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EXPERIMENTAL GENETICS

Modeling the Genetic Correction of Familial Hypercholesterolemia by Generating Cell Clones with Stable Expression of the Receptor for Low Density Lipoproteins

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At the present time, familial hypercholesterolemia (FH) is one of the most widespread autosomal dominant inherited human diseases [1]. In most populations the occurrence of homozygous and heterozygous FH is 1:1,000,000 and 1:500, respectively [2].

The primary biochemical defect in this disease is depletion of the receptor for low density lipoproteins (rLDL) [3,4]. Insertion of the normal gene coding for rLDL and providing for its stable and effective expression in the cells of FH patients might be a radical approach to the treatment of FH.

The aim of the present study was to model genetic correction of FH by generating clones of animal cells with stable expression of the human rLDL gene inserted into the cells by genetic engineering techniques.

MATERIALS AND METHODS

CHOLA3 cells were cultured in HAM'S F12 medium supplemented with 10% fetal calf serum.

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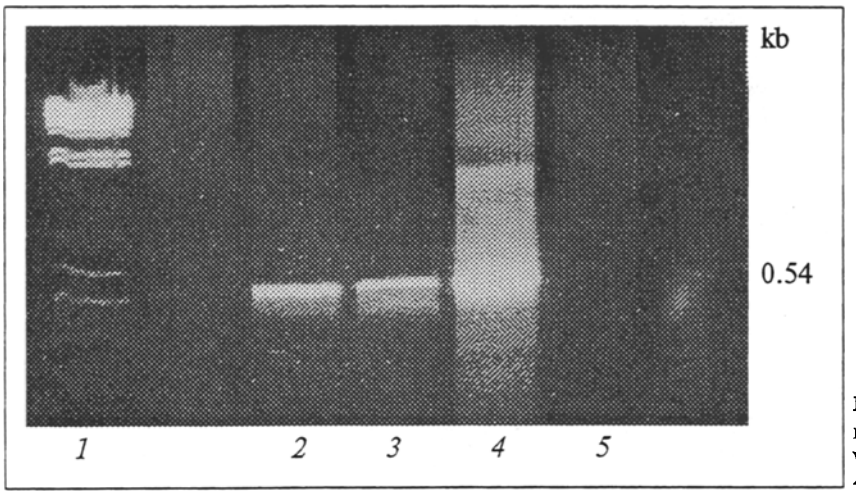

Fig. 1. Electrophoresis of polymerase chain reaction products. *1*) λ phage DNA restricted with Hind III; *2, 3*) G−418−resistant clones; *4*) pMSLV; *5*) native CHOLA3 cells.

Transformation was performed by calcium phosphate precipitation [5]. DNA from transformed cells was isolated as described [5]. Integration of human cDNA containing the rLDL gene in the genome of cultured cells was analyzed by the polymerase chain reaction (PCR) [6] with primers specific for exons 7-11 of the human rLDL gene (the expected size of the amplification product was 546 nucleotide pairs). The expression of the rLDL gene was evaluated by RNA-cDNA blot hybridization and fluorescent analysis [7]. The PCR products were analyzed by electrophoresis in 2% agarose.

RNA was isolated by guanidine-thiocyanate extraction with subsequent CsCl gradient centrifugation [5]. Electrophoresis was carried out under denaturing conditions (formaldehyde); blot hybridization (16 h at 65°C) of the RNA with ^{32}P-labeled cloned cDNA probes for the rLDL gene was done after alkaline fixation on Hybond N+ membrane filters (Amersham) [5]. A fragment of rLDL cDNA labeled with ^{32}P to a specific activity of 10 cpm using DNA polymerase I (Klenow fragment) and random primer labeling technique [8] was employed as a probe and positive control. Autoradiography was carried out for 7 days at -70°C with an intensifier screen. Immunofluorescent analysis was performed using the murine monoclonal antibody IgGC7 to human rLDL (Amersham) and FITC-conjugated anti-mouse IgG antibodies (Sigma) with the control for nonspecific antibody binding. The preparations obtained were embedded in glycerol and examined and photographed under a LYuMAM I2 microscope.

