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Specific Features of Gene Expression in Human Myoblasts. Analysis of Cells from Primary and Cloned Cultures

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Primary cultures of postnatal human myoblasts are obtained. Their purity is assessed by cytochemical determination of alkaline phosphatase activity and electrophoretic analysis of the expression of muscle proteins in comparison with postnatal human fibroblasts.

Key Words: *human myoblasts; alkaline phosphatase; tissue-specific muscle proteins*

Cultured mononuclear muscle cells (myoblasts, MB) obtained from various organisms are a convenient model for the investigation of cell differentiation and other biological processes [8,12,13]. So far, cultures initiated from rat or mouse muscles provided essentially stable lineages [11,12]. Meanwhile, human MB are of specific interest. In addition, considerable attention has been focused on these cells after successful cellular or gene therapy of Duchenne's muscular dystrophy by grafting donor MB in patient's muscles [8,9]. Donor's cells fused with patient's multinuclear myofibrils, which triggered the synthesis of dystrophin in patient's myofibrils.

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The presence of fibroblasts (FB) whose content varies in a wide range hampers the use of primary MB cultures as a model system in experimental studies and as a graft in cellular therapy.

We studied some specific features of gene expression in human MB and evaluated some protein markers for the determination of the MB/FB ratio in primary cultures.

MATERIALS AND METHODS

Primary MB cultures were initiated from human muscle specimens (1 g) obtained after treatment of the operative field. The tissue was washed with normal saline containing 1000 U/ml ampicillin and 200 U/ml streptomycin and treated with 0.1% collagenase (Sigma) and 0.1% trypsin for 1 h at 37°C. Cells

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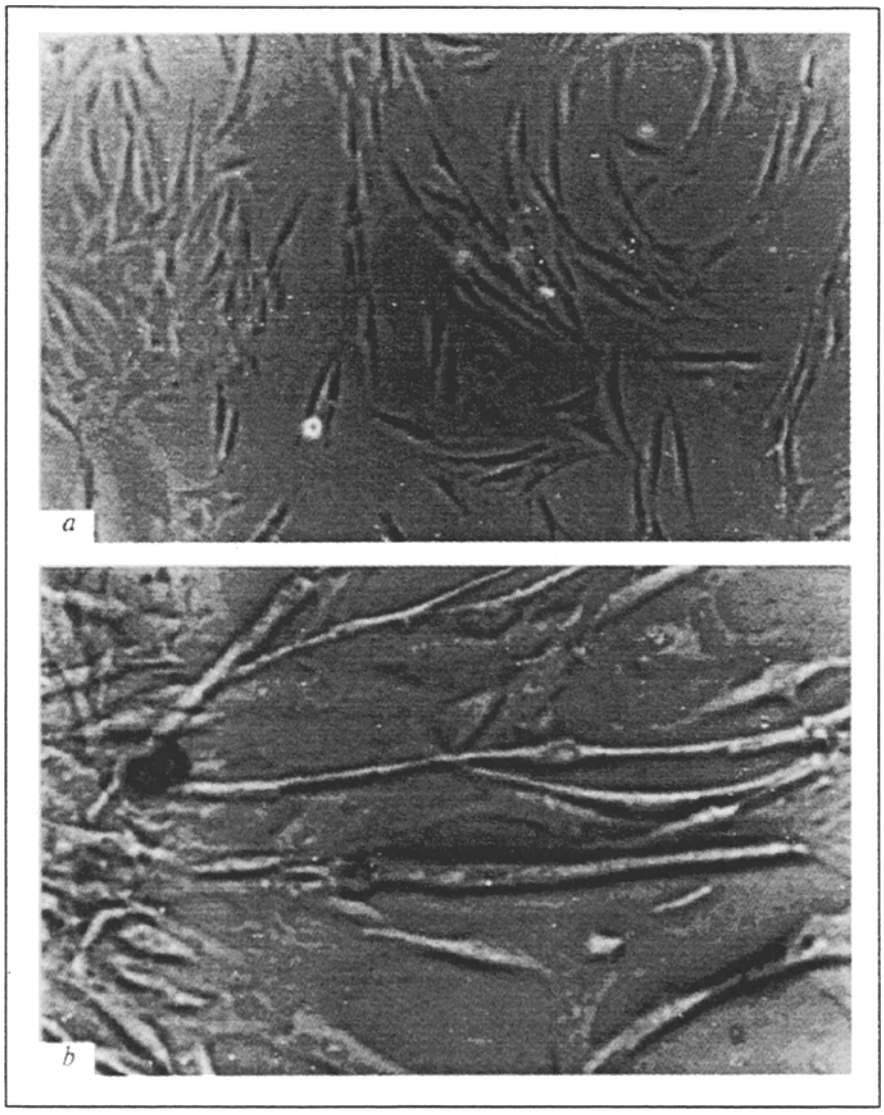

