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# Therapeutic Effect of Bone Marrow Mesenchymal Stem Cells on Rat Bladder Cancer

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**Abstract:** *Objective:* To analyze the effect of bone marrow mesenchymal stem cell therapy on rats with bladder cancer and provide a feasible direction for the treatment of human bladder cancer. *Methods:* An animal model was constructed, and Model 1 was used as an example. Two groups of rats were injected with anti-upconversion nanoparticles (UCNPs) (experimental group) and 0.9% normal saline (control group), respectively. *In vivo* imaging was performed to determine the accuracy of the anti-UCNPs method. *Results:* There were 15 rats in the experimental group with obvious bladder swelling. Among them, 11 rats had cauliflower-like and partially brown bladder tumors, whereas the other four rats had hard, nodular-like protrusions, with indistinct borders and adhesions to the anterior wall of the rectum. Small papillary masses were observed in two rats, local mucosal thickening without tumor formation was observed in two rats, and bladder stones were observed in six rats. The bladder specimens of 15 rats in the control group were pink and shiny, without any tumors. Fourteen rats in the experimental group and 12 rats in the control group had bladder cancer lesions, accounting for 93.33% and 80%, respectively. The detection accuracy of the experimental group was significantly better than that of the control group. *Conclusion:* Multimodal nanoprobes targeting bladder cancer stem cells i*n vivo* were used to image the orthotopic tumor and lymph node metastasis models of animals by anti-UCNPs imaging to observe the distribution, migration, and differentiation process of bladder cancer stem cells in model mice. It is clear that rare earth upconversion luminescent nanomaterials, modified by BCMab1 and CD44 monoclonal antibodies, can be used as probes for the detection of bladder cancer, the tracking of lymph node metastasis in bladder cancer, and the comprehensive evaluation of the overall efficacy of nanoprobe-targeted therapy for bladder cancer stem cells.

**Keywords:** Bone marrow; Stem cells; Bladder cancer

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## 1. Introduction

Bladder cancer (BC) is one of the most common malignant tumors with high incidence and high postoperative recurrence rate. A series of inflammatory mediators secreted by inflammatory cells, such as

interleukin (IL) and tumor necrosis factor (TNF), has an effect on tumor metastasis and invasion by causing oxidative stress damage and changes in the tumor microenvironment. This effect is mediated by neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and other blood inflammatory response markers [1,2]. PLR, NLR, *etc*. are independent factors that affect the prognosis of tumors [3]. It has been found that systemic inflammatory response (SIR) is also closely related to tumors. In this study, a rat model was used to analyze the effect of bone marrow mesenchymal stem cell therapy on rats with bladder cancer and provide a feasible direction for the treatment of human bladder cancer.

## 2. Materials and methods

### 2.1. Experimental materials

Normal mice and immunodeficient mice (nonobese diabetic/severe combined immunodeficiency, NOD/SCID).

### 2.2. Methods

#### 2.2.1. Construction of animal models

(1) Normal mice.
(2) Model 1: *In situ* tumor formation in immunodeficient mice (NOD/SCID): using the bladder chemical injury (1% HCl solution) and cell suspension transurethral perfusion method.
(3) Model 2: A distant metastasis model of immunodeficient mice (NOD/SCID): using the tail vein injection method of stably transfected cell lines.
(4) Model 3: An immunodeficient mice (NOD/SCID) subcutaneous tumorigenesis model.

#### 2.2.2. *In vivo* imaging of animals

Taking Model 1 as an example, two groups of rats were injected with anti-upconversion nanoparticles (UCNPs) (experimental group) and 0.9% saline (control group), respectively. Then, *in vivo* imaging detection was performed on all 15 rats in each group.

The *in vivo* imaging was performed as follow: (1) reagents were injected through the tail vein of mice; (2) 980 nm wavelength infrared light was used as the excitation source, and the upconversion luminescence signal was detected at a wavelength of 800 ± 12 nm; (3) signal images were detected separately at 30 min, 2 h, and 24 h after injection.

