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MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF GENETIC ANALYSIS OF OSTEOGENESIS IMPERFECTA IN CULTURED DERMAL FIBROBLASTS

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The modern approach to the study of hereditary diseases presupposes analysis and comparison of all stages of their pathogenesis – from the action of mutant genes to the appearance of the phenotypic picture of the disease at the cell, organ, and whole body levels, as ways of realization of genetic information. The cell is a special apparatus, which constitutes the structural-topographic basis of interaction between genes. Many pathological processes, which are undoubtedly based on molecular defects, are realized through a genetically determined disturbance of cell functions and of the behavior of cellular communities. Under these circumstances, the use of somatic cells cultured in vitro is a unique contribution to the analysis of the pathogenesis of hereditary diseases, for such cells preserve the genetic information of the original organism, while at the same time, they are descended from it. Osteogenesis imperfecta (OI) is a generalized disease of connective tissue, the chief manifestation of which is pathological fragility of the bones. As a result of the clinical variability of OI, four clinical types of it are distinguished [9]. The basic defect in all types of OI is now associated with a disturbance of synthesis and/or metabolism of type I collagen, and for this reason the corresponding changes may be exhibited by cultures of dermal fibroblasts (CDF), which produce this type of collagen sufficiently constantly [8]. Considering the role of the cell in the realization of genetic information and the correlations found between the molecular defects and clinical phenotype of OI, we attempted to discover the cellular mechanisms of the disturbed process of morphogenesis in the space between the molecular defect and the clinical phenotype of OI.

EXPERIMENTAL METHOD

Skin biopsy specimens were obtained from the medial surface of the forearm of 10 patients, eight of whom had type I OI and two had type III OI [9], and also from three clinically healthy volunteer donors. Diploid strains of dermal fibroblasts were obtained by the method developed by Kukharenko and co-workers [2]. A dynamic analysis of growth of CDF was undertaken by a modified method in [6]. Cell strains were compared with respect to the following parameters: 1) days needed by the CDF to reach the stationary phase of growth; 2) the cell density of CDF in the stationary phase of growth; 3) the length and width of the cells with calculation of the ratio between them. The index

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TABLE 1. Functional Parameters of Activity of Cultured Dermal Fibroblasts from Patients with Osteogenesis Imperfecta

Serial No.	Diagnosis—type of OI according to Sillence's	Age, years	Passage	Density of monolayer in stationary phase of growth	Ratio of length to width of cells in stationary phase of growth	Time (days) to reach stationary phase of growth	Index of ^{3}H-thymidine-labeled cells (%)	Cloning efficiency (%)	Fibronectin concentration (μg/ml)
1	OI I	1,5	7	1,0	19,9	Over 16	69,0	14,0	148,0
2	OI I	29	12	0,95	21,2	«—»	50,5	10,0	—
3	OI I	9	8	0,95	17,8	«—»	52,0	11,6	154,0
4	OI I	36	8	0,85	20,7	«—»	45,3	14,1	295,0
5	OI III	15	8	0,78	17,6	«—»	89,5	—	—
6	OI I	15	7	1,1	10,5	«—»	84,7	—	—
7	OI III	14	4	1,05	12,1	«—»	64,0	—	—
8	OI I	15	11	0,85	16,9	«—»	87,0	—	217,0
9	OI I	49	11	1,28	25,6	«—»	80,0	24,3	27,1
10	OI I	2,5	7	1,2	19,6	«—»	69,0	—	—
11	Control	33	11	1,6	22,9	Up to 13 days	72,0	—	—
12	"—"	27	10	1,35	33,9	«—»	73,0	18,3	1,2
13	"—"	11	9	1,45	26,6	«—»	38,5	24,0	0,8
$M\pm m$ (HO, n=10)				1,0±0,09	18,19±1,66		71,5±12,6	14,8±3,0	168,2±50,3
$M\pm m$ (Control n=3)				1,47±0,105	27,8±4,62		55,8±30,4	23,2±8,5	1,0±0,3
t; p				t=3,401; $p<0,01$	t=1,96; $p<0,05$		t=0,2; $p>0,5$	t=0,92; $p>0,25$	t=2,97; $p>0,05$

