Int J Legal Med (2001) 115:61–63

ORIGINAL ARTICLE

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Sequence of melanocyte migration into human scar tissue

Received: 17 December 2000 / Accepted: 14 March 2001

Abstract Scars of human skin can on occasions provide a very useful ancillary method of identification of an unknown deceased. If the age of any visible scars can be estimated objectively, then this might be of some assistance in the identification procedure. Melanocytes migrate into scar tissue as it ages and their number within the epidermal basal layers alters during the maturation of a scar. A total of 64 scar samples, all from previous surgical sites, were taken in the course of autopsies. Each scar was stained by the H & E method and by an immunohistochemical method using polyclonal S100 antibody. The number of melanocytes in the basal layer was counted in the epidermis overlying the scar and in the adjacent epidermis. This ratio was matched with the documented age of the scar and a statistical evaluation was carried out matching the chronological age of the scars to the melanocyte/basal epidermal cells ratio. Scars with a duration between 1 and 3 years showed a mean ratio of 1.85 and a maximum ratio of 1.94, 1.8 years after a surgical operation. The number of basal melanocytes declined thereafter and reached that of the adjacent epidermis after about 10 years. The immunohistochemical detection of melanocytes can be used for the diagnostic ageing of scars which may be a valuable contribution to improve the identification of unknown deceased persons.

Keywords Melanocytes · Ageing of scars · Identification · Immunohistochemistry

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Introduction

Human melanocytes (HMs) originate from the neural crest, migrate to the skin and can be detected in skin biopsies of human fetuses as early as 11 weeks gestational age. During the post-natal life, HMs reside in the basal layer of the epidermis. Laminin-5 is a ligand that attaches HMs at the lamina lucida of the basement membrane [9]. Because of their clear cytoplasm, melanocytes are readily identifiable by light microscopy as “clear cells” in haematoxylin & eosin (H&E) stained preparations. After trauma, HMs migrate from the immediately adjacent epidermis into the replenished epidermal defect and recolonise the epidermis [3, 6].

The purpose of this study was to establish if there is a correlation between the ratio of melanocytes to basal cells within the basal layer of the epidermis and the age of scars [5, 10] and also to ascertain whether this correlation can be used in a scar of unknown age to accurately estimate the time that has elapsed since the wound was inflicted to objectively improve the ageing of scars. This can occasionally prove useful and assist other methods used in the identification of unknown deceased persons, perhaps mutilated, from the scars that are present on the body.

Material and methods

A total of 64 scar samples were taken from healed surgical scars in the course of autopsies carried out in the Forensic Medicine Unit of the Department of Pathology of the University of Edinburgh. The autopsy samples were available from different age groups and anatomical sites, all in white Caucasians and the corpses were kept at 4 °C in a refrigerated chamber for a maximum of 3 days prior to autopsy.

The age of scars sampled varied between 10 days and 39 years. The surgical scars that were chosen had all healed by primary intention with no documented evidence of associated wound infection or other delayed healing. The scarred skin, including a 1.0 cm margin of adjacent epidermis, was excised and the margin acted as an in-built control for the skin constituents for the specific anatomical part of the body. The samples were flattened on to cardboard and fixed in buffered 10% formalin solution.

Samples were stained with H & E or by the elastic/van Gieson method. The indirect avidin-biotin-complex method (ABC) was used on deparaffinised sections which were hydrated and pretreated in a microwave (1000 W, 3 × 5 min) and incubated with the rabbit anti-cow S100 antibody (polyclonal antibody, Z0311, Dako, Hamburg) at a 1/2,500 dilution.

The number of melanocytes in the basal layer of the epidermis was counted microscopically under × 180 magnification in 5 visual fields both in the epidermis overlying the scar and in the adjacent epidermis. From these the ratio (number of HMs in the scar/number of HMs in the adjacent epidermis) was estimated. The TBS buffer was substituted for the primary antibody in the negative control test. Samples of melanocyte lesions [2] which were stained with the S100 antibody and ABC kit of the same batch, were used as the positive control.

The ratio of HMs were matched with the known age of the scars and statistically evaluated.

Results

Melanocytes in normal skin

Human melanocytes (HMs) can be specifically detected with the antibody S100 (Fig. 1a). The mean number counted microscopically in five visual fields chosen at random under this power of magnification was compared with the number of HMs in the scar. Cells of Langerhans within the epidermis exhibited an immunohistological staining reaction, but their characteristic shape and general appearance allowed a reliable differentiation and accurate counting.

Ratio of melanocytes to basal epidermal cells in scars

The ratio of melanocytes to basal cells in the scar increased 10 days after a surgical operation. Scars where the duration was between 1 and 3 years (Fig. 1b) showed a mean ratio of 1.85 (± 0.41) and a maximum ratio of 1.94, 1.8 years after a surgical operation. The mean ratio of scars aged 3–10 years was 1.36 (± 0.27), scars aged 10 years or more had a mean ratio of 0.92 (± 0.38). The number of melanocytes declined thereafter and reached that of the adjacent epidermis after about 10 years.

A model for the prediction of the most probable scar age

A cubic regression analysis was used to generate a model for the prediction of the most probable scar age with the help of the estimated ratio of melanocytes. The predictor quality with a 95% confidence interval for the individual cubic regression was satisfactory ($P = 0.0001$, $r^2 = 0.8227$).

