Antitumor and Immunomodulating Effects of a Fragment of HL-60 Cell Differentiation Factor in Mice with Lewis Pulmonary Carcinoma

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A six-member HLDF6 peptide, fragment of HL-60 cell differentiation factor, exhibited antimetastatic and immunomodulating effects.

Key Words: *differentiation factor; carcinoma; metastases; macrophages; proliferation*

Search for agents with antitumor and particularly antimetastatic effects is one of the priority trends in modern oncology. The development of tumor process is associated with disorders in the immunity system, playing the key role in the formation of antitumor resistance. Hence, use of therapeutic agents, which, in addition to their antitumor effect, restore the activity of immune reactions of tumor host, can lead to improvement of remote results of antitumor therapy. Substances of endogenous (natural) origin characterized by low toxicity and pronounced regulatory effects, are promising in this sphere. Among these substances are differentiation factors.

Study of transformed promyelocytic human leukemia HL-60 differentiation factor (HLGF) resulted in identification of a six-member HLDF6 fragment [2]. The HLDF6 peptide retained the capacity of the full-size factor to induce differentiation in parallel with arrest of cell proliferation. Experiments on mice with transplanted NSO myeloma showed that HLDF6 was an effective antitumor agent [1]. However it is unknown whether this peptide possesses an antimetastatic effect.

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We evaluated the antitumor and antimetastatic effects of HLDF6 peptide and its effects on some immunological parameters of animals on the model of Lewis metastatic pulmonary carcinoma.

MATERIALS AND METHODS

Peptide HLDF6 (Thr-Gly-Glu-Asn-His-Arg), used in the study, was synthesized by the method of activated esters in solution. Experimental studies were carried out on C57Bl/6 mice (18-23 g) from Breeding Department of Vector Center.

Transplanted Lewis pulmonary carcinoma served as the experimental model for study of anticarcinogenic effect of the peptide. Tumor cells (10^6 per mouse) were transplanted intramuscularly (0.1 ml) in the thigh.

In experimental series I the animals received 5 and 10 daily injections of agent in doses of 5, 25, and 50 mg/kg.

In experimental series II HLDF6 peptide was injected in a dose of 25 mg/kg 5 times at 24-h intervals. One day after the end of peptide treatment (day 10 after tumor cell transplantation) half of experimental animals were injected with cyclophosphamide (CP) (a single intraperitoneal injection of 50 mg/kg). Controls were injected with saline (negative control) or CP (positive control).

Antitumor and antimetastatic effects of the drug were evaluated by comparing the weight of the primary tumor node, number of metastases in the lungs, estimation of tumor growth inhibition (TGI) percentage and metastasis inhibition percentage.

The TGI percentage was estimated by the formula:

$$TGI=(W_k-W_e)/W_k\times100,$$

where W_k is the mean tumor weight in the control group and W_e mean tumor weight in experimental group.

In experimental series III the antitumor and immunomodulating effects of HLDF6 were studied. Three groups of animals were formed: 1) intact mice; 2) mice with tumors, receiving 5 injections of saline (control); and 3) mice with tumors, receiving 5 injections of the peptide (25 mg/kg) at 24-h intervals (experiment).

On days 6, 10, and 20 after transplantation (24 h after the 3rd and 5th injections of the peptide and 10 days after the end of treatment) the animals were sacrificed by cervical dislocation. The weights of the tumor, lymphoid organs (thymus, spleen, inguinal lymph nodes) were measured, the spleen and peritoneal exudation were collected for analysis.

Spontaneous and induced proliferative activities of splenocytes were evaluated by 3-(4,5-dimethylthiasolyl-2)-2,5-diphenyltetrasoleum bromide reduction by the cells (MTT test) [4]. Proliferation was induced by mitogen (concanavalin A; ICN Biomedicals Inc.) in a concentration of 10 μg/ml.

Macrophage function was studied in a short-term cell culture by the level of nitroblue tetrasoleum reduction test (NBT test) [5]. Phagocyte activity was evaluated in the spontaneous test or in test with zymosan (macrophage metabolic respiration activator; Sigma), which was added to the formed cell monolayer in a concentration of 3 mg/ml.

On day 20 after tumor transplantation the tumor was weighed and the TGI percentage and number of metastases in the lungs were estimated.

The data were statistically processed using Statgraphics 5.0 software. The significance of differences between the groups was evaluated using Student's *t* test.

RESULTS

Antitumor efficiency of HLDF6 was evaluated in monotherapy and in combination with CP. The drug effect on the development of the blastomatous process depended on the treatment protocol. Five and ten daily injections of the peptide in doses of 5, 25, and 50 mg/kg virtually did not modify the tumor growth and its metastasizing into the lungs.

Five injections of HLDF6 peptide in a dose of 25 mg/kg at 24-h intervals did not modify the growth of the primary tumor node, but caused a significant reduction in the number of metastases in the lungs, this effect being comparable to that of CP in a dose of 50 mg/kg (Table 1). In addition, a trend to inhibition of metastatic process in mice treated by HLDF6 and CP in comparison with mice treated by CP alone was noted.