Fig. 1. Primary culture of human myoblasts. Phase contrast microscopy, ×300. Myoblasts at the early (*a*) and late (*b*) stages of differentiation.

were pelleted by centrifugation, washed from the enzymes, resuspended in Ham's F-10 medium supplemented with 20% fetal calf serum, and seeded on 6-well plates. Myoblasts attached and flattened within 3-4 days after seeding. The cells were grown in medium A containing Ham's F-10, 10 mM MOPS, pH 7.2, vitamins, sodium pyruvate (Sigma), 20 U/ml streptomycin, 50 U/ml ampicillin, and 10% fetal calf serum. Cells were cloned on Petri dishes (10 cm in diameter, Nunk) and 96-well plates covered with polylysine. For this purpose medium A with human umbilical cord serum (final concentration 10%) was used.

Postnatal human FB (IMG796) served as controls. The cells were grown in DMEM with 5% bovine serum and 5% human umbilical cord serum.

Proteins from cell cultures were analyzed by one- and two-dimensional electrophoresis in polyacrylamide gel [2]. Specific muscle proteins were identified by immunoblotting and other methods with the use of monoclonal antibodies [7]. The activity of alkaline phosphatase (AP) was measured using naphthol AS-BI phosphate and Fast Red P [3].

RESULTS

Several essentially stable cultures growing for at least 1 month were obtained by culturing 26 specimens of the skeletal muscle. Such cultures grew predominantly from the specimens obtained from donors below forty. Cells with different length/width ratio were present in the culture (Fig. 1, *a*). Their morphology is consistent with that described by others [8,12]. Multinuclear structures containing 2-15 nuclei (Fig. 1, *b*) and resembling myotubes [8] were formed in prolonged stable cultures.

Two-dimensional electrophoresis of proteins isolated from these cultures revealed a distribution pattern similar to that of muscular proteins; however, the contents of major contractile proteins were lower than

in mature muscle. This is consistent with the observation that the expression of the main muscle proteins starts at the late stages of differentiation.

Immunoblotting with antibodies against the light myosin chain 1 [1] and against desmin (DE-U-10, Sigma) revealed these tissue-specific proteins in primary cultures of MB. In FB cultures (control), these proteins were not identified or were present in trace amounts.

Previously, it was reported that cultured human MB display considerable AP activity [6]. Cytochemical analysis of our cultures showed that 50-70% of MB have AP activity.

According to the results of cloning experiments, the clones were divided into two groups: with (specific staining of more than 90% of cells in a culture) and without AP activity. Control FB cultures were not stained in the AP assay. From these findings it can be suggested that this simple cytochemical test can be useful in the differentiation of cultured MB and FB.

In order to test this suggestion several mixed cultures were grown and analyzed for AP activity. Cultures with various MB:FB ratios — 20:80, 30:70, 50:50; 70:30, and 80:20 — were seeded on cover slips. The initial MB and FB cultures served as control. After fixation and staining, percent ratio between stained and unstained cells was calculated. The ratio between AP-positive and AP-negative cells was proportional to the MB/FB ratio in a mixed culture

