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Bladder Cancer

Real-Time Cancer Cell Tracking by Bioluminescence in a Preclinical Model of Human Bladder Cancer Growth and Metastasis

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Abstract

Background: Bladder cancer is the fifth most common malignancy in the Western world and the second most frequently diagnosed genitourinary tumor. In the majority of cases, death from bladder cancer results from metastatic disease. Understanding the multistep process of carcinogenesis and metastasis in urothelial cancers is pivotal to the development of new therapeutic strategies. Molecular imaging of cancer growth and metastasis in preclinical models provides the essential link between cell-based experiments and clinical translation.
Objective: Develop preclinical models for sensitive bladder cancer cell tracking during tumor progression and metastasis.
Design, setting, and participants: A human transitional cell carcinoma UM-UC-3 cell line was generated that stably expresses luciferase 2 (UM-UC-3luc2), a mammalian codon-optimized firefly luciferase with superior expression. Preclinical models were developed with human UM-UC-3luc2 cells xenografted into the bladder (orthotopic model with metastases) or inoculated into the left cardiac ventricle (bone metastasis model) of immunocompromised mice.
Measurements: Noninvasive, sensitive bioluminescent imaging of human firefly luciferase 2–positive bladder cancer in mice using the IVIS100 imaging system.
Results and limitations: In the orthotopic model (intravesical inoculation), tumor growth could be followed directly after inoculation of UM-UC-3luc2 cells. Importantly, micrometastatic lesions originating from orthotopically implanted cancer cells could be detected in the locoregional lymph nodes and in distant organs. In addition, the superior bioluminescent indicator firefly luciferase 2 allows the detection and monitoring of micrometastatic lesions in real time after intracardiac inoculation of human bladder cancer cells in mice. The main disadvantage is the lack of T-cell immunity in the preclinical models.
Conclusions: The new bioluminescence-based preclinical bladder cancer models enable superior, noninvasive, and real-time tracking of cancer cells, tumor progression, and micrometastasis. Because of the significant improvement in detection of small cell numbers, the presented models are ideally suited for functional studies dealing with minimal residual disease as well as real-time imaging of drug response.

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1. Introduction

Bladder cancer is the fifth most common malignancy in the Western world and the second most frequently diagnosed genitourinary tumor after prostate cancer. Bladder cancer occupies the 10th position of estimated cancer death causes. In the majority of cases, death from bladder cancer results from metastatic disease. Understanding the different steps of carcinogenesis and the closely linked mechanisms of angiogenesis, invasion, and metastasis is pivotal to the development of new therapeutic strategies. The animal model is the essential link between cell-based experiments and translation of novel therapeutics into patients with bladder cancer.

A number of preclinical models are currently used to study bladder cancer growth and metastasis, including intravesical implantation of cancer cells that may lead to invasive orthotopic growth and lymph node involvement [1]. Bladder cancer metastasis can also be studied using experimentally induced metastasis by direct inoculation into the blood circulation. Using this model, initial steps of invasion and intravasation are bypassed, and colonization of the distant organs by the cancer cells can be studied [2]. New molecular imaging modalities including ultrasound, magnetic resonance imaging (MRI), or optical imaging (bioluminescence or fluorescence) are pivotal to assess tumor growth and metastasis in real time [1,3–6]. Sensitive, noninvasive molecular imaging permits longitudinal and quantitative real-time gene expression, cellular localization, and drug-response studies in vivo. We have successfully used firefly luciferase in gene function studies and the evaluation of drug response in orthotopic growth and metastasis of other solid cancers, demonstrating the usefulness and power of optical imaging in preclinical models [5,7–11].

Experimentally-induced bladder cancer metastasis can be accomplished by different routes of cancer cell inoculation (ie, to the lateral tail vein or the left cardiac ventricle) [2,12]. Bioluminescent imaging (BLI) in orthotopic bladder cancer models has been reported in different xenograft models [6,12–16]. However, in these orthotopic bladder cancer models, no metastases could be detected by BLI. Furthermore, Lodillinsky et al. showed bladder cancer metastasis to the lung in a murine orthotopic bladder cancer model (MB49-I cells), but noninvasive optical imaging was not used [17].

