Localization of tenascin in human skin wounds — an immunohistochemical study

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Summary. A total of 56 surgically treated human skin wounds with a wound age between 8 h and 7 months were investigated. Tenascin was visualized by immunohistochemistry and appeared first in the wound area pericellularly around fibroblastic cells approximately 2 days after wounding. A network-like interstitial positive staining pattern was first detectable in 3-day-old skin wounds. In all wounds with an age of 5 days or more, intensive reactivity for tenascin could be observed in the lesional area (dermal-epidermal junction, wound edge, areas of bleeding). In wounds with an age of more than approximately 1.5 months no positive staining occurred in the scar tissue. In conclusion, for forensic purposes, positive staining for tenascin restricted to the pericellular area of fibroblastic cells indicates a wound age of at least 2 days. Network-like structures appear after approximately 3 days or more. Since tenascin seems to be regularly detectable in skin wounds older than 5 days, the lack of a positive reaction in a sufficient number of specimens indicates a wound age of less than 5 days. The lack of a positive reaction in the granulation tissue of wounds with advanced wound age indicates a survival time of more than about 1.5 months, but a positive staining in older wounds cannot be excluded.

Key words: Tenascin — Wound age — Immunohistochemistry

Introduction

Wound repair is a series of biological processes involving cellular proliferation, migration, differentiation, and tissue remodeling by the synthesis of structural components of the extracellular matrix such as various collagen subtypes.

However, proteins of the extracellular matrix are not only important for advanced structural remodeling of the tissue, but are also involved in the early phases of wound healing as previously shown for fibronectin [13].

Tenascin is a glycoprotein of the extracellular matrix with a six-armed structure [9] which gave rise to the term hexabrachion. According to previous findings, tenascin has been isolated from various sources leading to a variety of synonyms such as myotendinous antigen, glioma mesenchymal extracellular matrix antigen, brachionectin, J1 and cytotactin [21].

Tenascin interacts with fibronectin [10, 18], supports the adhesion of a variety of cell types including fibro-
blasts and endothelial cells [5], appears to act as a cell adhesion molecule in particular in the central nervous system [17], and seems to exert immunomodulatory activities [25].

Tenascin is produced by cultured fibroblasts and mesenchymal tissues [21] and is often co-localized with fibronectin, but its occurrence is much more restricted than that of fibronectin [7]. It is found in normal skin in association with epithelial and endothelial cells. Immunohistochemical studies provide evidence for positive staining of the papillary dermis immediately beneath the basal lamina, within the walls of blood vessels, and in smooth muscle bundles of the musculi arrectores pilorum. Strong reactivity can also be found around the cuboidal cells of the basal layer of sweat gland ducts [19, 26].

Since a time-dependent appearance in experimental wound healing has been shown [11, 21, 23], this study was performed to investigate whether the immunohistochemical localization of tenascin may be useful for the time estimation of human skin wounds.

Material and methods

A total of 56 human skin wounds (surgical wounds, stab wounds and lacerations after surgical treatment) with a wound age between 8 h and 7 months were investigated. Specimens were obtained at autopsy within a postmortem interval of less than 3 days. Subjects had died in traumatic events (car accidents, falls, homicides) and their ages ranged between 15 and 92 years (average age 54 years). Only subjects without malnutrition or severe diseases which could have an influence on wound healing, such as cancer or metabolic disorders, were selected for this study. According to clinical reports no substances which could influence wound healing, such as glucocorticoids or cytotoxic agents, had been administered during therapy.

From each skin wound at least 2 specimens were fixed in 4% PBS-formaldehyde solution and then embedded in paraffin wax. Sections (thickness of 2–3 μm) were prepared, and after enzyme pretreatment, tenascin was visualized using a monoclonal antibody (Locus-Genex, Helsinki, Finland) according to the ABC-method [14].

Undamaged skin from the same subjects and specimens without the inclusion of the primary antibody were used as controls.

Results

Undamaged skin

In normal skin, a strong positive reaction for tenascin was found in the walls of blood vessels, muscle bundles of the musculi arrectores pilorum, nerve bundles, and the basal cell layer of skin appendages, in particular in sweat gland ducts and in papillary layer of the dermis adjacent to the basement membrane. Strong reactivity was also observed around the bulb of hair follicles.

Skin wounds

Positive staining for tenascin in the wound area was first detectable around fibroblastic cells in skin wounds approximately 2 days old. The development of network-like structures positive for tenascin could be localized in the lesional area as early as about 3 days after wounding. Such a distinct positive reaction could be observed in 3 out of 10 cases (30%) with a wound age between 2 and 3 days. In all skin wounds with a survival time of 5 days or more, a strong positive reaction was observed in the form of a network-like structure with the exception of the oldest wounds investigated (wound age 2.5 or 7 months). Negative results were obtained in 2 out of 6 (33%) skin wounds aged between 4 and 5 days, in one of these cases a questionable reaction was found.

Tenascin was also detectable in the granulation tissue of older wounds (wound age 1.5 months) but in reduced amounts. In 3 out of 5 cases (60%) with a wound age of 1.5 months or more, no reactivity for tenascin was detectable in the scar tissue. However, the capillaries in the scar tissue showed positive staining.

There were no relevant differences in the time-dependent localization of tenascin between stab wounds, surgical wounds and lacerations.

In our series, no significant delay in the earliest appearance of tenascin was detectable in skin wounds obtained from older subjects, as compared to lesions from younger subjects, even though in some cases of advanced individual age a somewhat reduced staining intensity for this protein occurred.