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3T3 VARIANTS UNABLE TO BIND EPIDERMAL GROWTH FACTOR CANNOT COMPLEMENT IN CO-CULTURE

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A Swiss-Webster 3T3 variant, called 3T3-ENR7, unable to divide in response to epidermal growth factor was isolated by the mitogen-colchicine selection technique. Like the other EGF non-proliferative variants (3T3-NR6 and 3T3-TNR2), 3T3-ENR7 was unable to bind ^{125}I-EGF. Pairwise co-culture of the three independently isolated EGF non-responsive variants did not restore mitogenic responsiveness to EGF.

We have previously described the isolation from Swiss-Webster 3T3 cells of two variant cell lines unable to mount a mitogenic response to the polypeptide growth factor, epidermal growth factor (EGF) (1,2). In both cases the basis for this non-proliferative response was the inability of these cells to bind EGF; they are functionally missing EGF receptor activity. In an attempt to extend the range of variant phenotypes unable to demonstrate a mitogenic response to EGF we have now isolated a third EGF non-responsive variant, 3T3-ENR7. Like the previous two variants (3T3-NR6 and 3T3-TNR2) 3T3-ENR7 cells are unable to bind iodinated EGF. They, too, are functionally missing EGF receptors.

Krieger et al. (3) have used a different type of selection system to isolate variants of CHO cells that are unable to bind low density lipoprotein (LDL). These CHO variants are phenotypically similar to our EGF-nonbinding 3T3 variants in that they are unable to bind the ligand used for selection, but are capable of binding and internalizing unrelated ligands. Recently, Krieger (4) demonstrated that co-cultivation of several of these CHO mutants defective in

Abbreviations Used:
EGF, epidermal growth factor TPA, 12-O-tetradecanoylphorbol-13-acetate

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LDL binding is able to restore the ability to bind and internalize LDL. The CHO mutants can be divided into two complementing classes; cbc mutants are complemented by co-cultivation and display LDL receptors while icc cells are inducer cells in co-cultivation, but do not themselves display LDL receptors in mixed culture. In this report we describe attempts to complement by co-cultivation the EGF non-responsive phenotype of our three independently isolated 3T3 variants.

MATERIALS AND METHODS

Procedures for variant selection and cell culture have been described previously (1,2). The newest EGF non-responsive cell line, 3T3-ENR7, was selected in a fashion identical to 3T3-NR6 (1). EGF was purified as described by Savage and Cohen (5). Binding of iodinated EGF has been described previously (6). Co-cultivation studies were performed by plating identical numbers of cells (1.25×10^5) in 60 mm culture plates. After cells reached confluence and stopped dividing, mitogen was added and wells were scored for incorporation of tritiated thymidine (2) following a one-hour pulse 24 hours after mitogen addition.

RESULTS

Isolation and Characterization of the EGF-Nonresponsive Variant 3T3-ENR7:

Swiss-Webster 3T3 cells were subjected, using EGF as the mitogen, to the mitogen/colchicine selection procedure we have previously described for the selection of mitogen-specific, non-proliferative variants (1,2). After several rounds of selection clones were picked and tested for response either to increased serum, 12-O-tetradecanoyl-13-phorbol acetate (TPA) as a non-selected mitogen, or EGF. Confluent, non-dividing 3T3 cells were able to respond to all three agents (Figure 1). In contrast, 3T3-ENR7 cells were unable to respond to EGF, but were competent to mount a mitogenic response to serum or TPA.

To characterize the biochemical basis for the lack of responsiveness of 3T3-ENR7 cells to EGF we first examined the ability of the variant and parental line to bind EGF. The 3T3 parent was able to bind iodinated EGF (Figure 2). In contrast, 3T3-ENR7 cells demonstrated the same phenotype as our other EGF non-proliferative variants (3T3-NR6 and 3T3-TNR2); they are defective in their ability to bind EGF.

Variants Defective in EGF Binding Are Unable to Complement in Co-Cultivation:

Krieger's recent observation of complementation in co-culture of CHO variants unable to bind and internalize LDL (4) suggested that the independently

