Metastatic Rate of Liver Tumors Induced by Diethylnitrosamine in Mice

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SUMMARY

The metastatic capability of liver tumors induced in 220 C57BL/6J X C3HeB/FeJ F1 mice by diethylnitrosamine was studied. Microscopically primary liver tumors showed a predominantly trabecular pattern with varying degrees of cellular atypia. The invasion of the portal vessels by tumor cells was seen occasionally. Detailed histological examination of the lung tissue revealed metastatic foci in 22% of animals bearing primary liver tumors. Tumors metastasized mainly by the hematogenous route; the metastases were multifocal, often of microscopic size, with isolated foci showing a tendency to coalesce and form larger tumor masses. Areas of diffuse infiltration of the lung parenchyma were also seen. The metastatic capability of diethylnitrosamine-induced liver tumors further substantiated their frank neoplastic nature. Detection of high rate of lung metastases was made possible only through the detailed examination of the whole lungs, regardless of whether or not macroscopic changes were present at autopsy.

INTRODUCTION

The question of the nature of liver tumors induced by chemical carcinogens in mice has been raised frequently by many investigators (1, 3, 10, 14). This is in contrast to the information available regarding the development of liver tumors in rats from nodular hyperplasia to frank neoplasia (2, 6, 8, 9).

However, the assessment of mouse liver tumors ranges from that of nonspecific regenerative lesions to that of frank malignant tumors (11–13, 15).

One argument frequently raised against the neoplastic nature of liver lesions in mice is the absence of signs of local invasion and the relatively rare observation of metastases to distant tissues, particularly to the lungs (7, 14). Our interest in mouse hepatocarcinogenesis led us to make a thorough investigation of the metastatic behavior of liver tumors in the primary host. This was considered to be of particular concern, as our recent studies demonstrated that chemically induced liver tumors in mice were transplantable, characterized by uncontrolled growth, local invasion, and distant metastases via the lymphatic and blood vessels (5). Consequently, it was thought that the infrequent detection of lung metastases in the primary host was due to the dissemination of the primary tumor late in life and/or to the very small size of the metastatic foci that caused them to remain undetected at autopsy and to be missed even during routine histological examination. Thus, it was decided to conduct a systematic histological examination of the lungs of mice bearing liver tumors for the presence of metastases. This paper pertains to such a study, the results of which have been presented recently (4).

MATERIALS AND METHODS

The investigated liver tumors were induced by i.p. injections of diethylnitrosamine in C57BL/6J X C3HeB/FeJ F1 mice bred in our laboratory. The purity of the carcinogen was tested spectrophotometrically. It was then dissolved in trioctanoid (Eastman Kodak Co., Rochester N. Y.) and administered in 4 treatments at 3- (2nd) and 6- (3rd and 4th) day intervals. Animals received a total of 12 μg/g body weight. Both sexes were used, and treatments were initiated at 1 or 15 days of age. The animals were fed Purina laboratory chow and had free access to water during the entire experiment. Animals were observed throughout their life-span and were killed when moribund. On the average, 72% of the diethylnitrosamine-treated mice developed liver tumors, regardless of sex and age at treatment, with a latent period for induction of 73 weeks for males and 96 weeks for females. Autopsies were performed on all animals, and the grossly visible pathological changes were recorded. Both liver and lungs from 220 mice bearing liver tumors were taken for this study. In order to secure the whole lung, this organ was removed at autopsy en bloc with the trachea, paratracheal lymph nodes, thymus, and thyroid, and were fixed in 10% buffered formalin solution. Liver slices containing both grossly visible liver tumors and surrounding normal liver tissue were also taken for histological examination. Lungs were further processed as a whole. They were cut 5 μm thick along their long axis at 2 different levels, 1 of which was passing through the hilar region, including in most cases the thyroid, trachea, thymus, and regional lymph nodes. All paraffin sections (lung and liver) were stained with hematoxylin and eosin and were subjected to systematic histological examination. In addition, in 40 mice bearing liver tumors, the histological examination of the lungs was confined to individual lung lobes only. This was done in order to assess the validity of examining selected lung segments instead of the entire lung.

RESULTS

Histologically, the primary liver tumors appeared as...
multifocal, solitary, or confluent growths. They were composed of small rounded or large polygonal cells arranged occasionally in cords, but primarily in trabecular formations (Fig. 1). In places, they formed solid masses, while in many areas blood-filled sinusoid-like spaces were separating cords and trabeculae of tumor cells (Figs. 2 and 3). The tumor cells appeared polygonal or rounded, demonstrating a moderate to severe degree of anisocytosis and pleomorphism. Their cytoplasm was basophilic and, in certain areas, amphophilic or eosinophilic. Nuclear atypia of varying degrees, mitotic activity (Fig. 4), and intranuclear and intracytoplasmic inclusion bodies were observed (Fig. 5). Tumor masses within large and medium-sized branches of the portal vessels were seen occasionally (Fig. 6).