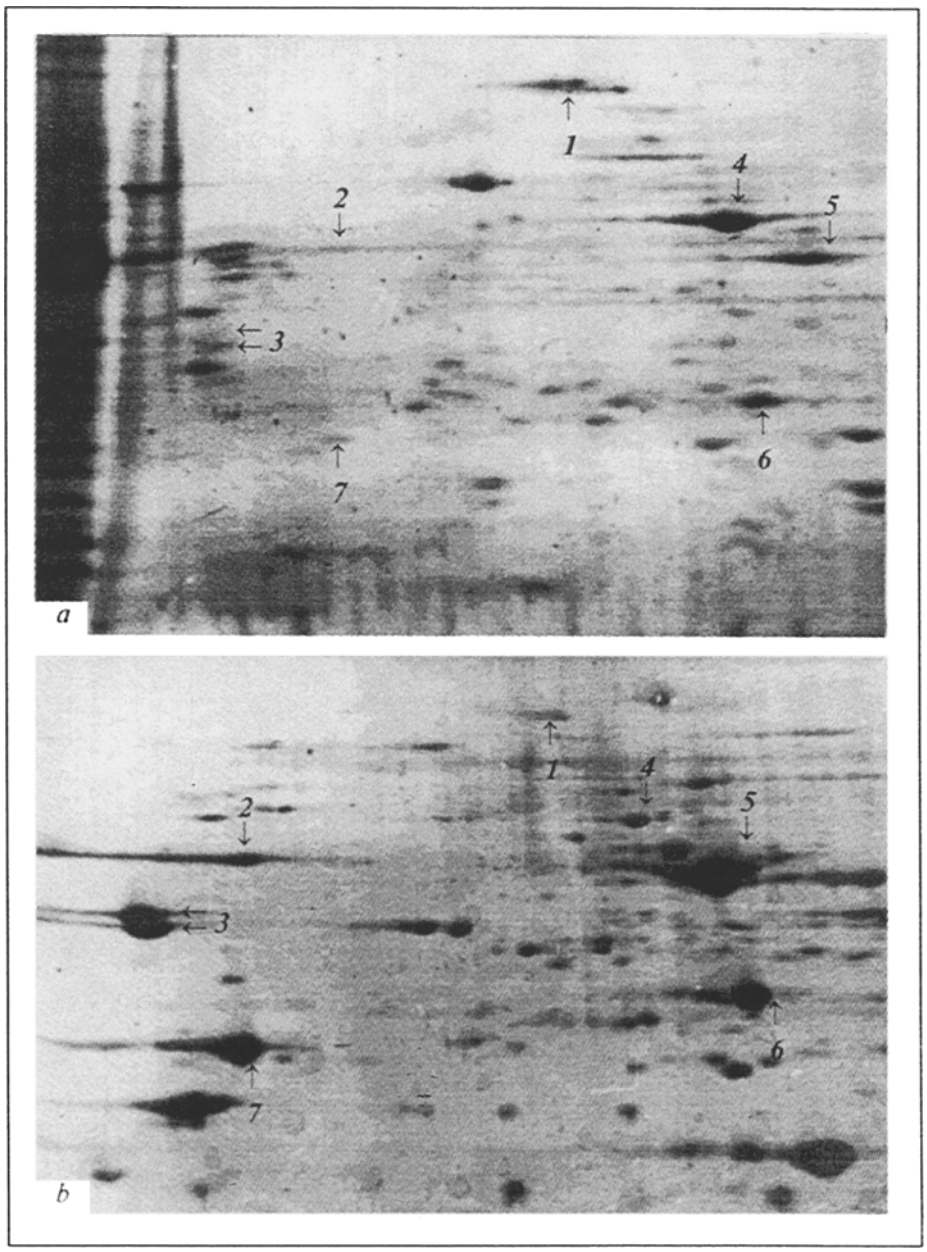

Fig. 2. Two-dimensional electrophoresis of proteins isolated from cultured human fibroblasts (*a*) and adult skeletal muscle (*b*). Some proteins expressed in the skeletal muscle are indicated with arrows. *1*) transferrin; 2) actin; *3*) α- and β-tropomyosins; *4*) α-ATP synthase; *5*) M-subunit of creatine phosphokinase; *6*) muscle isoform of carboanhydrase III; *7*) light myosin 1 chain.

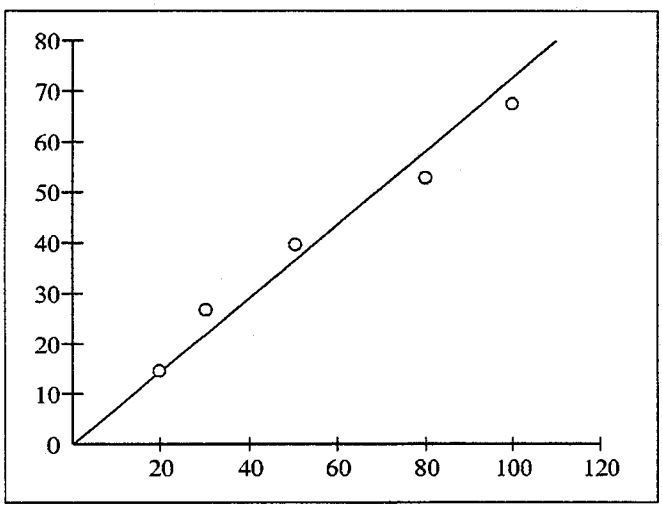

Fig. 3. Relationship between the number of AP-positive cells and the number of myoblasts in a mixed culture. Ordinate: percent of AP-positive cells; abscissa: percent of myoblasts.

(Fig. 3). This relationship was observed in TM1 and TM5 independent cultures of MB.

Six human genes coding for different AP isoforms were mapped. One of these isoforms (ALPIF) is expressed during embryogenesis [10]. High AP activity has been recently detected in pluripotent murine and bovine cells [4,5]. Thus, it can be suggested that one of AP isoforms is synthesized in various cell types at the early stages of differentiation. Presumably, this enzyme is synthesized in cultured MB which can differentiate into myotubes. Our findings support this hypothesis. It is noteworthy that the myotubes did not stain for AP.

The results obtained indicate that human MB and FB can be differentiated in a culture using tests based on specific features of gene expression in these cells. However, it should be stressed that specific tests are necessary for the identification of FB in cultures where MB predominate.

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