QUANTITATIVE ANALYSIS OF ULTRASTRUCTURE OF TUMOR CELLS IN TISSUE CULTURE

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The fine structure of the mitochondria, ergastoplasm, and polysomes of cells of primary and transplanted tumor tissue cultures was studied by morphometry. In both primary and transplanted cultures considerable differences were found in the fine structure of cells of different strains. On the whole, however, these groups differed significantly in the organization of their cytoplasmic protein-synthesizing apparatus. Primary cultures were characterized by a more reduced ergastoplasm and a more highly developed polysome system. For tumor cells, culture in vitro can be presumed to be a factor facilitating further reorganization of the protein-synthesizing system toward the synthesis of substrates necessary for growth and multiplication of the cell mass.

KEY WORDS: culture of tumor cells; morphometry; culture on coverslips.

Various exogeneous factors, especially a factor such as prolonged cultivation in vitro, may have a considerable influence on the morphological and functional state of tumor cells. It was therefore decided to study the ultrastructural features of tumor cells in primary trypsinized and transplanted cultures, i.e., to attempt to analyze the character of ultrastructural reorganization of tumor cells during culture in vitro. It is most logical to use ultrastructural morphometry in investigations of this sort [1, 3, 6, 8]. No information could be found in the accessible literature on investigations of this type using methods of quantitative analysis of cell ultrastructure.

A comparative quantitative study was accordingly made of the ultrastructural organization of cells of primary trypsinized and transplanted tumor tissue cultures.

EXPERIMENTAL METHOD

A comparative morphometric analysis was made of two-day cultures of primary trypsinized tumors: rat reticulosarcoma 321 (KRS-321) [1] and hamster sarcoma induced by human adenovirus of serotype 12 SKhA-12), and also cells of transplantable lines HEp-2 (carcinoma of the human larynx) and CaVe (carcinoma of the human stomach). Cells for electron-microscopic investigation were detached from the coverslip with 0.02% versene solution, centrifuged at 4000 rpm for 8 min, and then fixed with 3% glutaraldehyde and 1% OsO_4 by the usual methods. After dehydration in alcohols and embedding in a mixture of epoxy resins (Epon–Araldite), ultrathin sections were cut on the LKB-111 ultrasome, stained with uranyl acetate and lead citrate, and examined in the IEM-7A electron microscope. For morphometric analysis 30 cells were chosen by random selection in each experimental group. In this way 120 objects (30 × 4) were investigated. The following morphometric parameters were obtained from photographic prints with an enlargement of 27,000 × [1, 3]: the surface area of the mitochondrial cristae (S_V^{cr}), the surface–volume ratio of the mitochondria (S_V^m/V_V^m), the coefficient of fragmentation of the mitochondrial cristae (K_f^{cr}). The fraction of conventionally active mitochondria (AM) determined as the sum of mitochondria of classes 2, 3, and 4 by size [7], the volume of the cisterns of the ergastoplasm (V_V^e), the number of ribosomes per micron length of the ergastoplasm membranes (N_L^{re}), and the coefficient of fragmentation of the ergastoplasm (K_f^e).

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TABLE 1. Morphometric Parameters and Coefficients of Level of Morphological Organization of Cells from Cultures of Tumors KRS-321 and SKhA-12 and of Transplantable Tumor Strains HEp-2 and CaVe

Parameters	Primary culture of tumor cells		P	Transplantable culture of tumor cells		P
	KRS-321	SKhA-12		HEp-2	CaVe	
K_m	6,1±1,16	11±2	<0,001	15±3	5±1,2	<0,001
S_v^{cr}	2,2±0,5	2,6±0,4	<0,05	3,9±0,7	2,2±0,54	<0,001
S_v^m/V_v^m	11,4±1	13,8±1,2	<0,001	11,9±0,6	13,6±1,2	<0,02
K_f^{cr}	2,2±0,22	1,7±0,12	<0,001	1,7±0,12	2,1±0,2	<0,001
AM	0,53	0,57	—	0,48	0,35	—
K_e	1,5±0,34	0,4±0,28	<0,001	0,09±0,035	0,6±0,15	<0,001
S_v^e	1,5±0,2	1,8±0,2	<0,05	0,06±0,14	1±0,13	<0,001
N_L^{re}	22±2	15±1,2	<0,001	16±1,8	15±1,6	<0,05
V_v^e	0,056±0,008	0,055±0,013	<0,05	0,006±0,0032	0,05±0,008	<0,001
K_f^e	1,35±0,15	0,9±0,08	<0,001	1,5±0,2	1,2±0,16	<0,05
K_p	63±12	122±15	<0,001	153±13,6	94±13	<0,001
K_e/K_p	0,03±0,008	0,008±0,0022	<0,001	0,0006±0,00026	0,011±0,004	<0,001

