Int J Legal Med (2008) 122:409–414
DOI 10.1007/s00414-008-0255-1

ORIGINAL ARTICLE

Expression of oxygen-regulated protein 150 (ORP150) in skin wound healing and its application for wound age determination

Y. Ishida · A. Kimura · T. Takayasu · W. Eisenmenger · T. Kondo

Received: 6 February 2008 / Accepted: 19 May 2008 / Published online: 18 June 2008

Abstract Immunohistochemical study combined with morphometry was carried out to examine the expression of oxygen-regulated protein 150 (ORP150) using 58 human skin wounds of different ages (group I, 0–12 h; II, 1–5 days; III, 7–14 days; and IV, 17–21 days). In human wound specimens aged 4–12 h, neutrophils recruited at the wound showed no positive signals for ORP150. With the increase in wound age of ≥7 days, granulation tissue and angiogenesis were observed, with the migration of macrophages and fibroblasts with ORP150-positive reactions. In semi-quantitative analysis, the average of ORP150-positive ratios in group III was highest. In group III, all samples had an ORP150-positive ratio of >40%, and 17 samples showed >50%. In group IV, three out of ten samples showed a positive ratio of 40–45% and the remaining seven cases less than 40%. Collectively, with regard to the practical applicability with forensic safety, these observations suggest that an ORP150-positive ratio of >50% strongly indicates a wound age of 7–14 days.

Y. Ishida · A. Kimura · T. Kondo (✉)
Department of Forensic Medicine, Wakayama Medical University,
811-1 Kimiidera,
Wakayama 641-8509, Japan
e-mail: kondot@wakayama-med.ac.jp

T. Takayasu
Department of Forensic and Social Environmental Medicine,
Kanazawa University Graduate School of Medical Science,
Kanazawa, Ishikawa, Japan

W. Eisenmenger
Institute for Legal Medicine, University of Munich,
Frauenlobstrasse 7a,
80337 Munich, Germany

Keywords Forensic pathology · Wound age determination · Immunohistochemistry · Hypoxia · ORP150

Introduction

In forensic practices, forensic pathologists encounter autopsy cases with fresh and old skin wounds. In such cases, wound age determination is essential because pathologists are required to judge how wounds are related to the cause of death [1–10]. Conventionally, the detection of Berlin-blue depositions in macrophages may give useful information for wound age determination [8]. Wound healing is a basic biological response, and it is well known that wound healing is composed of three phases: inflammation, proliferation, and maturation [11, 12]. Various biological substances, such as growth factors, cytokines, and adhesion molecules, are known to be closely involved in each phase of the wound healing process [11–17]. Some of these substances have been applied to the determination of wound age, including wound vitality [18–43].

Hypoxic conditions induce various polypeptides to prevent serious and eventual fatal damage. Kuwabara et al. [44, 45] identified a novel oxygen-regulated protein with a molecular weight of 150 kDa (ORP150) from the primary culture of rat astrocytes. Several lines of accumulating evidence have demonstrated that ORP150, a member of the stress protein family, plays a role as an endoplasmic reticulum (ER) chaperone in pathological conditions such as ischemic brain damage [46], atherosclerotic plaques [47], hyperglycemia [48], malignant tumors [49], and glutamate toxicity [50]. Moreover, Ozawa et al. [51] demonstrated that overexpression of the ORP150 gene by adenovirus vectors accelerated wound healing by modulating intracellular vascular endothelial growth factor transport. These

observations implied that ORP150 was essentially involved in skin wound healing.

In the present study, we immunohistochemically examined ORP150 expression in human skin wounds of different wound ages and discuss the practical availability of ORP150 as a marker for wound age determination.

Materials and methods

Antibodies

The following monoclonal or polyclonal antibodies (mAbs or pAbs) were used for immunohistochemical and immunofluorescence analyses in the present study: rabbit anti-ORP150 pAbs (a kind gift from Prof. Ogawa, Kanazawa University), mouse anti-human CD 68 mAb (clone PG-M1; Dako Cytomation, Kyoto, Japan), mouse anti-α-smooth muscle actin (αSMA, clone 1A4; Dako Cytomation), cyanine dye 3 (cy3)-conjugated donkey anti-mouse IgG pAbs, and flourescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG pAbs (Jackson ImmunoResearch, West Grove, PA, USA).

