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THE CYTOSOL POLYPEPTIDES OF EHRLICH ASCITES TUMOR CELLS AND THEIR EFFECT ON DNA SYNTHESIS IN BALB c/3T3 CELLS

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SUMMARY. ^{3}H-arginine-labeled polypeptides from cytosol of Ehrlich ascites tumor cells have been isolated and characterized. The acidic polypeptide / pI-5.0 /, containing sugar components, stimulated ^{3}H-thymidine incorporation to DNA of Balb c/3T3 cells, whereas the other polypeptides of basic nature inhibited this process. These results suggest that the polypeptides from tumor cells can play an important role in the regulation of cell growth.

INTRODUCTION

The cell growth is dependent on the presence of polypeptides called generally stimulating factors. They are produced and secreted by various normal / 1, 2 / and neopastic cells / 3, 4, 5, 6 /. They are important factors not only during carcinogenesis process but for maintenance of the tumor cell phenotype as well / 3 /. These substances have molecular weight in the range from 7 000 to 20 000 daltons / 4 /.

It seemed of interest to isolate and characterize the polypeptides from Ehrlich ascites tumor cells and to study the effect of these substances on the incorporation of ^{3}H-thymidine into DNA of mouse cells Balb c/3T3 in culture.

MATERIALS AND METHODS

Tumor growth. Male swiss mice weighing 50 g were used for the experiments. Ehrlich ascites tumor cells were grown in the mice after intraperitoneal injection of 0.2 ml of cell suspension in ascites fluids.

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Preparation of cytosol ^{3}H-arginine-containing polypeptides. The tumor bearing mice were fasted 18 - 20 hours before i.p. injection of 0.2 ml 20 μCi / L-5-^{3}H / - arginine, specific radioactivity 16.7 Ci/mM, Radiochemical Centre Amersham, England. Thirty mice were killed 2.5 hours after injection of arginine. Further procedure of cytosol preparation was the same as described previously / 7 /.

The cytosol was submitted to the gel filtration on Sephadex G-25 in order to separate the high molecular from low molecular substances. Peak II, containing the low molecular substances, was pooled and desalted on the column with Bio-Gel P-2 / Bio-Rad Laboratories, Richmond, California /. The fractionation of polypeptide mixture was carried out by chromatography on the cationic exchanger SP-Sephadex C-25. Full details are given elsewhere / 8 /. Homogeneity of polypeptides was studied by isoelectric focusing / 9 / and polyacrylamide disc electrophoresis / 10 /.

Molecular weight determination. Molecular weight of polypeptides was determined by electrophoresis on 12.5% polyacrylamide slab gels in discontinuous buffer system according to Laemmli / 10 /.The following polypeptides were used as standards : 1. insulin, m.w. 6 000 / Reanal, Budapest Hungary /, 2. ribonuclease A, m.w. 13 700 / Pharmacia, Fine Chemicals, Sweden /, 3. chymotrypsynogen A, m.w. 23 500 / Pharmacia, Fine Chemicals, Sweden /. The gels, were stained with Coomassie Blue R-250 according to Fairbanks / 11 /.

Isoelectric focusing. The isoelectric focusing of polypeptides was performed according to Vesteberg and Svensson / 9 / at 4° using 2.5% Pharmalyte gradient / pH 4.0-6.0 and 6.5-9.0 /. The position of polypeptides in pH gradient was identified by determination of ^{3}H-arginine radioactivity.

Hexose and hexosamine analysis. Hexoses and hexosamines were identified and quantitatively estimated by gas liquid chromatography / 12 /. The instrument used was a Beckman G-C 2 chromatograph equipped with a flame ionisation detector and Honeywell-Brown 0-1 mV recorder.

Assay for growth-promoting activity was performed on sister cultures of mouse cells Balb c/3T3 according to the method described by De Larco and Todaro / 3 /. Particular polypeptides were added in concentration of 150 μg of α-amino nitrogen per ml. After 16 hours of incubation the cells were exposed for 8 hours to 100 μCi of ^{3}H-thymidine. Further procedure was the same as described by De Larco and Todaro / 3 /. Each experiment was done in triplicate. The results were expressed in cpm of incorporated radioactive thymidine per 10^{6} cells.

RESULTS AND DISCUSSION

Five polypeptides from Ehrlich ascites tumor cells were obtained. They were homogeneous during electrophoresis and isoelectric focusing. Their molecular weights / m.w. / and isoelectric points / pI / are presented in Table 1.

Peptide I of the m.w. 18 500 and pI 5.0 is the most abundant in cytosol. It contains some carbohydrate components / see Table

Table 1 : Some physico-chemical properties of cytosol polypeptides of Ehrlich tumor cells.

Material	pI	Molecular weight	Carbohydrate component[x]				
			Glucose	Galactose	Mannose	Galactos-amine	N-acetylne-uramic acid
Peptide I /glycopeptide/	5.0	18 500	150.07	69.16	161.13	80.22	33.20
Peptide II	7.7	10 500	-	-	-	-	-
Peptide III	7.7	11 000	-	-	-	-	-
Peptide IV	8.7	10 000	-	-	-	-	-
Peptide V	8.9	8 500	-	-	-	-	-

[x] μg of carbohydrate component per mg of α-amino nitrogen

Table 2 : The effect of particular polypeptides on the incorporation of ^{3}H-thymidine to DNA of mouse Balb c/3T3 cells.

Material	Mean values and variation range, cpm x 10^{-3} per 10^{6} cells	% stimulation of thymidine incorporation	% inhibition of thymidine incorporation
Control	75 /55 - 92/	100	-
Peptide I /glycopeptide/	193 /173 - 212/	257.4	-
Peptide II	40 /35 - 44/	-	42.9
Peptide III	44 /36 - 50/	-	39.7
Peptide IV	48 /37 - 58/	-	36.0
Peptide V	26 /22 - 29/	-	68.0

x The cells were counted with the use of microscopic net ocular.

1 /. The remaining four polypeptides of lower molecular weight have basic character. The last peptide of the lowest molecular weight is rich in arginine. In comparison to other peptides it occurs in lowest amount in cytosol.

All the investigated polypeptides affect the incorporation of ^{3}H-thymidine to DNA / Table 2 /.

Only glycopeptide of acidic nature / peptide I / was able to stimulate thymidine incorporation. The polypeptides of basic character and lower molecular weight were inhibitors of the thymidine incorporation. In the presence of these peptides in the cell culture medium the incorporation was inhibited in high degree.

It is not known whether the glycopeptide isolated by us from Ehrlich ascites tumor cells is identical factor as that produced by other neoplastic and transformed cells. The growth factor isolated from MSV-transformed cells by Todaro et al. / 4 / was not completely purified and characterized. However, the factors isolated by Roberts et al. / 6 / from MSV-transformed cells and from

various tumor cells had the molecular weight of 7 000 - 10 000 daltons. It is worthy of note that there are fluctuations in the production of specific cellular peptides during / 13 / metabolically distinct states : growing and resting.

We think that growth-stimulating glycopeptide isolated by us is not the same as that described by Todaro et al. / 4 / and Roberts / 6 /. The mechanism by which the glycopeptide causes the stimulation of cell growth is not presently known. It is possible that released factor may evoke the "auto-stimulation" of cell growth, as suggest Todaro and De Larco / 14 /. It can be suggested that the polypeptides are released from tumor cells and they stimulate the growth of other cell.

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