Hepatocarcinoma-29, a Metastasizing Transplantable Mouse Tumor Inducing Cachexia

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Here we describe an experimental tumor, hepatocarcinoma-29: transplantable strain of this tumor is maintained in an ascitic form in CBA/LacYIcgn mice in Institute of Cytology and Genetics of SD of RAS. After inoculation into the thigh muscles, the tumor induces anorexia, progressing loss of fat and muscle tissues, and physiological changes specific for cachexia: leukocytosis, hypoglycemia, and hypercorticism. The tumor metastasizes to all vital viscera and leads to animal death from renal failure.

Key Words: *mice; transplantable hepatocarcinoma-29; metastasizing; cachexia*

Malignant tumors affect the host body in different ways due to their non-specific features (size, location) and their ability to ectopic synthesis of hormones and other regulatory molecules. Neuroendocrine shifts, including psychogenic changes, aggravate pathological manifestation caused by the presence of the tumor in the body, which leads to the development of depressive states. The latter aggravates negative development of the degenerative process, accompanied by anorexia and loss of fat and muscle tissues (cachexia syndrome). The incidence of this syndrome at the terminal stage of the disease reaches 80%, and in 20% cases cachexia is the main cause of death in cancer patients [1,2]. Analysis of the major causes of cachexia sometimes allows to apply adequate therapy and to improve the state and quality of life of cancer patients [2,3].

For evaluation of the causes and development of appropriate methods of treatment, various experimental models reflecting this or that clinical condition are required. Despite some progress in this field [2], the spectrum of these models is limited. In experimental studies in oncology, primarily fast-growing mouse and rat tumors are used [4,5]; cachexia symptoms appear only at the late stages, when the tumor reaches 10% body weight or more. This is not typical of cancer patients with a relatively low rate of tumor growth; only in rare cases tumor weight reaches 5% body weight [6].

We obtained hepatocarcinoma-29 (H-29) inducing considerable loss of body weight in tumor-bearing animals and marked signs of cachexia. Here we studied growth kinetics of solid transplants of this tumor, the pattern of metastasizing, and its effect on the organism, which would be useful for modeling of cancer cachexia.

MATERIALS AND METHODS

Experiments were conducted on 3-4-month-old male CBA/LacYIcgn (CBA) mice. The animals were kept in plastic trays (32×23×15 cm, 8-10 mice per tray) with wood chips at daylight illumination and free access to water and food (pelleted forage PK 120-1, Laboratorsnab).

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H-29 developed spontaneously in a female CBA mouse in 1987 г. Being transplanted into thigh muscles of syngeneic mice, it grew slowly, the 2nd passage was successfully performed after 4 months. Further tumor growth was more rapid, the 3rd passage was performed 45 days later, the 4th and subsequent passages were made less then in 3 weeks. In order to transfer the tumor into ascitic form, suspension of tumor cells was transplanted into abdominal cavity of a group of mice. Ascitic fluid was transplanted intraperitoneally until growth parameters were stabilized. At present time, tumor cells are frozen in the bank of tumor strains in the Institute of Cytology and Genetics of SD of RAS.

For experimental purposes, thawed H-29 cells were transplanted into abdominal cavity of CBA mice, ascitic fluid was aspirated after 10 days, suspended in 10-fold volume of physiological saline, and then injected into right thigh muscles of intact animals in a volume of 0.1 ml. The mice were monitored with daily weight and food consumption control until natural death. The developed tumor nodes were measured with a caliper and their weight was calculated. Mouse lifetime from the moment of tumor transplantation, body weight, and weight of hindlimbs cut at the level of hip joints were determined. Tumor weight was determined as the difference between the weights of hindlimb with and without tumor. The mice with transplanted Ehrlich tumor receiving equivalent amount of food and water were used as controls.