Human skin wound specimens

A total of 58 human skin wounds with different post-infliction intervals ranging from a few minutes to 21 days (18 stab wounds, 12 incised wounds, 20 surgical wounds, and eight lacerations) were removed at forensic autopsy (Institute for Legal Medicine, University of Munich). The individual ages ranged from 8 to 75 years (mean age, 48.6 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, which may possibly influence wound healing, were administered during medical treatment. According to wound ages, the wound specimens were classified into four groups as follows: group I, 0–12 h (n=12); group II, 1–5 days (n=16); group III, 7–14 days (n=20); and group IV, 17–21 days (n=10). Uninjured skin from the same individuals was also taken as a control.

Immunohistochemistry

The wound specimens were fixed in 4% formaldehyde solution with phosphate-buffered saline (PBS; pH 7.2) and embedded in paraffin, followed by sectioning at a thickness of 4 μm. After deparaffinization, the sections were immersed in 0.3% H_2O_2–methanol for 30 min and incubated with anti-ORP150 pAbs (1:100) at 4°C overnight. Thereafter, Envision+™ (Dako Cytomation) for rabbit or mouse immunoglobulin was reacted at room temperature for 30 min, and positive reactions were visualized with diaminobenzidine.

Double-color immunofluorescence analysis

Double-color immunofluorescence analysis was also performed to determine the types of ORP150-expressing cells during skin wound healing, as described previously [16, 17]. 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 in pairs of anti-ORP150 (1:100) and anti-CD68 (1:00) for human macrophages, or anti-ORP150 (1:100) and anti-αSMA (1:100) antibody at 4°C overnight. After incubation with cy3-conjugated anti-mouse IgG pAbs (1:50) and FITC-conjugated anti-rabbit IgG pAbs (1:25) at room temperature for 1 h, the sections were observed under a fluorescence microscope. Fluorescent images were digitally merged.

Morphometrical analysis

According to the methods of previous studies [27, 28, 33, 38], morphometrical analysis was performed for semi-quantitative evaluation of the immunohistochemical findings by two different investigators without prior knowledge. Briefly, in each section, ten high power microscopic fields (square=0.016 mm^2) were randomly selected, and the ratio of the number of ORP150-positive infiltrating cells, such as leukocytes and fibroblasts, to the total number of infiltrating cells was estimated in each microscopic field. The average ratio of the ten selected microscopic fields was evaluated as the ORP150 expression score of each wound specimen.

Statistical analysis

In each group, the mean values of the ORP150-positive ratios and standard errors (SE) were calculated. Statistical analyses were performed using one-factor analysis of variance to determine whether differences existed among the group means, followed by Scheffé's F test to identify significantly different means.

Results

Immunohistochemical and double-color immunofluorescence analyses

In unwounded specimens, ORP150-positive signals could not be detected. In wound specimens aged 4 h to 1 day,

polymorphonuclear neutrophils were predominantly observed at the wound site; however, those neutrophils showed no immunopositive reaction to ORP150. With an increase in wound age, the infiltration of mononuclear cells was obviously dominant compared to neutrophils, followed by concomitant migration of fibroblasts. Immunopositive signals for ORP150 were localized at the cytoplasm of these mononuclear cells and fibroblastic cells (Fig. 1). There was no influence of autolysis on the staining intensity in human skin wound samples used in the present study. Furthermore, the ORP150 expression was not enhanced in the postmortem skin wounds. In the next series, we performed double-color immunofluorescence analysis to determine the cell type producing ORP150 using a combination of anti-ORP150 and anti-CD68 (a marker for macrophages) or anti-ORP150 and anti-αSMA (a marker for myofibroblasts). As shown in Fig. 2, macrophages and myofibroblasts expressed ORP150 in human skin wounds.