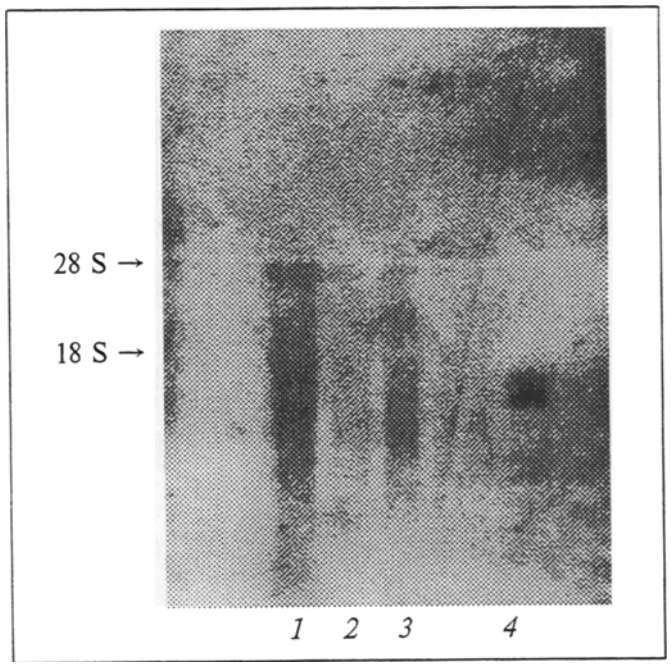

Fig. 2. Blot hybridization of RNA. *1, 3*) RNA from G−418−resistant clones; *2*) native CHOLA3 cells; *4*) fragment of human rLDL cDNA containing exons 1−8 of human rLDL gene.

RESULTS

Previously we constructed a recombinant plasmid pMSVL containing a full-length rLDL cDNA under the control of a hybrid promoter consisting of regulatory sequences from the early SV-40 genes and enhancer of the Molony sarcoma virus [9]. This plasmid was inserted into cultured CHOLA3 cells by calcium-phosphate coprecipitation with the plasmid pSV2neo. Transformed cells were grown on a selective medium containing the antibiotic G-418. The polymerase chain reaction of DNA from six G-418-resistant clones showed that two of them carry human rLDL cDNA containing the 7-11 exon sequence (Fig. 1). In this experiment, DNA from the pMSVL plasmid was used as a positive control and DNA from native cells served as a negative control. Blot hybridization of RNA (10 μg) from transformed cells with rLDL cDNA also revealed a signal which was absent in the

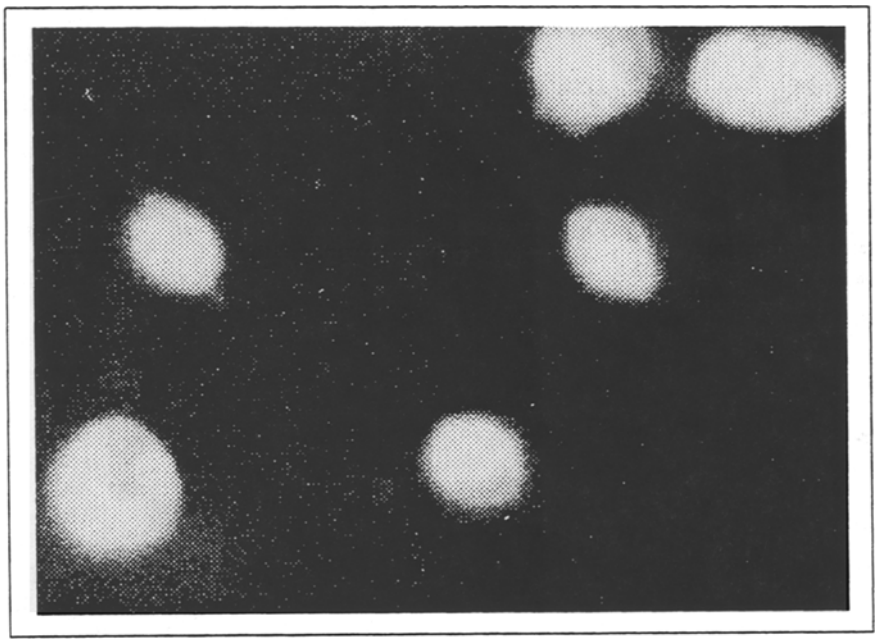

Fig. 3. Immunofluorescent analysis of cells expressing human rLDL mRNA.

control of transformation, although there was slight degradation of rLDL mRNA (Fig. 2). Hybridization was performed with cDNA (0.82 kb) containing exons 1-8 of the rLDL gene. The major region of nondegraded mRNA has a size of 5.3 kb, which corresponds to the full length of human rLDL mRNA. These data confirm a stable expression of human rLDL mRNA by transformed CHOLA3 cells.

We also performed immunofluorescent analysis to pinpoint more accurately the functionally active LDL receptors on the cell surface. The fluorescence pattern observed in our experiments probably reflects the typical distribution of the receptor on the plasma membrane (Fig. 3). Thus, the results obtained lead us to conclude that we have generated cell clones with stable expression of the human rLDL gene.

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