These tumors metastasized to the lungs mainly by the hematogenous route. Metastatic foci were usually multifocal (Fig. 7), composed of small groups of cells or of larger tumor masses showing a close morphological resemblance to the primary tumor cells. They occupied mainly the alveolar capillaries (Fig. 8) and the small and medium-sized branches of the pulmonary artery, adjacent to the small bronchi and bronchioles (Fig. 9). The metastatic foci were growing concentrically, displacing the surrounding lung tissue (Fig. 10) and occasionally distorting the bronchiolar lumen. Frank infiltration of the lung parenchyma was observed in certain areas (Fig. 11), but the most frequent feature was the formation of larger tumor masses by the confluence of adjacent metastatic foci (Fig. 12).

The metastatic rate of these tumors to the lungs was first evaluated in a preliminary study during which the histological examination of randomly selected lung lobes was performed. Forty animals (males and females) were used in this group. It was shown that the metastatic rate was only 4.3 and 0.0% for males and females, respectively.

Following these preliminary studies, the "whole" lungs from 102 1-day-old and 118 15-day-old treated mice were examined histologically (Table 1). The incidence of metastases of primary liver tumors was 21.6% in the 1-day-old and 22.9% in the 15-day-old group. Since there was no statistically significant difference in the rate of metastasis between the 1- and 15-day-old groups, the data were arranged according to the sex of the animals. Data showed no sex-associated difference in the metastatic rates of chemically induced primary liver tumors. However, although females developed liver tumors with a frequency similar to that observed in males, they emerged approximately 20 weeks later in life.

**DISCUSSION**

The foregoing data demonstrated that the described liver tumors were capable of local invasion of blood vessels, and that they metastasized readily to the lungs of the primary host, regardless of sex. This observation was made possible through the detailed examination of the whole lung, a procedure that is crucial in such a study, whether or not macroscopic changes are present. As anticipated, in many cases metastases were of relatively small size, although multifocal in origin, which prevented their detection by gross examination of lungs. Their small size might be attributed to the short time interval between lung metastasis and death of the animal, the low growth capabilities of the lesions per se, and/or a certain degree of immunological surveillance induced by the primary tumor. The last possibility is indirectly supported by the fact that transplantable liver tumors grew rapidly and metastasized readily, leading to the death of the hosts (5).

The data presented have clearly demonstrated the capability of diethylnitrosamine-induced mouse liver tumors to metastasize in the primary host with a relatively high incidence. This result brings to light another pathognomonic parameter, indicating the frank neoplastic nature of the liver tumors described here. Similar hepatomas have been seen in mice exposed to a variety of carcinogens, such as benzidine (unpublished data), aflatoxin B<sub>1</sub> (16), benzo(a)pyrene (unpublished data), dimethylnitrosamine (13), and N,N'-2,7-fluorenylidenbacacetamide (11, 12). It should, therefore, be accepted that induction of such liver tumors cannot be ignored, but that they can serve as biological indicators of carcinogenicity.

**REFERENCES**


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Figs. 1 to 6. Morphology of primary liver tumors.

Fig. 1. Small tumor cells arranged in trabeculae separated by sinusoid-like spaces. The nuclei are moderately hyperchromatic and occupy a large portion of the basophilic cytoplasm. H & E, x 325.

Fig. 2. Large tumor cells in a trabecular configuration. Trabeculae are surrounded by a well-developed capillary basement membrane. Blood-filled spaces are prominent. Many of the tumor cells demonstrate a delicately foamy cytoplasm. H & E, x 130.

Fig. 3. Plump tumor cells are closely packed in broad trabecular configurations. H & E, x 250.

Fig. 4. Solid liver tumor of the trabecular type. The tumor cells are relatively large, with intense basophilia, moderate nuclear atypia, and a high mitotic activity. H & E, x 325.

Fig. 5. Liver tumor of the trabecular type. The tumor cells are large and pleomorphic, with striking intranuclear and intracytoplasmic inclusion bodies. H & E, x 325.

Fig. 6. Tumor embolus in a portal blood vessel. Note the finger-like appearance and the preservation of the endothelial investment. H & E, x 100.

Figs. 7 to 12. Morphology of lung metastases of liver tumors.

Fig. 7. Microscopic multifocal lung metastases occupying blood vessels adjacent to terminal bronchioles. Arrows, blood vessels occupied by tumor masses. H & E, x 68.

Fig. 8. Tumor cells within alveolar capillaries. Note anisocytosis and cellular pleomorphism. H & E, x 352.

Fig. 9. Large tumor masses occupying medium-sized branches of the pulmonary artery. H & E, x 170.

Fig. 10. Multifocal metastatic foci displacing and in places infiltrating the surrounding lung parenchyma. H & E, x 170.

Fig. 11. Higher magnification of metastasis presented in the lower left corner of Fig. 10. Metastatic liver tumor cells infiltrate the lung parenchyma. The cells are large and basophilic, showing high mitotic activity. Intranuclear and intracytoplasmic inclusion bodies are evident. H & E, x 325.

Fig. 12. Two metastatic foci tend to coalesce by displacing the lung parenchyma and form a larger tumor mass. H & E, x 130.
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