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ORIGINAL ARTICLE

Detection of fibrocytes in human skin wounds and its application for wound age determination

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Abstract Fibrocytes, a newly identified cell type, are bone marrow-derived mesenchymal progenitors that coexpress hematopoietic cell antigens and fibroblast products. In this study, a double-color immunofluorescence analysis was carried out using anti-CD45 and anti-collagen type I antibodies to examine the time-dependent appearance of fibrocytes, using 53 human skin wounds with different wound ages (group I, 0–3 days; group II, 4–7 days; group III, 9–14 days; and group IV, 17–21 days). In wound specimens with an age of less than 3 days, $CD45^{+}$/collagen type I^{+} fibrocytes were not detected. The fibrocytes were initially observed in wounds aged 4 days, and their number increased in lesions with advances in wound age. In a semiquantitative morphometrical analysis, the average number of fibrocytes was highest in the wounds of group III. These findings imply that human skin wounds containing fibrocytes are at least 4 days old. Moreover, a fibrocyte number of over 10 indicates a wound age between 9 and 14 days (i.e., group III). Based on the average number of fibrocytes in each group, a fibrocyte number of over 15 more strongly suggests a wound age of 9–14 days. Together, our observations indicate the participation of fibrocytes in wound healing of human skin inducing the accumulation of extracellular matrix components, and therefore, detection of fibrocytes could be a useful marker for wound age determination.

Keywords Fibrocytes · Wound age determination · Immunohistochemistry · Forensic pathology

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Introduction

In forensic practice, wound examination is one of the most important tasks for forensic pathologists. It is mandatory to discriminate antemortem wounds from postmortem damage on wound examination [1–4]. Furthermore, in vital wounds, wound age determination is essential in judging how wounds are related to a cause of death. The chronological histopathological alterations that characterize the different phases of wound healing have been applied to wound age determination because objective scientific evidence is always required in forensic practice [1–8].

Wound healing is a basic biological response that involves soluble mediators, extracellular matrix (ECM) components, resident cells, and infiltrating leukocytes, which participate differently in three sequential phases: inflammation, proliferation, and maturation [9, 10]. The molecular mechanisms of wound repair are not fully understood but involve growth factors, cytokines, adhesion molecules, and matrix metalloproteases [9–13]. Some of these substances have been used for the determination of wound age and wound vitality [14–28].

Recently, a unique cell population called fibrocytes has been reported to contribute to tissue fibrosis [29–36]. Fibrocytes are bone marrow-derived mesenchymal progeni-

tors that coexpress hematopoietic cell antigens and fibroblast products such as CD45 and collagen type I (Col I), respectively [29–38]. They constitutively produce ECM components as well as ECM-modifying enzymes and can further differentiate into myofibroblasts both in vitro and in vivo under permissive microenvironmental conditions [30, 34–37, 39–41]. Therefore, they may participate in tissue repair after injury. In the present study, we immunohistochemically detected fibrocytes in human skin wounds with different wound ages and discussed the practicality of fibrocytes as a marker for wound age determination.

Materials and methods

Human skin wound specimens

A total of 53 human skin wounds (15 stab wounds, eight incised wounds, 23 surgical wounds, and seven lacerations) with different post infliction intervals ranging from a few hours to 21 days were removed during forensic autopsies (Institute of Legal Medicine, University of Munich, Germany). The ages of the victims ranged from 8 to 75 years (mean age 40.6 years), and the postmortem interval was less than 3 days in each case. None of the cases had suffered from severe malnutrition, malignant diseases, or metabolic disorders, and no substances such as cytostatic agents or glucocorticoids that could have influenced wound repair were administered during medical treatment. The wound specimens were classified into four groups according to wound age as follows: I, 0–3 days (n=15); II, 4–7 days (n=10); III, 9–14 days (n=16); and IV, 17–21 days (n=12). Uninjured skin from the same individual was also taken as a control.

