Organ distribution of experimental metastases of a human colorectal carcinoma injected in nude mice

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(Received 18 November 1987; accepted 11 February 1988)

The metastatic behavior of the HT-29 human colorectal carcinoma cell line was studied following injection into nude mice by different routes. After intrasplenic injection, experimental metastases formed in the livers of most mice. Variant lines were established in culture from the liver lesions and from tumors growing at the site of injection, the spleen. Cells of the HT-29 LMM line exhibited slightly enhanced ability to form liver metastases compared with cells of the non-selected parent line. When injected i.v., the HT-29 cells produced only a few small experimental metastases in the lungs, but in most of the mice macroscopic tumors were found in various lymph nodes and the interscapular fat. Analyses of the distribution of IdUrd-labeled cells did not reveal a preferential localization of the HT-29 cells in sites where metastases subsequently formed. This suggested that the growth of the human colon carcinoma cells in those sites might be the result of a stimulatory interaction between the tumor and host cells as opposed to growth in sites such as the lungs, where numerous cells arrested after i.v. injection but only a few, small metastases were seen 60 days later.

Abbreviations

MEM, minimum essential medium, Eagle’s modification with Earle’s salt; HBSS, Hank’s balanced salt solution; s.c., subcutaneous; i.s., intrasplenic; IdUrd, [125I]-5-iodo-2’-deoxyuridine; EDTA, ethylenediaminetetraacetic acid.

Introduction

An increasing number of experimental studies use athymic nude mice as recipients of xenografts of human tumors (see [8] for a review). The morphological and biochemical characteristics of the xenografted tissues are frequently maintained in the nude mouse. Many reports, however, document a very low incidence of metastasis, even with tumor cells originally derived from metastatic lesions [33]. Kozlowski et al. proposed suppression of the endogenous natural killer cell activity in the nude mouse as one way to enhance metastasis formation by cells of human tumor lines [16]; they also suggested that the route of tumor cell injection influenced metastasis formation since tumor cells injected into the spleens of nude mice produced tumors in the liver, lymph nodes, and lungs of the recipient animals,

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whereas s.c. or intramuscular injection of the cells gave rise to tumors that did not disseminate to distant sites. More recently, we reported work with cells isolated from surgical specimens of human tumors. Our studies emphasized the importance of the site for tumor cell inoculation for analysis of the malignant potential of the xenografts [6, 7, 22].

Intrasplenic injection of cells is especially appropriate for studies of the malignant behavior of colorectal carcinomas, as this technique commonly produces experimental metastases in the liver, the most common site of metastases of this carcinoma [14, 16, 18]. Intrasplenic injection of human colorectal cells resulting in tumor formation in the nude mouse livers was used to distinguish carcinoma specimens with different malignant properties. In contrast, although all of the colon carcinoma specimens grew readily when injected s.c. or in the leg muscle of nude mice, no metastases were found in these animals [6].

The process of metastasis is generally regarded as selective. Populations of cells with enhanced metastatic properties have been isolated from low-metastatic rodent tumor lines by harvesting the metastatic lesions [1, 34, 35]. Similar techniques have been used with human tumor lines in nude mice to generate variants with higher metastatic potential than the starting population on reinjection into the nude mouse host [2, 15]. In this study we isolated cells from liver tumors of the HT-29 human colorectal carcinoma to determine whether the process of liver colonization following i.s. injection imparted a selective pressure on the human tumor cell population, resulting in variants with higher liver-colonizing ability than the nonselected parent line. Our purposes were to analyze the metastatic process of human tumor cells in the nude mouse host and to derive tumor variants with enhanced malignant properties that could be used, for example, as experimental targets for therapies specifically directed against human metastatic colorectal carcinoma cells.

The concept that the organ environment may play a role in the course of metastasis formation has prompted interest in the interactions between tumor cells and normal 'host' cells. Recent experimental studies of the effects of organ-conditioned media in modulating tumor cell proliferation and possibly influencing the distribution of metastases in vivo [10, 12, 20, 22, 23, 25] echo the 'seed and soil' hypothesis proposed in 1889 by Paget [26], to explain the nonrandom patterns of human cancer metastases [36]. In this study we analyzed the distribution of the human colorectal cells shortly after inoculation into nude mice, for comparison with the results of experimental metastasis development. The results showed that the arrest of the HT-29 cells in an organ did not reliably predict where the cells would proliferate. A remarkable affinity for growth in lymph nodes was noted, making the HT-29 human colorectal carcinoma cell line a model system with great potential for the analysis of growth regulation of human carcinoma cells in different organ sites.

**Materials and methods**

**Animals**

Athymic NCrNu/Nu mice, 5–6 weeks old, were obtained from the Animal Production Area of the NCI-Frederick Cancer Research Facility, Frederick, MD. Within each experiment the mice were of the same age and gender. The mice were housed under specific pathogen free conditions.