Induction of Blebbing in Ehrlich Ascitic Adenocarcinoma Cells during *In Vitro* Hyperthermia

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We studied the effect of *in vitro* hyperthermia on induction of blebbing in cultured Ehrlich ascitic adenocarcinoma cells. Hyperthermia (42-48°C) promotes induction of blebbing in cell culture, but this induction is reversible, and cessation of hyperthermia leads to almost complete recovery of the morphological composition of cell suspension.

Key Words: *Ehrlich ascitic adenocarcinoma; hyperthermia; blebbing*

Relationships between various types of tumor cell death is one of the most pressing problems in modern experimental oncology. The pathway of cell death (apoptosis or necrosis) largely determines the outcome of antitumor therapy [2,6].

Blebbing (formation of vesicular processes of the cytoplasm) is the earliest morphological sign of apoptosis [4,5]. Hyperthermia is a promising approach to the treatment of oncological diseases: therefore we studied the effect of hyperthermia of different intensity on induction of blebbing in Ehrlich ascitic carcinoma cells.

MATERIALS AND METHODS

Nonsynchronized culture of Ehrlich ascitic adenocarcinoma in the stationary growth phase was used [3]. Cells isolated from the abdominal cavity of mice were three times washed by centrifugation in a 5-fold volume of medium 199.

Hyperthermia was modeled by *in vitro* incubation of cells in medium 199 in a thermostat at 40, 42, 44, 46, and 48°C for 30 min. After heating the cells were washed again and incubated at 37°C for 90 min in medium 199 (recovery period). In the control, the recovery period was preceded by a 30-min incubation at 37°C. The concentration of adenocarcinoma cells in medium 199 during incubation was 1.5×10^{10}/ml.

Immediately after hyperthermia and after 30- and 90-min recovery the percentage of adenocarcinoma cells at the stage of blebbing was evaluated in a Goryaev chamber. To this end, the cells were stained with methylene blue (10 μl cell suspension and 40 μl 0.5% stain in 0.85% NaCl were mixed with 800 μl medium 199). The presence of vesicular processes on the plasma membrane served as the criterion of blebbing.

The results were processed using Student's *t* test.

RESULTS

The intensity of blebbing of Ehrlich ascitic adenocarcinoma cells increased in all variants of hyperthermic exposure (Table 1). Immediately after 42°C hyperthermia, the percentage of cells with blebbing 12.3-fold surpassed the control. After incubation at 44°C the intensity of blebbing was 5.3-fold higher than in the control, but significantly lower than at 42°C. Such a trend persisted with further increase of temperature, and after incubation at 48°C the intensity of blebbing virtually did not differ from the control. This can be due to the fact that hyperthermia at 44°C and higher disturbs mechanisms responsible for induction of the initial stages of apoptosis in cells.

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TABLE 1. Percentage of Ehrlich Ascitic Adenocarcinoma Cells with Signs of Blebbing at Various Temperatures and Duration of Posthypothermic Recovery ($X \pm m$)

Duration of recovery	Control (n=12)	Hyperthermia, °C				
		40 (n=13)	42 (n=11)	44 (n=11)	46 (n=14)	48 (n=10)
0 (initial)	2.84±0.71	3.85±0.97	35.00±16.81*++	14.96±5.49*++oo	6.4±1.7**	2.7±1.2+++o
30 min	2.75±0.69	3.2±1.2	7.53±4.73***+++	11.44±3.91*+++oo	4.89±2.90	2.1±0.4ooo
90 min	2.53±0.80	2.83±0.60	5.66±2.31***+++	1.86±0.76***+++oo	1.46±0.60***+++oo	1.2±0.3***+++oo

Note. *p<0.001, **p<0.01, ***p<0.005 *vs.* the control; +p<0.001, ++p<0.01, +++p<0.05 *vs.* 40°C hyperthermia; °p<0.001, °°p<0.01, °°°p<0.05 *vs.* 42°C hyperthermia.

After 30-min recovery period the intensity of blebbing in control cells and cells incubated at 40°C remained at the previous level, while in cells incubated at 42°C it decreased 4.6-fold compared that recorded immediately after hyperthermia (p<0.01). After 30-min recovery period the intensity of blebbing in cells incubated at 44°C was higher than after incubation at 42°C. Hence, cells subjected to mild hyperthermia more rapidly restore their temperature sensitive metabolic and structural components and, therefore, the signal inducing blebbing is arrested more rapidly, which promotes recovery of morphological composition of cell suspension.

After 90-min recovery, the intensity of blebbing continued to decrease. At this term the intensity of blebbing only negligibly differed from the control (Table 1). It seems that the structure of plasma membranes in adenocarcinoma cells recovered completely by the 90th min, at least at the morphological level, which is obviously a result of adequate development of their adaptation mechanisms aimed at maintenance of cell homeostasis and viability.

Hence, incubation at 42-48°C leads to activation of intracellular mechanisms aimed at realization of the initial stages of apoptosis (induction of blebbing) in Ehrlich ascitic adenocarcinoma cells. However, removal of cells from the medium with unfavorable thermal conditions inactivates these mechanisms and cancels blebbing, which results in almost complete restoration of the morphological composition of cell suspension.

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