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Establishment of orthotopic transplantation model of human bladder cancer and detection by MRI*

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Abstract ***Objective:*** To establish an orthotopic bladder cancer model bearing human bladder cancer for experimental research, and monitor tumor progression by magnetic resonance imaging (MRI). ***Methods:*** The mucosa was mechanically damaged transurethrally under direct vision, and then human bladder cancer cell line T24 was inoculated into the bladders of BALB/c nude mice to establish orthotopic bladder cancer model. To find a suitable concentration of Gd-DTPA for this research. MRI was performed weekly to assess tumor growth, using Gd-DTPA as contrast agent. The pathologic morphology of the bladders and other specimens were observed with HE stain. ***Results:*** All the 25 mice developed bladder cancer after inoculation. The best concentration of Gd-DTPA was 1.408 mg/mL. On MRI, no change in the bladders was observed on day 7 after inoculation, filling defect in the bladders, accordant to actual tumor size, was detected on days 14, 21 and 28. Pathologic examination showed that tumor grew in the mucosa or superficial muscle of bladder on day 7, confined in muscle layer on days 14–28, and invaded serosa on day 35. ***Conclusion:*** Transurethrally damaged bladder mucosa under direct vision and instilled bladder cancer cell T24, we successfully established an orthotopic bladder cancer model. Tumor growth simulated the progression of human bladder cancer approximately. MRI was a reliable way for dynamic detection of murine orthotopic bladder tumor.

Key words bladder neoplasms; animal model; magnetic resonance imaging (MRI)

Bladder cancer is the most common urological cancer in China, and patients with superficial (non-muscle invasive) tumors account for 55%–60% of all newly diagnosed cases. The first choice of treatment for superficial cancer is transurethral resection (TUR), assisted with intravesical chemotherapy or immunotherapy. A major problem in the treatment of superficial TCC of the bladder is the high incidence of tumor recurrence following TUR. New methods to apply intravesical therapy and new therapeutic agents were adopted to make intravesical treatment effectively. A few studies showed that perform intravesical treatment immediately post-operation or combination of different agents may improve anti-tumor effect [1]. Development of molecular biology also impulsed researches in bladder cancer. The technique small interference RNA has been used to intravesical gene therapy in experimental study, which may be a potential therapy to superficial bladder cancer [2]. A suitable bladder tumor model that resembles human disease is essential for evaluating new therapeutic agents and modalities. There were kinds of way to establish orthotopic bladder cancer model, and the ideal one simulating human bladder cancer for gene therapy was orthotopic transplantation animal model. As follow, human bladder cancer cell line T24 was inoculated into the bladders of nude mice which mucosa were mechanically scraped, and MRI was performed to assess tumor growth. The model was feasible for preclinical studies on experimental intravesical therapies.

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Materials and methods

Cell line and animals

Human bladder cancer cell line T24 were obtained from Institute of Biochemistry and Cell Biology of CAS, Shanghai, and bred in Iscove's Modified Dulbecco's Media (IMDM) containing 10% fetal calf serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd.) at 37 ℃ in a humidified atmosphere containing 5% CO_2. All

mice were cared in compliance with the institution's guidelines for the care and use of laboratory animals in research. Four to six week female BALB/c nu/nu mice, weighting approximately 16–20 g, were purchased from Shanghai Slac Laboratory Animal Co. LTC. All the mice were maintained in a laminar air-flow cabinet under pathogen-free conditions.

Tumor cell implantation

After trypsinization, human bladder cancer cell line T24 in logarithmic growth phase were rinsed twice with PBS solution. Cell number was determined with a Coulter counter. For orthotopic implantation, 2×10^6 cells in 0.1 mL PBS were instilled intravesically. The mice were anaesthetized with intraperitoneal injection of pentobarbitone (45 mg/kg weight), and sterilized with iodine. A lower midline incision about 0.8 cm long was performed to expose the bladder. The bladder of mouse was catheterized via the urethra with a 22-gauge arterial puncture needle cannula (TERUMO corporation, Japan). A needle was inserted through the catheter to scrape left side of bladder wall for twice or three times. The bladder was lift with a pincette when scraped. Then the bladder was flushed twice with PBS. The cells in 0.1 mL PBS were instilled and left indwelling for at least 1 h, when a metal pin was used to temporary close the external urethral meatus. Immediately removed the catheter 1 h later. Abdominal incision was sutured with thread.

Observation after implantation

The mice were monitored daily for general health status and haematuria. Record the implantation day as day 0, and perform MRI (Philips Eclipse 1.5 T superconductive MRI system, small joint coil) on days 7, 14, 21 and 28 to assess tumor growth, 5 mice once. The mice were sacrificed within 24 h after MRI detection. To identify hydrops or expansion of renal pelvis and ureter with gross observation, and take the organs such as bladder, lung, liver, kidney, ureter, tissue surrounding bladder and uterus for microscopic examination. The remaining 5 survival mice were observed till 35 days after implantation.

