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The Spine Journal 13 (2013) 1597–1606

THE SPINE JOURNAL

Basic Science

A rat model of metastatic spinal cord compression using human prostate adenocarcinoma: histopathological and functional analysis

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Received 14 February 2012; revised 28 September 2012; accepted 4 May 2013

Abstract

BACKGROUND CONTEXT: Cancer is a major global public health problem responsible for one in every four deaths in the United States. Prostate cancer alone accounts for 29% of all cancers in men and is the sixth leading cause of death in men. It is estimated that up to 30% of patients with cancer will develop metastatic disease, the spine being one of the most frequently affected sites in patients with prostate cancer.

PURPOSE: To study this condition in a preclinical setting, we have created a novel animal model of human metastatic prostate cancer to the spine and have characterized it histologically, functionally, and via bioluminescence imaging.

STUDY DESIGN: Translational science investigation of animal model of human prostate cancer in the spine.

METHODS: Luciferase-positive human prostate tumor cells PC3 (PC3-Luc) were injected in the flank of athymic male rats. PC3-Luc tumor samples were then implanted into the L5 vertebral body of male athymic rats (5 weeks old). Thirty-two rats were randomized into three surgical groups: experimental, control, and sham. Tumor growth was assessed qualitatively and noninvasively via bioluminescence emission, upon luciferin injection. To determine the functional impact of tumor growth in the spine, rats were evaluated for gait abnormalities during gait locomotion using video-assisted gait analysis. Rats were euthanized 22 days after tumor implantation, and spines were subjected to histopathological analyses.

RESULTS: Twenty days after tumor implantation, the tumor-implanted rats showed distinct signs of gait disturbances: dragging tail, right- or left–hind limb uncoordination, and absence of toe clearance during forward limb movement. At 20 days, all rats experienced tumor growth, evidenced by bioluminescent signal. Locomotion parameters negatively affected in tumor-implanted rats included stride length, velocity, and duration. At necropsy, all spines showed evidence of tumor growth, and the histological analysis found spinal cord compression and peritumoral osteoblastic reaction characteristic of bony prostate tumors. None of the rats in the sham or control groups demonstrated any evidence of bioluminescence signal or signs of gait disturbances.

FDA device/drug status: Not applicable.

Author disclosures: ***RS-E:*** Nothing to disclose. ***PLZ:*** Nothing to disclose. ***CAM:*** Nothing to disclose. ***IJ-E:*** Nothing to disclose. ***MLG:*** Nothing to disclose. ***ZLG:*** Stock Ownership: US Spine (C), Spinal Kinetics (C); Board of Directors: JNS Spine (C), Spine Journal, Journal of Spinal Disorders (C), European Spine Journal (C), Nature Reviews (C), World Neurosurgery (C), Journal of Surgical Oncology (C), US Spine (C); Scientific Advisory Board: NREF (C); Research Support: AO Spine (C), NREF (C); Grants: DePuy (C); Fellowship Support: AO Spine (C), NREF (C). ***AB:*** Research Support: Johnson & Johnson (E, Paid directly to institution/employer. ***TFW:*** Eli Lilly (unknown, Paid directly to institution/employer). ***J-PW:*** Nothing to disclose. ***DMS:*** Speaking/Teaching Arrangements: Globus (B, Paid directly to institution/employer), DePuy (B, Paid directly to institution/employer); Grants: DePuy (F, Paid directly to institution/employer).

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

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1529-9430/$ - see front matter
http://dx.doi.org/10.1016/j.spinee.2013.05.021

CONCLUSIONS: In this project, we have developed a novel animal model of metastatic spine cancer using human prostate cancer cells. Tumor growth, evaluated via bioluminescence and corroborated by histopathological analyses, affected hind limb locomotion in ways that mimic motor deficits present in humans afflicted with metastatic spine disease. Our model represents a reliable method to evaluate the experimental therapeutic approaches of human tumors of the spine in animals. Gait locomotion and bioluminescence analyses can be used as surrogate noninvasive methods to evaluate tumor growth in this model.