For evaluation of the dynamics of tumor growth and somatic disorders produced by the tumor, the mice were decapitated under ether anesthesia on days 5, 8, 12 and 16 after inoculation of tumor cells, absolute and relative tumor weight were recorded. Blood samples collected after completion of experimental protocol were used for detection of cachexia markers: leukocyte count and glucose and corticosterone concentrations. The lung, liver, spleen, kidneys, heart, and hindlimb with transplanted tumor were taken for morphological examination. The organs were fixed in 10% neutral formalin and processed routinely using a MICROM histological complex (Carl Zeiss), 3-4 μ sections were stained with hematoxylin and eosin, PAS-hematoxylin-orange G staining was also used.

The data were processed using Statistica 6.0 software; the difference was significant at $p<0.05$.

RESULTS

The weight of control mice (transplantation of Ehrlich carcinoma) progressively increased throughout the observation period and after 1.5 months reached 147.6% of initial value. This increase was determined exceptionally by the tumor: mouse body weight without tumor weight remained at the initial level until animal death. Food consumption remained unchanged throughout the experiment: 149±6 mg/g body weight per day during the first 3 weeks after transplantation, 146±7 mg/g during week 5, and 145±15 mg/g during week 7. Unchanged daily food consumption was observed despite progressive increase in tumor weight (up to 38% at terminal stage). In this experimental series, the mice died on day 57.0±2.1 after transplantation, tumor weight was 13.8±1.2 g. These findings suggest that solid Ehrlich tumor does not induce signs of cachexia in bearers.

Starting from the 5th day after transplantation of H-29 tumor, body weight of recipients decreased and by the time of death (on day 14.1±0.6 day) reached 86% of the initial value with the tumor and 77.8% without the tumor. Food consumption progressively decreased: 145±2 mg/g body weight per day on 1-3 days after transplantation, 107±7 mg/g on 4-6 days after transplantation, 86±3 mg/g on 7-9 days after transplantation, 79±8 mg/g on 10-12 days after transplantation and <36 mg/g by the "terminal" days 13-14. H-29 tumor constituted <10% of body weight and caused a more than 20% decrease in body weight.

TABLE 1. Leukocyte Count and Blood Concentrations of Corticosterone and Glucose in Male CBA Mice after Intramuscular Transplantation of H-29 and Ehrlich tumor

Parameter	Control (Ehrlich tumor)	Time after transplantation of H-29, days			
		5	8	12	16
Number of animals	5	5	5	4	3
Tumor weight, g	—	0.40±0.03	1.10±0.06***	2.80±0.27***	3.10±0.22***
Glucose concentration, μmol/ml	7.35±0.26	7.37±0.21	5.66±0.13***	5.30±0.31***	3.13±0.05***
Leukocyte count, 10^3/ml	6.49±0.90	5.50±1.31	9.70±1.45	17.40±3.66*	39.0±7.0**
Corticosterone concentration, ng/ml	45.00±3.33	70.4±7.2*	46.30±8.18	99.4±19.5*	80.00±9.97*

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to the control.

In the course of H-29 growth we also measured some parameters (leukocyte count and concentration glucose and corticosterone) typically changed in cachexia [8]. Glucose concentration in the blood decreased, while leukocyte count and corticosterone concentration increased compared to the control (Table 1).