After the mice were sacrificed, tumor tissue sections were taken for histopathological examination (hematoxylin and eosin staining) and immunohistochemical experiments. The experimental results were compared to judge the correctness of the guessed mechanism and verify the accuracy of the anti-UCNPs method.

### 2.3. Observation indicators

The accuracy of the anti-UCNPs method.

### 2.4. Statistical analysis

SPSS 25.0 was used for data analysis. The count and measurement data were expressed as n/% and $\bar{x} \pm s$, respectively, and $\chi^2$ and t tests were performed. $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Morphological observation of rat bladder

In the experimental group, 15 rats had obvious bladder tumors, of which 11 rats had bladder tumors that

were cauliflower-like and partially brown, while the other four rats had hard nodular protrusions in their bladder mucosa with blurred borders and adhesion to the anterior wall of the rectum. Small papillary masses were observed in two rats, local mucosal thickening without tumor formation was observed in two rats, and bladder stones were seen in six rats. The bladder specimens of 15 rats in the control group were pink and shiny, without any observable tumors.

### 3.2. Accuracy of anti-UCNPs method

Through histopathological examination, it was found that 15 mice in the experimental group and the control group were all bladder cell cancer variants. Immunochemical experiments were performed on the two groups of mice, respectively. Following immunohistochemical experiments, bladder cancer cells appeared in the experimental group. There were 14 rats with bladder cancer lesions in the experimental group, accounting for 93.33%, and 12 rats with bladder cancer lesions in the control group, accounting for 80%. The accuracy of detection in the experimental group was significantly higher than that in the control group.

## 4. Discussion

Bladder cancer is one of the most common malignant tumors of the urinary system. It ranks tenth among the most common tumors in humans. It often occurs in male, in which its incidence rate is 4:1. The incidence of bladder cancer is affected by various factors, including heredity, environment, diet, genes, and other factors. At present, the clinical treatment of bladder cancer is mainly based on the combination of surgery, radiotherapy, systemic chemotherapy, and immunotherapy. However, the prognosis remains relatively poor [5,6]. In recent years, the prevention and treatment of tumors by traditional Chinese medicine has gradually attracted widespread attention. The disease name “bladder cancer” has not been clearly stated in ancient Chinese medicine books, but it can be classified according to its clinical manifestations. It belongs to the categories of “long closure,” “hematuria,” “drowned blood,” and “blood stranguria” [7-9]. The active ingredients of traditional Chinese medicine have low toxicity, less side effects, multiple targets, and multiple pathways in the treatment of tumors. In the treatment of bladder cancer, the focus should be on each molecular change involved in the tumor and the regulation of signaling pathways, which is closely related to the occurrence and development of tumors [10-12].

BCMab1+/CD44+ bladder cancer stem cells were sorted by flow cytometry and cultured *in vitro*; multimodal nanoprobes (anti-UCNPs) with different concentration gradients were added to study the interaction between multimodal nanoprobes and bladder cancer stem cells and observe the endocytosis effect of cells on the probe, the subcellular localization of the probe, as well as the effect of the probe on cell proliferation and differentiation. It is clear that the upconversion luminescence *in vivo* imaging system can be used to detect BCMab1 and CD44 monoclonal antibody-modified rare earth upconversion luminescent nanomaterials that have high luminescence efficiency and bladder cancer specificity *in vivo* [1,2].

In this study, it was found that (1) aberrantly glycosylated integrin α3β1 in bladder cancer cells can be specifically recognized by the monoclonal antibody BCMab1; (2) BCMab1 can significantly inhibit tumor proliferation, migration, and adhesion; (3) 1% of the total BCMab1+/CD44+ subset has strong self-renewal ability and differentiation potential; (4) GALNT1-mediated glycosylation of sonic hedgehog (SHH) protein is necessary for Hedgehog signal activation; (5) intravesical instillation of *GALNT1* siRNA and SHH-targeted inhibitors can effectively inhibit the occurrence and development of bladder tumors; (6) bladder cancer stem cells originate from bladder epithelial stem cells and bladder cancer non-stem cells. We use this feature of BCMab1 monoclonal antibody to design and prepare upconversion luminescent nanomaterials (anti-UCNPs) linked to the antibody. Using this material as a biological probe, the diagnosis of bladder cancer and the tracking and detection of lymph node metastasis can be performed non-invasively,

targetedly, and efficiently in animals by means of *in vivo* fluorescence imaging.