Immunofluorescence microscopy

The wound specimens were fixed in 4% formaldehyde solution with phosphate-buffered saline (PBS) and embedded in paraffin before being sectioned at a thickness of 4 μm. Wounded and unwounded skin sections were analyzed by double-color immunofluorescence staining to determine the presence of fibrocytes as described previously [42]. Briefly, deparaffinized sections were incubated with PBS containing 1% normal donkey serum and 1% bovine serum albumin to reduce nonspecific reactions. Thereafter, the sections were further incubated with a pair of anti-CD45 (rabbit polyclonal; dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-human Col I (mouse monoclonal, clone I-8H5; dilution 1:100; MP Biomedicals, Solon, OH, USA) overnight at 4°C. After incubation with cyanine dye (Cy)3-conjugated donkey anti-rabbit IgG polyclonal antibodies and fluorescein isothiocyanate (FITC)-conjugated donkey anti-mouse IgG polyclonal antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at room temperature for 1 h, the sections were observed under a fluorescence microscope. The immunofluorescent images were digitally merged. We could confirm that these primary antibodies were available for the detection of fibrocytes by the use of isolated human fibrocytes. Normal rabbit or mouse IgG was used instead of primary antibody as negative control.

Morphometrical analysis of fibrocytes

According to the methods of previous studies [22, 27, 28, 43], morphometrical analysis was performed for a semiquantitative evaluation of the immunohistochemical findings by two different investigators who had no prior knowledge of the specimens. Briefly, 10 high-power microscopic fields (0.4×0.4 mm each) were randomly selected in each section, and the number of $CD45^+$/Col I^+ fibrocytes was counted in each microscopic field. The average fibrocyte number from the 10 selected microscopic fields was evaluated in each wound specimen.

Morphometrical analysis of collagen deposition

The specimens were also stained with Masson's trichrome for the detection of collagen accumulation at the wound sites. The collagen deposition was semiquantitated as the area staining blue in sections with Masson's trichrome [44]. The area of collagen deposition in the wound bed was evaluated by NIH image and expressed as the percentage of wound bed. Histopathological changes were evaluated by two investigators who had no prior knowledge of the samples.

Statistical analysis

In each group, the means of the $CD45^+$/Col I^+ fibrocyte numbers and the standard error (SE) were calculated. Statistical analyses were performed using 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.

Results

Double-color immunofluorescence analysis

In unwounded specimens, $CD45^+$/Col I^+ fibrocytes were not detected. Although $CD45^+$ leukocytes were detected, no dual-positive ($CD45^+$/Col I^+) cells were observed in the wound specimens of group I (wound age 0–3 days)