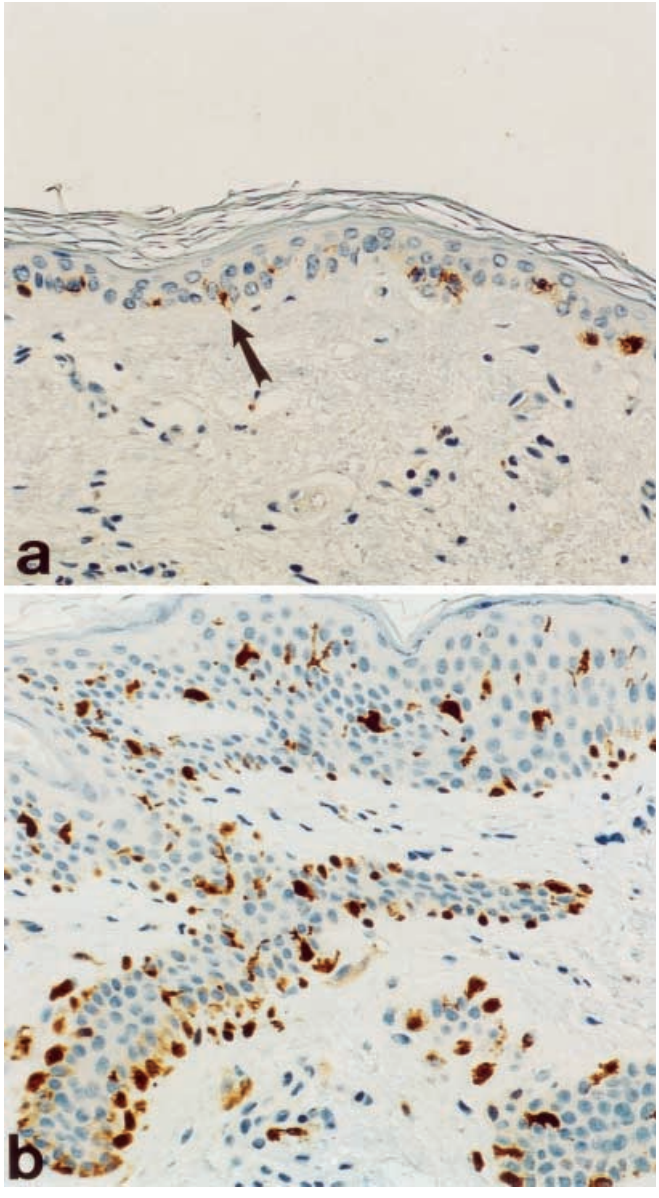

Fig. 1 A sample of 1.8-year-old scar tissue showing **a** low number of human melanocytes (HMs, *arrow*) in the basal layer of the normal epidermis (paraffin, ABC × 250) and **b** increased number of HMs in the epidermis of the scar/ratio 1.94 (paraffin, ABC × 250)

Discussion

The characterisation of the age of scars in skin by estimating the ratio of human melanocytes (HMs) to basal epithelial cells, has not been described previously. Most of the recent studies which deal with the estimation of the wound age, have utilised various immunohistochemical techniques with the emphasis being directed at the connective tissue and vascular changes within the wound [1, 4, 7, 8].

Botella-Estrada et al. [3], in one of the very few histological reports on scars, described melanocytic pigmentation in excision scars of melanotic and non-melanotic skin

tumours. These authors estimated that the scarring process itself seems to modulate the pigmentation, with the likely existence of a chemically-mediated induction process through which scar tissue acts on HMs of the local epidermis and directs their migration. Toyoda and Morohashi [11] examined the pathological changes of HMs in light-damaged skin and in sun-exposed facial skin they found a statistically significant increase in the number of HMs, a marked nuclear heterogeneity (shown in ultrastructural studies), signs of cell activation, close apposition to light-damaged degenerate keratinocytes, degenerative changes represented by large intracytoplasmatic vacuoles and frequent direct contact with the cells of Langerhans. Swope et al. [10] demonstrated a statistically significant decrease in colour strength and number of HMs from healed skin in cultured skin substitutes after flow cytometric separation.

These findings do not address the same matter being investigated in our study in that no precise data about the age-dependant number of HMs in scars have been published and the materials and methods for studying HMs used in these studies were quite different from the current human autopsy-based study.

In our investigations, the ratio of HMs in the scar increased 10 days after a surgical operation. Scars with a duration of between 1 and 3 years, showed a mean ratio of 1.85 and a maximum ratio of 1.94, 1.8 years after a surgical operation. The mean ratio of HMs in scars with a known duration of 3–10 years was 1.36. Scars as old as 10 years had a mean ratio of 0.92. The number of melanocytes declined thereafter and reached that of the adjacent epidermis after about 10 years.

These results have therefore closed a gap in the diagnostic aspects of the ageing of scars. The migration pattern of melanocytes may be a phenomenon which varies between individuals and perhaps also in different parts of the body and our investigations are unable to address any such interindividual differences.

In conclusion, the ratio of HMs to basal epithelial cells can be used for the estimation of the age of scars. The method is simple, easy to carry out and well within the reach of most histopathology laboratories. The results could be important for the identification of unknown dead bodies particularly when this finding is added to other circumstantial evidence already available in cases where other more classical forms of identification are not available or possible.

Acknowledgements The authors are very much obliged to Tim Ingman and Francis Rea from the Department of Pathology of the University of Edinburgh for their excellent technical assistance.

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