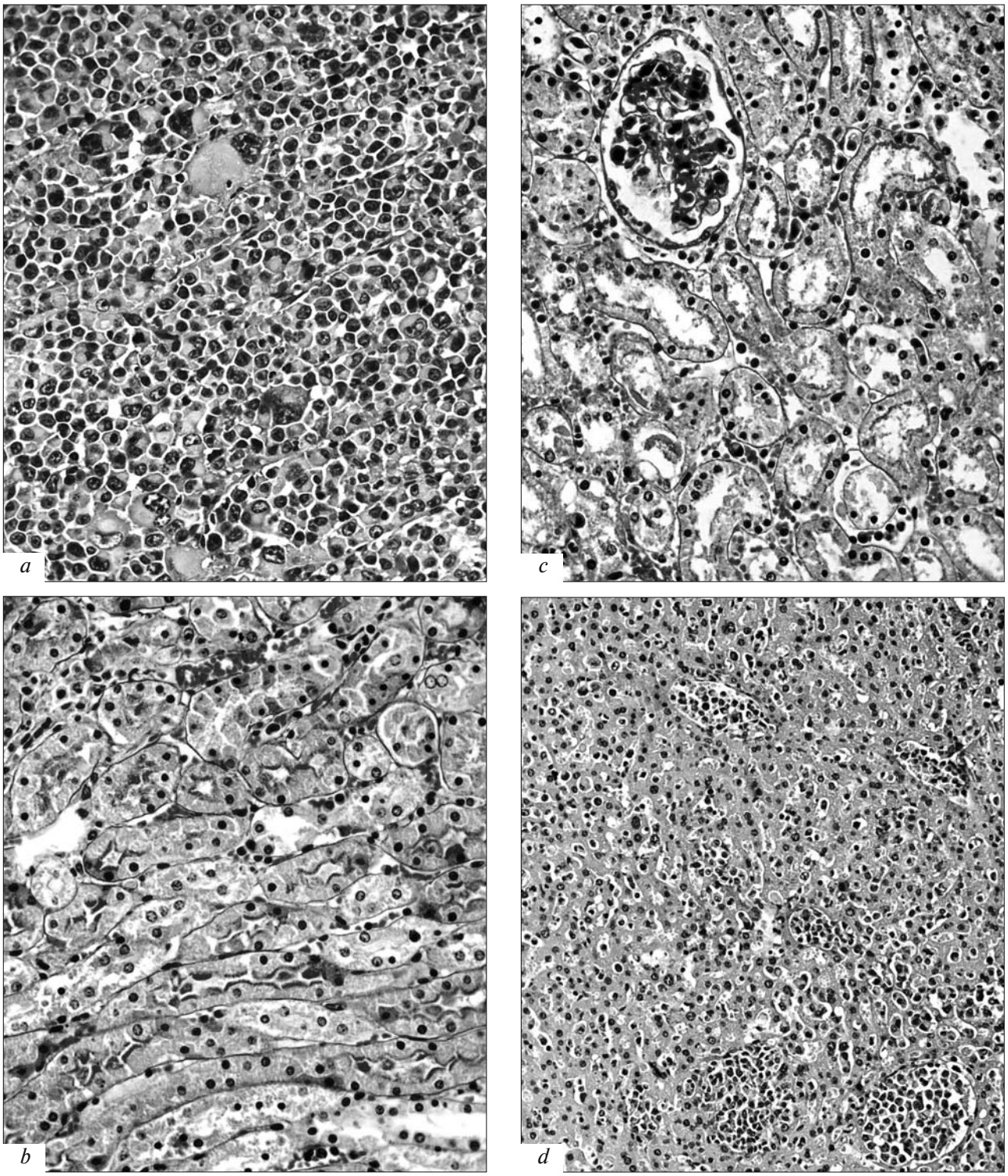

Fig. 1. Morphological changes in transplanted H-29 and internal organs after humor metastasizing. *a*) primary tumor node in mouse thigh muscles: trabecules formed by tumor cells separated by thin sinusoid septa, pronounced cellular and nuclear polymorphysm; *b*) necrosis of epithelial cells in distal and proximal renal tubules 16 days after H-29 transplantation; *c*) deposits of PAS-positive substance in capillaries of renal glomerulus 16 days after H-29 transplantation; *d*) macrofocal and microfical metastases in liver sinusoids 16 days after H-29 transplantation, ×250. *a*, *d*) hematoxylin and eosin staining; *b*, *c*) PAS-hematoxylin-orange G staining, ×400 (*а-в*).