Morphometry

Figure 3a demonstrates the distribution of the ratios of ORP150-positive infiltrating cells in relation to wound age. ORP150-positive ratios were very low in wound samples aged 0–12 h (group I), with most of the wound specimens in group I giving values of less than 10% (mean ± SE, 4.2± 1.1%). In wound specimens with a postinfliction interval of 1–5 days (group II), the ratio of ORP150-positive cells apparently increased (mean ± SE, 21.7±2.8%). Moreover, in wound samples aged 7–14 days (group III), the ORP150-positive ratio considerably increased, and in 17 out of 20 samples, the ratio was over 50% (mean ± SE, 58.8±2.0%). A 14-day-old wound in group III showed the highest value (75.6%) among all of the 58 human skin wound specimens in the present study. Although the ORP150-positive ratio significantly decreased compared with group III in wound specimens with a postinfliction interval of 17–21 days (group IV), it remained moderately high (mean ± SE: 28.1± 3.5%). Statistical analysis revealed significant differences between group I and the three other groups, between groups II and III, and between groups III and IV (Fig. 3b); however, there was no significant difference between groups II and IV.

Discussion

ORP150 has been identified as a novel stress protein from cultured rat astrocytes [44, 45]. ORP150 is induced under endoplasmic reticulum stress and acts as a molecular chaperone. ORP150, selectively expressed by astrocytes, may contribute to their adaptive response to ischemic stress, thereby ultimately contributing to the enhanced survival of neurons [44, 45]. Tamatani and colleagues [46] demonstrated that ORP150 transgenic mice were resistant to cerebral ischemia by preventing hypoxia-induced neuronal death. Moreover, ORP150 was involved in various pathophysiological conditions such as atherosclerosis [47], prevention from glutamate toxicity [50], and Purkinje cell survival [52].

Neovascularization is indispensable for tissue repair and tumor growth [11, 12]. VEGF is the most potent angiogenic cytokine [53–55]. ORP150 is closely involved in angiogenesis, with the adenovirus-mediated suppression of ORP150 expression inhibiting the growth of glioma by the reduction of neovascularization [49]. In contrast, overexpression of ORP150 accelerated skin wound healing in diabetic mice by enhancing angiogenesis at the wound sites [51]. In the molecular mechanism of how ORP150 enhances angiogenesis, it has been clarified that ORP150 modulates the intracellular transport of VEGF from the endoplasmic reticulum to the Golgi apparatus [51].

Consistent with a previous study [51], immunopositive signals for ORP150 were detected in the cytoplasm of macrophages recruited to wound sites, and αSMA-positive

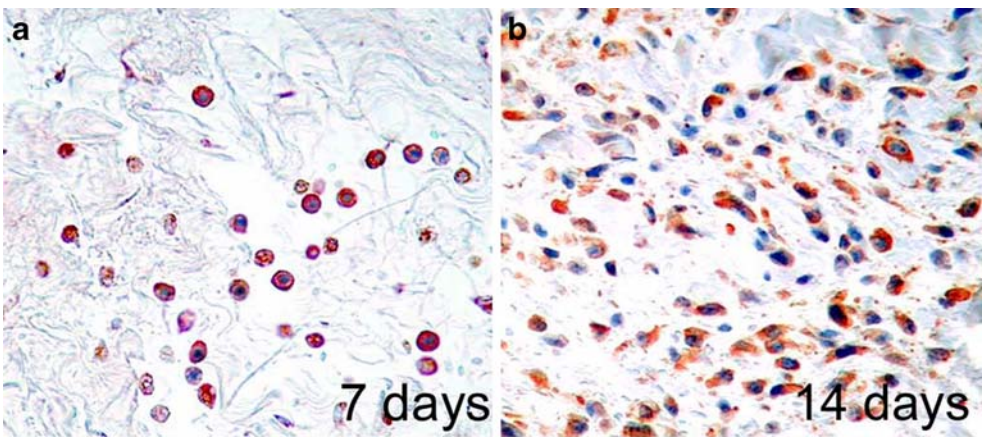

Fig. 1 **a** In this 7-day-old wound, phagocytic macrophages are immunostained with anti-ORP150 pAbs. **b** In this 14-day-old wound, spindle-shaped fibroblastic cells were positively immunostained with anti-ORP150 pAbs. Original magnification ×200