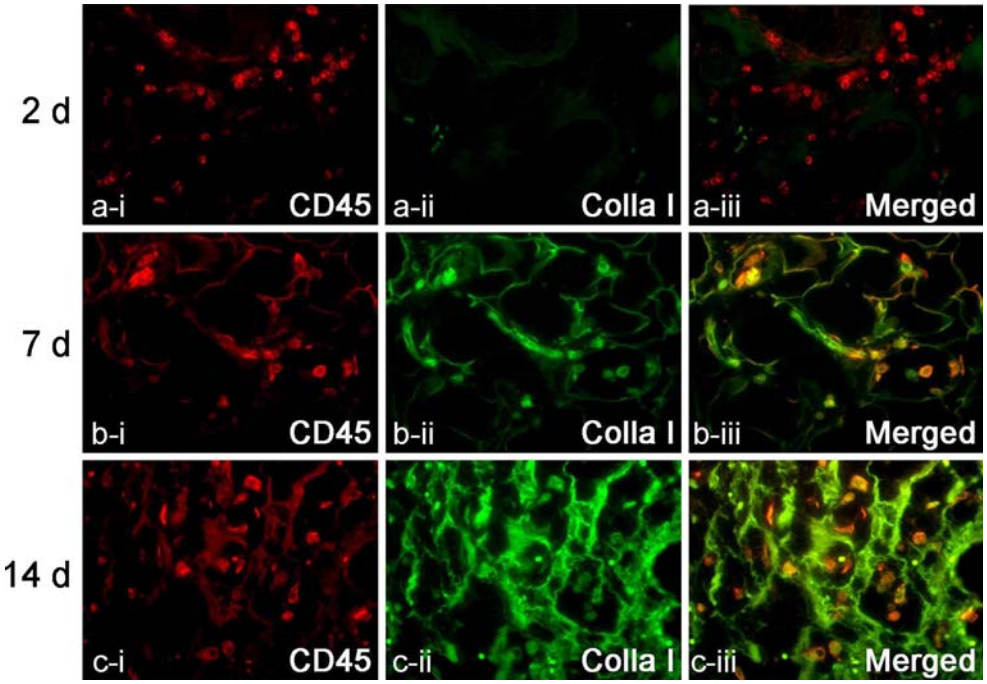

Fig. 1 CD45$^+$/Col I$^+$ fibrocytes emerge in human skin wounds. Double-color immunofluorescence analysis of human wounds was performed using anti-CD45 (*i* Cy3) and anti-Col I (*ii* FITC). The fluorescence images in *i* and *ii* were digitally merged as panel *iii*. Representative results from three independent experiments are shown (**a** 2-day-old, **b** 7-day-old, and **c** 14-day-old wound). Original magnification ×400

(Fig. 1a). A dual-positive reaction was initially detected in skin wounds with a post infliction interval of 4 days (Fig. 2a). With advances in wound age, more dual-positive fibrocytes were detectable at the wound site (Fig. 1b, c).

Morphometrical analysis of fibrocytes

Figure 2 further shows the distribution of the number of CD45$^+$/Col I$^+$ fibrocytes in relation to wound sites. No fibrocytes were detected in wound specimens aged 0–3 days (group I). In wound specimens with a post infliction interval between 4 and 7 days (group II), fibrocytes were present but only in very small quantities with all of the wound specimens giving numbers of less than 10 (mean ±SE=5.4±0.9). On the contrary, 14 out of 16 wounds aged between 9 and 14 days (87.5% of group III) showed a fibrocyte number of over 10 (mean±SE=17.3±1.5), and 10 samples had over 15 fibrocytes. A 14-day-old wound from group III showed the highest number of fibrocytes (28) among all of the 53 human skin wounds in the present study. Although the average number of fibrocytes decreased significantly in group IV compared with that of group III, it remained moderately high (mean±SE=8.9±1.4). Statistical analysis revealed significant differences between group III and the other three groups individually; however, there was no significant difference between groups II and IV (Fig. 2b).

Morphometrical analysis of collagen deposition

Because it has been reported that fibrocytes can promote tissue fibrosis in various organs [29–36], we next

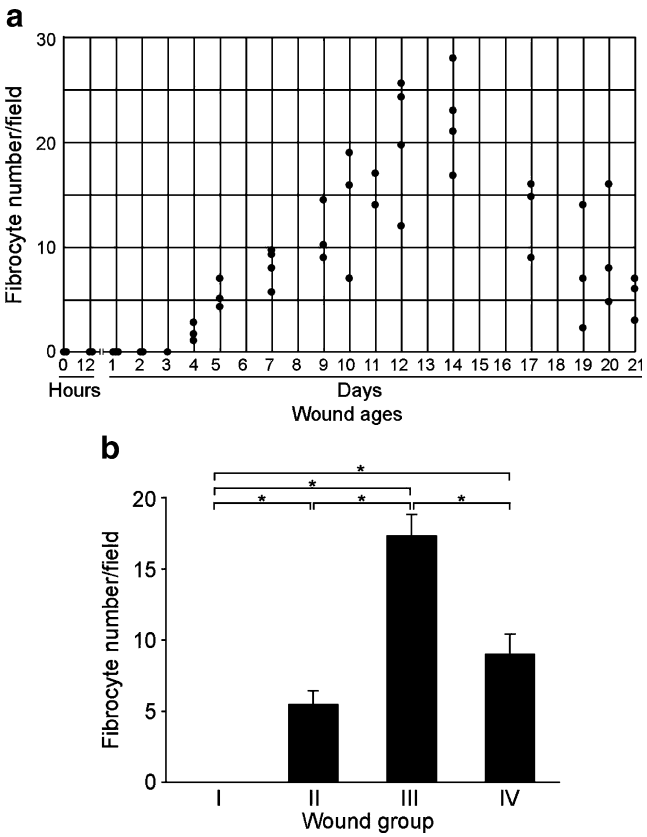

Fig. 2 The group III wound specimens (wound age 9–14 days) show the highest fibrocyte infiltration into wounds among all of the groups. Morphometrical analysis was performed to measure the fibrocyte number in each wound section as described in the "Materials and methods" section (**a**). Mean value and standard error of fibrocyte number in each wound group (**b**). *$p<0.01$, a statistically significant difference was observed

examined collagen deposition in human skin wounds histopathologically. Masson's trichrome staining of the wound specimens demonstrated that collagen deposition was enhanced in the wound bed along with advances in wound age (Fig. 3). Furthermore, these increases almost always correlated with the extent of fibrocyte appearance in the wound (Fig. 3b). Therefore, fibrocytes may participate in collagen deposition and granulation tissue formation during wound healing of human skin.

