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Role of the Implantation Site on Metastatic Ability of the Murine MBT-2 Transitional Cell Carcinoma

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Summary. The influence of implantation site on the metastatic behavior of a murine transitional cell carcinoma line (MBT-2) was examined. MBT-2 cells were injected into one of four anatomic sites; subcutaneously, intramuscularly, intravenously or into the footpad, to evaluate the influence of implantation site on the formation and number of metastases. The MBT-2 cell line produced a low incidence of lung metastases after intravenous injection with a mean of 1.1 lung tumors per mouse. Injection of MBT-2 cells into the footpad or subcutaneously did not produce metastases from the primary tumor. Intramuscular implantation, however, resulted in a sixty percent incidence of metastasis with a mean of 8.2 lung nodules per mouse. This study demonstrated a definite implantation site influence on the metastatic ability of the MBT-2 line.

Key words: Bladder metastasis – Implantation site – MBT-2 cells

Introduction

Rodent transitional cell carcinomas induced by exposure to the chemical carcinogen, N-[4-(nitro-2-furyl)-2-thiazolyl] formamide (FANFT) have been used extensively for the study of bladder cancer [11]. A FANFT generated tumor in mouse bladder, the MBT-2 line, has been isolated and maintained as both an in vivo transplantable tumor line and as an in vitro cell line [6, 10–12]. Reports regarding the metastatic ability of the MBT-2 line however, have been varied and inconsistent [2, 3, 8, 10]. One variable that may influence the observed difference in the metastatic ability of the MVT-2 line may be the anatomic site of implantation of the cells. In other tumor models, the site of tumor cell injection has been shown to influence the growth [1] and metastatic behavior of the tumor cells [5, 7]. The present study reports on the effect that subcutaneous (SC), intramuscular (IM), footpad (FP) and intravenous (IV) implantation had on the metastatic propensity of MBT-2 tumor cells.

Materials and Methods

Animals

Male C3H mice (Charles River Breeding Company, Portage, MI) were maintained on certified Purina Laboratory chow and water, provided ad libitum. Mice were 8 weeks old at initiation of the experiments.

Tumor Cell Line and Culture

The MBT-2 murine transitional cell carcinoma line adapted for growth in tissue culture was obtained from Dr. D. Mickey (Department of Urology, University of North Carolina, Chapel Hill). This cell line was established from the MBT-2 solid tumor line, originally obtained from a FANFT-induced transitional cell carcinoma in a female C3H mouse. The MBT-2 cell line was maintained in our laboratory in 79S medium (a modification of Ham's F-12) supplemented with 0.1 mM calcium, 5% fetal bovine serum (FBS) and gentamicin. Cells were maintained in a humidified 3% CO_2-97% air incubator at 37 °C.

Assay of Tumorigenesis and Metastatic Ability

The tumorigenicity and metastatic ability of the MBT-2 cell line at various sites of implantation was assayed. A single cell suspension of MBT-2 cells was achieved by trypsinization of semi-confluent cultures. The cells were washed in Hank's balanced salt solution (HBS) three times and cell viability was assessed by trypan blue exclusion. Only single cell suspensions with viabilities greater than 90% were used for injection. Mice were injected with 1×10^5 cells in 0.05 ml of HBS into one of four anatomic sites:

1) subcutaneously (SC) on the dorsal surface of the right hind thigh midway between the knee and the hip;

2) intramuscularly (IM) into the right hind thigh;

3) beneath the skin of the dorsal surface of the right hind footpad (FP); or

4) intravenously (IV) into the lateral tail vein approximately 2 cm from the base of the tail.

