Effects of primary and recurrent sacral chordoma on the motor and nociceptive function of hindlimbs in rats: an orthotopic spine model

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OBJECTIVE Chordoma is a slow-growing, locally aggressive cancer that is minimally responsive to conventional chemotherapy and radiotherapy and has high local recurrence rates after resection. Currently, there are no rodent models of spinal chordoma. In the present study, the authors sought to develop and characterize an orthotopic model of human chordoma in an immunocompromised rat.

METHODS Thirty-four immunocompromised rats were randomly allocated to 4 study groups; 22 of the 34 rats were engrafted in the lumbar spine with human chordoma. The groups were as follows: UCH1 tumor-engrafted (n = 11), JHC7 tumor-engrafted (n = 11), sham surgery (n = 6), and intact control (n = 6) rats. Neurological impairment of rats due to tumor growth was evaluated using open field and locomotion gait analysis; pain response was evaluated using mechanical or thermal paw stimulation. Cone beam CT (CBCT), MRI, and nanoScan PET/CT were performed to evaluate bony changes due to tumor growth. On Day 550, rats were killed and spines were processed for H & E–based histological examination and immunohistochemistry for brachyury, S100 β , and cytokeratin.

RESULTS The spine tumors displayed typical chordoma morphology, that is, physaliferous cells filled with vacuolated cytoplasm of mucoid matrix. Brachyury immunoreactivity was confirmed by immunostaining, in which samples from tumor-engrafted rats showed a strong nuclear signal. Sclerotic lesions in the vertebral body of rats in the UCH1 and JHC7 groups were observed on CBCT. Tumor growth was confirmed using contrast-enhanced MRI. In UCH1 rats, large tumors were observed growing from the vertebral body. JHC7 chordoma-engrafted rats showed smaller tumors confined to the bone periphery compared with UCH1 chordoma-engrafted rats. Locomotion analysis showed a disruption in the normal gait pattern, with an increase in the step length and duration of the gait in tumor-engrafted rats. The distance traveled and the speed of rats in the open field test was significantly reduced in the UCH1 and JHC7 tumor-engrafted rats compared with controls. Nociceptive response to a mechanical stimulus showed a significant ($p < 0.001$) increase in the paw withdrawal threshold (mechanical hypalgesia). In contrast, the paw withdrawal response to a thermal stimulus decreased significantly ($p < 0.05$) in tumor-engrafted rats.

CONCLUSIONS The authors developed an orthotopic human chordoma model in rats. Rats were followed for 550 days using imaging techniques, including MRI, CBCT, and nanoScan PET/CT, to evaluate lesion progression and bony integrity. Nociceptive evaluations and locomotion analysis were performed during follow-up. This model reproduces cardinal signs, such as locomotor and sensory deficits, similar to those observed clinically in human patients. To the authors'

ABBREVIATIONS CBCT = cone beam computed tomography.

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knowledge, this is the first spine rodent model of human chordoma. Its use and further study will be essential for pathophysiology research and the development of new therapeutic strategies.

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KEY WORDS chordoma; spine; tumor; model; locomotion; nociception; rat; oncology

CHORDOMA is a tumor of the spinal column with an incidence of 0.08 cases per 100,000 people and a median survival of 6.29 years.²⁷ These characteristically slow-growing and locally invasive tumors most commonly present in the sacrococcygeal region (50%–60%), followed by the clivus/spheno-occipital region (15%–35%) and mobile spine (15%).^{7,42} Clival chordomas tend to cluster in a younger population, whereas sacrococcygeal chordomas present more frequently in older adults.^{16,29,50} The clinical presentation of patients suffering from chordoma is dependent on tumor location, and pain is the most common presenting symptom.²⁰ Patients with clival chordoma frequently present with cranial nerve palsies and headaches, whereas patients with sacral chordoma commonly present with sacral or flank pain and gait disturbances.^{29,50}