The complex of disorders in defense immune reactions plays an important role in the tumor dissemination process. For this reason, in the next series of experiments the immunomodulating effect of the drug was studied on mice with Lewis pulmonary carcinoma. Morphophysiological parameters of immunocompetent organs and cells of mice with transplanted Lewis carcinoma were evaluated.

The development of tumor was paralleled by enlargement of the spleen, inguinal lymph nodes, and shrinkage of the thymus in comparison with intact animals. Changes in the weights of the lymph nodes and thymus were detected as early as 6 days after tumor transplantation, splenomegalia developed 10 days after transplantation. No appreciable differences in the weights of the lymphoid organs in control and experimental animals were detected.

Spontaneous and mitogen-induced proliferative activity of splenocytes increased 10 days after transplantation in experimental mice in comparison with

TABLE 1. Effect of HLDF6 Peptide on the Growth and Metastases of Transplanted Lewis Pulmonary Carcinoma

Drug, dose	Tumor weight, g	TGI, %	Number of metastases per lung	% of control
Saline	6.41±0.33	—	23.80±1.50	—
CP, 50 mg/kg	5.86±0.29	8.6	15.71±2.02*	66.0
HLDF6, 25 mg/kg	6.53±0.24	0	14.28±2.79*	60.0
HLDF6, 25 mg/kg+CP, 50 mg/kg	6.39±0.22	0	11.14±2.25*	46.8

Note. *$p<0.05$ *vs.* control (saline).

TABLE 2. Levels of Spontaneous and Mitogen-Induced Proliferative Activities of Splenocytes of C57Bl/6 Mice with Transplanted Lewis Pulmonary Carcinoma during Different Periods after Tumor Transplantation under Conditions of Treatment by HLDF6 Peptide (*M*±*m*)

Group	Level of reduced MTT, rel. units	
	spontaneous	mitogen-induced
6 days after tumor transplantation (1 day after 3rd injection of drug)		
Intact	0.400±0.022	0.610±0.059
Control (saline)	0.380±0.018	0.580±0.017
HLDF6, 25 mg/kg	0.430±0.028	0.610±0.033
10 days after tumor transplantation (1 day after 5th injection of drug)		
Intact	0.430±0.036	0.92±0.07
Control (saline)	0.510±0.035	0.950±0.072
HLDF6, 25 mg/kg	0.560±0.031*	1.090±0.032*
20 days after tumor transplantation (10 days after 5th injection of drug)		
Intact	0.380±0.047	0.890±0.034
Control (saline)	0.610±0.063*	0.660±0.039*
HLDF6, 25 mg/kg	0.66±0.04*	0.680±0.029*

Note. *$p<0.05$ *vs.* intact animals.

TABLE 3. Functional Activity of Peritoneal Macrophages of C57Bl/6 Mice with Transplanted Lewis Pulmonary Carcinoma in Different Periods after Tumor Transplantation (*M*±*m*)

Group	Level of reduced NBT, rel. units×100					
	day 6		day 10		day 20	
	I	II	I	II	I	II
Intact	10.85±0.45	31.77±0.30	16.29±0.41	38.77±1.58	11.87±0.95	35.92±2.51
Control (saline)	12.22±0.35	47.18±4.74	13.01±0.90*	29.55±2.02*	12.62±0.87	33.50±0.10
HLDF6, 25 mg/kg	13.40±0.83*	53.95±3.57*	17.91±0.90**	54.79±2.88*,**	12.58±1.20	38.54±3.05

Note. I) spontaneous; II) zymosan-induced. $p<0.05$ *vs.* *intact, +control animals.

intact animals (Table 2). These changes were statistically significant in animals treated by HLDF6. During later periods this parameter was virtually the same in control and experimental groups.

Changes in the nonspecific resistance of animals were evaluated by the level of spontaneous and induced metabolic activities of peritoneal macrophages. Spontaneous metabolic activity of peritoneal macrophages 10 days after transplantation was lower in control mice with tumors than in intact animals, which can be attributed to a lower functional reserve of the myelocyte/monocyte component during tumor growth (Table 3). This hypothesis is confirmed by a significantly lower zymosan-induced redox activity of macrophages (Table 3). Peptide HLDF6 stimulated the activity of peritoneal cells in comparison with intact (days 6 and 10 after transplantation) and control animals (10 days after transplantation). The level of NBT, reduced by zymosan-induced macrophages of experimental mice, was 1.9 times higher than the control value.

Presumably, peptide activation of macrophages is one of the factors responsible for inhibition of the metastatic process in Lewis' carcinoma, which is in line with the data of other studies, indicating an important role of macrophages in suppression of the metastatic process, obtained on the same tumor model [3,6,7].

Hence, experiments on Lewis pulmonary carcinoma showed that HLDF6 peptide did not inhibit the growth of the primary tumor node, but exhibited a pronounced antimetastatic effect. These data indicate that HLDF6 peptide is characterized by immunomodulating activity, presumably underlying its antimetastatic effect.

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