A NUTRITIONAL TEST FOR THE LOW DENSITY LIPOPROTEIN RECEPTOR IN CHINESE HAMSTER FIBROBLASTS

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1. Introduction

The role of the low density lipoprotein (LDL) receptor in the uptake of serum cholesterol and the regulation of cholesterol biosynthesis has proved pivotal in understanding these two processes and their malfunction in the human congenital disease, homozygous familial hypercholesterolemia [1]. Furthermore, the uptake of LDL has proved to be an interesting model for general studies of receptor-mediated endocytosis [2]. In studies of the uptake of LDL, fibroblasts from patients with familial hypercholesterolemia have been of great value in providing test systems. These naturally occurring mutations fall into three allelic groups but the complexity of the LDL pathway suggests that other complementary mutations can exist. Thus, the full genetic analysis of the LDL pathway would seem to require application of the techniques of somatic cell genetics.

Probably the most useful somatic cell system for genetic analysis has proved to be cultured Chinese hamster cells, particularly the CHO-Kl line [3]. When suitable nutritional means are available for distinguishing between the presence or absence of a particular function, this cell can be used for the isolation of mutations defective in that function by the BudR visible light technique [3]. Furthermore, nutritional detection of a function in this cell allows the use of hybridization techniques in the analysis of complementation groups [3] and chromosomal mapping of human genes for that function [4]. Here we present a simple medium which allows nutritional detection of the low density lipoprotein receptor which could prove useful in such somatic cell genetic studies.

2. Materials and methods

The CHO-Kl cell is the standard culture established in these laboratories. The V79 cell and a 25-hydroxycholesterol resistant mutant of this cell, E5, were gifts of Dr J. L. Goldstein. Cells were grown in Ham's F12 [5] supplemented with 8% fetal calf serum or with 4% lipoprotein deficient serum (HDF) prepared by flotation of the lipoproteins at 100 000 × *g* at a density adjusted to 1.215 with KBr. 25-Hydroxycholesterol was purchased from Steraloids (Wilton, NH).

Low density lipoprotein was prepared from human chlyomicron-free serum by flotation at a density of 1.063 after removal of VLDC [6].

3. Results and discussion

The CHO-Kl line used in these experiments has been shown to express LDL receptors whereas the V-79 line is comparatively deficient in LDL receptors [7].

Our nutritional test for the LDL receptor is based on the ability of 25-hydroxycholesterol to inhibit the synthesis of cholesterol through a repression of HMG-CoA reductase activity [8]. Thus, at 0.1–0.5 μg/ml medium 25-hydroxycholesterol inhibits the growth of CHO-Kl cells in the absence of cholesterol but not in its presence [9]. When LDL is the only source of cholesterol for growing cells it would be expected that only cells that have the LDL receptor could grow in the presence of levels of 25-hydroxycholesterol that inhibit cholesterol biosynthesis. On the other hand,

both receptor-bearing and receptor-deficient cells would be expected to grow when cholesterol is supplemented to medium in unbound form. Since E5 is resistant to the inhibitory effects of 25-hydroxycholesterol on sterol biosynthesis it would be expected to grow in the presence or absence of this compound.

The results in fig.1 show the typical experimental results observed under these conditions. It can be seen that cholesterol supplementation permits the growth of CHO-Kl and V79 in the presence of 25-hydroxycholesterol but that LDL supplementation can only permit growth in the receptor-bearing CHO-Kl cell.

The ability of the 25-hydroxycholesterol resistant mutant, E5, to grow in these test media demonstrates that lack of growth of a cell is specifically due to the presence of 25-hydroxycholesterol and acts as a positive control on the HDF preparation. The outcome of this experiment is independent of cell density from 300–2000 cells/35 mm dish allowing nutritional tests to be made without careful counting of cells. Similar results have been obtained [7] using the drug compactin instead of 25-hydroxycholesterol.

