DISCUSSION

The present study has demonstrated that a significant proportion of the epidermal melanocytes can be arrested in mitosis by VCR, and that the frequen-

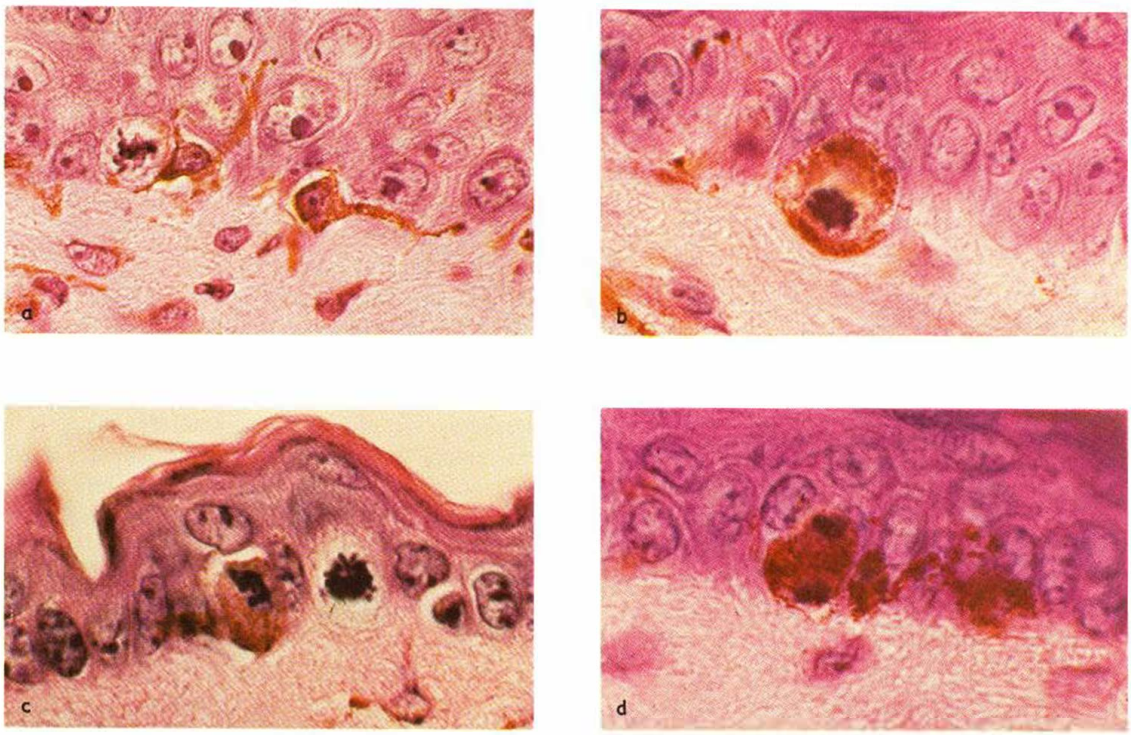

Fig. 1. Epidermal melanocytes in the mouse ear (×1 800). (*a*) Dendritic melanocytes in interphase, from UVB irradiated ear. (*b*) Melanocyte in metaphase, from UVB-irradiated ear. (*c*) Melanocyte in metaphase, from unstimulated control ear. The cells in *a*–*c* were obtained from animals injected i.p. with vincristine sulphate 2 mg/kg. ((*d*) Melanocyte in telophase from UVB-irradiated ear. Taken from animal not treated with vincristine sulphate.

cy of mitotic melanocytes is considerably higher in the UVB-stimulated skin than in the control skin. Cells classified as dividing melanocytes had no apparent proximal dendrites in single sections, but there can be no doubt about the identification of these cells as melanocytes. They were located at the basal lamina, were DOPA-positive, and contained large quantities of melanosomes. Thus, they were celarly different from dividing basal keratinocytes. Such dividing melanocytes were also observed in unirradiated control ears of animals not injected with VCR.

Stathmokinetic drugs, such as VCR, are often used in cell kinetic studies, since they have no apparent effect on the rate with which cells in a given population enter into mitosis. However, the mouse ear is not an ideal preparations for such cytokinetic studies of the melanocyte system, due to the small number and uneven distribution of these cells. Consequently, the present data represent only a single point observation, which makes it impossible to obtain a reliable estimate of the turn-over time of melanocytes, especially since nothing is known about possible diurnal rhythm or the proportion of cells taking part in mitosis. Despite this it might be of interest to consider some of the quantitative aspects of our observations. In the previous cumulative labelling study a considerable proportion of the melanocytes in the epidermis from the unstimulated ear had incorporated tritiated thymidine (11, 12). Since there was no concomitant change in the number of melanocytes it was concluded that there was a continuous renewal of the epidermal melanocyte population in unstimulated skin. The present finding of 1.6% of the melanocytes arrested in mitosis in the control epidermis suggests that the new melanocytes are formed by division of mature, pigment-producing melanocytes rather than by division of precursor cells. It is also notable that the proportions of melanocytes and basal keratinocytes in mitosis were about the same in the control ear. This might indicate that the epidermal melanocytes