Discussion

The skin wound healing process is a complex but well-organized biological response composed of three different phases: inflammation, proliferation, and maturation [8–10]. Analysis of time-dependent cellular and molecular reactions and/or the initial appearance of various cell types can provide significant information for the estimation of skin wound age. In practice, the molecular and cellular pathophysiology of wound healing has been applied for wound age determination with advances in biochemical and immunohistochemical techniques [1–7, 14–17, 45–47].

Fibrocytes are a novel leukocyte subset that expresses both hematopoietic and mesenchymal cell markers including CD45 and Col I, respectively [29–35, 37, 38]. Since fibrocytes express ECM proteins, such as Col I and fibronectin, they may be involved in connective tissue formation including skin wound healing [29, 30, 39, 48, 49]. Thus, we hypothesized that an analysis of the time-dependent appearance and/or disappearance of fibrocytes could be used to estimate wound age. In the present study, we found that $CD45^{+}$/Col I^{+} fibrocytes infiltrated into human skin wounds along with advances in wound age, suggesting that fibrocytes might be utilized in wound age determination.

There is increasing evidence that fibrocytes contribute to a new population of fibroblasts and myofibroblasts that emerge at tissue sites during repair processes and in some fibrotic lesions [29, 31–33]. On in vitro stimulation with profibrotic cytokines and growth factors, human fibrocytes further differentiate into cells identical to contractile myofibroblasts [30, 34, 36]. Since myofibroblast migration is indispensable in tissue repair process [9, 10], Betz and colleagues examined the time-dependent appearance of α-SMA^{+} myofibroblasts in human skin wounds and demonstrated that myofibroblasts were detected during the initial formation of typical granulation tissue in human skin wounds as early as approximately 5 days post wounding [46]. Consistent with this previous report, we found that fibrocytes initially appeared in 4-day-old wounds and subsequently increased at the wound site. These observations indicate that fibrocyte infiltration into wounds and their differentiation into myofibroblasts might be important in skin wound healing, and therefore, the time-dependent