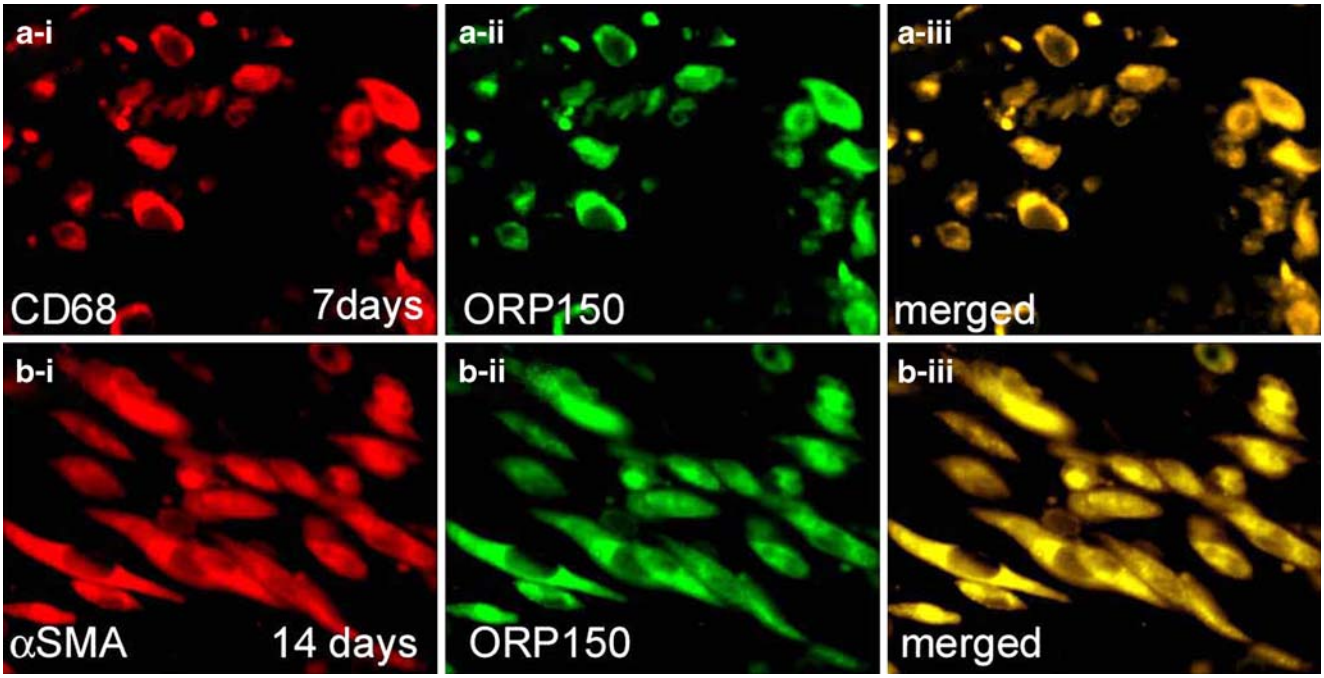

Fig. 2 Double-color immunofluorescence analysis was performed to determine ORP150-expressing cell types. The samples were immunostained with anti-CD68 [**a** (*i*), Cy3], anti-αSMA [**b** (*i*), Cy3], or anti-ORP150 [**a** (*ii*) and **b** (*ii*), FITC], as described in "Materials and methods," and observed under a fluorescence microscope. Signals in *i* and *ii* were digitally merged in *iii*. Representative results from three independent experiments are shown here (original magnification ×400)

myofibroblasts were also expressed. Betz et al. [56] examined the appearance of αSMA-positive myofibroblasts in human skin wounds, demonstrating that they appeared with the initial formation of typical granulation tissue as early as approximately 5 days post-wounding. Our previous study demonstrated that both macrophages and myofibroblasts were VEGF-producing cells during skin wound healing [38]. Actually, ORP150 and VEGF were colocalized in macrophages and myofibroblasts (data not shown). Thus, it is considered that macrophages and fibroblasts should be morphometrically analyzed, in accordance with our previous studies.

