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A novel murine model of human renal cell carcinoma spinal metastasis

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Abstract

There is currently no reproducible animal model of renal cell carcinoma (RCC) spinal metastasis that allows for laboratory study of the human disease. In this report, we describe an animal model that reliably reproduces RCC spinal metastasis using a human tumor cell line. A posterior surgical approach was used to implant tumor cells into the lamina of immunosuppressed mice. Histology sections were analyzed 12 weeks after tumor cell implantation to quantify the location and extent of tumor growth. RCC xenografts grew in treated animals (8 mice) according to a reproducible pattern of growth. After implantation, tumor growth occurred primarily in the antero-posterior dimension. At 8 weeks after tumor cell implantation, there was visible tumor growth in all treated mice. Histologic correlation at 12 weeks after tumor cell implantation confirmed tumor growth involving primarily the paraspinal region and lamina. Our investigation resulted in an orthotopic model of human RCC spinal metastasis. Ultimately this will allow testing of targeted therapies for RCC with spinal involvement. Furthermore, this model can be expanded to develop similar spinal metastasis models for other tumor cell lines.

Keywords

Murine model; Renal cell carcinoma; Spinal metastasis; Tumor

1. Introduction

Approximately 1.2 million individuals are diagnosed with cancer each year in the United States. Of those, about 5% will develop symptomatic spinal metastases. Symptoms may manifest as subtle pain or numbness but can also lead to severe neurologic compromise from spinal cord compression. Reports of spinal cord compression secondary to spinal metastasis exceed 20,000 cases per year in the United States.¹ Of the malignancies that metastasize to the spine, renal cell carcinoma (RCC) is particularly debilitating. About one-third of patients with metastatic RCC develop skeletal complications. They are relatively radiotherapy- and chemotherapy-resistant.^{2, 3}

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Disclosures/Conflict of interest

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Surgery and/or radiation are the mainstays of treatment for patients with RCC spinal metastasis. Unfortunately, local recurrence is common, and therapeutic options after recurrence are often limited. Clearly, there is an urgent need to identify methods to prevent tumor growth and provide more effective treatment. However, very little is currently known about the molecular mechanisms that lead to skeletal metastases in RCC.³ It has been suggested that the pathways involved in RCC bone metastasis are not the same as those identified in other cancers, such as in breast and prostate cancers.⁴ The lack of adequate animal models for RCC spinal metastasis has hindered advances in elucidating the pathophysiology of this disease and the development of effective treatment algorithms.

To study RCC, a suitable animal model should be developed from human cancer cells. The model should also demonstrate a reproducible pattern of growth. In this report, we describe the development of an animal model for human RCC by subcutaneous xenograft over the posterior spine. Our simplified surgical approach reproducibly creates spinal metastases. This model can be used to further study the pathophysiology and natural history of RCC spinal metastases.

2. Materials and methods

All animal studies were performed according to the protocol approved by the Institutional Animal Care and Use Committee of the University of Michigan.

2.1. Cell culture

The human renal carcinoma cell line, ACHN, was purchased from American Type Culture Collection (Manassas, VA, USA) and was cultured in Dulbecco's Modified Eagle Medium (DMEM)/F12 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco) in a humidified atmosphere of 5% carbon dioxide (CO₂) in air at 37°C, and used when in the log phase of growth.

2.2. Development of a subcutaneous xenograft of human renal carcinoma cells

Monolayers of ACHN cells were detached using 0.25% Trypsin-ethylenediaminetetraacetic acid (EDTA) (Gibco), washed, and suspended in 200 µL of serum-free **Hanks' Balanced Salt Solution** (HBSS)/Matrigel (BD Biosciences, San Jose, CA, USA) mixture (1:1 volume) at a concentration of 10⁷ cells per 100 µL. The cells were then injected subcutaneously into the lower abdominal area of NOD/SCID mice (Harlan Laboratories, Indianapolis, IN, USA). Approximately 10 to 14 days after injection, subcutaneous tumors were harvested by dissection, placed in a culture dish with DMEM/F12/10% FBS, and maintained on ice until implanted in the experimental animals.

2.3. Animal preparation

A total of 15 six-week to eight-week-old NOD/SCID mice were used for this experiment. Animals were randomized into two groups: 12 mice in the treated group underwent surgery and implantation of a small piece of the ACHN xenografted tumor, and three mice in the control group underwent a sham surgery without tumor implantation.

2.4. Development of spinal tumor

Animals were anesthetized and placed prone on a heating pad with their limbs affixed to the pad with tape. The back was shaved and prepared with solution (Triad Disposables, Brookfield, WI, USA). Subsequent surgery was performed by two surgeons using an operating microscope. A 1.5-cm skin incision was made using a scalpel blade, the underlying muscles were exposed, and a small superficial incision was made between the

muscles and extended deeper until the spinous process and lamina were identified. The muscles were stripped laterally to expose the lamina. Using a 1-mm bur attached to a hand drill, the spinous process and lamina were decorticated. A piece of the previously harvested tumor (~0.3 mm × 0.3 mm) was placed onto the decorticated spinous process and lamina in the treated mice. Nothing was placed on the decorticated lamina of the control mice. The muscles and the skin were closed separately using a running suture (Ethicon, Somerville, NJ, USA). A hand-held cautery device was used for hemostasis during surgery.