TABLE 2. Comparison of Mean Averaged Morphometric Parameters and Coefficients for Cells of Primary Trypsinized and Transplantable Cultures of Tumor Cells

Parameters	Mean averaged values for primary tumor cultures	Mean averaged values for transplantable tumor cultures	P
K_m	8,55±1,24	10±1,7	<0,05
S_v^{cr}	2,4±0,34	3,05±0,5	<0,05
S_v^m/V_v^m	12,6±0,76	12,75±0,6	<0,05
K_f^{cr}	2±0,13	1,9±0,1	<0,05
AM	0,55	0,42	—
K_e	0,95±0,24	0,35±0,06	<0,001
S_v^e	1,65±0,14	0,8±0,08	<0,001
N_L^{re}	19±1,3	15,5±1,2	<0,001
V_v^e	0,0555±0,008	0,028±0,006	<0,001
K_f^e	1,1±0,1	1,35±0,12	<0,01
K_p	92±11	124±8	<0,001
K_e/K_p	0,019±0,013	),006±0,005	<0,05

On the basis of these parameters conventional coefficients of the level of "morphological organization" were calculated [9]: K_m for the mitochondria as $S_v^{cr} \cdot (S_v^m/V_v^m) \cdot AM \cdot 1/K_f^{cr}$; K_e for the ergastoplasm as $S_v^e \cdot V_v^e \cdot N_L^{re} \cdot 1/K_f^e$; K_p for the polysomal apparatus as N_a^{rp}, the number of ribosomes per square micron of cytoplasm. The ratio K_e/K_p, reflecting the ratio between extracellular and intracellular protein synthesis, also was investigated. All the parameters indicated above were subjected to statistical analysis. The confidence limits of the mean values were determined with a reliability of 95%.

EXPERIMENTAL RESULTS

Comparison of the ultrastructure of the tumor cells of the primary trypsinized cultures with each other revealed differences in nearly all parameters. The values of K_m and S_v^m/V_v^m in the SKhA-12 cells were significantly higher than those in the KRS-321 cells, whereas the values of K_f^{cr} (the reciprocal of K_m) were lower. Meanwhile, the values of practically all parameters characterizing the degree of development of the ergastoplasm (except V_v^e) in the SKhA-12 cells were significantly lower than in the KRS-321 cells, whereas the values of K_p, on the other hand, were higher. Similar differences, but with higher levels of significance, were observed when HEp-2 and CaVe cells were compared (Table 1).

Because of the considerable individual differences between the various strains of both primary and transplantable cultures discovered, a comparative analysis was made of the averaged data allowing for dispersion between groups (Table 2). As Table 2 shows, the morphometric characteristics of the mitochondrial apparatus in the groups studied showed about equal mean tendencies. Against this background, a significant difference was found in the structural organization of the protein-synthesizing system. Cells of the primary cultures were characterized by lower values of K_e, S_v^e, V_v^e, and N_L^{re}, and the higher value of K_f^e. Meanwhile their polysomal apparatus was more developed (K_p). The value of K_e/K_p also was much lower, but because of the considerable scatter of the mean sample data characterized by serial values, the differences between the mean averaged values of the parameter in cells of the primary and transplantable cultures were not significant with respect to the statistical criteria used.

Quantitative analysis of the fine structure of the cells of these cultures showed that in both primary and transplantable tumor cultures considerable differences could be found in the structural organization of cells of the different strains, affecting their energy-forming and protein-synthesizing systems. On the whole, however, the primary and transplantable tumor cultures differed significantly in the organization

of their cytoplasmic protein-synthesizing apparatus. Primary cultures were characterized by a more reduced ergastoplasm which, in the modern view [4, 5], is responsible for the synthesis of highly specialized protein substrates. In addition, the value of K_p, reflecting the activity of nonspecific protein synthesis, was much higher. Presumably cultivation in vitro is a factor which, in the case of tumor cells, contributes to the further reconstruction of the protein-synthesizing system toward the synthesis of nonspecific substrates essential for growth and multiplication of the cell mass.

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