Modulation of carcinoembryonic antigen release by HT-29 colon carcinoma line in the presence of different agents

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Summary. In this study we followed the effects of various differentiating agents on the expression of carcinoembryonic antigen (CEA) released into the medium by a colon carcinoma cell line HT-29. Butyric acid 1 mM markedly increased the level of CEA (12-fold in comparison to control levels). 12-O-tetradecanoyl-phorbol-13-acetate (TPA) 50 ng/ml and 5-azacytidine 4×10^{-6}M increased the amount of CEA, 2- and 1.5-fold respectively. On the other hand retinoic acid 10^{-5}M, N methyl-formamide 1% and N,N hexamethylene bisacetamide 2.5 mM decreased CEA 2-, 4- and 3-fold respectively. Our results emphasize that various differentiating agents affect CEA levels differently. Thus changes in CEA levels appear not to be reliable as a marker of a more differentiated phenotype.

Key words. Carcinoembryonic antigen; colon carcinoma line; differentiating agents.

The relationship between carcinogenesis and cellular differentiation is among the most intriguing and fundamental questions in biology. In a number of systems, chemical compounds which induce differentiation have been found to be capable of reducing or inhibiting the tumoroigenicity of cancer cells[1-6]. Thus, HL-60 human promyelocytic leukemia cells and Friend mouse erythroleukemia cells become terminally differentiated in the presence of maturating agents[7,8]. On the other hand, differentiating agents do not induce terminal differentiation in solid tumors, but rather cause a change in phenotypic expression leading to partial normalization[6,9].

A number of markers have been proposed for monitoring the shift of cancer cells towards normality. In the present study we used as a marker CEA released into the culture medium by cells from the human colonic adenocarcinoma line HT-29, which is undifferentiated in standard culture conditions[10], in order to follow the effects of a number of chemical compounds representing different classes of differentiating agents. Although CEA is not a specific marker of colorectal cancer, its level in plasma helps to monitor the stage of the disease[11].

Materials and methods. The human colonic adenocarcinoma cell line HT-29 was originally established by Dr J. Fogh[12] and kindly supplied by Dr F. Doljanski of the Hebrew University, Jerusalem.

5×10^5 cells were cultured for 3 days in 9-cm tissue culture plates in 10 ml of RPMI supplemented with 10% fetal calf serum, glutamine and antibiotics (Biological Industries, Beth Haemek, Israel), and the medium was replaced by fresh medium before adding the different agents, which were all obtained from Sigma. TPA was prepared as a stock solution of 1 mg/ml ethanol and diluted with PBS prior to use. Other agents were dissolved in PBS except for retinoic acid, which was dissolved in DMSO (final concentration 1%). The agents in the concentrations used had no toxic effects on the cells as tested by trypan blue dye exclusion studies. After the addition of the agents the cells were allowed to grow for another 4–5 days until subconfluence (or confluence). The number of cells in each plate was determined and the supernatants were collected, centrifuged at $500 \times g$ for 10 min and dialyzed for 3 days against 2 daily changes of distilled water. They were then lyophilized and suspended in 0.5 ml distilled water. The CEA levels were determined by using an Elisa method with a kit from Abbott Company.

Results and discussion. Our results show that various differentiating agents exerted different effects on the levels of CEA released by cells of the HT-29 line into the medium (table), which presumably represent cellular CEA levels[13]. Butyric acid 1 mM had the most pronounced effect, increasing the CEA 12-fold. This is in agreement with the findings of Tsao et al.[6] who measured CEA levels in cells of the human rectal cancer line HRT-18. TPA, which is an inducer of differentiation of various cells, for example HL-60[14], had a relatively weak effect on CEA (2-fold). It is of interest that in preliminary experiments TPA and butyric acid were found to have a synergistic effect on CEA expression as observed also by others on EBV expression[15]. 5-Azacytidine, which causes inhibition of DNA methylation and influences gene expression[16], had a weak effect on CEA. Retinoic acid (RA) was found to decrease CEA 2-fold, which is in disagreement with the observation of Tsao et al.[6] who found no effect on CEA. RA, which acts as an antipromotor in many systems[17,18], partially inhibited the effect of TPA. In view of the role that the cell surface is assumed to play in regulation of cell growth, and because RA can modulate the glycosylation of cell surface glycoconjugates, it has been proposed that RA modulate cellular properties (towards suppression of transformed phenotype) by altering membrane structure and function[4]. DMSO 1%, which served as the solvent for retinoic acid, had no effect on the release of CEA. N-methylformamide and N,N hexamethylene bis acetamide, which are inducers of differentiation[7,19,20], inhibited CEA 4- and 3-fold respectively.

Reports in the literature claim that well-differentiated colorectal cancer lines have higher CEA levels[13,21,22], while others claim the opposite[23,24]. Our results emphasize that various differentiating agents affect CEA levels differently. Thus, in general, changes in CEA levels appear not to be reliable as a marker for a more differentiated phenotype.

