Some structural analyses of hyalin, extracellular matrices of juvenile hyaline fibromatosis

Kazumoto KATAGIRI, Shuuji TAKASAKI, Sakuhei FUJIIWARA, *Tomomichi ONO and Hiroshi SHINKAI

Department of Dermatology, Medical College of Oita
Department of dermatology, Kumamoto University Medical School

Juvenile hyaline fibromatosis is a rare mesenchymal dysplasia inherited in an autosomal recessive fashion. The histologic findings are a tumor-like lesion characterized by deposition of amorphous hyaline materials which reacted with PAS in extracellular spaces. These materials consist of few mature collagen fibrils and fibrillar filaments constituted with a regular cross-banded striation. To elucidate the nature of the hyaline, biochemical and immunological analyses were performed on extracellular matrices of tumor from the patient.

MATERIALS and METHODS

Extraction of extracellular matrices

Tumor tissues were cut into small pieces and homogenized in phosphate buffered saline by Ultra Torax at cold. The homogenates were extracted with 6M guanidium HCl containing 0.1% CHAPS and protease inhibitors in Tris-HCl buffer, pH 7.5 after removal of serum components. The supernatants were collected by centrifugation and CsCl density gradient centrifugation was performed followed by the addition of CsCl to a final concentration of 25%. Four fractions with a density of 1.36 - 1.31, 1.31 - 1.27 and 1.27-1.23 g/ml and protein aggregates which floated at the top of tube, were separated.

Purification of type VI collagen

Each fraction except a low buoyant fraction was chromatographed on a Sephacryl S-400 column (2.5 x 70 cm) in 4 M guanidium hydrochloride - 0.05M Tris-HCl buffer, pH 7.5. Kav= 0.08 - 0. 24 fractions were collected and chromatographed on a DEAE cellulose column (2.0 x 15 cm) equilibrated with 0.05M Tris-HCl buffer, pH 8.0 containing 6M urea after treatment with and without reduction and alkylation.

Preparation of globular regions and collagenous domains

Enzymatic treatments were carried out with purified collagenase or pepsin. These endproducts were separated on polyacryl amide gels by SDS-PAGE.

Analysis of amino acid sequences and N-linked oligosaccharides
Katagiri et al.: Hyaline

Amino acid sequences were determined with a protein sequencer (Applied Biosystems model 473A) from the bands on the PVDF membrane after electroblotting from SDS polyacrylamide gel. Con A bound oligosaccharide chains containing polypeptides were detected on the PVDF membrane with peroxidase-linked con A.

**Immunohistochemical analysis**

Monoclonal antibody or polyclonal antibodies against α2(VI) collagen were used for the detection of bands on the transblotted PVDF membrane.

**RESULTS and DISCUSSION**

Hyaline from a tumor-like lesion in the patient with hyaline fibromatosis was mainly composed of dermatan sulfate (72%) and hyaluronic acid (8%) and small amounts of chondroitin sulfate (16%) as glycosaminoglycan though major substances that extracted with GuHCl-CHAPS contained collagens, type I collagen and collagen-like-proteins. The collagen-like proteins reacted with anti-collagen type VI and gave 3 bands on SDS-PAGE after digestion with pepsin. When type I and type VI collagen rich fractions obtained from molecular sieve chromatography were subjected to ion exchange chromatography without reduction and alkylation, these collagens passed through the column, though carboxymethylated type VI collagen could bind to the gel. This indicated that type VI collagen co-associates with type I collagen. The intact molecule of the subcomponents of type VI collagen and collagen-like domains reacted with Con A and PAS staining though collagenase resistant domains did not. Non-helical regions obtained from collagenase digestion separated 7 components on 10% SDS-PAGE with a tricine buffer system. Amino acid sequence revealed that MW 50 Kd and 45 Kd band corresponded with the C-terminal region of the α1(VI) and α2(VI) chain, respectively.

---

**Fig. 1** Amino acid sequences of collagenase resistant peptides

MW 50Kd corresponds to C-terminal α2(VI) chain

| Gly-His-Gln-Gly-Pro-Pro-Gly-Pro-Glu-X-Glu-Leu-Asp-Ile-X-Ile-X | 1 | 5 | 10 | 15 | 20 |

MW 45Kd is identical with C-terminal α1(VI) chain

| Gly-Pro-Pro-Gly-Pro-Gly-Leu-Thr-Glu-X-Asp-Val-Met-Thr-Tyr-Val-Arg-X-X | 1 | 5 | 10 | 15 | 20 |