To the best of our knowledge, only a few studies have focused on ORP150 in the field of forensic pathology. Immunohistochemical analysis of ORP150 in the brain may be very useful to determine the duration of brain ischemia before death in forensic autopsy cases and to differentiate between sudden infant death syndrome and acute asphyxia [57, 58]. From the viewpoint of forensic pathological applications, the present study shows that ORP150 is a suitable marker for wound age determination. All wound samples in groups I and II showed an ORP150-positive ratio of <40%. In group III, all samples had an ORP150-positive ratio of >40%, and 17 samples showed >50%. In

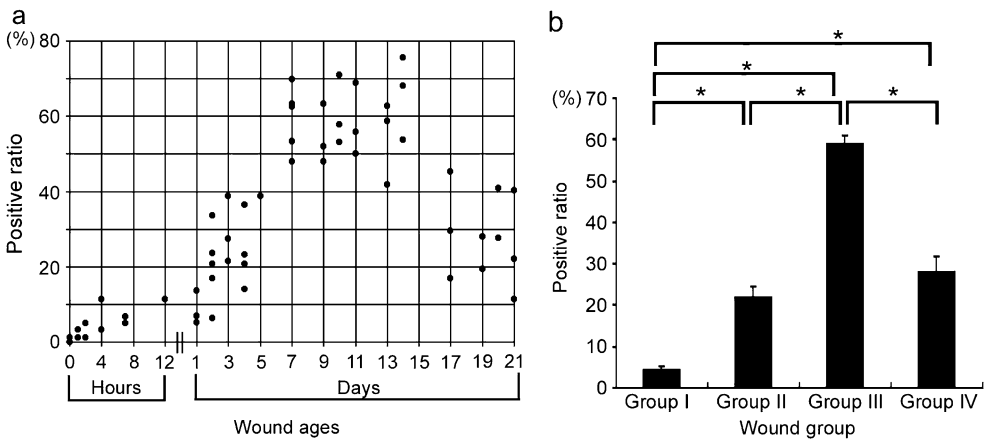

Fig. 3 **a** Ratio of ORP150-positive infiltrating cells in relation to wound age. **b** Mean value and standard error of ORP150-positive infiltrating cells in each wound group. *$p<0.05$, a significant difference was observed statistically

group IV, three out of ten samples showed a positive ratio of 40–45%, and the remaining seven cases were less than 40%. Thus, it was very difficult to determine the wound age in 40–50% from the aspect of forensic safety. Collectively, with regard to practical applicability with forensic safety, these observations suggested that an ORP150-positive ratio of >50% strongly indicates a wound age of 7 to 14 days.

Considering from the expression of ORP150 and other markers, fibronectin, adhesion molecules, and interleukin-1α were presumed to be sensitive markers for the determination of wound aged less than 1 day [10]. Chemokines are seemingly useful for wound age of one to several days [10]. Moreover, VEGF and ORP150 would be available markers for wound age determination at the later phase of wound healing process. Thus, the combination with several markers would give more corrective and objective information for wound age determination.

Acknowledgment This study was financially supported by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science, Sports and Culture of Japan. We sincerely thank Ms. Mariko Kawaguchi for her excellent assistant in preparing this manuscript.

