The study of fibrogenesis using a wound healing model

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The excess production of collagens and other ECM components causes tissue fibrosis. It is therefore important to elucidate the mechanism of production of collagen molecules in order to obtain a better understanding of fibrogenesis.

We examined the expression of collagen subtypes, the kinds of fibers and the phenotypes of fibroblasts in wounded skin using a mouse model. Two full-thickness wounds, which measured 5-mm diameter, were made on the dorsal skin of the ICR mouse. Thereafter, the wound and the surrounding areas thereof were cut at different time points, namely at 2, 4, 6, 10 and 15 days and 1 year after the wounds had been made. The excised tissue specimens were then used for in situ hybridization and electron microscopy analyses. The major types of collagen, namely types I and III, were initially expressed at 2 days in the post-wound granulation tissue specimens. Interestingly, the fibroblasts derived from the superficial fascia of connective tissue in body wall muscle, and not the dermal skin fibroblasts, were thus found to play a major role in the production of collagen and wound closure. The former one had a lot of vesicles and long filopodia, which did not appear to be fibroblastic. However, after making the wound, the cells showed a fibroblastic form that had an abundant rough endoplasmic reticulum and cytoskeleton fibers and which also induced collagen production. For cell culture, we obtained morphologically uniform fibroblastic cells from the subcutaneous superficial fascia in the connective tissue of the non-injured skin. When these cells were cultured with a medium containing 10% FCS, morphological changes which were similar to those in the wounded tissue specimens were thus observed. These cells were different from the circulating fibrocytes in the peripheral blood that have recently been reported in regard to their presence in connective tissues, while they have also been observed to be CD13 negative. These fibroblastic cells express a high ratio of Type V collagen in comparison to type I collagen and they also produce rather thin fibers. As a result, they may be newly categorized as fibrolastic cells. Therefore, further investigation is called for to find new specific marker and to elucidate biological function of these cells.

Monitoring of Pressure Ulcer Detecting ECM Fragments From Wound Surface

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Objective: Although pressure ulcer is defined as pressure induced skin ulcer, its clinical appearance is heterogeneous. The heterogeneity of pressure ulcer makes the treatment and prevention difficult. Therefore, we investigate the wound surface ECM in order to categorize pressure ulcer and to develop the biomarker for the wound. In this study, we focus on dermal matrix molecules such as versican, decorin, fibulin-2, latent TGF-beta binding protein-1 (LTBP-1) and fibronectin.

Methods: We sampled ECM from wound surface using absorbent cotton. Then the samples were extracted with 6 M guanidine hydrochloride buffer. We mainly employed dot blotting analysis using specific antibodies for the ECM molecules, since high molecular ECM aggregates do not enter the SDS-PAGE gel. Immuno histochemical study was also performed.

Results: Versican G3 fragments were detected from transitional granulation tissue between hydrated fragile matrix and stabilized epithelial connective tissue. Versican G3 fragments were also detected from granulation tissue with friction. Fibronectin was detected from wound surface in epithelial formation. Decorin was detected from almost all wound surfaces.

Conclusions: Combination of detecting these matrix molecules by dot blot assay can clarify the pathogenesis of pressure ulcer.