ORIGINAL ARTICLE

Immunohistochemical analysis of PDGFR-α for wound age determination

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Abstract

Immunohistochemical analysis of platelet-derived growth factor receptor-α (PDGFR-α) was performed on human skin wounds obtained from forensic autopsy cases. Thirty human skin wounds were collected at different post-infliction intervals as follows: Group I, 4 h to 3 days ($n = 16$); Group II, 4 to 7 days ($n = 7$); Group III, 9 to 10 days ($n = 3$); and Group IV, 14 to 20 days ($n = 4$). Immunopositive reactions for PDGFR-α were not observed in the uninjured human skin specimens. In a semi-quantitative morphometrical analysis, the number of PDGFR-α-positive cells was observed increased in Group II, with the average number of PDGFR-α-positive cells being the highest in Group II. Additionally, in Group II, all specimens showed PDGFR-α-positive cells, with an average number of > 200 cells in five fields of view, suggesting a wound age of 4 to 7 days. Taken together, the immunohistochemical detection of PDGFR-α in human skin wounds can be a useful tool for wound age determination.

Keywords Forensic pathology · Wound age determination · PDGFR-α · Immunohistochemistry

Introduction

The crucial role of forensic wound examinations in evaluating the connection between injuries and the cause of death has been well established. Moreover, forensic pathologists have to also ascertain the amount of time that has elapsed since a wound was is incurred, a process known as wound age determination [1–5]. Currently, histopathological and immunohistochemical alterations occurring during various stages of wound healing have been used for wound age determination [6, 7].

Wound healing occurs in three stages: inflammation, proliferation, and maturation [8, 9]. Numerous biological elements such as growth factors, cytokines, and adhesion molecules have been recognized to participate in every stage of the wound healing [10]. From a forensic standpoint, multiple strands of accumulating evidence indicate the potential utility of these substances as markers for wound age determination using immunohistochemical methods [11–21].

Platelet-derived growth factor (PDGF) plays a significant role in the cell division of fibroblasts, and is particularly prevalent in wound healing [22]. It can also promote cell proliferation, migration, and angiogenesis. PDGF also stimulates chemotaxis of fibroblasts, neutrophils, and macrophages [23–27]. Furthermore, PDGF facilitates the contraction of collagen matrices during wound healing, which affects wound contraction [28, 29]. The distinct variations of PDGF isoforms exert their biological effects on target cells by interacting with platelet-derived growth factor receptor (PDGFR)-α and β. Actually, reduced expression of PDGFs and their receptors in diabetic or glucocorticoid-treated mice resulted in the retardation of skin wound healing [30, 31], indicating that PDGFs was essential for skin wound healing. Moreover, over-production of PDGFs would cause hypertrophic scar or keloid formation.

In this study, we conducted an immunohistochemical analysis of PDGFR-α expression in human skin wounds at various stages of healing and investigated the potential applicability of PDGFR-α as an indicator of wound age.

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Materials and methods

Human skin wound specimens

A total of 30 human skin wounds (two stab wounds, three incised wounds, 14 surgical wounds, nine lacerations, and two gunshot wounds), with wound ages ranging from a few hours to 20 days, were obtained from forensic autopsies at Department of Forensic Medicine, Wakayama Medical University, Japan. The ages of the victims ranged from 18 to 94 years (mean age, 66.5 years), and the postmortem interval was < 3 days in each case. None of the case had severe malnutrition, malignant diseases, or metabolic disorders, and no substances such as cytostatic agents or glucocorticoids that could have influenced wound repair were administrated during medical treatment. Based on the wound ages, the wound specimens were classified into four groups as follows: Group I (inflammatory phase), 4 h to 3 days ($n = 16$); group II (early proliferative phase), 4 to 7 days ($n = 7$); group III (late proliferative phase), 9 to 10 days ($n = 3$); and group IV (maturation phase), 14 to 20 days ($n = 4$). Uninjured skin from the same individuals were use as control.

Immunohistochemistry

Immunostaining of PDGFR-α was performed using Ventana Discovery XT (Ventana Medical Systems, Inc., AZ, USA) using rabbit anti-PDGFR-α monoclonal antibody (#3174, clone D1E1E, Cell signaling technology, Danvers, MA). After incubating the specimens with an HRP-conjugated anti-rabbit multimer (518-102128, OmniMap HRP Multimer, Roche Diagnostics K.K., Tokyo, Japan), the immune complexes were visualized using the Chromomap kit DAB (518-100803, Roche Diagnostics K.K.) according to the manufacturer's instructions. As a negative control, sections were incubated with normal rabbit serum instead of the primary antibody, and no positive signal was detected, indicating the specificity of the antibody.