Keywords: Metastatic prostate cancer; Spine tumor; Spinal metastasis; Animal model; Animal gait locomotion

Introduction

Cancer is a major public health problem in the United States and many other parts of the world. Twenty-five percent of deaths in the United States are because of cancer. It is expected that in 2012, among men, cancers of the prostate, lungs, and colon will account for about half of all newly diagnosed cancers. Prostate cancer alone will account for 29% of incident cases [1]. In 2008, globally, prostate cancer was the second most frequently diagnosed cancer in men, the fifth most common cancer overall, and the sixth leading cause of death in men [2]. It is estimated that up to 30% of patients with cancer will develop metastatic disease [3–6].

Most prostate cancer–related deaths are because of advancement of systematic disease, which results from local or distant spread via blood and lymphatic flow [7–9]. The skeleton is one of the main sites of metastatic spread, with the bony spine being affected in 65% to 70% of patients with advanced prostate cancer [10–12]. Pelvic tumors metastasize to the axial skeleton as a result of venous reflux into the valveless veins bathing the vertebral bodies [13]. The latter occurring mainly via Batson plexus, which is a common pathway for metastatic embolization to the vertebral column [13–15].

Owing to advancements in late-stage disease treatment, patients with prostate cancer may survive for many years with substantial tumor burden [10–12,16]. Patients suffering from metastatic prostate cancer to the spine may experience radicular or cord compression. The latter presenting as a constellation of symptoms such as disabling pain, motor or autonomic dysfunction, and/or sensory dysfunction [5,17,18].

Virtually all treatments are palliative, and unfortunately, there are no truly curative options for patients with metastatic cancer. Radiation, vertebroplasty, and salvage chemotherapy are often offered to patients with cancer at the end of life to decrease pain, prevent paralysis and severe neurological dysfunction, and offer a few additional months of life [5,18–20]. As a result, targeted therapies for spine-specific tumor spread of prostate cancer are needed. To test therapeutic interventions (eg, surgery, radiation, chemotherapy, hormonal therapy) in the setting of metastatic prostate cancer to the spine, preclinical experiments require the use of reliable animal models that closely represent the human pathology.

In this study, we locally implanted solid human prostate tumors in the lumbar spine of immunosuppressed rats. Local orthotopic inoculation involving direct implantation of cancer cells into the desired site for the metastases overcomes some limitations of systemic administration approaches. In our laboratory, we have previously developed intravertebrally implanted tumor animal models using rat mammary adenocarcinomas [21–24]. In the present study, we have advanced this model to grow human-derived neoplasms. In addition, we have incorporated the use of bioluminescence and gait locomotion analyses as noninvasive methods to evaluate human prostate tumor growth in experimental animals. Noninvasive methods of monitoring tumor growth provide an opportunity to determine a therapeutic window for intervention. Determination of a therapeutic window, in terms of tumor growth, is essential for evaluating future therapeutic interventions.

Materials and methods

Prostate cancer cell line luciferase transduction

Prostate cancer cell line PC3 (CRL-1435) was purchased from the American Type Culture Collection (Manassas, VA, USA). The PC3 cell line was obtained from a Grade IV human prostate adenocarcinoma, collected from the site of bony metastasis. Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C at subconfluent levels in the Roswell Park Memorial Institute medium, supplemented with 10% fetal bovine serum and antibiotics. Cells were passaged every 2 to 3 days. To visualize tumor cell growth in vivo, we used a lentiviral vector containing the codifying sequence of the firefly luciferase and the green fluorescent protein (GFP) genes (generously donated by Linzhao Cheng and Colette Aprhys). Ten thousand PC3 cells were incubated in 1 mL of medium with 2 μL of virus plus polybrene for 24 hours. The GFP expression was evaluated with an Axiovert inverted microscope (Carl Zeiss, Berlin, Germany) connected to a digital camera. Image brightness and contrast were adjusted using Axiovision software (Carl Zeiss). Cell bioluminescence was performed using 1,000 cells/well in 96-well plates, 15-second exposure, and a 5% concentration of luciferin in serum-free media using the IVIS Spectrum Imaging System (Caliper Life