A Method for Reproduction of Metastases in the Liver
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A new method for reproduction of metastatic involvement of the liver in outbred albino rats is proposed. The use of the method rules out the effects of stress factors (surgical intervention, total anesthesia) on malignant tumor metastasizing, making it maximally similar to the natural process. The method allows visual evaluation of the time course of the primary tumor node growth. The metastatic involvement of the liver develops only by the most significant route, hematogenic. The method can be used for the development of experimental therapy for this condition.

Key Words: liver; metastases; model; rats

According to the data of the World Gastroenterological Society and the International Union for Prevention of Gastrointestinal Cancer, colorectal cancer is diagnosed in one million patients every year and annual mortality surpasses 500,000 [2]. In Russia, colorectal cancer ranks second among oncological diseases in men and third in women; it ranks second in the oncological mortality structure of the population of Russia [1]. According to expert predictions, the absolute number of colorectal cancer cases will increase in the world during the next two decades – as a result of increase in the population in general and the population aging in countries with well-developed and developing economy [2]. Metastases develop with time in 50-60% patients. About 15% patients have metastases by the moment of the primary diagnosis. The majority of patients (up to 80-85%) have metastases in the liver, and in half of these, the liver is the only organ with metastases [4].

Drug sensitivity of the primary tumor and metastases can be different and drugs effective for primary tumor can be ineffective in metastases.

The metastatic process is not confined to common transfer and multiplication of cells in sites of new disposition: the tumor and metastatic cells are still actively changing. For example, X. Wu, et al., conclude that only some tumor cells are capable of forming metastases at the early stage of tumor development. Later new mutations accumulate in the primary tumor and in metastases. Mutations in some genes disorder the routes of metabolism and signal transduction, and these pathways can serve as the targets for effective therapy [5]. Another group of scientists has found that metastasis “budded” from the primary tumor rather early, after which the tumors and metastases developed irrespective of each other, accumulating new mutations. Only 30-37% mutations are common for the tumor cells from the primary tumor and from its metastases [3].

We have developed a method for reproduction of metastases in the liver of outbred albino rats with visual control of the time course of primary tumor node growth and a high output of metastases shortly after transplantation of the primary tumor.

MATERIALS AND METHODS

The experiment was carried out on 40 outbred male albino rats (200-250 g). The animals were kept under standard vivarium conditions at fixed light/darkness regimen with free access to water and food. The animals were handled in accordance with the European Convention on Protection of Animals Used in Ex-
periments (Directive 86/609/EEC). Sarcoma-45 strain served as the tumor model. The strain was obtained at Laboratory of Combined Therapy of Tumors, Institute of Experimental Diagnosis and Therapy of Tumors, N. N. Blokhin Russian Cancer Research Center.

At least 2 weeks before the tumor transplantation, the spleen was brought under the skin. Two weeks later, after the operation wound healing, the tumor (sarcoma-45) cell suspension in normal saline ($1 \times 10^6$) was injected (0.1 ml) into the spleen.

Forty-five days after injection of sarcoma-45 cell suspension, CO$_2$ in a high concentration was injected to rats, after which they were sacrificed by decapitation. Median laparotomy was carried out and the primary tumor and the liver were removed and fixed in 10% formalin. After paraffin processing of the organs and tissues the blocks were sliced on a microtome, the sections (2-4 μ) were stained by hematoxylin and eosin and examined in transmitting light at ×100, ×400, and ×1000 (oily immersion).

RESULTS

Tumor growth in the spleen (tumor node of 9.76-16.2 mm$^3$) and metastatic involvement of the liver (1-2 tumor foci of 18.71-34.56 mm$^3$) was recorded 2.5-3 weeks after intralienal injection of sarcoma-45 cell suspension. Icteric staining of the skin integument and mucosa developed in some animals 45 days after intralienal transplantation of sarcoma-45, and total bilirubin level in the blood increased to 10 μmol/liter. The spleen lost its common structure, its tissue was presented by just scanty small islets on the tumor node surface. Solitary cysts filled with bile were scattered over the surface of the liver. Metastases in the liver developed in 95% rats.

Morphologically the primary tumor node in the spleen consisted of densely packed spindle cells with a moderate number of mitoses, without signs of injury, enveloped in a loose connective-tissue capsule, except the few degenerative elements, small necrotic foci, and small solitary islets of splenic tissue.

Microscopic examination of the liver showed the organ plethora with hemorrhagic foci, impaired cord and radial structure of the hepatic lobules, and degenerative changes in part of parenchymatous cells (Fig. 1). The picture of destructive changes in the liver was supplemented by abundant densely lying cells, most often round, with hyperchromatic large nuclei and numerous mitotic figures (10 and more per visual field), including atypical ones (Fig. 2). These undifferentiated cells could be referred to immature sarcoma, namely, round-cell sarcoma. The stroma was scanty in tumors of this kind. The tumor grew into hepatic tissue, destroying it and impairing its structure (Fig. 3, a). Tumor cells grew into adjacent tissue over the surface of the previous hepatic trabeculae, which were atrophic.

Vessels with tumor cells disseminated along their walls, with tumor conglomerations in the lumens were seen in some micropreparations (hematogenic dissemination of the tumor; Fig. 3, b).

Morphologic signs of cholestasis emerged presumably as a result of tumor cell growth in the bile ducts.

These facts indicated a higher tumor aggression of the hepatic metastases vs. the primary tumor.

Injection of tumor cell suspension into the spleen brought under the skin 2 weeks before allowed visual monitoring of the primary tumor growth and ruled out the effects of stress factors (surgical intervention, total narcosis) during the tumor transplantation; hence, the experimental metastatic process was maximally similar to the natural process. In addition, the method was

Fig. 1. Microscopic image of impaired cord and radial structure of hepatic lobules, dilatation of blood vessels, hemo- and plasmostasis. Hematoxylin and eosin staining, ×100.

Fig. 2. Numerous tumor cell mitoses, including atypical. Hematoxylin and eosin staining, ×1000.