Int J Legal Med (1998) 111 : 169–172

ORIGINAL ARTICLE

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The time-related expression of p53 protein in human skin wounds – a quantitative immunohistochemical analysis

Received: 14 October 1997 / Received in revised form: 22 December 1997

Abstract The time-dependent expression of p53 protein during wound healing has been investigated by immunochemistry in fibroblastic cells of skin wounds ranging between a few minutes and 11 weeks old. When compared to uninjured skin, an increased expression of p53 was found earliest in a wound with a postinfliction interval of 3 days. The ratio (r) of positively stained cells in relation to the total number of fibroblastic cells in the wound area of this specimen was about 0.2. A considerable increase in the expression of p53 ($r > 0.5$) was first found in a wound aged 8 days and in wounds with postinfliction intervals ranging between 3 and 11 weeks, where the ratio of positive cells was between 0.40 and 0.64. Therefore, it can be calculated that r-values of at least 0.5 indicate a postinfliction interval of approximately 1 week or more. Since comparably low numbers of positively stained fibroblastic cells were found in specimens with an advanced wound duration, reliable information for a forensic wound age estimation can only be provided by positive results.

Key words Protein p53 · Wound age · Immunohistochemistry · Quantitative image analysis

Introduction

The human p53 tumor suppressor gene encodes a 393 amino acid phosphoprotein that exhibits sequence-specific DNA binding and directly interacts with various cellular and viral proteins [19]. The current model of p53 function postulates that p53 senses DNA damage and arrests the cell cycle in either the G1 or G2 phases to allow DNA repair to take place [10, 11, 24]. If repair is not successful, p53 initiates programmed cell death thus preventing the propagation of genetic defects to successive generations of cells [16 17].

Loss of this cell cycle checkpoint may contribute to tumor development by increasing the number of genetic abnormalities in daughter cells following DNA damage [8]. Point mutations in the p53 gene are the most frequently identified genetic changes leading to the accumulation of mutant p53 protein [13, 14, 21]. Thus, in malignancies such as cancer of the colon, stomach, bladder, breast, lung, thyroid, testes, melanomas and soft tissue sarcomas, p53 protein can be demonstrated by immunohistochemistry [2, 5, 12, 19, 23]. Since a recent experimental animal study also demonstrated elevated p53 expression during normal tissue regeneration in response to acute cutaneous injury in porcine skin [1], a study was set up to determine whether the detection of p53 can possibly provide information on wound age in human skin lesions.

Material and methods

A total of 82 human skin wounds (lacerations, stab wounds, surgical wounds) with post infliction intervals ranging between a few minutes and 11 weeks were investigated as well as 20 specimens of normal skin (negative control) obtained at autopsy. The post-mortem interval was between 1 and 4 days. Only those specimens were investigated which showed no serious signs of autolysis such as post-mortem cell shrinkage or diminished nuclear stainability in hematoxilin-eosin stained preparations. The individual age of the wound tissue donor ranged between 17 and 75 years (average age 52 years). No therapy which could have a possible influence on wound healing such as the application of cytostatic agents or glucocorticoids had been performed. Furthermore, the patients showed no pathology conditions such as severe malnutrition, malignant diseases or metabolic disorders which could also have influenced wound repair.

After fixation of the specimens in 4% PBS-formaldehyde solution, paraffin sections (3–5 μm) were stained with H&E. In each case the specimens were investigated at one level of the block. The immunohistochemical staining procedure using the monoclonal primary antibody mouse anti-human p53 protein (DAKO, Code# N 1581) was performed according to the recommended protocol. The examination focussed on the fibroblastic cells which were identified by typical spindle shaped cell morphology. Only those cells which showed a distinct nuclear staining but no significant cytoplasm reaction were regarded as positive.

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Quantitative analysis was performed with an automatic image processing and analysis system (LEICA QWin). For data evaluation, the total number of p53 positive fibroblastic cells within a defined microscopical measurement frame were determined in skin wounds and negative control specimens. The ratio of positive fibroblasts to the total number of fibroblastic cells in the wound area was defined as "r". In order to obtain reliable informations for a forensic wound age estimation, the earliest time of p53 expression was recorded during the wound healing process. A more elaborate statistical analysis was thought to yield no further information under forensic aspects and has not been performed in this study.

