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SPECIFICITY OF THE INHIBITION OF DNA SYNTHESIS BY EXTRACTS FROM CLONED NORMAL, SARCOMA-VIRUS-TRANSFORMED AND REVERTANT 3T3 CELLS

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Summary.—Extracts containing tissue-specific DNA-inhibitory activity were prepared from normal FL (Swiss) and BALB/c 3T3 cells, from these cells transformed with sarcoma virus and from revertants cloned from the transformed cell lines. By testing all extracts on all cell lines we found that (1) production of and susceptibility to the inhibitors were decreased in transformed BALB/c cells, (2) specificity varied with expression of the transforming genome, as an extract from a given cell line inhibited the growth of its cell of origin, *e.g.* revertant, more than normal or transformed cells, and (3) there was also a DNA-synthesis stimulator.

REVERSIBLE DNA-inhibitory activity in extracts from normal and revertant mouse (FL (Swiss) and BALB/c 3T3) cells inhibits *in vitro* DNA synthesis in the cell of origin. In contrast, extracts from transformed BALB/c cells, which are highly malignant *in vivo*, have no inhibitory effect on their cell of origin (Ebbesen *et al.*, 1977). By testing all extracts on all cell lines we now find that both production and susceptibility are decreased in transformed BALB/c cells, that the specificity of action of the extracts varies with expression of the transforming genome, and that there is also a compound stimulating DNA synthesis.

MATERIALS AND METHODS

Cells.—Early passages of cloned murine 3T3 FL cells derived from outbred Swiss mice and 3T3 cells derived from inbred BALB/c mice, Moloney sarcoma virus-transformed, sarcoma-genome positive, leukaemia-virus negative (S^+L^-) 3T3 cells, Kirsten sarcoma virus-transformed, non-producer (NP) 3T3 cells, and flat revertants cloned from the transformed cultures (Nomura *et al.*, 1972; Fischinger *et al.*, 1974) were used. Tests for tumour-virus production and contamination by bacteria and mycoplasm Ebbesen *et al.*, (1977) were negative.

Preparation of extract.—Cells were harvested at 50% confluence. After mechanical disruption the cell fragments were centrifuged at 105,000 *g* at 4°C for 1 h, the supernatant heated to 70°C for 15 min, and centrifuged at 105,000 *g* at 4°C for 1 h. The last supernatant was then separated by filtration into mol.-wt fractions $< 20{,}000$, 20–50,000, 50–100,000, 100–300,000 and $> 300{,}000$. The inhibitory and stimulatory activity was found in the 20–50,000 fraction, and this fraction was used throughout (Ebbesen *et al.*, 1977; Olsson and Ebbesen, 1977). The amount of protein and nucleosides was determined spectrophototometrically in each sample, which then was lyophilized and stored at −70°C. The amount of nucleosides was $< 1\%$ w/w of the protein in the extracts used.

IN VITRO *test.*—The extract from 10^6 cells was added to a subconfluent culture with 5×10^5 cells $\pm 2 \times 10^5$ (s.e.) in a 30 cm^2 bottle. Following incubation at 37°C for 18 h, 10 μCi [^{3}H]TdR was added, and 4 h later the cells were harvested, counted, and

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TABLE I.—*Percentage inhibition or stimulation (—) of DNA synthesis in subconfluent cell cultures after addition of various cell extracts, as registered by [^{3}H]TdR incorporation*

	Extracts from subconfluent cell cultures of the various lines										
	Swiss mouse							BALB/c mouse			
Target cells	3T3-554 (N)	3B11 (T)	SR448 (R)	2143 (R)	3382 (T)	F131 (R)	F271 (R)	B-3T3 (N)	K-B-23 (T)	KSR12121 (R)	KSR4111 (R)
Swiss mouse											
3T3-554 (N)	86	3	24	−23	−51*	25	49*	−8	11	17	−3
3B11 (T)	−9	84*	−14	−37	48*	−7	−19	−3	18	17	−10
SR448 (R)	−24	−235*	91*	−84*	−71*	4	−1	−19	−22	−77[a]	−124*
2143 (R)	36*	46*	−23	65*	50*	−3	−19	67*	9	−4	−16
3382 (T)	−7	−112*	−8	19	81*	29*	33*	−18	−2	1	−14
F131 (R)	−94*	64*	64*	−23	−24	69*	−23	−14	−335*	−21	−5
F271 (R)	−17	−268*	39*	46*	−159*	54*	86*	−164*	−11	37[a]	36*
BALB/c mouse											
B3T-3 (N)	59*	14	63*	49*	31*	22	37*	78*	24	−19	22
K-B-23 (T)	−19	16	−21	−11	0	18	19	8	21	4	−11
KSR12121 (R)	35*	−1	−17	−15	−17	13	21	31*	−12	68*	41*
KSR 4111 (R)	49*	−7	−19	0	19	14	12	46*	−3	−16	−46*

N = normal.
T = transformed.
R = revertant.
* = significantly different from control cell lines ($P < 0{\cdot}01$, Student's t test).