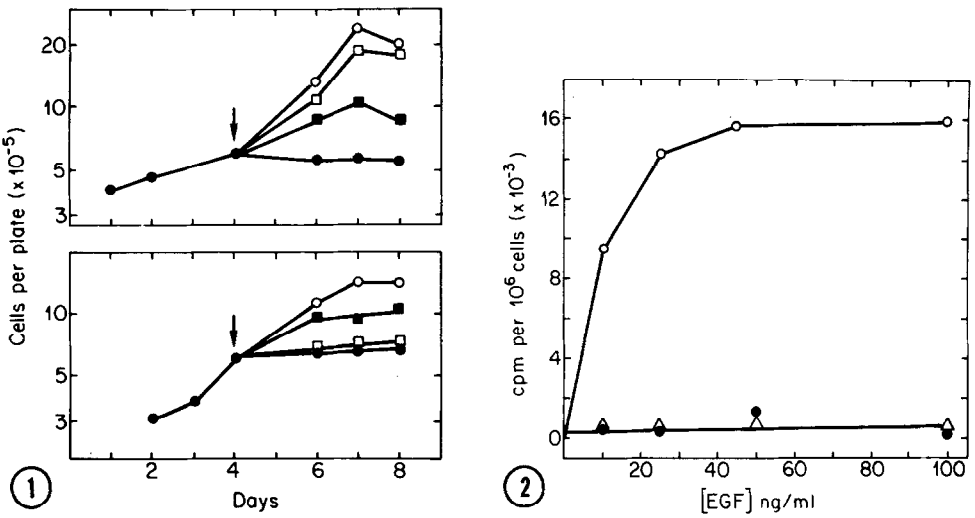

Figure 1. Response of 3T3 and 3T3-ENR7 to Mitogenic Stimulation. Cells were grown to confluence in medium containing 5% fetal calf serum. Mitogens were added at the arrow, and cell numbers were subsequently determined. (●), control; (○), 10% serum; (■), 10 ng TPA/ml; and (□), 10 ng EGF/ml. Top panel, 3T3; bottom panel, 3T3-ENR7.

Figure 2. Binding of ^{125}I-EGF to 3T3, 3T3-ENR7, and 3T3-NR6 Cells. Confluent monolayers of cells were incubated with increasing amounts of ^{125}I-EGF at 4° for 60 minutes. Unbound radioactivity was washed away, cells were solubilized, and radioactivity was measured. (○), 3T3 cells; (△), 3T3-ENR7 cells; (●), 3T3-NR6 cells.

isolated 3T3 variants unable to bind and respond to EGF might similarly complement. However, when 3T3-NR6, 3T3-TNR2 and 3T3-ENR7 cells were co-cultivated pairwise in all possible combinations the mixed cell monolayers did not demonstrate increased incorporation of tritiated thymidine in response to EGF, although elevated serum concentration was able to increase DNA synthesis (Figure 3). Thus, unlike the CHO LDL receptor variants, the 3T3 EGF receptor variants are unable to complement in co-culture.

DISCUSSION

The 3T3 NR6 variant that is unable to bind EGF has been used for a variety of biological purposes, including a biological definition of receptor specificity for EGF (7) and for other mitogens (8-10), characterization of the mode of action of sarcoma growth factor (11), as a receptorless substrate for transfer of the EGF receptor (12), etc. We want to emphasize that three independently isolated 3T3 variants with a similar phenotype (EGF nonresponsive,

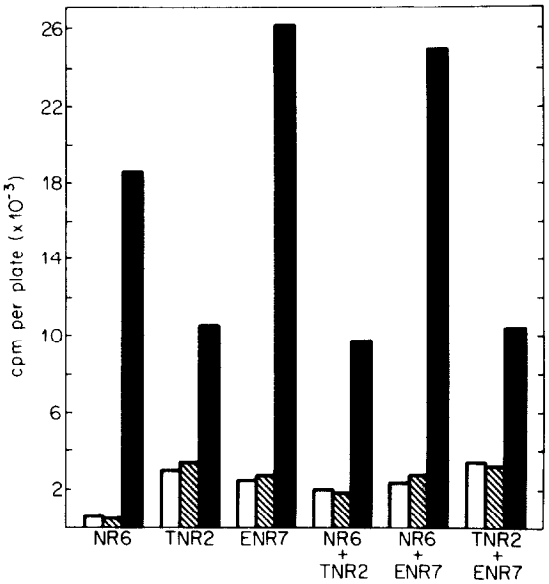

Figure 3. Mitogenic responses of mixed cultures of 3T3-NR6, 3T3-TNR2 and 3T3-ENR7. Equal numbers of cells were co-cultured pairwise and grown to confluence and density arrest. Mitogens were added and incorporation of tritiated thymidine was determined in a one-hour pulse 24 hours after stimulation. Open bars, control; striped bars, 20 ng EGF/ml; solid bars, 10% serum.

unable to bind EGF, and responsive to other mitogens) are now available from our laboratory.

In each case where we have obtained EGF nonresponsive variants we have isolated cells unable to bind EGF. Similar studies with platelet derived growth factor (PDGF) have resulted in a similar phenotype; cells have greatly reduced PDGF receptors. These data are consistent with, but do not prove, the idea that the polypeptide mitogens have a common cellular mediator of their mitogenic activity. (Only an alteration in the receptor function would result in variants defective in response to individual polypeptide mitogens. Variants altered in subsequent steps would be pleiotypic in their phenotype.) In contrast, variants unable to respond to TPA still bind the ligand (13) and demonstrate early responses such as glucose transport (14) and arachidonic acid release (15). The data suggest (but again do not prove) that the TPA mitogenic pathway has some steps, subsequent to ligand binding, not shared by the polypeptide mitogens.

Our inability to demonstrate complementation by co-cultivation with the three EGF nonresponsive 3T3 variants prevents us from subdividing these variants

into classes. We are currently marking the variants and the parental cell line with drug-resistance plasmids, in order to test the dominance or recessiveness of the "receptorless" phenotype and to determine their capability to complement in heterokaryon and somatic cell hybrid experiments.

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