CHANGES IN THE ISOZYME SPECTRUM OF SOME ENZYMES OF ENERGY METABOLISM AND IN MITOCHONDRIAL ULTRASTRUCTURE IN VIRUS-INDUCED CARCINOGENESIS

A. I. Ageenko, A. S. Yagubov,
Yu. E. Vitorgan, and V. A. Kats

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The dynamics of redistribution of the isozyme spectrum of lactate and malate dehydrogenases in rat embryonic fibroblasts (REF) in contact with type 12 human adenovirus was studied and compared with the dynamics of changes in the fine structure of the mitochondria of the same cell models. The action of the oncogenic virus was shown to produce changes in the isozyme spectra of lactate and malate dehydrogenases in both the nucleus and cytoplasm. These disturbances began after the first days of interaction between the REF culture and the oncogenic virus and were not connected with proliferative growth of the cell culture, but were due to the action of the virus on the cell. Comparison of the kinetics of changes in the enzymes studied with the results of quantitative analysis of the ultrastructure of the mitochondrial apparatus showed that the biochemical changes arise much sooner, virtually at the moment of infection.

KEY WORDS: rat embryonic fibroblasts; lactate dehydrogenase; isozymes; human type 12 adenovirus.

Much attention has recently been paid to the study of changes in the isozyme spectrum of several enzymes in the course of carcinogenesis. The reason is that isozymes, while functionally homogeneous, are characterized by some degree of genetic individuality, i.e., the synthesis of molecular forms of a particular enzyme is controlled by different genes [9, 10, 14]. Since the process of virus-induced carcinogenesis is accompanied by considerable changes in the genetic apparatus of the cell as a result of integration of the viral and cell genomes, it can be postulated that the specific redistribution of isozymes characteristic of tumor cells and observed during virus-induced carcinogenesis, is a regular process.

The dynamics of redistribution of the isozymes of the enzymes of energy metabolism, notably lactate (LD) and malate (MD) dehydrogenases, during virus-induced carcinogenesis have so far hardly been studied, despite the fact that their presence in the cytoplasm and in the nucleus has frequently been demonstrated.

The object of this investigation was to study changes in the isozyme spectrum of LD and MD in cells of an intact culture and of a culture in contact with type 12 human adenovirus (A-12) and also to compare the dynamics of changes in the isozyme spectrum with the dynamics of changes in the mitochondrial ultrastructure in the same cell model.

EXPERIMENTAL METHOD

A culture of rat embryonic fibroblasts (REF), studied on the 1st, 3rd, 5th, 8th, 18th, and 24th days after infection with A-12 virus, was used as the model. An intact REF culture was studied at the same time.

The nuclei were isolated by the method of Chauveau et al. in the modification of Zbarskii and Georgiev [4]. Proteins were fractionated by disk electrophoresis in polyacrylamide gel [11], after which activity of the

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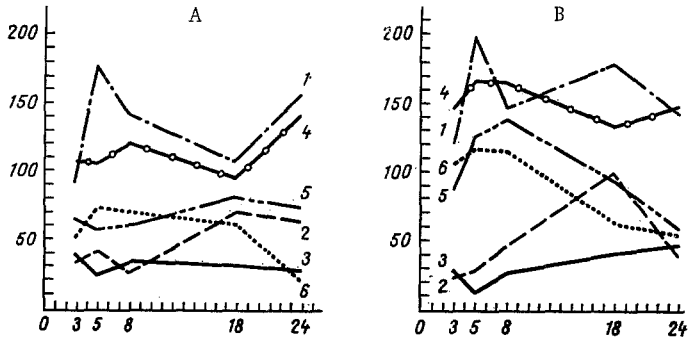

Fig. 1. Dynamics of changes in LD and MD isozyme activity in globulin fraction of cell nuclei of REF culture. A) Control, B) experiment. Abscissa, isozyme activity in relative units; ordinate, days after infection of REF culture with adenovirus A-12. Curves 1-3 represent activity of LD-1, LD-2, and LD-3, respectively; curves 4-6 represent activity of MD-1, MD-2, and MD-3, respectively.

LD and MD isozymes was determined [9] from their relative density recorded by densitometry. The isozymes were numbered in accordance with increasing electrophoretic mobility from cathode to anode [13]. The experimental results were subjected to statistical analysis [7].

Material for electron-microscopic investigation was fixed, dehydrated, embedded in epoxide resins, and stained by the usual methods and examined in the I(i)EM-7A electron microscope. Mitochondrial structure was analyzed by morphometry, with determination of the surface to volume ratio of the mitochondria (S_V^M/V_V^M), the ratio of surface area of the cristae to volume of the mitochondria (S_V^{cr}), the coefficient of fragmentation of the cristae (K_f^{cr}), calculated as N_A^{cr}/L_A^{cr}, where N_A^{cr} is the number of cristae and L_A^{cr} the length of the cristae per unit area of section through the cytoplasm. Confidence intervals of the mean values were obtained with a probability of 95% [5].