Results

Normal skin

In uninjured skin, positively stained cells were regularly found in the epidermal layer (Fig. 1), in hair follicles, sebaceous and sweat glands, representing fast proliferating cells in the skin. In contrast only a few fibroblastic cells of the dermis showed a positive nuclear reaction. The average ratio (r) of stained fibroblastic cells in relation to the total number of fibroblasts was about 0.1. The quantitative analysis revealed no relevant influence of individual age, mode of death or postmortem interval which must be taken into consideration for the interpretation of the results obtained on the skin wounds.

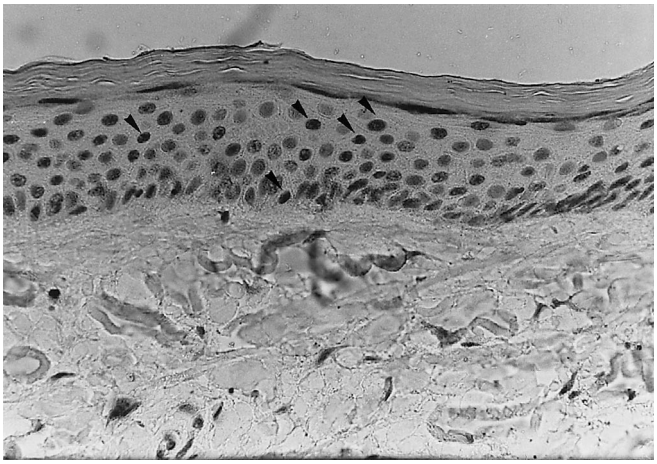

Fig. 1 Normal skin: p53 positive nuclear staining in the epidermal layers (paraffin, 370 ×)

Skin wounds

In lesions with a postinfliction interval of a few minutes up to 1 day the ratio of positive fibroblasts was comparable to that of uninjured skin ($r \leq 0.14$). The wound areas showed intensive hemorrhages and some granulocytic infiltrates with increasing postinfliction interval. The earliest positive reaction defined as $r \geq 0.2$ was observed in a wound with a postinfliction interval of 3 days. In a total of 7 out of 11 wounds aged between 8 and 21 days, the ratio of positively stained fibroblastic cells exceeded 0.5 (Fig. 2). On the other hand, in the postinfliction interval between 2 and 21 days the ratio of positive fibroblastic cells was below 0.2 in 11 cases and thus not significantly different from values for uninjured control specimens. The skin wounds aged 3 weeks or more showed a ratio of at least 0.4. The maximum value was observed in a skin wound with a postinfliction interval of 14 days (r = 0.71, Fig. 3).

Discussion

The tumor suppressor p53 has been found to affect the initiation of programmed cell death (apoptosis) in a dose-dependent manner when induced by agents that cause DNA strand breakage. Therefore, the p53 protein can be demonstrated in a variety of human cancers [2, 7, 19, 23] as

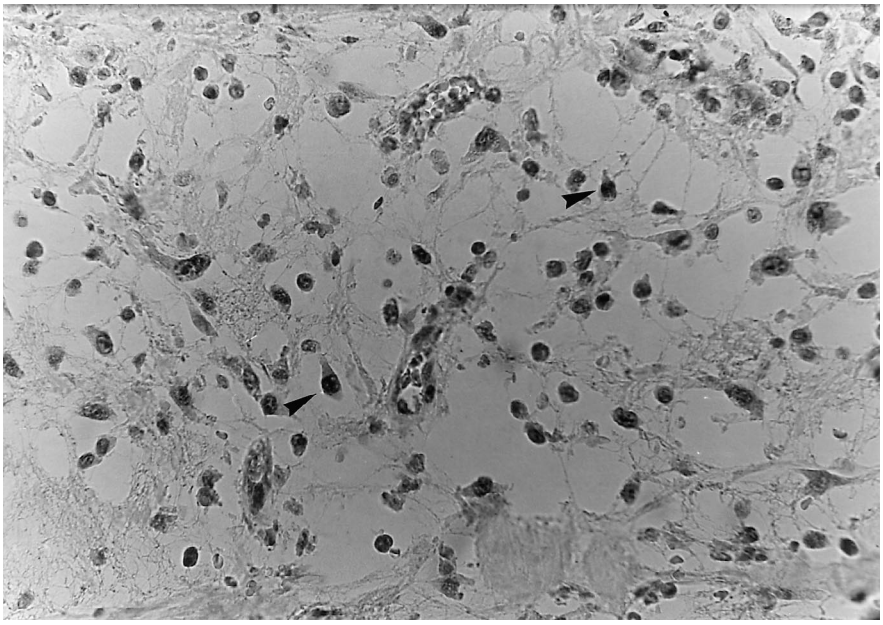

Fig. 2 Skin wound with a postinfliction interval of 8 days: numerous positively stained fibroblastic cells in the granulation tissue (paraffin, 440 ×)