of labeled cells was determined by an autoradiographic method after incubation of the cells for 24 h with ^{3}H-thymidine, after addition of the isotope to the culture 24 h after seeding. The index was expressed in percent as the ratio of the number of labeled cells to the total number of cells analyzed (not fewer than 5000). Experiments on cell cloning after preliminary keeping of the CDF in Eagle's medium with 1% fetal calf serum were carried out in flasks from "Nunclon" in medium 199 with the addition of 10% bovine blood serum and 10% human umbilical cord blood serum in an atmosphere of 95% air and 5% CO_2. The cloning efficiency was determined as the fraction of cells capable of forming colonies, after 18 days in culture, consisting of 16 or more cells [3]. The concentration of fibranectin (Fn) in cultural samples was analyzed by a modification of enzyme immunoassay [1]. To construct a calibration curve, a double dilution of native Fn was prepared. Samples of CDF for transmission electron microscopy were fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.2-7.4, followed by postfixation in 1% OSO_4 solution. After dehydration the material was embedded in Araldite. Ultrathin sections were examined in the JEM-7A electron microscope. Morphometric investigations were carried out with the aid of a grid [4].

EXPERIMENTAL RESULTS

Characteristic growth parameters of the cultured fibroblasts are given in Table 1 and Fig. 1. Fibroblasts from patients with OI reached the stationary phase of growth later (more than 16 days) than control cells (up to the 13th day). 2. All samples of CDF from patients with OIL (with both I and III types of OI) showed a significantly lower monolayer density in the stationary phase of growth compared with normal. 3. The shape of the cells in OI also was disturbed: the ratio of length to width of the cells was significantly lower in OI than in the control. A significant difference between the second and third parameters in OI was observed despite the marked individual differences affecting these parameters normally.

None of the three parameters depended on the patients' age, on the passage No. (between the 4th and 12th), or on the type of OI. As Table 1 shows, the lowered density of the CDF monolayer in the stationary phase compared with normal and the anomalies of size and shape of the cells in OI did not depend on the proliferative potential of the cultured cells (index of ^{3}H-thymidine-labeled cells and their cloning efficiency in OI and in the normal individual do not differ significantly). Meanwhile the concentration of Fn in CDF in OI was significantly higher than in the control, and electron-microscopic investigation of CDF (Fig. 2) revealed dilated cisterns of the rough endoplasmic reticulum (RER) and a statistically significant increase in the fraction of RER in CDF in OI compared with normal

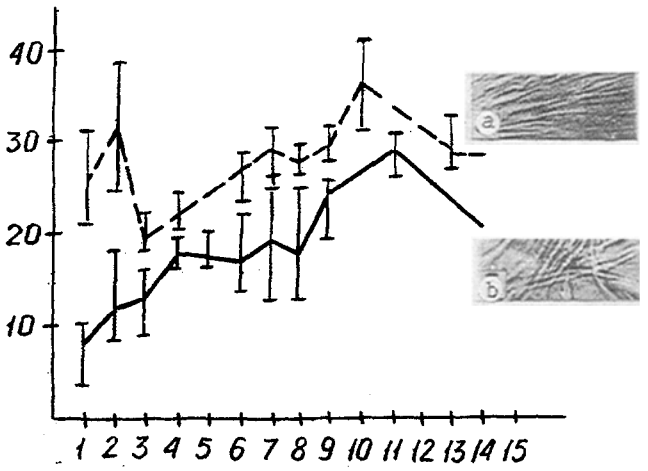

Fig. 1. Time course of growth of cultures of dermal fibroblasts, and density of cell monolayer in stationary phase: a) CDF in control, b) CDF from patients with OI. Phase contrast. 70×. Abscissa, days of culture; ordinate, ratio of length to width of cells.