Morphometrical analysis

Morphometrical analysis was performed for the semi-quantitative evaluation of the immunohistochemical findings by two investigators without prior knowledge, as described previously [18, 32–35]. Five high-power microscopic fields (magnification: x 200) were randomly selected in each section, and the number of PDGFR-α-positive cells were counted in each microscopic field. The average of the five selected microscopic fields was used as the PDGFR-α expression score for each wound specimen.

Statistical analysis

In each group, the means of the PDGFR-α-positive cell numbers and the standard error (SE) were calculated. Statistical analyses were performed using a one-factor analysis of variance to determine whether differences existed among the group means, followed by Scheffe's F test to identify significantly different means. $P < 0.05$ was considered as significant. All statistical analyses were performed using the Statcel3 software under the supervision of a medical statistician.

Results

Immunohistochemical analysis

Only a small number of PDGFR-α-positive cells were found in intact skin and wounds which were < 1 day old. In contrast, PDGFR-α positive signals were detected in dermal cells of wounds > 2 days (Fig. 1).
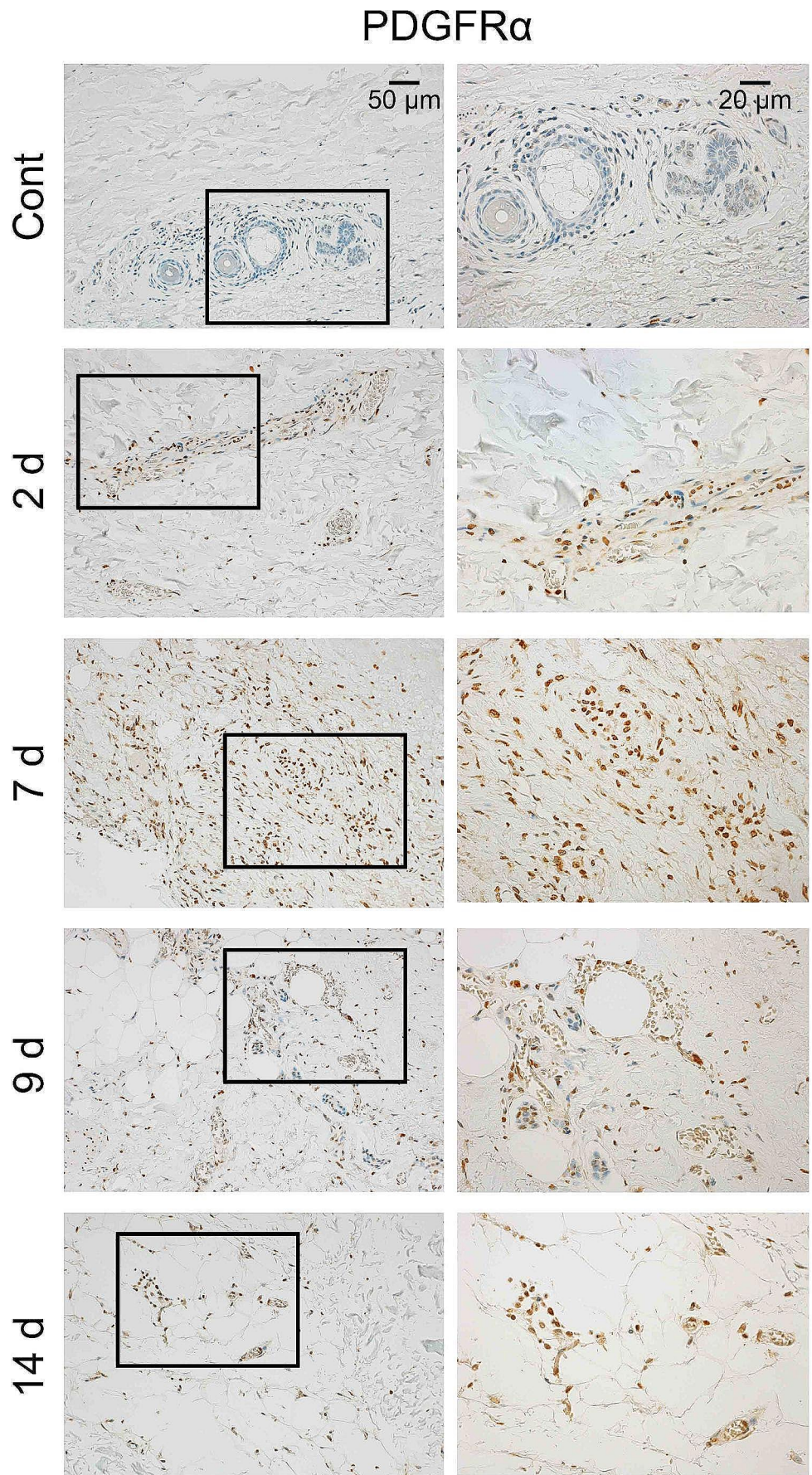
Morphometrical analysis