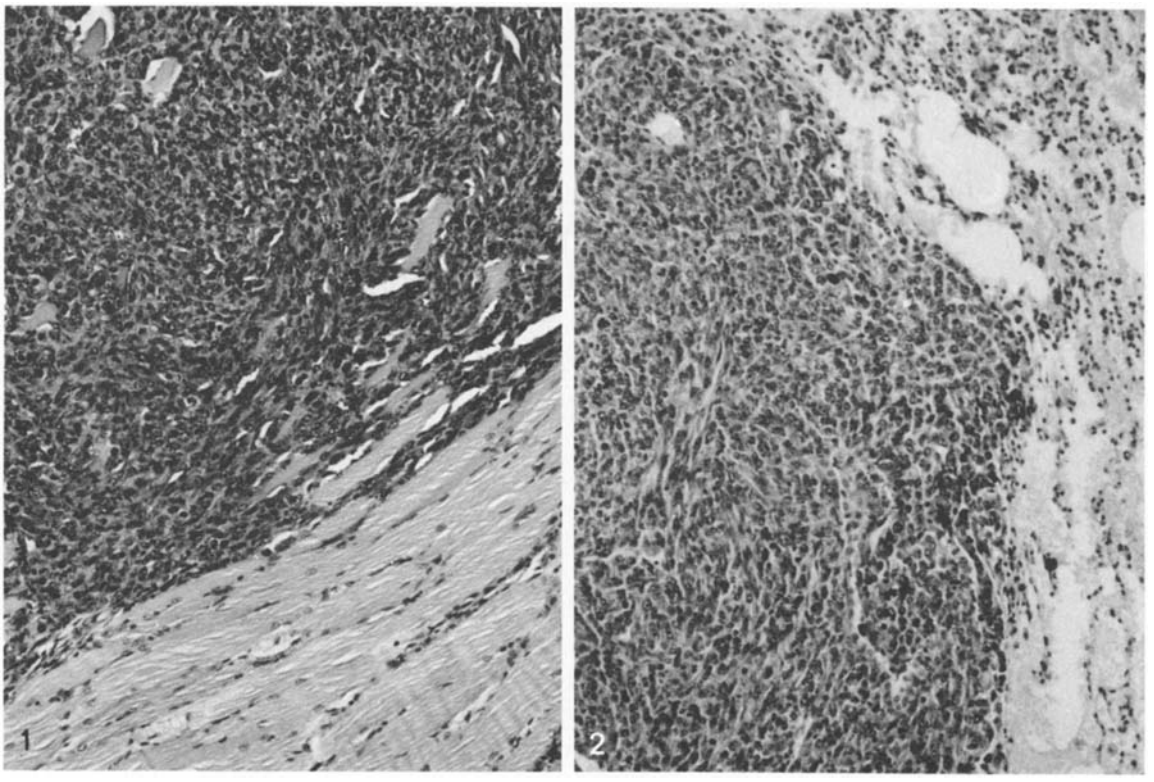

Fig. 1. Photomicrograph of a hematoxylin and eosin stained section of a primary MBT-2 tumor injected intramuscularly. Tumor was sampled five weeks after inoculation of MBT-2 cells (x100)

Fig. 2. Photomicrograph of a hematoxylin and eosin stained section of a lung metastases from an intramuscularly transplanted MBT-2 cell tumor sampled five weeks after inoculation (x100)

Table 1. Primary and metastatic growth of MBT-2 cell line

Implantation site[a]	Weight of primary tumor[b]	Incidence of mice with metastases	Mean number of lung tumors[c]
SC	7.28 ± 1.11	0/10	0
IM	10.29 ± 0.99	6/10	8.20 ± 3.64
FP	1.37 ± 0.33	0/10	0
IV	–	5/10	1.10 ± 0.59

[a] 1×10^5 MBT-2 cells were injected either subcutaneously (SC), intramuscularly (IM), intravenously in the tail vein (IV) or in the footpad (FP) of male C3H mice

[b] Values represent the mean ± S.C. of primary tumor weight (g). The MBT-2 generated 100% incidence of primary tumors at the SC, IM and FP sites

[c] The number of lung tumors reflect the mean ± S.D. of the number of gross and histologically discernible lung tumors per mouse for all 10 sampled mice (all mice at risk). Metastases were found only in the lung

Ten mice were injected at each site. Mice were sampled five weeks after tumor cell implantation. At sampling, the mice were killed by fatal anaesthesia with sodium pentobarbital and necropsied. The primary tumor weight was determined after its excision from the animal. Visceral organs were removed, grossly analyzed for tumors, fixed in 10% buffered formalin, dehydrated in ethanol, and embedded in paraffin. Five micrometer thick paraffin sections of the visceral organs and the primary tumors were stained with hematoxylin and eosin (H&E). Skip sections, 50 microns apart were cut from lung tissue for quantitation of metastatic lesions. Care was taken to ensure that the same tumor was not counted more than once when the tumor appeared in adjacent skip sections. The incidence and number of metastases were quantified by histology. In addition, H&E sections of the primary tumor were studied with light microscopy.