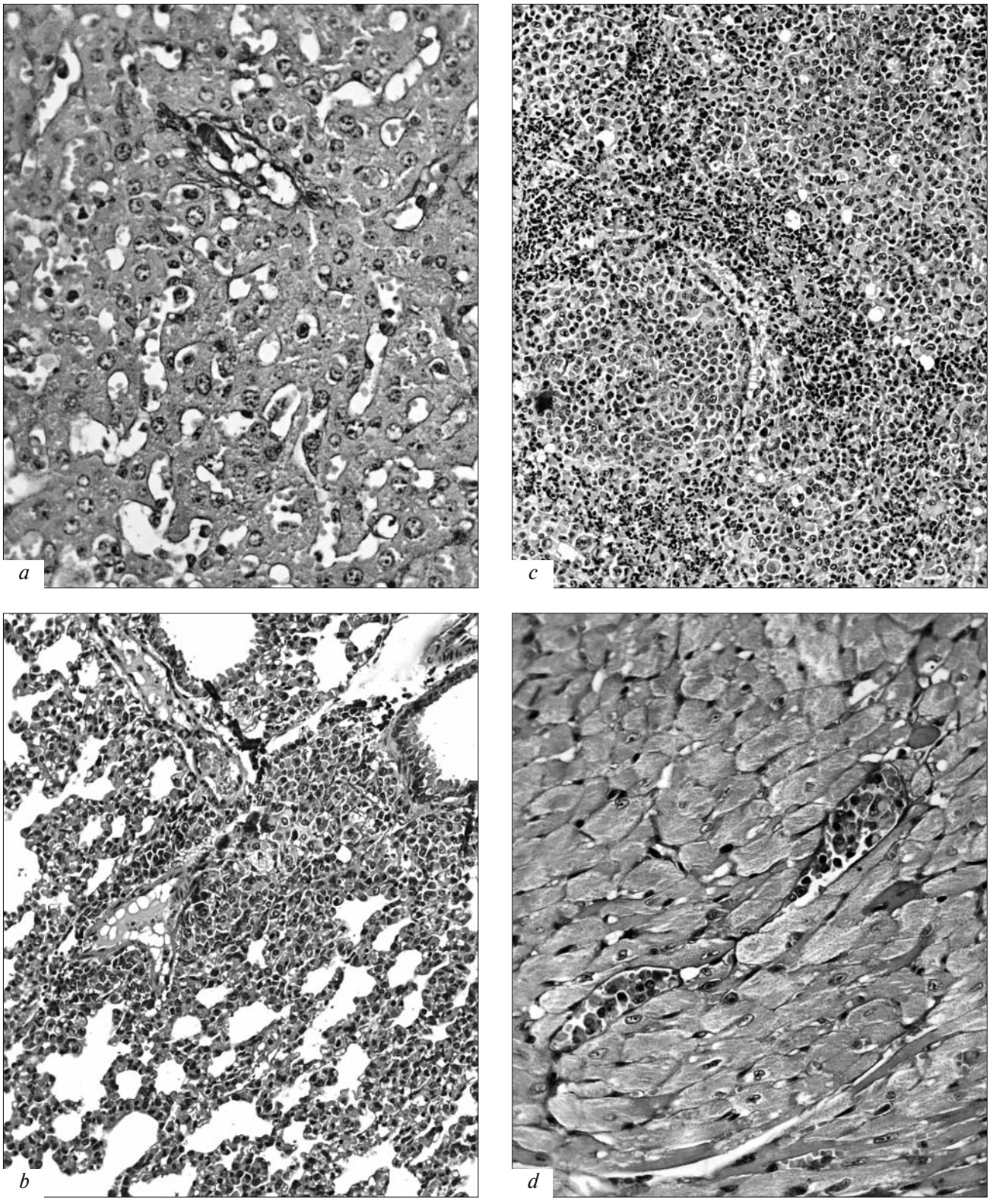

Fig. 2. Morphological changes in internal organs 16 days after H-29 transplantation. *a*) perisinusoid PAS-positive substance deposits in the liver; *b*) macrofocal metastasis of the tumor in the lung with bronchial wall damage; *c*) total replacement of lymphoid tissue in the spleen by macrofocal metastases; *d*) tumor infiltration of myocardium vessels. *a*, *d*) PAS-hematoxylin-orange G staining, ×400; *b*, *c*) hematoxylin and eosin staining, ×250.

