Human Transitional Cell Carcinoma in the NMRI-nu/nu Mouse Bladder: A New Animal Model for the in Vivo Use of Monoclonal Antibodies and Cytotoxic Agents

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ABSTRACT

Human transitional cell carcinoma of the bladder was successfully transplanted into the bladder of the NMRI-nu/nu mouse. Transplantation of a single-cell suspension from a human Grade II transitional cell carcinoma had an acceptance rate of 33%. The tumors showed invasive growth and could be identified by an anti-human monoclonal antibody. This tumor model can be valuable as an in vivo test system for the local use of cytotoxic agents and monoclonal antibodies in diagnosis of and therapy for human bladder carcinoma.

INTRODUCTION

The introduction of MAB2 directed against tumor-associated antigens of TCC of the urinary bladder (1–4) underlines the need for a reliable animal model to study the use of MAB in diagnosis and therapy. Animal bladder tumors such as the FANFT model have been used to test chemotherapeutic agents (5–7) and to study the development of diagnostic methods, e.g., in cytology (8). However, the use of MAB implies that experimental tumors should be from humans and they should also mimic the multifocal nature of TCC.

Our s.c. transplantation of human bladder tumor into the NMRI-nu/nu mouse had an acceptance rate of 60% (9), which indicated that such transplantation is possible. We describe here a new method to establish multifocal growth of human bladder TCC in the bladder of the NMRI-nu/nu mouse in only 10 weeks.

MATERIALS AND METHODS

Tumor Cell Transplantation. Seventeen NMRI-nu/nu mice, 5–6 weeks old, were anesthetized, their bladders were exposed, and 1 mg of MNU was instilled into each bladder to produce urothelial inflammation (10). Two days later we injected into each bladder 0.1 ml of a single-cell suspension prepared by trypsin dissociation of a fresh specimen of human TCC that had been serially transplanted into NMRI-nu/nu mice in passage 17 (11). Cell viability had been determined by the exclusion of trypan blue; a solution of 107 cells/cm3 was prepared with 95% viability. Two mice received an additional 0.1 ml s.c. as an internal control to demonstrate viability of the injected cells in case none of the intravesical injected cells would grow in the bladder. After the injection voiding was inhibited for 2 h simply by ligation of the urethra to ensure that tumor cells had enough time to adhere. Mice that developed abdominal masses were killed and examined for tumor growth. The remaining mice were killed 10 weeks after tumor cell implantation and examined for tumor growth. The bladders of the mice were frozen and then dissected and stained with May-Grünwald-Giemsa.

Immunohistology. The monoclonal antibody H2 6/5 produced in our laboratory was developed by immunization of a mouse with the same TCC in passage 12 of an NMRI-nu/nu mouse that was used for tumor cell transplantation. It is an IgG antibody that selectively reacts with nuclear structures of bladder carcinoma cells and with cells from virus-infected tissue, such as condyloma acuminatum (12). The antibody reacts with the nuclei of the tumor used for transplantation. Peroxidase-conjugated goat anti-mouse IgG was used as a second antibody. Peroxidase-conjugated swine anti-goat IgG was used as a third antibody. Peroxidase activity was demonstrated with diaminobenzidine. The preparations were counterstained with hemalum and mounted.

RESULTS

Five of 15 mice developed abdominal masses and were killed 6–8 weeks after implantation. They showed sessile and invasive bladder tumors with an average size of about 1 cm3. The microscopic appearance was of a polymorphic tumor with hyperchromatic nuclei and numerous mitoses (Figs. 1 and 2). Three of the 5 accepted tumors showed multifocal tumor growth in the bladder (Fig. 1); in 4 of the 5 accepted tumors the tumor growth showed muscle invasion. The tumor cells were differentiated to Grade II as was the originally human transitional cell carcinoma from a patient with a Grade II T2 bladder carcinoma. They were microscopically similar to the tumor used to prepare the single-cell suspension and to the original tumor of the patient (Fig. 3). Two mice died before the instillation (one had bowel necrosis, probably due to an MNU leak into the abdomen and one had an abscess).

The two mice that had simultaneous s.c. and intravesical tumor instillation did not develop bladder carcinomas. However, both developed s.c. tumors that were histologically similar to the original tumor.

Antibody H2 6/5 stained the nuclei of the tumor cells in the bladder of the five mice. It did not stain nuclei of the cells in normal bladder or nuclei of normal cells in the animals that received transplants. The original tumor reacted with MAB H2 6/5. The MAB H2 6/5 selectively stains nuclei of bladder tumor patients and nuclei of papilloma virus-infected cells (12).

DISCUSSION

Histological and immunohistological examination confirmed that the original tumor, rather than mouse bladder tumors, grow in the bladders of the mice. A single dose of MNU never produced bladder carcinoma, even when the dose was higher than the ones we used to induce denudation and inflammation of the epithelium (10). Hicks and Wakefield (10) showed that a single intravesical dose of MNU produces toxic damage and hyperplasia of the epithelium but is not carcinogenic. Whereas bladder tumors caused by MNU instillation appear from 12 weeks on, but not earlier (10), the tumors we found in this study appeared in 6–8 weeks. Monoclonal antibody H2 6/5 is specific for nuclei of human bladder carcinoma cells and human papilloma virus-infected cells. That the antibody reacted with the nuclei of the tumor cells before and after implantation proves that the antigen structure of the nuclei of the original tumor was conserved.
Three elements of our procedure made tumor cell adherence and tumor cell growth in the murine bladder possible: denudation and inflammation of the epithelium with MNU; use of the human bladder carcinoma that had been successfully transplanted s.c. into NMRI-nu/nu mice; and inhibition of voiding of the tumor cells for 2 h after injection.

Our model has several advantages. The use of monoclonal antibodies in diagnostic and therapeutic in vivo studies now is possible only with human antigen structures. Our model makes animal studies possible. Thus far chemotherapeutic tests are being done only with FANFT tumors, which are murine tumors (5, 6, 13, 14). It will now be possible to do these tests with a relevant human cell population.

One hypothesis related to human bladder cancer is that exfoliated cells are responsible for tumor recurrence in that they implant and grow on areas of denuded epithelium (6). Our animal model offers an ideal system for examining implantation phenomena. It is also possible to imitate the multicentricity of neoplasia seen in human bladder carcinoma, which is cited as a basis for recurrent tumor development.

The acceptance rate of tumor cell implantation in FANFT tumors in inbred mice with a comparable method was 60% (6). We had an acceptance rate of 33% (5 of 15 implants). Human tumors still seem to be more difficult to implant into the mouse bladder than do murine tumors. For immunological tests it will be necessary to use human tumor material; also for chemotherapeutic and other tests our model constitutes an improvement.

REFERENCES

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