Results

The tumorigenic and metastatic behavior of the MBT-2 murine bladder transitional cell carcinoma line at various implantation sites is shown in Table 1. The MBT-2 cell line readily formed primary tumors at the SC, IM and FP sites (100% incidence for all three sites). No significant differences were noted in the mean tumor weights of the SC and IM implanted cells after 5 weeks. Primary tumor growth (as measured by tumor weight) in the FP was less than that observed in the IM and SC sites. The MBT-2 line when injected either SC or in the FP failed to metastasize. When implanted IM, the MBT-2 cells produced pulmonary metastases in 60% (6/10) of the animals with a mean of 8.20 tumors per mouse. Direct injection of the MBT-2 cells into the tail vein (IV) produced a lower incidence of lung meta-

stasis (50%) with a mean of 1.10 tumors per animal. Metastatic tumors were detected only in pulmonary tissue.

Histologic examination of the SC, IM, and FP implanted tumors displayed similar morphology (Fig. 1). Small areas of necrosis, centrally located in the primary tumor were observed in all tumors at the IM, SC, and FP sites at the 5 week sampling time. Both SC and IM primary tumors showed local muscle invasion. Lung metastases in the IM and IV tumors were also similar in morphology and in size (Fig. 2).

Discussion

In the MBT-2 tumor model, the site of primary implantation appears to be an influencing factor in the metastatic propensity of the tumor cells. In the current study, the ability of MBT-2 cells to produce metastases was examined in four different sites of implantation. The sites of SC, IM, FP and IV were examined as these are most commonly used implantation sites for transplantable tumors. The SC and FP sites failed to produce metastases while IM and IV injection sites proved "permissive" for metastasis in the present study. Differences in the metastatic ability of the MBT-2 cells have also been noted by other investigators using this model. Babayan et al. reported that SC implantation of the MBT-2 tumor lines did not result in metastases but implantation in the FP produced metastasis [2]. Crabtree et al. demonstrated that IM implantation of MBT-2 cells resulted in metastasis [3]. In studies by Murphy et al., metastases from SC implantation of this tumor line were found [8–10].

In the present study, the weights of the SC and IM implanted primary tumors were not significantly different from each other, thus suggesting that the site rather than the size of the primary tumor is important in the metastasis of the MBT-2 tumor. No encapsulation of the SC implanted tumor was noted and local muscular invasion was evident in all SC tumors.

Intravenous injection of tumor cells has been utilized as an indicator of a tumor cell's ability to complete several metastatic steps [4]. However, IV injection is an artificial metastasis since primary tumor growth and invasion is omitted when using this site. In our study, the intravenous injection of MBT-2 cells produced a low incidence of metastasis and low number of metastases. The MBT-2 cell line therefore, appears to have little or no ability to metastasize except when implanted IM. Enhancement of the metastatic ability of the MBT-2 cells at the IM site may be related to this site's selection and enhancement of a metastatic subpopulation in the cell line and/or through other, as yet undetermined, factors. The process of intramuscular injection results in damage to blood vessels. These damaged vessels may provide rapid access of the MBT-2 cells to the vascular system. However, while the metastatic incidence of IM injected tumors (60%) and IV injected tumors (50%) was similar, the mean number of metastatic tumors per lung in the IM injected cells was over seven times that of the IV injection. This suggests that other factors may be responsible for the enhanced metastatic behavior of MBT-2 cells from this site. Previous studies have documented both regional influences on the growth of primary tumors and/or site-influenced differences in metastatic ability [1, 5, 7, 13]. The present study demonstrated a definite site influence on the ability of MBT-2 cells with IM implantation promoting metastatic ability. Use of this "permissive" primary site for the metastasis of MBT-2 cells allows for the study of the mechanisms of bladder metastasis using this tumor model.

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