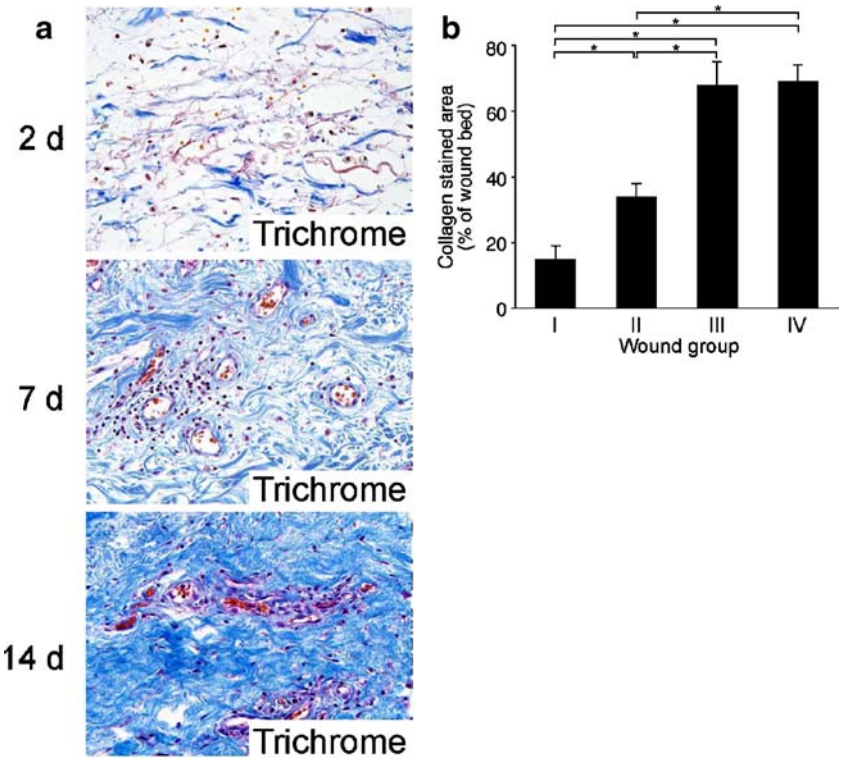

Fig. 3 Fibrocyte infiltration seems to be correlated with increases in collagen accumulation in the skin wound bed. **a** Histopathological analysis. Masson's trichrome staining was performed to determine collagen deposition at skin wound sites. Representative results from three independent experiments are shown (2-, 7-, and 14-day-old wound). Original magnification ×200. **b** Kinetics of fibrotic change at wound sites. Fibrotic changes were quantified with trichrome-stained sections. *$p<0.01$, a statistically significant difference was observed

appearance of fibrocytes at a wound site could be used to estimate wound age.

It has been reported that the synthesis of several subtypes of collagen associated with myofibroblasts could be detected during the proliferative phase of skin wound healing [15, 16, 46]. Thus, myofibroblasts are presumed to play a key role in granulation tissue formation related to collagen synthesis in skin wound healing [9, 10]. An immunohistochemical localization of collagen types I, III, V, and VI could provide some useful information for forensic age estimation of human skin wounds [6, 15, 46, 50]. We found fibrocytes in human skin wounds aged older than 4 days along with increases in collagen deposition, indicating that fibrocytes, in addition to myofibroblasts, may modulate skin wound healing by producing collagen.

Of interest is that fibrocytes isolated from peripheral blood and cultured ex vivo secrete a unique profile of cytokines, growth factors, and chemokines [31, 33, 35, 41]. Based on the presence of fibrocytes in healing wounds and their ability to secrete numerous biological substances, fibrocytes have been postulated as a modulator of tissue repair owing to their regulation of the activities of local fibroblasts as well as infiltrating cells, although further investigation is needed [33, 41, 51].

In the forensic practice, the determination of wound age including wound vitality is always required to clarify the relationship between wounds and the cause of death. The aim of this prospective study was to establish reference data concerning the age of skin wound when the age of the injury is unknown. The present study provided evidence for the involvement of fibrocytes in wound healing of human skin and furthermore showed that fibrocyte appearance is available as a marker for wound age determination from the viewpoint of forensic pathology. Our data demonstrate that the appearance of fibrocytes in human skin wounds indicate at least a 4-day post infliction interval. Moreover, a fibrocyte number of over 10 within a high-power microscopic field (0.4× 0.4 mm) might indicate a wound age between 9 and 14 days (i.e., group III). Based on the average number of fibrocytes in each group, a fibrocyte number of over 15 strongly suggests a wound age of 9–14 days. Although it is difficult to differentiate wounds aged 4–7 days (group II) from wounds aged 17–21 days (group IV) using the extent of fibrocyte appearance alone, the degree of collagen accumulation would be different between these two wound ages. Therefore, with regard to its practical applicability, a combination of fibrocyte appearance and other markers including collagen accumulation would give more reliable information for wound age determination. Thus, it is considered that the evaluation of fibrocytes can achieve wound age determination with a high degree of accuracy and objectivity.

