EHLERS-DANLOS SYNDROME TYPE II
A CONTROLLER GENE DISEASE IN COLLAGEN

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The Ehlers-Danlos syndrome (EDS) is one of a group of inherited connective tissue disorders and is clinically characterized by hyperextensibility and fragility of skin, hypermobility of joints and easy bruisability. It has a very heterogeneous etiology and is now subdivided into ten types, by clinical and genetic criteria¹. Molecular defects of only some types of EDS have been reported, which include defects in type III collagen synthesis and secretion (type IV EDS) and in lysyl hydroxylase (type VI EDS). Defects of type I collagen in EDS (type VII EDS) involve a failure in the processing of procollagen to collagen.

Recently, we found a patient whose clinical diagnosis was EDS, but whose molecular defect appeared to involve the α2(I) chain⁴. Here we investigated the collagen metabolism of fibroblasts from this patient at the protein level, mRNA level, and genomic level in order to clarify the etiology of this particular variant of EDS as well as to understand the functions of collagen in the body.

Clinical data ⁴,⁵) The patient was born full-term by normal delivery and was noted to have moderate hyperelasticity of the skin and hyperextensibility of the joints. During childhood, she bruised easily and formed occasional cigarette-paper scars, but did not suffer from bone fractures. From the age of 35, she sometimes noticed formation of purpura or ecchymosis. At the age of 38, she became aware of palpitations, dyspnea and easy fatigue on exertion. When she visited the hospital, mitral valve insufficiency was discovered.

Methods Details of the method were described previously.⁶-⁸)

Collagen synthesis by normal and EDS fibroblasts
The relative rate of collagen synthesis to that of total protein synthesis was 7.6±0.5% for three strains of the control cells and 4.0±0.2% for the EDS fibroblasts (Fig. 1A).
Recently we found that supplementation of the culture medium with ascorbic acid 2-phosphate (Asc 2-P), a long acting vitamin C derivative, stimulates collagen synthesis, cell growth and formation of tissue-like substance by fibroblasts). The same effects were observed in the EDS fibroblasts as in the control fibroblasts, although the effects were less obvious in EDS fibroblasts (Fig. 1A and B). Under these culture conditions, the relative rate of collagen synthesis of the EDS fibroblasts (10.8±0.2%) was still approximately one-half of that of the control fibroblasts (21.9±0.9%).

Collagen components produced by control and EDS fibroblasts

Normal fibroblasts in culture produced type I [α1(I) and α2(I) chains], type III [α1(III) chain] and type V [α1(V) and α2(V) chains] collagens with the ratio of 88:9:3, as determined by densitometry of the fluorogram of the pepsin-treated samples (Fig. 2). The ratio of α1(I) to α2(I) was 2.5±0.3, which was quite similar to the theoretical value (2.3) of type I collagen labelled with radioactive proline. On the other hand, there was no α2(I) chain in the collagen prepared from EDS fibroblasts (Fig. 2), even when the same amount of collagenous material was loaded. When procollagen fraction was analyzed, there was no component corresponding to α2(I) chain and its precursors in the preparation from EDS fibroblasts.

Messenger RNA for proα1(I) and proα2(I) collagen chains in control and EDS fibroblasts

The amount of mRNA for proα2(I) collagen chain in EDS fibroblasts was less than that of control cells as observed by the densities of the dot blots for proα2(I) collagen mRNA, even though the amount of proα1(I) collagen mRNA prepared from both cells was essentially the same (Table I).

In vitro transcriptional activity of type I collagen genes

Nuclei were isolated from control fibroblasts and from EDS fibroblasts and in vitro transcriptional activities of proα1(I) and proα2(I) collagen genes were determined. The activity of the proα2(I) gene in EDS fibroblasts was two-thirds of the control cells when the nuclei were incubated for 10 min with radioactive RNA precursors (Table II) and one-fourth when those were incubated for 30 min. The activities of proα1(I) gene were essentially the same with both control and EDS fibroblasts (Table II).

Genes for proα2(I) collagen chain in control and EDS fibroblasts

Southern-blot analysis of EcoRI digests of DNA from both control and EDS fibroblasts showed three DNA fragments as reported previously for a normal individual. The density of all three bands of DNA obtained from both the control and the
patient's cells were essentially the same\(^6\), suggesting that the concentration of genomic molecules for \(\text{pro\alpha}_2(\text{I})\) collagen is the same in both.

**Conclusion**

Collagen synthesis was examined in skin fibroblasts from a patient with type II Ehlers-Danlos syndrome. Alpha two chain and its precursors were not found in the patient fibroblasts. Dot-blot and Northern-blot analyses showed that the patient's fibroblasts to contain less than 10% of the cytoplasmic mRNAs for \(\text{pro\alpha}_2(\text{I})\) found in control fibroblasts. Nuclear run-off experiments showed that *in vitro* transcriptional activity of \(\text{pro\alpha}_2(\text{I})\) collagen gene in EDS fibroblasts was less than that of control cells, indicating transcriptional defects of the gene and/or instability of primary transcripts of \(\text{pro\alpha}_2(\text{I})\) collagen gene is responsible for the failure of the patient's fibroblasts to synthesize \(\text{pro\alpha}_2(\text{I})\) collagen chains.

Recently instability or defect in splicing of mRNA precursors for \(\text{pro\alpha}_2(\text{I})\) collagen chain was suggested in fibroblasts obtained from a male patient with quite similar clinical symptoms\(^9\).

These data suggest that type II Ehlers-Danlos syndrome is a controller gene disease in collagen.

**References**

8) Kurata, S. & Hata, R. submitted to press
Fig. 1. Effects of L-ascorbic acid 2-phosphate (Asc-P) on the relative rate of collagen synthesis to total protein synthesis (A) and on the cell growth (B) of fibroblasts obtained from three normal controls (Cont) and a patient with Ehlers-Danlos syndrome (EDS).

Fig. 2. Fluorogram of SDS/polyacrylamide gel of collagen obtained from fibroblasts of a normal control (Cont) and a patient with Ehlers-Danlos syndrome (EDS). Collagen samples were prepared from the cell layer and the medium and were separated by SDS/5% polyacrylamide gel electrophoresis in the presence (+DTT) or absence (-DTT) of dithiothreitol.
### Table I

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<th>Cell Chain</th>
<th>Control</th>
<th>EDS-I</th>
<th>EDS-II</th>
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<tbody>
<tr>
<td>a1/g-actin</td>
<td>6.30±0.64</td>
<td>6.41±0.09</td>
<td>100%±0.92</td>
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<tr>
<td>a2/g-actin</td>
<td>100%±0.64</td>
<td>100%±0.5</td>
<td>100%±0.5</td>
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### Table II

<table>
<thead>
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<th>EDS-II</th>
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<tr>
<td>a1/g-actin</td>
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<td>a2/g-actin</td>
<td>0.92±0.06</td>
<td>100%±0.5</td>
<td>100%±0.5</td>
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**HYBRIDIZATION ASSAYS OF MESSENGER RNAs FOR PRO alpha 1 (I) AND PRO alpha 2 (I) COLLAGEN CHAINS FROM NORMAL CONTROL AND THOSE FROM THE PATIENT WITH ELDERS-DANLOWS SYNDROME TYPE II OF FIBROBLASTS OBTAINED FROM CONTROLS AND THE PATIENT WITH ELDERS-DANLOWS SYNDROME TYPE II IN VITRO TRANSCRIPTIONAL ACTIVITY OF TYPE I COLLAGEN CHAINS.**