Morphometric analysis was performed to semi-quantitatively evaluate the immunohistochemical findings. Figure 2a shows the distribution of the PDGFR-α-positive cells with respect to wound age. The average number of PDGFR-α positive cells were lowest in wound specimens from group I (wound age 4 h to 3 day), compared to the other groups (mean ± SE: 76.3 ± 51.3) (Fig. 2b). Group II (wound age 4 to 7 days) had the highest mean PDGFR-α-positive cell count among all the groups (mean ± SE: 370.9 ± 148.6). Moreover, all wounds in Group II showed PDGFR-α-positive cells, with an average number of > 200 cells in five fields of view (Fig. 2a). The average number of PDGFR-α-positive cells were decreased significantly in Group III (wound age 9 to 10 days; mean ± SE: 128.7 ± 51.8) and Group IV (wound age 14 to 20 days; mean ± SE: 97.6 ± 51.9) compared with that of Group II. Statistical analysis revealed significant differences between Group II and the other three groups individually (Fig. 2b).

Discussion

In forensic practices, forensic pathologists must consistently establish diagnoses regarding the correlation between wounds and the cause of death and determine factors such as wound vitality or post-infliction intervals. In particular, the estimation of the wound ages can provide significant scientific information for the investigation of criminal cases.

Fig. 1 Immunohistochemical analyses were performed by using anti-PDGFR-α antibody in human skin wounds obtained from forensic autopsy cases
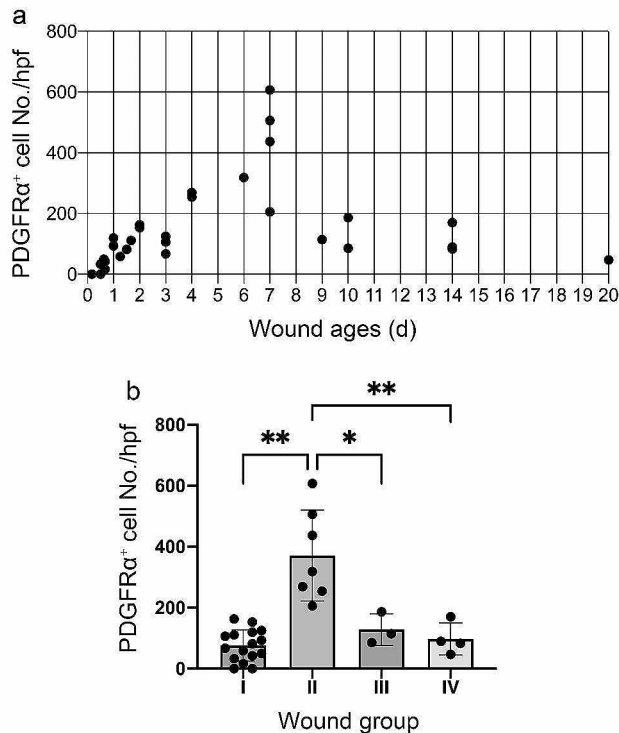
Fig. 2 Morphometrical analysis of PDGFR-α-positive cells. (a) The distribution of immunopositive cells in relation to wound ages. (b) Group I, 4 h to 3 days ($n=16$); group II, 4 to 7 days ($n=7$); group III, 9 to 10 days ($n=3$); and group IV, 14 to 20 days ($n=4$). Mean value and standard error of PDGFR-α-positive cell numbers in each wound group. $*p < 0.05$, $**p < 0.01$

PDGFR-α is a receptor for the PDGF family, which regulates cellular processes through the activation of specific signaling pathways, and plays an important regulatory role in cell growth, migration, angiogenesis and collagen synthesis [23–29]. Therefore, we hypothesized that PDGFR-α could be used as an effective marker for the estimation of wound ages and performed this study. We found that the average number of positive infiltrated cells was the highest at 4 to 7 days of wound age, imply that PDGFR-α could be utilized in wound age determination.