Multiple metastases in visceral organs were detected in dead mice; this was the ground for detailed pathomorphological investigation.

Primary H-29 tumors represented solid nodes surrounded by a connective tissue capsule with fibrinoid necroses in the center. Peripheral areas of the necrotized tissue were formed by polymorphic hepatocyte-like cells with central location of the nucleus and dark-blue nucleoli and with signs of mitotic division. Tumor cells formed trabecules of varying width separated by thin sinusoid septa (Fig. 1, *a*). PAS-reaction revealed no glycogen in tumor cells. The stroma of the tumor was formed by loose connective tissue with small capillaries and sinusoids.

The most pronounced pathological changes were observed in the kidneys. In most animals, total necrosis of epithelial cell in distal and proximal tubules was observed on day 16 (necrotic nephrosis; Fig. 1, *b*). Enlargement of mesangial matrix was observed in glomeruli, tumor cells and depositions of PAS-positive substance were observed in loops of glomerular capillaries (Fig. 1, c).

After 16 days, disturbances in liver architectonics caused by the development of multiple vast micro-, macrofocal, and nodular metastases were seen in all animals (Fig. 1, *d*). Fibrinoid necroses were observed in the center of macronodular metastases, similarly to primary node. Pronounced degenerative changes in hepatocytes were observed, they contained no glycogen. Sinusoid lumens were enlarged, plethoric, and contained polymorphonuclear leukocytes and tumor cells. PAS assay showed perisinusoidal deposits of PAS-positive substance (Fig. 2, *a*).

In the lungs, vast confluent and diffuse cellular metastases growing into the walls of lobar bronchi and alveoli were seen after 16 days against the background of pronounced plethora (Fig. 2, *b*). Lumens of remained alveoles had scalloped outline; capillary loops contained fibrin (PAS-positive substance) and erythrocytes. Total replacement of the lymphoid tissue with the tumor and formation of follicular structures was observed in the spleen (Fig. 2, *c*).

In the heart, examination in normal and polarized light revealed muscle fibers contractures. The walls of small arteries were hyalinized. In some cases, focal necroses of the muscular tissue with hemorrhages and lymphoid infiltration appeared. Tumor metastases were seen in capillaries and intermuscular connective tissue (Fig. 2, *d*).

Thus, intensive metastasizing of H-29 into the lungs, liver, kidneys, and heart led to pronounced polyorgan failure at the terminal stage of tumor development, which led to metabolic disturbances, rapid cachexia, and animal death. However, cachexia in animals with H-29 was determined by not only redistribution of plastic substances between rapidly growing tumor cells and cell populations in internal organs, but also to a some extent by toxic effect of tumor degradation products [9].

The first sign of cachexia, anorexia, appeared in mice during first days after tumor transplantation: food consumption steadily decreased starting from day 4. Significant increase in blood corticosterone concentration typical of cachexia was noted on day 5 after tumor transplantation. First metastases were revealed on day 8 in the liver (against the background of total disappearance of glycogen in hepatocytes) and in the lungs. After 12 days, macrofocal and microfocal metastases appeared in all examined organs. By this time, the weight of primary tumors surpassed 1 g and was higher than the summarized weight of all metastases. It can be assumed that pronounced body weight loss and aggravation of other cachexia signs at subsequent terms of the experiment is caused not only by metastases in vital organs, but also by humoral factors synthesized by tumor cells or other cell populations in response to tumor growth and producing suppressive effect on the organism.

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