References

1. Raekallio J (1972) Determination of the age of wounds by histochemical and biochemical methods. Forensic Sci Int 1:3–16
2. Laiho K (1998) Myeloperoxidase activity in skin lesions. I. Influence of the loss of blood, depth of excoriation and thickness of the skin. Int J Legal Med 111:6–9
3. Laiho K (1998) Myeloperoxidase activity in skin lesions. II. Influence of alcohol and some medicines. Int J Legal Med 111:10–12
4. Eisenmenger W, Nerlich A, Glück G (1988) Die Bedeutung des Kollagens bei Wundaltersbestimmung. Z Rechtsmed 100:79–100
5. Oehmichen M (1990) Die Wundheilung. Springer, Berlin, pp 5–67
6. Oehmichen M, Cröpelin A (1995) Temporal course of intravital and postmortem proliferation of epidermal cells after injury—an immunohistochemical study using bromodeoxyuridine in rats. Int J Legal Med 107:257–262
7. Betz P (1994) Histological and enzyme histochemical parameters for the age estimation of human skin wounds. Int J Legal Med 107:60–68
8. Betz P, Eisenmenger W (1996) Morphometrical analysis of hemosiderin deposits in relation to wound age. Int J Legal Med 108:262–264
9. Dressler J, Busuttil A, Koch R, Harrison DJ (2001) Sequence of melanocyte migration into human scar tissue. Int J Legal Med 115:61–63
10. Kondo T (2007) Timing of skin wounds. Legal Med 9:109–114
11. Martin P (1997) Wound healing—aiming for perfect skin regeneration. Science 276:75–81
12. Singer AJ, Clark RA (1999) Cutaneous wound healing. N Engl J Med 341:738–746
13. Ishida Y, Kondo T, Takayasu T, Iwakura Y, Mukaida N (2004) The essential involvement of cross-talk between IFN-g and TGF-b in the skin wound-healing process. J Immunol 172:1848–1855
14. Lin ZQ, Kondo T, Ishida Y, Takayasu T, Mukaida N (2003) Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. J Leukoc Biol 73:713–721
15. Mori R, Kondo T, Ohshima T, Ishida Y, Mukaida N (2002) Accelerated wound healing in tumor necrosis factor receptor p55-deficient mice with reduced leukocyte infiltration. FASEB J 16:963–974
16. 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
17. 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
18. Betz P, Nerlich A, Wilske J, Tübel J, Wiest I, Penning R, Eisenmenger W (1992) Immunohistochemical localization of fibronectin as a tool for the age determination of human skin wounds. Int J Legal Med 105:21–26
19. Betz P, Nerlich A, Wilske J, Tübel 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
20. Betz P, Nerlich A, Wilske J, Tübel J, Penning R, Eisenmenger W (1993) Immunohistochemical localization collagen types I and VI in human skin wounds. Int J Legal Med 106:31–34
21. Betz P (1995) Immunohistochemical parameters for the age estimation of human skin wounds. A review. Am J Forensic Med Pathol 16:203–209
22. Betz P, Nerlich A, Tübel 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
23. Dreßler J, Bachmann L, Kasper M, Hauck JG, Müller E (1997) Time dependence of the expression ICAM (CD-54) in human skin wound. Int J Legal Med 110:299–304
24. Dreßler J, Bachmann L, Koch R, Müller E (1998) Enhanced expression of selectins in human skin wounds. Int J Legal Med 112:39–44
25. Dreßler J, Bachmann L, Koch R, Müller E (1999) Estimation of wound age and VCAM-1 in human skin. Int J Legal Med 112:159–162
26. 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
27. Kondo T, Ohshima T, Eisenmenger W (1999) Immunohistochemical and morphometrical study on the temporal expression of interleukin-1a (IL-1a) in human skin wounds for forensic wound age determination. Int J Legal Med 112:249–252
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. Ohshima T, Sato Y (1998) Time-dependent expression of interleukin-10 (IL-10) mRNA during the early phase of skin wound healing as possible indicator of wound vitality. Int J Legal Med 1998:251–255
30. Guan D, Ohshima T, Kondo T (2000) Immunohistochemical study on Fas and Fas ligand in skin wound healing. Histochem J 32:85–91
31. Sato Y, Ohshima T (2000) The expression of mRNA by proinflammatory cytokines during skin wound healing in mice: a preliminary study for forensic wound age estimation (II). Int J Legal Med 113:140–145