EXPERIMENTAL RESULTS

Investigation of LD and MD at different periods in cells of the intact REF culture showed some increase in the activity of each LD isozyme in the cytoplasm of the REF cells in the late stationary phase (the changes were not statistically significant). No significant changes in LD isozymes were found in the nuclear fraction. The study of the MD isozyme spectrum showed a significant increase in MD-2 activity in the cytoplasm of the REF in the late stationary phase. In the nuclear fraction a significant increase in the content of MD-1 was observed.

It follows from data in the literature that the character and distribution of LD and MD isozymes are specific for each organ and tissue under normal conditions in the various systems of animals and man [2, 8, 13, 15]. Changes discovered in the LD and MD isozymes in the cytoplasm and nucleus of intact REF were therefore probably connected with growth of the cells in vitro.

In the next series of experiments in which the REF culture was exposed to the action of oncogenic adenovirus A-12 a significant increase in activity of isozyme LD-1 in the nuclear fraction of the REF cells was observed on the 5th and 18th days (Fig. 1). Isozyme LD-5 disappeared from the cytoplasm, and later so also did LD-4. A substantial fall in LD-1 activity and a considerable decrease in the LD-3 content were observed on the 24th day.

Oncogenic A-12 virus caused a significant increase in the MD-1 content in the nucleus on the 5th and 18th days. MD-3 activity was significantly increased on the 3rd and 24th days (Fig. 1). An increase in the MD-3 level in the cytoplasm was found on the 3rd and 24th days.

It can accordingly be concluded from the results of these experiments that exposure to the oncogenic virus caused changes in the isozyme spectra of LD and MD in both the nuclear fraction and the cytoplasm. These disturbances began from the first days of interaction between the REF culture and the virus. The results agree with those of previous investigations on the REF system during exposure to human adenoviruses A-12, A-3, and A-6 [1], in which aerobic and anaerobic glycolysis and total aldolase and LD activity in the

TABLE 1. Morphometric Parameters of Mitochondrial Ultrastructure of Cells of Intact REF Culture (I) and of Culture Transformed by Human Type 12 Oncogenic Adenovirus (A-12) (M ± m)

Parameter		Day of investigation					
		1st	3rd	5th	8th	18th	24th
S_v^{cr}	I	2,3±0,4	1,3±0,2	2,4±0,4	1,7±0,3	0,8±0,18	1,9±0,5
	A-12	1,8±0,4	2,3±0,32	2,5±0,5	1,7±0,34	1,5±0,28	1,0±0,24
S_v^M/V_v^M	I	10,9±1,1	13,6±0,8	14,2±1,0	14,3±0,98	15,0±1,2	13,1±1,5
	A-12	10,9±0,74	10,3±0,7	15,6±1,4	15,2±1,1	15,9±1,6	15,2±1,2
K_f^{cr}	I	2,2±0,15	1,9±0,14	2,0±0,1	2,2±0,19	2,1±0,16	2,1±0,16
	A-12	1,9±0,13	2,2±0,13	2,2±0,11	2,3±0,15	2,2±0,14	2,4±0,14

cytoplasm were determined, and also with the results of a study of some indices of carbohydrate metabolism in the cytoplasm, including changes in the LD isozyme spectrum in a system consisting of hamster embryonic fibroblasts infected with human adenoviruses A-12 and A-1 [3, 6]. Changes discovered in the regulation of synthesis of the isozymes studied were not connected with proliferative growth of the cell culture, but were due in fact to the action of the virus on the cell. They perhaps were the result of changes in the epigenome produced by the action of the oncogenic virus.

Infection of the REF culture with oncogenic A-12 adenovirus subsequently led to a small increase in the surface area of the mitochondrial cristae (S_V^{cr}) on the 3rd day followed by a decrease on the 24th day of the investigation. A significant decrease in the surface to volume ratio of the mitochondria (S_V^M/V_V^M) was found on the 3rd day, and a small increase in this ratio on the 24th day (Table 1).

Comparison of the kinetics of the changes in LD and MD in cells of the REF culture after transformation by A-12 virus with the results of quantitative analysis of the mitochondrial ultrastructure of these cells showed that the biochemical changes began to appear virtually from the moment of infection, whereas changes in the ultrastructure of the mitochondria in the early stages could not be detected even by quantitative methods.

To detect specific changes in the pattern of energy metabolism of the cell in the early stages of virus-induced carcinogenesis the most accurate and penetrating methods can be regarded as those which study the kinetics of total activity and redistribution of the isozyme spectrum of the key enzymes of the energy metabolism of the cell, notably LD and MD.

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