Modulation of CEA release by HT-29 cells in the presence of different agents

Conditions	CEA[a] pg/10^6 cells	Cell growth[b] %
Control	407 ± 26	100
TPA 10 ng/ml	627 ± 58	90
TPA 25 ng/ml	836 ± 198	92
TPA 50 ng/ml	869 ± 218	82
Retinoic acid 10^{-5}M	190 ± 65	89
Retinoic acid 10^{-6}M	230 ± 113	92
Retinoic acid 10^{-6}M + TPA 25 ng/ml	564 ± 84	69
DMSO 1%	380 ± 155	82
5-Azacytidine 4×10^{-6}M	610 ± 138	80
N-Methyl-formamide 1%	113 ± 49	41
N,N-Hexamethylene bis acetamide 2.5 mM	137 ± 85	86
Butyric acid 0.5 mM	3980 ± 1174	55
Butyric acid 1 mM	4993 ± 1410	30

[a] Mean of 3–6 experiments ± standard deviation. [b] Control was taken as 100%. 5×10^5 cells were cultured initially and after 7–8 days the number of cells in the control was around 7×10^6.

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Accumulation of drastic mutants in selection lines for resistance to the insecticides dichlorvos and malathion in *Drosophila melanogaster**

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Summary. Viability tests were performed on second and third chromosomes from lines of *Drosophila melanogaster* selected for increased resistance to the organophosphorus insecticides dichlorvos and malathion, in order to evaluate the accumulation of drastic alleles. Our results show that malathion reduces significantly the relative viability of chromosome 3 and also increases the frequency of drastic alleles in this chromosome, while dichlorvos increases significantly the frequency of drastic alleles in chromosome 2.
Key words. Drosophila melanogaster; dichlorvos; malathion; viability tests; drastic mutants.

Lethal alleles have repeatedly been found at high frequencies in experimental populations subjected to selection for quantitative characters[1,2]. In these laboratory populations, lethals arise by mutation and could increase its frequency by different means, for example, by heterotic effects of lethal heterozygotes[3] or by epistatic interactions between linked lethal genes[4]. When the selected character is the resistance to a potentially mutagenic compound, the response must be higher due to the induced increase in the mutation frequency. Therefore, studies on the occurrence of drastic mutants in these selected lines could provide useful information on the genotoxicity of tested compounds.

There is no doubt that natural insect populations contain many pesticide-resistant variants and, when challenged by selection, quickly develop resistance. In this way, positive responses in selection experiments for increased resistance to several insecticides have been reported by different authors[5-7] but, up to now, no data have been published on the genetic load induced in the selected lines by analyzing quantitatively and qualitatively the effects of detrimental alleles accumulated.

Organophosphorus compounds generally show alkylating properties[8] and, consequently, they are potentially mutagenic agents. Dichlorvos and malathion are two organophosphorus insecticides fairly well studied from the mutagenic point of view but, unfortunately, no general agreement has been reached on their genotoxicity because both positive and negative results were found using different organisms and mutagenicity assays[9-13].

The present report deals with the occurrence of drastic mutants in lines of *Drosophila melanogaster* selected, during the adult stage, for increased resistance to dichlorvos and to malathion. The question whether the results of this kind of experiment would be interesting from the point of view of mutagenicity studies is considered.

Materials and methods. 1) Chemicals and treatment procedure. Dichlorvos, or 0,0-dimethyl-0-(2,2-dichlorovinyl) phosphate, with a purity of 97%, was supplied by Productos Cruz Verde S.A. (Barcelona) and was diluted in an aqueous solution which contained 5% sucrose. Malathion, or S-[1,2-di(ethoxycarbonil) ethyl] dimethyl phosphorothiolothionate, 50% emulsifiable concentrate (50% active ingredient, 50% xylol, dispersing and emulsifying agents), obtained from Agrocrós S.A. (Barcelona), was first dissolved in dimethyl sulfoxide (DMSO, Panreac) and then diluted with a 5% sucrose solution to give a final DMSO concentration of 1%. In both cases, the insecticide concentrations used for treatment were based on the active ingredient. For adult feeding treatment, 3–4-day-old flies were starved for 4 h and then fed the test solution in special glass filter feeding units[14].

2) Selection experiments. Selection experiments were performed on a population of *Drosophila melanogaster* designated MRA, previously selected for increased resistance to malathion[15]. The selection procedure was simple. In the line selected for increased resistance to malathion, flies surviving after adult treatment were used as parents of the next generation. The selection was practised during 8 generations, increasing the concentration of malathion from 50 to 100 ppm. In the line selected for increased resistance to dichlorvos the experiment lasted for 20 generations, but the selection was applied in alternate generations because of the high toxicity of dichlorvos. In this line, the concentrations applied ranged