32. 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
33. 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
34. Hausmann R, Betz P (2001) Course of glial immunoreactivity for vimentin, tenascin and alpha1-antichymotrypsin after traumatic injury to human brain. Int J Legal Med 114:338–342
35. Hausmann R, Betz P (2000) The time course of the vascular response to human brain injury—an immunohistochemical study. Int J Legal Med 113:288–292
36. Hausmann R, Riess R, Fieguth A, Betz P (2000) Immunohistochemical investigations on the course of astroglial GFAP expression following human brain injury. Int J Legal Med 113:70–75
37. Hausmann R, Kaiser A, Lang C, Bohnert M, Betz P (1999) A quantitative immunohistochemical study on the time-dependent course of acute inflammatory cellular response to human brain injury. Int J Legal Med 112:227–232
38. 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
39. Takamiya M, Saigusa K, Nakayashiki N, Aoki Y (2003) Studies on mRNA expression of basic fibroblast growth factor in wound healing for wound age determination. Int J Legal Med 117:46–50
40. 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
41. Hausmann R, Seidl S, Betz P (2006) Hypoxic changes in Purkinje cells of the human cerebellum. Int J Legal Med 12:175–183
42. Hausmann R, Vogel C, Seidl S, Betz P (2006) Value of morphological parameters for grading of brain swelling. Int J Legal Med 120:219–225
43. Hausmann R, Biermann T, Wiest I, Tübel J, Betz P (2004) Neuronal apoptosis following human brain injury. Int J Legal Med 118:32–36
44. Kuwabara K, Matsumoto M, Ikeda J, Hori O, Ogawa S, Maeda Y, Kitagawa K, Imuta N, Kinoshita T, Stern DM, Yanagi H, Kamada T (1996) Purification and characterization of a novel stress protein, the 150-kDa oxygen-regulated protein (ORP150), from cultured rat astrocytes and its expression in ischemic mouse brain. J Biol Chem 271:5025–5032
45. Ikeda J, Kaneda S, Kuwabara K, Ogawa S, Kobayashi T, Matsumoto M, Yura T, Yanagi H (1997) Cloning and expression of cDNA encoding the human 150 kDa oxygen-regulated protein, ORP150. Biochem Biophys Res Commun 230:94–99
46. Tamatani M, Matsuyama T, Yamaguchi A, Mitsuda N, Tsukamoto Y, Taniguchi M, Che YH, Ozawa K, Hori O, Nishimura H, Yamashita A, Okabe M, Yanagi H, Stern DM, Ogawa S, Tohyama M (2001) ORP150 protects against hypoxia/ischemia-induced neuronal death. Nat Med 7:317–323
47. Tsukamoto Y, Kuwabara K, Hirota S, Ikeda J, Stern D, Yanagi H, Matsumoto M, Ogawa S, Kitamura Y (1996) 150-kD oxygen-regulated protein is expressed in human atherosclerotic plaques and allows mononuclear phagocytes to withstand cellular stress on exposure to hypoxia and modified low density lipoprotein. J Clin Invest 98:1930–1941
48. Ozawa K, Miyazaki M, Matsuhisa M, Takano K, Nakatani Y, Hatazaki M, Tamatani T, Yamagata K, Miyagawa J, Kitao Y, Hori O, Yamasaki Y, Ogawa S (2005) The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes. Diabetes 54:657–663
49. Ozawa K, Tsukamoto Y, Hori O, Kitao Y, Yanagi H, Stern DM, Ogawa S (2001) Regulation of tumor angiogenesis by oxygen-regulated protein 150, an inducible endoplasmic reticulum chaperone. Cancer Res 61:4206–4213
50. Kitao Y, Ozawa K, Miyazaki M, Tamatani M, Kobayashi T, Yanagi H, Okabe M, Ikawa M, Yamashima T, Stern DM, Hori O, Ogawa S (2001) Expression of the endoplasmic reticulum molecular chaperone (ORP150) rescues hippocampal neurons from glutamate toxicity. J Clin Invest 108:1439–1450
51. Ozawa K, Kondo T, Hori O, Kitao Y, Stern DM, Eisenmenger W, Ogawa S, Ohshima T (2001) Expression of the oxygen-regulated protein ORP150 accelerates wound healing by modulating intracellular VEGF transport. J Clin Invest 108:41–50
52. Kitao Y, Hashimoto K, Matsuyama T, Iso H, Tamatani T, Hori O, Stern DM, Kano M, Ozawa K, Ogawa S (2004) ORP150/HSP12A regulates Purkinje cell survival: a role for endoplasmic reticulum stress in cerebellar development. J Neurosci 24:1486–1496
53. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N (1989) Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246:1306–1309
54. Ferrara N, Henzel WJ (1989) Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 161:851–858
55. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z (1999) Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 13:9–22
56. Betz P, Nerlich A, Wilske J, Tübel 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
57. Ikematsu K, Tsuda R, Kondo T, Kondo H, Ozawa K, Ogawa S, Nakasono I (2004) The expression of '150-kDa oxygen regulated protein (ORP-150)' in human brain and its relationship with duration time until death. Legal Med 6:97–101
58. Ikematsu K, Tsuda R, Kondo T, Kondo H, Ozawa K, Ogawa S, Nakasono I (2003) The expression of a novel stress protein '150-kDa oxygen regulated protein' in sudden infant death. Legal Med 5:15–19