

# Human Tumor Bone Metastasis Model in Athymic Nude Rats

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Metastasis is the principal cause of the morbidity and death of cancer patients. Consequently, there is intense interest in the development of human tumor model systems appropriate for the study of the biology and therapeutic response of various metastatic cancers. Clinicians can use such information to design therapeutic strategies to treat or inhibit the formation of metastatic lesions.

The biology of skeletal metastasis is poorly understood, even though the skeleton is the third most common site of metastasis. As many as 60% of cancer patients are found to have bone metastasis at autopsy (1,2). The major impediment to research on this clinically important problem has been the lack of an appropriate animal model. Animal models that closely mimic the clinical experience of metastasis to bone and bone marrow are urgently needed. Most of the widely accepted concepts on mechanisms involved in the development of metastasis are based on data obtained with rodent tumors that metastasize in the lung. First, important differences exist between rodent and human tumors; second, the profound differences in the milieu between bone and bone marrow and lung tissue suggest that the formation of metastases in the two divergent tissues is likely to be mediated by different cellular events (2).

The athymic mouse has been developed over the past 21 years as an excellent host for the study of biology and chemosensitivity of human cancers, usually grown as subcutaneous xenografts (3,4). However, a shortcoming of the nude mouse model is that metastasis rarely occurs spontaneously; most often, it appears in young, immunologically immature or immunosuppressed mice. Moreover, subcutaneous implantation represents an anatomical site that bears little resemblance to the organ of origin for the neoplasm. Results of clinical studies have suggested that the response of cancer metastasis to anticancer drugs is influenced by the anatomical location of the lesions. However, the development of human tumor metastasis models in nude mice represents a significant improvement over earlier studies in syngeneic murine and rat tumor systems. Several human tumors reproducibly metastasizing in adult mice have been developed (5,6) by injection of tumor cells at sites relevant for specific tumors, e.g., the gut for colon cancer and the kidney for renal cancer.

Shevrin et al. (7) first reported skeletal metastasis in an animal model using a human tumor by injecting poorly differentiated human prostate adenocarcinoma cells (PC-3 line) into the tail vein of athymic mice while the inferior vena cava was occluded. With this approach, bone metastasis occurred in 53% of the animals given injections. The subcutaneous injection of PC-3 cells resulted in metastasis to the draining lymph nodes and lungs, but not to the skeleton (8).

A colony of athymic rats that can be maintained in conventional, germ-free and specific-pathogen-free conditions was established in 1975 (9). The nude rat may be more convenient than

the nude mouse model for the conduct of experiments requiring technically difficult procedures such as surgery, cannulation, or intracardial injections because of its larger size and greater resistance to infection. Unfortunately, detailed studies on human tumor metastasis formation in nude rats are just beginning to appear. For example, Kjønniksen et al. (10) recently described the use of athymic rats as a model for the metastasis of LOX human melanoma cells to the lung. However, because the outcome of metastasis depends on both tumor and host properties, many important questions will have to be answered after a large variety of human tumors have been used before one can conclude that the nude rat model will be highly useful in cancer biology. That is, the conditions for the proper use of this system must be defined so that useful and reliable data on the biology and therapy of human cancers can be generated.

Several potential problems with the nude rat must be taken into consideration by investigators planning to use it as a model for human cancers. These include experiments during which they will (a) assess the effect of variables such as age, sex, site of tumor inoculation, method of processing of fresh tumors, and tissue culture conditions of cell lines on tumor growth and metastasis; (b) determine whether the phenotypic characteristics of transplanted tumors are profoundly altered by the host; (c) determine whether the patterns of metastasis and chemosensitivity of transplanted tumors are retained, and (d) detect whether the growth rates of transplanted tumors are significantly altered. It is likely that various factors influence the growth and metastasis of xenografts in the nude rat, as was found with the nude mouse model. It is important that one rigorously characterize metastasis in the nude rat, so that the mistakes involved in the development of the nude mouse model will not be repeated.

The influence of immunologic competence on the growth and metastasis of the human tumor xenografts in nude rats is a critical area for investigation, because natural killer cell activity is likely to be important in modulating tumor progression (4,11). Therefore, treatment effects with chemotherapeutic agents must be evaluated. Do they enhance the cytolytic activity of this effector cell population? The few reports on the growth of human tumors in the nude rat are inconsistent. Some investigators have reported poor "take rates" and spontaneous regression of human cancers transplanted subcutaneously in nude rats (12-14), whereas others (15) report high take rates for several human tumors. In one study (10), the correlation is clear between the age of the nude rats and metastasis; metastasis was much higher and survival much shorter in 4-week-old rats than in 6- to 10-week-old animals.

A comparison of the nude mouse and the nude rat with respect to patterns of metastasis with various human tumors should prove to be interesting. The LOX human malignant melanoma cells had similar patterns of metastasis in the two species after intravenous injection (10). Although comparative studies with other human tumors are still too preliminary for one to reach definitive conclusions, they do suggest that for some tumors the tissue preferences are different in the mouse versus the rat species (10).

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Received January 30, 1990; accepted January 30, 1990.

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Such differences between the two species can potentially be exploited for identification of possible new factors that influence metastasis.

The paper of Kjønniksen and co-workers (16) is based on the earlier findings of Arguello et al. (17) that B16 melanoma cells injected into the left ventricle reproducibly colonize specific sites in the skeletal system of syngeneic C57BL/6 mice. Kjønniksen et al. describe a nude rat model of skeletal metastases based on left ventricle injection of LOX human malignant melanoma.

The direct introduction of tumor cells into the arterial circulation recapitulates the colonization phase of bone metastasis, a process consisting of arrest, extravasation, and proliferation. Arrest and extravasation in the highly specialized and morphologically modified microvasculature of bone marrow are likely to be very different from the arrest and extravasation reported for lungs. Researchers can use this model to investigate the molecular events involved in bone metastasis and also to examine the effect of specific agents on the metastatic process. Therefore, progress is being made in the development of appropriate models to study the biology and therapy of human tumors in animals. However, much remains to be learned, as the reports of several interesting models recently developed have demonstrated.

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