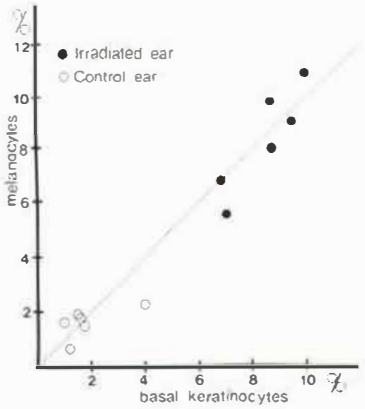

Fig. 2. Demonstrates a positive correlation ($p<0.05$) between the mitotic frequency of the basal keratinocytes and the epidermal melanocytes in C57Bl mice irradiated daily for 6 days with 0.1 Joule/cm² and injected with vincristine sulphate 2 mg/kg body weight.

are normally renewed at about the same rate as the keratinocytes.

Repeated UVB irradiation induces a marked increase in the epidermal melanocyte population in the mouse ear. Using the present irradiation schedule and dose, the increase is approximately exponential from day 2 to day 10 with a doubling of the population in about 2 days (12). Observations with tritiated thymidine incorporation have demonstrated that this increase can be accounted for by mitosis (12). If the new melanocytes were supplied by divisions of mature melanocytes, about 2% of these cells would have to enter mitosis every hour to account for this proliferation rate. In this rough estimate the mitotic frequency which is required to maintain the original number of melanocytes is ignored, since the population is already 5–7 times larger at day seven. Following VCR treatment a mean of 8.3% of the melanocytes were arrested in mitosis in the irradiated ear. Assuming a resorption time for the VCR of 30 minutes (2, 4, 18) the mitotic cells were collected over a 3 hour period. This short exposure time excludes significant metaphase degeneration. Consequently, about 2.8% of the melanocytes entered mitosis per hour in the irradiated ear compared with a predicted value of 2%. This comparison should be interpreted with caution, however, since the mitotic rate might undergo diurnal variations or be phased by UVB irradiation. Nevertheless, the mitotic rate found is so high that there seems to be no need to postulate that tyrosinase-negative precursor cells participate in the formation of new melanocytes.

The rare observations of dividing melanocytes reported in the literature might be explained by the low density of melanocytes compared with basal keratinocytes, and by the difficulty of detecting mitotic melanocytes in split skin preparations.

There was a large variation in the frequency of mitotic melanocytes in the irradiated ear of different animals. It is unlikely that these variations are due mainly to differences in the blocking efficiency of the injected VCR, as there was no positive correlation between the mitotic rate in the stimulated and the control ear of the same animal. The fact that no ana-telophases were observed in treated animals also indicates that the VCR dose was sufficient to give a complete arrest of metaphases during the exposure time. Therefore, other factors must be responsible for the variations found, such as differences in UVB sensitivity or timing of the proliferative response in individual animals. The striking parallelism in the mitotic frequency of the melanocytes and basal keratinocytes in the same animal might indicate that the mitotic frequency of the two cell types is regulated by a common factor(s). Interestingly, several earlier investigators have stressed the relationship between high mitotic activity or hyperplasia of th epidermis and hyperpigmentation (for ref. see 10).

The present observations, together with previous findings of Rosdahl & Szabó (12), demonstrate that the epidermal melanocyte system is a dynamic

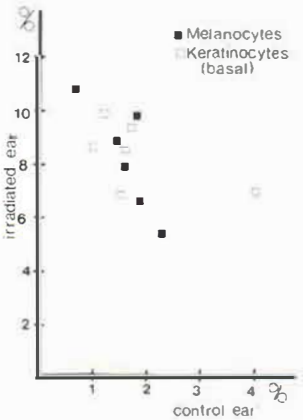

Fig. 3. Demonstrates statistically a lack of positive correlation between the mitotic frequency of the epidermal melanocytes or basal keratinocytes in the irradiated and control ear from the same animal.

system under continuous renewal. In this respect the epidermal melanocytes differ from other cells derived from the neural crest, such as ganglion cells and sympathetic neurons. The fact that mitosis of melanocytes is a normal phenomenon of the epidermis makes it easy to understand why the population density remains constant in early life, despite the enlargement of the body surface (7, 15, 17). Temporary changes in the number of melanocytes induced by factors other than UV light might also be due to changes in the mitotic rate (15, 16). Disturbances in the proliferation rate of the melanocyte system must be considered when analysing the pathogenicity of several pigmentary disorders of the skin.

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