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References

1. Laiho K (1998) Myeloperoxidase activity in skin lesions. I. Influence of the loss of blood, depth of excoriations and thickness of the skin. Int J Legal Med 111:6–9
2. Laiho K (1998) Myeloperoxidase activity in skin lesions. II. Influence of alcohol and some medicines. Int J Legal Med 111:10–12
3. Oehmichen M, Cropelin A (1995) Temporal course of intravital and postmortem proliferation of epidermal cells after mechanical injury. An immunohistochemical study using bromodeoxyuridine in rats. Int J Legal Med 107:257–262
4. Betz P (1994) Histological and enzyme histochemical parameters for the age estimation of human skin wounds. Int J Legal Med 107:60–68
5. Dressler J, Hanisch U, Kuhlisch E, Geiger KD (2007) Neuronal and glial apoptosis in human traumatic brain injury. Int J Legal Med 121:365–375
6. Eisenmenger W, Nerlich A, Glück G (1988) Die Bedeutung des Kollagens bei Wundaltersbestimmung. Z Rechtsmed 100:79–100
7. Betz P, Eisenmenger W (1996) Morphometrical analysis of hemosiderin deposits in relation to wound age. Int J Legal Med 108:262–264
8. Kondo T (2007) Timing of skin wounds. Leg Med (Tokyo) 9:109–114
9. Martin P (1997) Wound healing—aiming for perfect skin regeneration. Science 276:75–81
10. Singer AJ, Clark RA (1999) Cutaneous wound healing. N Engl J Med 341:738–746
11. Gillitzer R, Goebeler M (2001) Chemokines in cutaneous wound healing. J Leukoc Biol 69:513–521
12. Martin P, Leibovich SJ (2005) Inflammatory cells during wound repair: the good, the bad and the ugly. Trends Cell Biol 15:599–607
13. Ishida Y, Gao JL, Murphy PM (2008) Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. J Immunol 180:569–579
14. Ishida Y, Kimura A, Takayasu T, Eisenmenger W, Kondo T (2008) Expression of oxygen-regulated protein 150 (ORP150) in skin wound healing and its application for wound age determination. Int J Legal Med 122:409–414
15. Betz P, Nerlich A, Wilske J, Tubel J, Penning R, Eisenmenger W (1993) Analysis of the immunohistochemical localization of collagen type III and V for the time-estimation of human skin wounds. Int J Legal Med 105:329–332
16. Betz P, Nerlich A, Wilske J, Tubel J, Penning R, Eisenmenger W (1993) Immunohistochemical localization of collagen types I and VI in human skin wounds. Int J Legal Med 106:31–34
17. Betz P (1995) Immunohistochemical parameters for the age estimation of human skin wounds. A review. Am J Forensic Med Pathol 16:203–209
18. Betz P, Nerlich A, Tubel J, Wiest I, Hausmann R (1997) Detection of cell death in human skin wounds of various ages by an in situ end labeling of nuclear DNA fragments. Int J Legal Med 110:240–243
19. Dressler J, Bachmann L, Kasper M, Hauck JG, Muller E (1997) Time dependence of the expression of ICAM-1 (CD 54) in human skin wounds. Int J Legal Med 110:299–304
20. Dressler J, Bachmann L, Koch R, Muller E (1999) Estimation of wound age and VCAM-1 in human skin. Int J Legal Med 112:159–162

