

Growth Kinetics of Fibroblasts from a Patient with Ehlers-Danlos Syndrome

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A 6-year-old girl with Ehlers-Danlos syndrome (type I) who shows hyperelastic skin, hypermobile joints and delayed wound healing is reported. The growth kinetics of fibroblasts derived from her skin was examined using ³H-thymidine autoradiography. Our study showed that these fibroblasts had poor proliferative activity, compared with normal age-matched fibroblasts. Thus, we could suggest that the pathogenesis of Ehlers-Danlos syndrome was partially attributed to the lack of proliferative activity of the fibroblasts. Key word: Cell culture.

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Ehlers-Danlos syndrome (EDS) is a generalized connective tissue disorder characterized by hyperelastic, thin, velvety and fragile skin, poor wound healing with abnormal scars, easy bruising and joint hypermobility (1, 2). EDS is recognized to be clinically, genetically and biochemically heterogeneous and the essential defect is a quantitative deficiency of collagen (3–7). Abnormalities of lysyl oxidation (3), of cleavage of the N-terminal peptide from procollagen (4), of cross-link formation (3), of synthesis of type III collagen (5, 6) and of activity of procollagen peptidase (7) have been observed in some forms of EDS. However, the growth kinetics of fibroblasts derived from patients with EDS have not been previously reported.

In this paper, we report on the growth kinetics of fibroblasts from a girl with EDS (type I), examined *in vitro* using ³H-thymidine autoradiography.

CASE REPORT

A six-year-old girl met with a traffic accident on June 7, 1983 and was brought to the emergency department at our hospital for trauma of the upper and lower extremities and face. At the initial presentation, all wounds were pronouncedly open and her skin was hyperelastic, velvety and fragile. Also, all joints were hypermobile. Later, her wound healing was delayed and fish-mouth or cigarette paper like scars developed. Histologically, the dermal collagen was scanty and disorderly. Elastica

Van Gieson stained specimens showed that the elastic fibers were increased in number in the dermis but there were no abnormalities in shape.

She had a father and younger brother with the same phenotype, and so was diagnosed as having Type I EDS.

Methods

Cell culture. A skin specimen from a damaged portion of the left arm was taken using a scalpel under sterile conditions. Normal skin specimens were obtained as control samples from six healthy age-matched patients after pigmented or epidermal nevi were removed.

All skin specimens were cut into small pieces, and cultured on coverslips in 35 × 15 mm plastic dishes (Falcon plastic, Oxford, California, USA) containing 2–3 ml of Eagle's minimal essential medium (MEM) (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% fetal calf serum, and 60 µg/ml of Epocelin (Fujisawa Seiyaku, Osaka, Japan). All cultures were maintained at 37°C in a high humidity incubator with a mixture of 5% CO₂ in air. Culture medium was routinely changed every three days.

Using 14 to 21 days-old cultures of exponential growth, the cells were continuously labeled in fresh culture medium containing 1 µCi/ml of ³H-thymidine (New England Nuclear, specific activity 24.7 Ci/mM). At 2, 24 and 48 h after the labeling procedure, the cells were washed three times in cold phosphate buffered saline (pH 7.2), fixed in absolute methanol and air-dried. The labeled specimens were then processed for autoradiography.

Autoradiography. Cells labeled with ³H-thymidine were treated with 5% trichloroacetic acid at 37°C for 1 h, rinsed in distilled water, coated with photographic emulsion (NR-M2, Konishiroku, Tokyo, Japan) and exposed in a desiccated box at 4°C for 7 days.

The labeling index (LI) was determined by counting the number of labeled nuclei per 500 cells. A cell with five or more grains over the nucleus was considered to have a labeled nucleus.

The comparative growth kinetics of fibroblasts derived from normal skin and EDS were evaluated statistically using Student's *t*-test.

RESULTS

All data are summarized in Table I. EDS-derived fibroblasts showed a significant decrease in LI at 2, 24 and 48 h compared with control fibroblasts ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively).

Table I. Growth kinetic study of fibroblasts derived from EDS and normal skin

Results of normal skins are means \pm SD (n =number of cultures) and those of EDS are means \pm SD of triplicate cultures

	Normal skins ($n=6$) (%)	EDS (type I) (%)
2 h	23.2 \pm 9.1*	11.9 \pm 2.5
24 h	49.6 \pm 8.1**	23.3 \pm 6.1
48 h	73.1 \pm 10.3***	34.2 \pm 4.8

Significantly different from EDS; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's t -test).

DISCUSSION

Although it has been reported that the fibroblasts from EDS patients have some defects of collagen synthesis (3–7), little has been known about growth kinetics. It was noticeable that the EDS-derived fibroblasts had poor proliferative activity compared with normal age-matched fibroblasts. EDS histologically showed scanty collagen in dermis and clinically delayed wound healing (8). These characteristics may be due not only to metabolic disturbances of collagen synthesis, but also to the lack of proliferative activity of the fibroblasts. Therefore, further studies of fibroblasts proliferation will be necessary to uncover the pathogenesis of EDS.

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