Attempts to apply this test medium to human cells deficient in the LDL receptor have not yet been

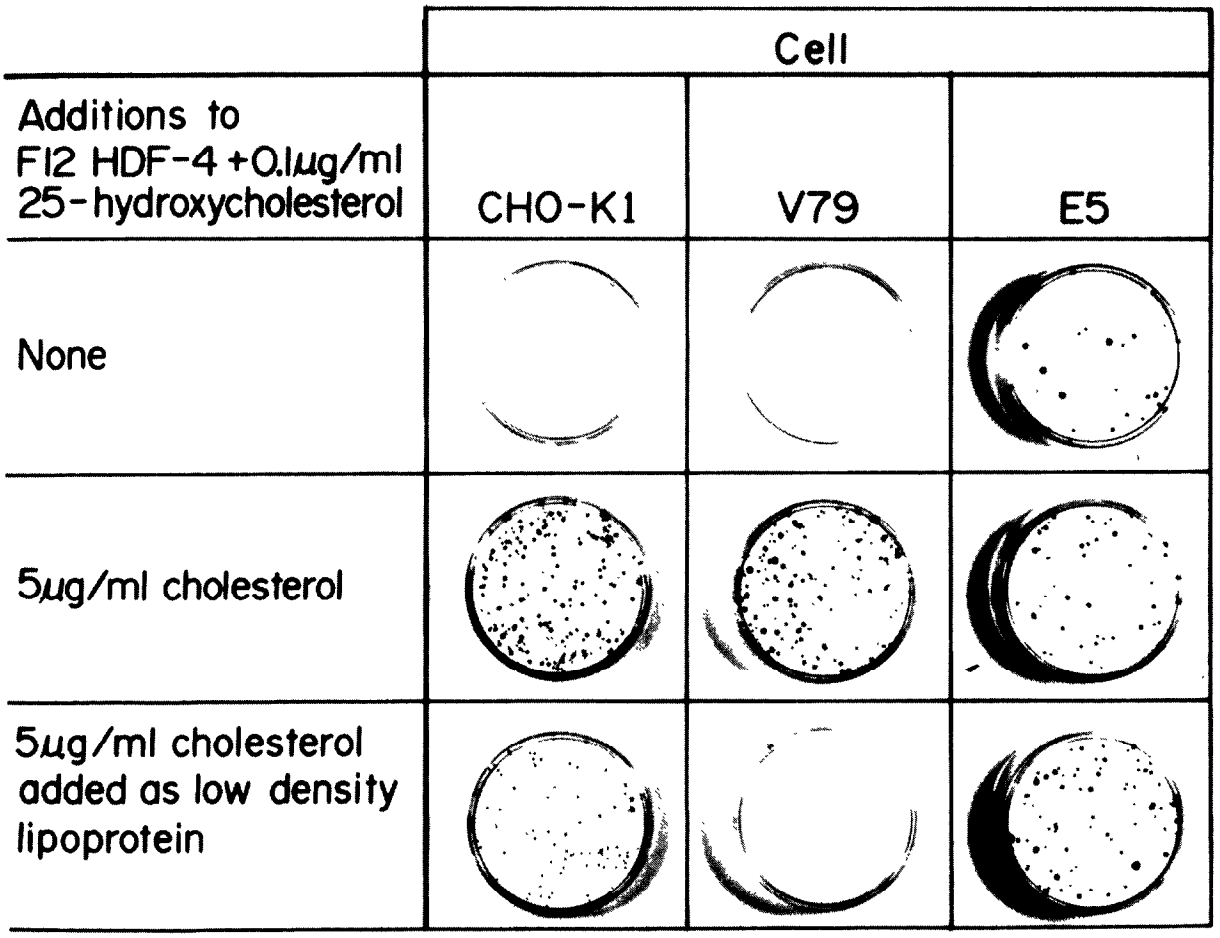

Fig.1. Growth of LDL receptor bearing cell line (CHO-Kl) and a receptor-deficient cell line (V79) in the test medium. Cells were grown for 72 h in test medium and then the medium was changed to F12 supplemented with 8% fetal calf serum and incubated for 3–4 days. Colonies were stained with crystal violet after fixation with 10% formalin. Cells (300) were innoculated into 35 mm plates.

reproducible, primarily because of the poor growth of diploid human fibroblasts on medium F12 at low density. Experiments with media particularly designed for use with these cells, such as those in [10] might be more successful. Such a test would be useful in diagnosis of type II familial hypercholesterolemia.

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