MRI detection

The scan parameters were set as follows: TR = 500 ms, TE = 12 ms, slices = 2 mm, FOV = 80 mm × 80 mm, matrix = 256 × 384 for T1-weight image (T1WI) at the transversal and sagittal levels; TR = 4029 ms, TE = 99.4 ms, slices = 2 mm, FOV = 80 mm × 80 mm, matrix = 512 × 512 for T2-weight image (T2WI) at the transversal level. Gd-DTPA (Guangzhou Kangchen Pharmacy Co., Ltd) was modulated to its optimal concentration at T1 transversal level, and diluted to 0.469 mg/mL, 0.939 mg/mL, 1.408 mg/mL and 2.112 mg/mL with normal saline. The diluted solution was taken using a 2 mL syringe and the signal intensity of the interested areas was measured. The subsequent evaluations would be performed with the optimal Gd-DTPA concentration. The nude mice were anaesthetized and the bladders were gently pushed to expel the urine completely; then a 22-gauge arterial puncture needle cannula was inserted and 0.1 mL Gd-DTPA of the optimal concentration was instilled; the external urethral meatus was ligated with thread and the nude mice were placed on the small-joint coil to conduct MRI scanning. The anesthetic was sufficient to anesthetize the mice for the entire MRI period and the thread on the urethra was released immediately after completing the examination. MRI images were then analyzed.

Histological analysis

Tissue from bladders and other organs were fixed in 10% phosphate buffer formalin, embedded in paraffin, serially sectioned at 4 μm, and were stained with haematoxylin eosin (HE) for histological examination.

Results

General status observation

The stitches dropped off automatically on days 5–7, or were taken out on day 7. Gross hematuria occurred in a few mice at day 15 when pressing the bladders bearing tumor. The diet and mental status of the most mice was normal within 3 weeks, except two younger mice at the implantation day. The two mice had weight loss and reduced movement on days 16–18. On day 25, the majority survival mice were depressed, weight lost and back arched. Three mice died of dyscrasia after 28 days, and the two remaining mice were dying at day 35.

MRI findings

Various concentrations of contrast agent in syringes were performed T1WI scanning. The optimal signal was obtained on T1-weight image when the concentration of the contrast agent was 1.408 mg/mL and the signal began to decrease when the concentration increased to 2.112 mg/mL (Fig. 1). Therefore the subsequent evaluations was performed with 1.408 mg/mL Gd-DTPA. On MRI, no obvious change in bladders was observed at day 7, filling defect in the bladders was detected at days 14, 21 and 28. The MRI findings showed the procedure of tumor growth, from the early stage to occupying the whole bladder cavity finally. Fig. 2 showed the typical images. After 35 days after inoculation MRI was terminated because of the obstruction of bladder tumor.

Histological characteristics

Hydronephrosis and ureterectasia at various extents was found on days 28 and 35, but not on days 7, 14 and 21. In observation period, tissue surrounding bladder and

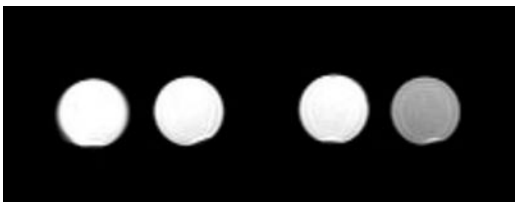

Fig. 1 Images of different concentrations of Gd-DTPA (T1WI). The concentration of Gd-DTPA from left to right was 0.469 mg/mL, 0.939 mg/mL, 1.408 mg/mL and 2.112 mg/mL respectively

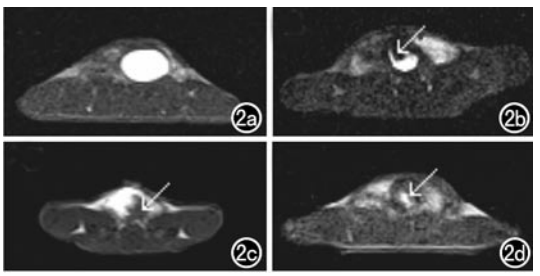

Fig. 2 MRI findings of bladder tumor at different times after inoculation. (a) No apparent abnormal findings on day 7. (b) Small filling defect (arrow) on day 14. (c) The filling defect (arrow) occupied 50% of bladder volume on day 21. (d) Only a little contrast agent (arrow) could be instilled into bladder on day 28

uterus were not tumor invaded, liver and lung were not tumor metastasized (Fig. 3). On day 7, there was not gross mass but thickening of bladder wall, but microscopic observation showed that tumor grew in mucosa or superficial muscle layer. We observed that the tumor formed visible lump in bladder cavity on day 14, filled 50% of the bladder cavity volume on day 21, and almost occupied the whole bladder cavity on day 28. Tumor size on MRI correlated well with actual tumor size seen from gross observation. Microscopic observation confirmed that all the established tumors were stage II–III urological bladder cancer. The tumors infiltrated different muscle layer but not serosa on days 14–28, and invaded serosa membrane 35 days after inoculation.