TABLE II.—*Significant inhibitory (○) and stimulatory (×) effect on DNA synthesis of the various target-cell: extract combinations*

	Extract from									
	Swiss mouse							BALB/c mouse			
Target	3T3-554 (N)	3B11 (T)	SR448 (R)	2143 (R)	3382 (T)	F131 (R)	F271 (R)	B-3T3 (N)	K-B-23 (T)	KSR12121 (R)	KSR4111 (R)
Swiss mouse											
3T3-554 (N)	●				×						
3B11 (T)		●	×	×	○		○				
SR448 (R)		×	●	×	×					×	×
2143 (R)	○	○		●	○			○			
3382 (T)		×			●	○	○				
F131 (R)	×	○	○			●			×		
F271 (R)		×	○	○	×	○	●	×		○	○
BALB/c mouse											
B-3T3 (N)	○		○	○	○		○	●			
K-B-23 (T)											
KSR12121 (R)	○							○		●	○
KSR4111 (R)	○							○			●

N = normal.
T = transformed.
R = revertant.
● = strongest inhibition obtained with a given extract.

the [^{3}H]TdR incorporation determined. The reversibility, absence of cytotoxicity and specificity of the extracts were also tested (Ebbesen *et al.*, 1977).

RESULTS

The cross-board study (Tables I and II shows that the strongest DNA-synthesis inhibition of a given extract is invariably found when tested on the cell from which the extract was obtained. The total number of *cell lines* that were significantly inhibited in DNA synthesis in relation to the total number of cell lines tested was 8/22 (36%) for normal cells, 22/66 (33%) for revertant cells, and 6/33 (18%) for transformed cells. The total number of *extracts* inhibiting DNA synthesis in relation to the total number of tested extracts from all cell types, was 9/22 (41%) for extracts from normal cells, 20/66 (30%) for extracts from revertant cells, and 7/33 (21%) for extracts from transformed cells. The tendency—not statistically significant—of transformed cells both to contain low inhibitory activity and to be less susceptible to otherwise inhibitory extracts, is specially pronounced for cell lines derived from the BALB/c mouse.

Tables I and II further show that some extracts, when added to certain cell lines, may stimulate DNA synthesis. The total number of *cell lines* stimulated in relation to the total number of cell lines tested was 1/22 (4·5%) for normal cells, 10/66 (15%) for revertant cells, and 3/33 (9%) for transformed cells; and the total number of stimulatory *extracts* in relation to the total number of extracts tested was 2/22 (9%) for normal cells, 5/66 (8%) for revertant cells, and 7/33 (21%) for transformed cells.

The Figure shows dose-response curves of typical inhibitory and stimulatory extracts respectively, tested on 2 cell lines. Inhibitory extracts had no stimulatory effect at any concentration tested. Stimulatory extract had no inhibitory effects, as the inhibition observed in

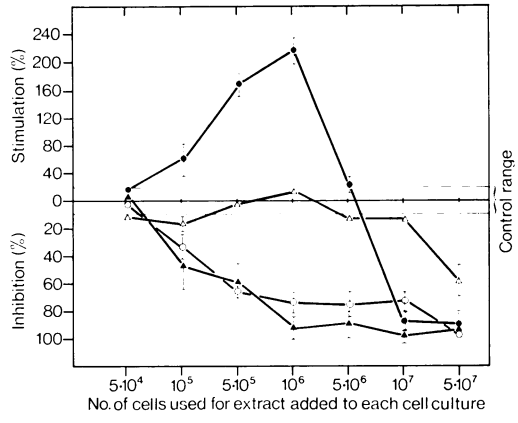

FIG.—Dose-response curves of the effect on [^{3}H]TdR incorporation in cells from 2 cell types treated with extracts from the same 2 cell types. Each point represents the value of the mean ± s.e.

	Extract from	Target cells
○——○	3B11	3B11
●——●	3B11	SR448
△——△	SR448	3B11
▲——▲	SR448	SR448

experiments with stimulatory extracts was due to increasing cell death as measured by trypan-dye exclusion. Thus extract from 5×10^6 cells induced no increased cell death, whereas extract of 10^7 and 5×10^7 cells induced 80–90% cell death. The cytotoxic effect of inhibitory extract was also only observed with extract doses above 5×10^6 cells and again with a cytotoxicity level about 80–90%.

DISCUSSION

Transformed K-BALB 23 cells are not *inhibited* by their own extract (Ebbesen *et al.*, 1977) and, as has been shown for some other malignant cells (Bullough & Deol, 1975; Rytömaa & Kiviniemi, 1968; Todaro & De Larco, 1976), these malignant cells are here found "deficient" both with respect to production of active inhibitory material and with respect to sensitivity to the various inhibitory extracts tested here.

Each of the 11 different cell extracts

studied had its most pronounced inhibitory effect on its cell of origin. Since all cultures of a given strain were derived from one another by cloning, this indicates for the first time that there may be a link between a certain degree of *specificity* and expression of the transforming genomes. Such a change in specificity would add a new way of escaping normal regulation (Lord *et al.*, 1974).

Another unexpected finding was the *stimulatory* effect exerted by some extract : target-cell combinations. The inhibitory and stimulatory effects are most probably due to different compounds, as different responses were obtained with different extracts on the same cell type, and as indicated by the dose-response curves. Our extracts were harvested from washed trypsinized cells, but this does not exclude the possibility that they contain a mitogen from the calf serum that was adsorbed to the cells. Calf serum contains a compound mitogenic for human fibroblasts, but this mitogen is removed by ultrafiltration (Houck *et al.*, 1972), as done by us. Mouse cells, however, are also stimulated by other serum factors of unknown molecular weight (Rudland *et al.*, 1974; Brooks, 1976). The stimulatory effect of some, but not all, cell extracts thus could derive from preferential binding/uptake of exogenous mitogen by some cells, although we favour the hypothesis that we are dealing with an enhanced endogenous production of mitogen.

Our finding of both quantitative and qualitative (specificity) differences, covarying with expression of malignant phenotype within cultures derived from each other by cloning, supports the assumption that such extracts contain regulatory compounds relevant to cancer biology, and justifies further work on purification.

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