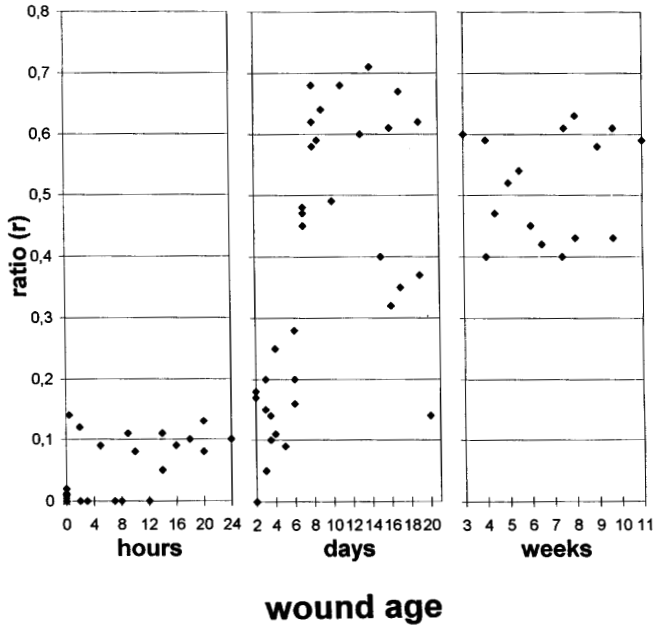

Fig. 3 Ratio of p53-positively stained fibroblastic cells to non-positively stained cells (r) in relation to the postinfliction interval (n = 82)

well as in tissue following DNA damage caused by exposure to radiation [9, 15, 18, 22] or toxic agents [20]. Furthermore an increasing p53 expression has been demonstrated in epithelial layers of injured skin during wound healing in experimental animals [1]. As the p53 protein is regularly detectable in the epidermal cell layers of normal skin, but is seen only in a very few fibroblasts of the dermis (corium), an evaluation of an increased p53 expression in fibroblastic cells of the wound area seemed to be superior to an investigation of re-epithelialisation under forensic aspects. When compared to uninjured skin, a slightly increased amount of p53-positive fibroblasts occurred earliest after a postinfliction interval of 3 days. A considerable increase in the fibroblastic p53 expression ($p > 0.5$) was found earliest in a wound aged 8 days. In wounds with postinfliction intervals between 3 weeks and 11 weeks, the ratio of positive cells was between 0.40 and 0.64 and thus considerably exceeded the average values found in normal skin or in wounds with shorter postinfliction intervals. With regard to the physiological function of p53, it was of interest to compare the time-dependent increase in the p53 expression with the detection of apoptotic cells in human skin wounds [6]. As expected the results provided evidence of an earlier increase in the p53 expression in fibroblastic cells of the wound area which are assumed to be involved in the contraction of the granulation tissue [4] due to the physiological function of the p53 protein as a promotor of apoptosis. The increase of p53 expression in fibroblastic cells also corresponds to the time-dependent re-epithelialisation of skin wounds as described in previous investigations [5]. Therefore, the findings obtained by the immunohistochemical detection of p53 easily fit into the concept of wound repair physiology and can provide additional information for a forensic wound age estimation.

Although an intra- and interindividual variability was observed as expected in biological processes, the detection of p53 by immunohistochemistry can complement the parameters used to determine the age of a wound in the advanced postinfliction interval. The accurancy of such morphological methods depends on the number of evaluable parameters providing reliable information on the postinfliction interval due to their earliest, regular, or latest appearance during the wound healing process. To avoid misinterpretation of immunohistochemically findings a critical evaluation is necessary as required previously [3]. Taking these aspects into consideration, the following conclusions can be drawn:

1. In uninjured skin, no significant numbers of p53-positive fibroblastic cells can be found ($r \leq 0.1$).
2. Significant numbers of fibroblastic cells with a positive reaction to p53 antigen ($r \geq 0.2$) can be expected at the earliest in wounds aged at least 3 days.
3. A ratio of positively stained fibroblasts exceeding values of 0.5 indicates a postinfliction interval of at least 8 days.
4. Because low numbers of p53-positively staining fibroblastic cells can be observed in specimens with advanced postinfliction intervals, these should be taken as providing reliable information on wound age only if positive results can be obtained.

Acknowledgements Special thanks to Mrs. Tübel and Mrs. Wiest for their excellent technical assistance.

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