Discussion

Subcutaneous tumor model was common in oncology research, which was easy to be established and observed. But orthotopic transplantation model was more closely to the microenvironment at the implantation site of the host organ. Orthotopic transplantation model was superior to transgenic mouse and subcutaneous tumor model in tumor metastasis and chemo-sensitivity studies [3]. Intravesical therapy is important for superficial bladder cancer. Therefore, orthotopic bladder cancer model was an essential experimental tool for assessing potential therapeutic agents against bladder cancer.

There were kinds of way to establish orthotopic bladder cancer model, but each had their drawbacks. The techniques of inducing bladder tumors by carcinogens in food or drinking water or by the intravesical instillation of carcinogens have been well established. But tumors require a long period to develop, and tumors can grow anywhere on the bladder surface. Orthotopic implantation of tumor cells by injection directly into the bladder wall yields 100% tumor incidence. However, the tumors were not superficial; they were often covered with a layer of normal or hyperplasic urothelium; there may be tumor implantation in abdominopelvic cavity because of cells leakage. The way instillating human bladder cancer cells into bladder cavity of nude mice usually require chemical or mechanical urothelial lesion. Sometimes, the lesion might brought severe complications for immunodeficient mice. And it need too much handling experience by transurethrally electric coagulation or abrasion. Bisson and his fellows [4] manufactured a special copper abrader, which could be inserted via the PE catheter into the bladder cavity. Push the abrader until the resistance of the posterior bladder wall was felt, then abraded the bladder wall. The instillation of tumor cells into the bladder after urothelial abrasion result in a 99.5% (206/207) successful tumor growth rate. We made a slight improvement to Bisson's technique. Though transurethrally scraped the bladder wall and instilled the tumor cells, we exposed the bladder through a small incision. Therefore the abrasion would be controlled under direct vision; no vesical perforation and cells leakage to abdominopelvic cavity was likely to occur. Meanwhile, the lesion was exact and located, which not only improved success rate but also avoided over damage to bladder wall. The tumors confined in mucosa or superficial layer of muscle at early stage, invaded the muscles and serosa at late stage, which simulated the progression

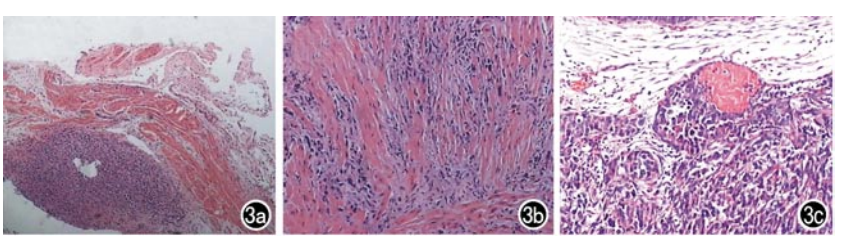

Fig. 3 Pathologic changes of bladder tumor. (a) The tumor grew in mucosa 7 days after inoculation. (HE × 40). (b) The tumor confined in muscle layer 14 to 28 days after inoculation (HE × 100). (c) The tumor invaded serosa 35 days after inoculation (HE × 100)

of human superficial bladder cancer approximately. In conclusion, this model system was reproducible and ideal for the study of intravesical therapy.

Deep in the body, accurate noninvasive assessment of orthotopic bladder tumor was more difficult compared with subcutaneous tumor. At present, we can observe the orthotopic tumor by MRI, ultrasonography, ultrathin cystoscope, and optical in vivo imaging system. But, some of them require special equipment or special handling to tumor cells before implantation. Xiao *et al* [5] utilized MRI to assess murine orthotopic bladder tumor, when keen coil was used. The images were capable of monitoring tumors nominally 2 mm in diameter. Kikuchi *et al* [6] also utilized MRI to detect bladder tumors, with homemade coil and 50 μL Gd-DTPA plus 50 μL air as contrast agent. With that technique small bladder tumors 1.5 mm in diameter as well as larger ones could be easily detected. At early stage (10 and 14 days after tumor inoculation) MRI detected 19 of 22 tumors (86.4%). As available for the majority of institutes, MRI was still of use, though new technique had been developed to observe murine orthotopic bladder tumor [7]. We optimized scanning parameters, and found the proper concentration of contrast agent for this study. MRI reliably revealed "filling defect" 14 days after inoculation, and could detect tumors nominally 0.2 mm (80/384 mm) in diameter. Together with the comprehensive analysis at sagittal and coronal sections, a more accurate location was obtained. So MRI can be used for the early detection and dynamic observation of the murine bladder tumor.

We successfully established an orthotopic model bearing human bladder cancer with high incidence. Tumors grew expansively into the bladder cavity from the surface (mucosa) and gradually invaded the muscles and serosa, which simulated the progression of human bladder cancer approximately. The animal model provided a feasible tool for the study of intravesical therapy. MRI was a reliable way for noninvasive monitoring tumor growth in the murine orthotopic bladder tumor model.

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