The process of wound healing is carefully coordinated across three distinct stages: inflammation, proliferation, and maturation. PDGF can promote the formation of new blood vessels, which is an important aspect of tissue repair [36], and induce the increased formation of granulation tissue during wound healing [37, 38]. PDGF is a major factor for the mitogen of fibroblasts, smooth muscle cells, and other cells, and can stimulate contraction of collagen matrices in vitro which can increase the rate of reepithelialization and of neovascularization [28, 29, 39, 40].

Furthermore, in a study of mice lacking PDGFR-α, it was suggested that PDGFRα signaling supported proliferation of fibroblast progenitors and increased their numbers during the early stages of wound healing, followed by downregulation of PDGFR-α to promote differentiation from fibroblasts to myofibroblasts [41]. Individuals with diabetes experience hindered skin wound healing, which can progress into persistent ulcers. Yu et al. discovered that lncRNA-H19 (*lncH19*) expressed in cutaneous PDGFR-α+ fibroblasts expedite the wound healing process by enhancing dermal fibroblasts proliferation and macrophage infiltration into the wounded skin [42]. These studies suggest that PDGFR-α plays essential roles in wound healing, especially in the early healing process.

In the inflammation stage of wound healing, platelets secrete growth factors, for example: epidermal growth factor (EGF), TGF-β, PDGF, and several chemokines [3]. These substances can recruit leukocytes to the wound sites. PDGF accelerates the proliferation and migration of various cell types, such as fibroblasts, smooth muscle cells, and endothelial cells to fill wound defects and promote tissue repair [43]. The functions and roles of PDGF are highly coordinated during wound healing. After wound formation, PDGF is released and initiates cell signaling pathways, such as PI3K-Akt, MAPK, and Ras, by binding to PDGFR-α and/or -β, which in turn promotes the processes of cell proliferation, migration, angiogenesis, and collagen synthesis [44–46]. In this study, we have found that the PDGFR-α expressed strongly during the 4-7days after wound formation, and weakly in the stages of 9–20 days.

Considering its relevance to forensic pathology, the current study demonstrates that PDGFR-α proves to be a suitable indicator for wound age determination. All of wound samples in Group II showed PDGFRα-positive cells of >200 PDGFR-α-positive cells, suggesting a wound age of 4–7 days (early proliferative phase). Several lines of accumulating evidence indicated that the determination of wound vitality is sometimes more relevant issue in forensic practices [20, 21, 47]. Based on our observations, PDGFRα is unlikely to be available as an indicator for determining wound vitality or extremely short interval after injury. Notably, growing evidence to suggests that IL-1α may be a valuable tool in determining wound ages within a time frame of 4 h to 1 day (inflammatory phase) [20, 21]. In the hierarchy of PDGF expression control, inflammatory cytokines such as IL-1α play pivotal roles as upstream regulators [48, 49]. Collectively, we propose that the immunohistochemical identification of PDGFRα combined with additional indicators can enhance the dependability of wound age determination.

Finally, it is needless to say that the estimation of wound vitality or ages is indispensable in forensic pathology. The results obtained from only animal experiments would be important but sometimes be insufficient from the aspects of forensic application. Thus, we would like to emphasize

to perform practical study using human samples as well as animal experiments.

Declarations

Ethical approval This study was approved by the Research Ethics Committee of Wakayama Medical University (No. 3229). All the procedures were performed in accordance with the Declaration of Helsinki Principles. Moreover, this study was conducted using autopsy records from the past, and we could not obtain informed consent from the bereaved family for the use of these records. Therefore, we conducted this study in accordance with the "Ethical Guidelines for Medical Research Involving Human Subjects (enacted by the Ministry of Health, Labor, and Welfare in Japan), Sects. 12–1(2) (a) (c)." This was a de-identified study using archived tissue obtained from judicial autopsy cases, and the information on the implementation of the study was posted on our website (https://www.wakayama-med.ac.jp/dept/igakubu/160420/index.html). If there was a request to refuse the use of the samples for research, they were excluded from samples of this study. In addition, the review board of the Research Ethics Committee of Wakayama Medical University waived the need for written informed consent from the relatives of the individuals studied in accordance with the national legislation and the institutional requirements.

Conflict of interest The authors declare that they have no conflict of interest.

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