Some studies have found that smoking is the main pathogenic factor for bladder cancer, and about 50% of bladder cancer is caused by smoking [12]. Although the impact of smoking on the prognosis of bladder cancer is controversial, a number of studies believe that smoking increases the incidence and mortality of bladder cancer with significant gender differences; moreover, the incidence of women is lower than that of men. Previous studies have shown that when the stage of the cancer is the same, the prognosis of men is better than that of women. Several studies have shown that bladder cancer is more common in the elderly, in which most of them are over 70 years old [14,15]. For Ta~T1 bladder tumors with longer life expectancy, due to the higher risk of tumor progression, such as when the tumor is large or numerous, total cystectomy is usually recommended. Studies have reported that the long-term cancer-specific survival rate of patients undergoing immediate total cystectomy is as high as 85% to 90%. The aforementioned studies have confirmed the safety and efficacy of total cystectomy in the treatment of patients with bladder cancer. It has been reported that postoperative intravesical chemotherapy in patients with bladder cancer has a positive effect on preventing the recurrence of postoperative bladder cancer. The recurrence rate of bladder tumors within 2 years was found to be lower than those who did not receive intravesical instillation.

Bladder cancer can be divided into muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC) [13,14]. The primary treatment of bladder cancer is still surgery or the former in combination with other comprehensive treatments [15]. However, 70–80% of NMIBC patients relapse or progress within 5 years after surgery [16], with 10–20% of patients progressing to MIBC or distant metastatic disease [17]. Both radiotherapy and chemotherapy have significant side effects and lack selectivity. The emerging second-line treatments, such as immunotherapy and targeted therapy, have yet to be accepted due to their high cost and low patient response rate [18,19].

Nanomaterials have become one of the new directions of tumor therapy due to their strong ability to penetrate tumor tissue, low immunogenicity, and long circulation time in the blood. ROS is a general term for oxygen-containing free radicals and peroxides that easily form free radicals, which are related to oxygen metabolism in the body. Low concentrations of ROS play an important role in regulating signaling pathways, eliminating pathogens, regulating inflammation, and promoting cell proliferation. However, when ROS concentrations are high, they may damage nucleic acids, proteins, or cell membranes, thus leading to cell death. Glutathione (GSH) is one of the most important antioxidants in cells, which can protect cells from ROS damage. The level of ROS in tumor cells is often higher than that in normal cells, which leads to an adaptive increase in the level of antioxidants, such as GSH, in the body. As a result, ROS and GSH maintain a dynamic balance at high concentrations, and the cells tend to be in a state of oxidative stress.

The inherent ROS concentration in tumor cells is high. When both tumor cells and normal cells are exposed to the same amount of external ROS, the ROS level in tumor cells will easily reach the threshold of triggering cell death. On the other hand, the inherent ROS concentration in normal cells is low, and thus able to buffer a certain amount of ROS without triggering cell death.

In conclusion, multimodal nanoprobes-targeted *in vivo* tracking of bladder cancer stem cells in animal models of orthotopic tumor and lymph node metastases were used for anti-UCNPs imaging to observe the distribution, migration, and differentiation of bladder cancer stem cells in model mice. It is clear that rare earth upconversion luminescent nanomaterials, modified by BCMab1 and CD44 monoclonal antibodies, can be used as probes for the detection of bladder cancer, the tracking of lymph node metastasis in bladder cancer, and the comprehensive evaluation of the overall efficacy of nanoprobe-targeted therapy for bladder cancer stem cells.

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**Disclosure statement**

The authors declare no conflict of interest.

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