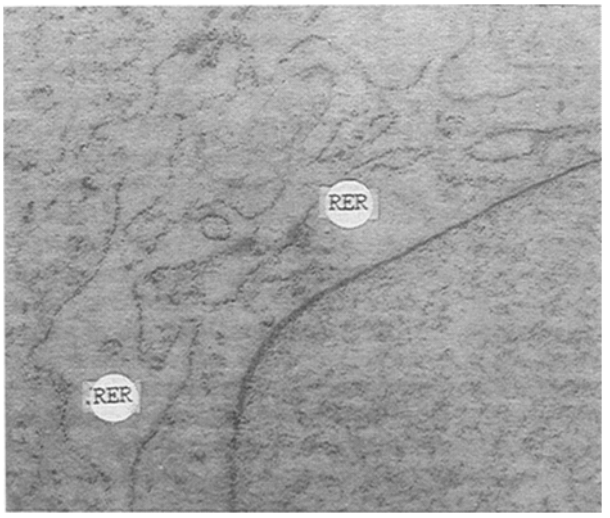

Fig. 2. Ultrastructure of cultured dermal fibroblast from patient with OI. Dilated cisterns of rough endoplasmic reticulum (RER) visible in cytoplasm. 23,000×.

($M \pm m = 0.09 \pm 0.01$ and 0.04 ± 0.01 respectively, $t = 3.53$, $p < 0.05$), probably indicating increased biosynthetic activity of the cells in OI. The fundamental processes responsible for the phenomena examined above are not yet sufficiently clear. However, we know that disturbances of fibroblast morphology are evidently connected with a defect of cellular adhesion and of spreading of the cells on a flat surface [6, 7]. In the normal process of attachment, proliferation, migration, and differentiation of fibroblasts an extremely important role is played by their interaction with the substrate, containing extracellular glycoproteins, including fibronectin and collagen [9]. Their combined action ensures that connective tissue cells perform their physiological functions. Disturbances of the structure of one of these components by mutation therefore involves a change in structure and/or content of the other components. Since a primary defect of collagen production is present in OI and its interaction with other components of the matrix is disturbed, it can be tentatively suggested that the increased Fn concentration in CDF is the response

reaction of a cellular apparatus producing excess of Fn, in accordance with the feedback principle. The increased biosynthetic activity of CDF in OI is evidence in support of this suggestion.

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EFFECT OF DIFFERENT HYPERBARIC OXYGENATION SCHEDULES ON TRANSCRIPTION ACTIVITY AND MORPHOLOGY OF FRONTAL CORTICAL NEURONS IN RATS WITH OCCLUSION OF THE COMMON CAROTID ARTERY

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The problem of optimization of therapeutic schedules of hyperbaric oxygenation (HBO) for patients with stroke is due partly to the complexity of the pathogenesis of cerebral ischemia and partly to inconstancy of the action of different HBO schedules. A comparative study of the efficacy of different HBO schedules for the treatment of stroke has been undertaken, but only fragmentarily, with no attempt to analyze small doses of hyperoxia such as we ourselves have developed [3]. The discrepancy between the results of clinical and experimental studies of the efficacy of HBO in the treatment of stroke and the absence of any research into optimization of HBO schedules depending on the severity and duration of cerebral ischemia necessitated an investigation such as that described below [7-9, 11, 12].

EXPERIMENTAL METHOD

To study the mechanisms of action of different HBO schedules in the treatment of cerebral ischemia a combined study was undertaken on noninbred male albino rats weighing 200 g with unilateral and bilateral ligation of the common carotid arteries below the thyroid cartilage under pentobarbital anesthesia (0.02 g/100 g body weight). This communication describes the results of an investigation of transcription activity and morphology of frontal cortical neurons after occlusion of the right common carotid artery, accompanied by early (3 h of ischemia) and late

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