Taken together, there is a clear need for a reliable, sensitive, bioluminescence-based bladder cancer model of orthotopic growth and resulting metastasis that allows real-time cancer cell tracking and drug response (functional and experimental therapeutic studies, respectively).

In this paper, we generated a human transitional cell carcinoma UM-UC-3 cell clone that stably expresses firefly luciferase 2 (UM-UC-3luc2), a mammalian codon-optimized bioluminescence reporter with improved expression levels allowing superior cancer cell tracking (tumor growth and metastasis). This cell line subsequently was used to develop novel preclinical models of bladder cancer progression and metastasis. Our in vivo models enable sensitive detection of tumor progression and metastatic disease by state-of-the-art optical imaging, as exemplified by metastasis to the lymph nodes and the lungs from the primary tumor, closely resembling the clinical situation of invasive urothelial carcinoma.

2. Materials and methods

2.1. Cell lines and culture conditions

The transitional cell carcinoma cell line UM-UC-3 was obtained from the American Type Culture Collection (ATCC; catalog no. CRL-1749). Cells were routinely cultured in ATCC Eagle's Minimal Essential Medium (ATCC catalog no. 30-2003) supplemented with 10% fetal bovine serum and penicillin/streptomycin. Cultured cells are regularly tested for mycoplasma contamination.

2.2. Stable transfection

Stable cell lines expressing pCAGGS3.1 luciferase 2 (modified pGl4 luciferase 2 vector [Promega, the Netherlands] containing the CMV early enhancer/chicken beta actin CAG promoter) were generated using Fugene HD, according to manufacturer's protocol (Roche, the Netherlands), followed by selection for single clones by supplementing 0.8 mg/ml neomycin to the medium (Invitrogen, the Netherlands). Luciferase activity was measured with luciferase assay (Promega).

2.3. Preclinical models

Eight- and five-week-old female nude (Balb/c nu/nu) mice were used for the orthotopic and experimental metastasis model, according to the Dutch guidelines for animal welfare (DEC no. 09189).

2.3.1. Subcutaneous inoculation

Subcutaneous inoculation was performed with 10 000, 1000, 100, or 10 UM-UC-3luc2 cells at the indicated sites on the dorsal side of the mice ($n = 2$; two mice per group) with a volume of 10 μl of 1:1 cell suspension (Matrigel Becton Dickenson growth factor reduced and cell suspension; for schematic representation, see Fig. 1B).

2.3.2. Orthotopic inoculation

Prior to inoculation of the cells, the bladder was pretreated with 100 μl of 0.1 mg/ml poly-L-lysine (Sigma, the Netherlands) for 20 min. Thereafter, the bladder was flushed with phosphate-buffered saline (PBS), and a single-cell suspension of 2×10^6 UM-UC-3luc2 cells per 35 μl was inoculated into the bladder via the urethra with an angiocatheter (24G; Jelco, Southington, CT, USA). During the entire surgical procedure, mice were kept under anesthesia for 3 h before the catheter was removed [18] ($n = 3$; 10 mice per group).

2.3.3. Intracardiac inoculation

A single-cell suspension of 1×10^6 UM-UC-3luc2 cells per 100 μl of PBS was inoculated into the left ventricle using a 30G needle, as described previously [7–9] ($n = 3$; 10 mice per group).

2.3.4. Bioluminescent imaging

BLI was performed using the IVIS100 imaging system (Caliper Life-Sciences, Hopkinton, MA, USA) after intraperitoneal administration of D-luciferin (Perbio Science Nederland, Etten-Leur, the Netherlands). Values are expressed as relative light units (RLU) [8,9]. The number of distinct metastases was calculated based on the number of distinct bioluminescent foci at distant sites.