21. Kondo T, Ohshima T (1996) The dynamics of inflammatory cytokines in the healing process of mouse skin wound: a preliminary study for possible wound age determination. Int J Legal Med 108:231–236
22. Kondo T, Ohshima T, Eisenmenger W (1999) Immunohistochemical and morphometrical study on the temporal expression of interleukin-1α (IL-1α) in human skin wounds for forensic wound age determination. Int J Legal Med 112:249–252
23. Ohshima T, Sato Y (1998) Time-dependent expression of interleukin-10 (IL-10) mRNA during the early phase of skin wound healing as a possible indicator of wound vitality. Int J Legal Med 111:251–255
24. Guan DW, Ohshima T, Kondo T (2000) Immunohistochemical study on Fas and Fas ligand in skin wound healing. Histochem J 32:85–91
25. Sato Y, Ohshima T (2000) The expression of mRNA of proinflammatory cytokines during skin wound healing in mice: a preliminary study for forensic wound age estimation (II). Int J Legal Med 113:140–145
26. Rebolledo Godoy M, Rebolledo Godoy AP, Oehmichen M (2000) AgNORs during the process of wound healing. Time dependency as evaluated in vital and postmortem biopsy. Int J Legal Med 113:244–246
27. Hayashi T, Ishida Y, Kimura A, Takayasu T, Eisenmenger W, Kondo T (2004) Forensic application of VEGF expression to skin wound age determination. Int J Legal Med 118:320–325
28. Kondo T, Ohshima T, Mori R, Guan DW, Ohshima K, Eisenmenger W (2002) Immunohistochemical detection of chemokines in human skin wounds and its application to wound age determination. Int J Legal Med 116:87–91
29. Metz CN (2003) Fibrocytes: a unique cell population implicated in wound healing. Cell Mol Life Sci 60:1342–1350
30. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN (2001) Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. J Immunol 166:7556–7562
31. Bellini A, Mattoli S (2007) The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab Invest 87:858–870
32. Gomperts BN, Strieter RM (2007) Fibrocytes in lung disease. J Leukoc Biol 82:449–456
33. Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R (2004) Circulating fibrocytes: collagen-secreting cells of the peripheral blood. Int J Biochem Cell Biol 36:598–606
34. Kisseleva T, Uchinami H, Feirt N, Quintana-Bustamante O, Segovia JC, Schwabe RF, Brenner DA (2006) Bone marrow-derived fibrocytes participate in pathogenesis of liver fibrosis. J Hepatol 45:429–438
35. Chesney J, Metz C, Stavitsky AB, Bacher M, Bucala R (1998) Regulated production of type I collagen and inflammatory cytokines by peripheral blood fibrocytes. J Immunol 160:419–425
36. Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S (2003) Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. J Immunol 171:380–389
37. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, Belperio JA, Keane MP, Strieter RM (2004) Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. J Clin Invest 114:438–446
38. Ishida Y, Kimura A, Kondo T, Hayashi T, Ueno M, Takakura N, Matsushima K, Mukaida N (2007) Essential roles of the CC chemokine ligand 3-CC chemokine receptor 5 axis in bleomycin-induced pulmonary fibrosis through regulation of macrophage and fibrocyte infiltration. Am J Pathol 170:843–854
39. Yang L, Scott PG, Giuffre J, Shankowsky HA, Ghahary A, Tredget EE (2002) Peripheral blood fibrocytes from burn patients: identification and quantification of fibrocytes in adherent cells cultured from peripheral blood mononuclear cells. Lab Invest 82:1183–1192
40. Pilling D, Tucker NM, Gomer RH (2006) Aggregated IgG inhibits the differentiation of human fibrocytes. J Leukoc Biol 79:1242–1251
41. Wang JF, Jiao H, Stewart TL, Shankowsky HA, Scott PG, Tredget EE (2007) Fibrocytes from burn patients regulate the activities of fibroblasts. Wound Repair Regen 15:113–121
42. Ishida Y, Kondo T, Kimura A, Matsushima K, Mukaida N (2006) Absence of IL-1 receptor antagonist impaired wound healing along with aberrant NF-κB activation and a reciprocal suppression of TGF-β signal pathway. J Immunol 176:5598–5606
43. Kondo T, Tanaka J, Ishida Y, Mori R, Takayasu T, Ohshima T (2002) Ubiquitin expression in skin wounds and its application to forensic wound age determination. Int J Legal Med 116:267–272
44. Furuichi K, Gao JL, Murphy PM (2006) Chemokine receptor CX3CR1 regulates renal interstitial fibrosis after ischemia-reperfusion injury. Am J Pathol 169:372–387
45. Betz P, Nerlich A, Wilske J, Tubel J, Penning R, Eisenmenger W (1992) Time-dependent appearance of myofibroblasts in granulation tissue of human skin wounds. Int J Legal Med 105:99–103
46. Betz P, Nerlich A, Wilske J, Tubel J, Wiest I, Penning R, Eisenmenger W (1992) Time-dependent pericellular expression of collagen type IV, laminin, and heparan sulfate proteoglycan in myofibroblasts. Int J Legal Med 105:169–172
47. Kondo T, Ohshima T, Sato Y, Mayama T, Eisenmenger W (2000) Immunohistochemical study on the expression of c-Fos and c-Jun in human skin wounds. Histochem J 32:509–514
48. Mori L, Bellini A, Stacey MA, Schmidt M, Mattoli S (2005) Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. Exp Cell Res 304:81–90
49. Wang J, Jiao H, Stewart TL, Lyons MV, Shankowsky HA, Scott PG, Tredget EE (2007) Accelerated wound healing in leukocyte-specific, protein 1-deficient mouse is associated with increased infiltration of leukocytes and fibrocytes. J Leukoc Biol 82:1554–1563
50. Betz P, Nerlich A, Wilske J, Tubel J, Wiest I, Penning R, Eisenmenger W (1992) The time-dependent rearrangement of the epithelial basement membrane in human skin wounds—immunohistochemical localization of collagen IV and VII. Int J Legal Med 105:93–97
51. Hartlapp I, Abe R, Saeed RW, Peng T, Voelter W, Bucala R, Metz CN (2001) Fibrocytes induce an angiogenic phenotype in cultured endothelial cells and promote angiogenesis in vivo. FASEB J 15:2215–2224