2.5. Tissue processing

At 12 weeks after tumor cell implantation, the animals were euthanized. The spines were harvested, fixed in 10% formalin for 24 hours, and then placed in decalcifying solution for one to two days. When the bone was soft, the tissues were embedded in paraffin, sectioned at a thickness of 7 µm, and stained with hematoxylin and eosin.

3. Results

Of the 12 mice in the treated group, four suffered a neurologic injury during surgery and were paraplegic postoperatively. All four mice subsequently expired and all underwent autopsy with the finding of an enlarged, presumably neurogenic, bladder. No complications were noted in the control mice. Of the eight surviving treated mice, all were noted to have tumor growth as evidenced by a visible dorsal mass by eight weeks after tumor implantation (Fig. 1). No growth was observed in the control mice.

3.1. Histological examination

Histological analyses were performed on euthanized animals 12 weeks after tumor cell implantation (Figs 2, 3). Examination of cross-sections from each mouse spine showed large paraspinal tumors with involvement of the adjacent lamina and spinous process (Fig. 4). The tumors exhibited cellular atypia, and cells could be seen infiltrating the venous foramina that connect the marrow to the epidural venous plexus. Encroachment into the epidural space was evident (Fig. 5).

4. Discussion

The biologic behavior and response to therapy by spinal metastases varies depending on the type of primary tumor. Currently there is no reproducible animal model for human RCC. In this report, we describe a mouse model of RCC spinal metastasis derived from human cancer cells. Specifically, we show that xenografts of human RCC can be established in the posterior spine of mice, and that these xenografts grow in predictable patterns. This model is valuable because it represents an orthotopic model of the human disease, and thus can be used to evaluate novel therapeutic approaches developed specifically for RCC spinal metastases.

Arguello et al. and Strube et al.⁵⁻⁷ established a nude mouse model that relied on intracardiac injection of tumor cells to create spinal metastases. This model had the advantage of using human tumor cells that mimicked the pathophysiology of metastasized cancer. A significant disadvantage, however, was that this model lacked predictability, since the location and the number of metastases were highly variable. This variability makes it difficult to test therapeutic interventions. In contrast, Mantha et al.⁸ directly implanted tumors into the lumbar vertebral body of rats, thereby establishing a predictable tumor location. Unfortunately these investigators used tumors derived from a rat breast cancer cell line. It has been well-established that the pathophysiology of tumors can vary between

different species. Therefore, this model may not be applicable in developing therapies for human cancers.

Recently, Tatsui et al.⁹ developed an orthotopic murine model of lung spinal metastasis that addressed the shortcomings of previous models. In their model, Tasui et al. surgically implanted cells derived from human lung cancer into the L3 vertebral body of immunocompromised nude mice. Their surgical implantation was performed via an anterior transperitoneal exposure, taking care to preserve the iliopsoas and nearby vasculature. In their initial cohort, they experienced a large percentage of immediate postoperative paraplegia, likely from over-manipulation of the iliopsoas or peripheral nerve injury. Although further studies minimized this postoperative morbidity, they emphasized the skill necessary for that microsurgical exposure.

Unlike the anterior transperitoneal approach for tumor implantation, we hypothesized that a posterior approach would be less technically demanding. Since a posterior approach to establish an animal spinal metastasis model has not been previously described, we designed a straightforward procedure to replicate the equivalent clinical condition in which a patient develops a posterior spinal metastasis. Although we also noted neurologic injury resulting in paraplegia in the initially treated mice, the learning curve appeared short, and subsequent surgeries were uncomplicated. Tumor growth was consistent in all treated mice, suggesting that this model can reliably replicate a posterior spinal metastasis. Another advantage of this posterior spinal tumor model is that potential therapeutic interventions can be more easily tested compared to a vertebral body tumor, which is less accessible. Although not yet studied, this posterior spinal tumor model could also be used to study other metastatic or even primary bone tumors.

5. Conclusion

In this study, we describe a novel posterior approach for tumor implantation that results in a consistently reproducible mouse model for human RCC spinal metastases. Ultimately, this model may be utilized to develop or test therapies for human RCC with spinal involvement.

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Figure 1.
Photograph of treated mouse eight weeks after tumor cell implantation, showing visible growth of tumor.

Figure 2.
Photograph of sacrificed mouse 12 weeks after tumor cell implantation, showing gross tumor attached to posterior spine.

Figure 3.
Photograph of harvested spine with attached tumor.

Figure 4. Axial cross-section photomicrograph showing posterior tumor (asterisks) with infiltration of the lamina on the left (hematoxylin and eosin staining, $\times 40$).

Figure 5.
Axial cross-section photomicrograph showing tumor (asterisk) growing into the epidural space (hematoxylin and eosin staining, $\times 40$).