Chordomas seldom metastasize, but when they do, the distal lesions occur late in the course of the disease (rates of chordoma metastasis reported in the literature range from 5% to 43%).^{7,11,28,48,51} The current standard of care includes a combination of resection and radiotherapy.^{13,14,33} However, due to the aggressive pattern of local invasion and risks to adjacent vital structures, complete tumor resection is balanced against the potential risks of neurological deficits and the potential for a high incidence of recurrence.²⁷ The major cause of death in patients with chordoma is associated with local aggressiveness.²

Despite the evolution of surgical techniques and advancements in medical and radiotherapies, the development of therapeutic strategies to combat this disease is limited by the availability of orthotopic in vivo models that recapitulate the histological and clinical features of chordoma. In 2014, Burger et al. developed a novel zebrafish model of chordoma driven by notochordal-specific green fluorescent protein–tagged HRASV12 expression. Their model is histologically comparable to human chordoma and has a rapid onset, which is well suited for preliminary drug screening.⁶ However, development of a clinically relevant spine chordoma model to study the surgical and clinical effects on this tumor in a human xenograft and mammalian-based system would increase our understanding of the biology of these rare malignancies and allow for the translation of potential therapies to human patients. Thus, it is of utmost interest to develop a tumor model system that closely resembles the etiology and pathophysiology of the disease in patients.

To this end, we present an orthotopic human chordoma rat model that replicates the histological and clinical features present in patients. We used 2 patient-derived primary and recurrent chordoma cell lines (UCH1 and JHC7, respectively) to characterize the development of intraspinal chordomas in rats. Following tumor implantation, imaging studies and neurological evaluation were conducted to assess nervous system dysfunction in the form of pain responses and behavioral changes. Our study will allow

researchers to test experimental therapy strategies in a clinically relevant system, thus providing predictive models of treatment efficacy.

Methods

Animal Procedure

All procedures were performed according to the *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee (Johns Hopkins University). Forty adult female, 5-week-old, immunocompromised rats (Cr:NIH-RNU, Charles River Laboratories) were maintained in standard cages in groups of 2 or 3 in a controlled colony room, under standard environmental conditions, with free access to food and water. The animals were randomly allocated to different experimental groups.

Human Chordoma Cell Lines

We used 2 primary-cultured human chordoma cell lines: UCH1 and JHC7. UCH1 is a human chordoma cell line established from a recurrent sacral chordoma (ATCC). UCH1 cells were cultured and expanded using Iscove's (Roswell Park Memorial Institute medium [4:1] + 10% fetal bovine serum) with additional 1% L-glutamine.³⁹ The JHC7 cell line was derived from clinical specimens obtained from a female patient with primary sacral chordoma who had no history of treatment for oncological disease.¹⁹ The JHC7 chordoma cell line was maintained using MesenPRO RS medium + MesenPRO RS Supplement (Thermo Fisher Scientific), GlutaMAX 1x, and antibiotic and antimycotic 1x. Cells were maintained in a humidity incubator in an atmosphere of 5% CO₂ at 37°C.¹⁹

Xenograft Establishment

To establish a subcutaneous chordoma, JHC7 or UCH1 cells were trypsinized and centrifuged at 180g for 5 minutes at 4°C. Cells were counted and 6×10^6 cells were resuspended in their respective media mixed with Growth Factor Reduced Matrigel (Corning) at a 2:1 ratio, to a final volume of 200 μ l. The cell suspension was injected subcutaneously into the flank of anesthetized rats ($n = 3$ for each cell line, Fig. 1A). Rats were maintained in vivarium conditions until a detectable tumor was appreciated (approximately 6–8 months).

Establishment of Chordoma in the Lumbar Spine

After flank tumors were established, the lesion was excised and either partially processed for histopathological analysis or used to establish the orthotopic chordoma model. To establish an orthotopic chordoma model in the lumbar spine, 34 female immunocompromised rats (5 weeks old) were randomly allocated into 4 surgical groups: 1) UCH1 tumor–engrafted ($n = 11$), 2) JHC7 tu-

FIG. 1. Human orthotopic chordoma model in the spine of immunocompromised rats. **A:** UCH1 or JHC7 cells were injected in the flank of 5-week-old female rats. After 6–8 months, subcutaneous tumors showed a multilobulated morphology, and physaliferous cells were observed between the tissues in the flank. H & E sections, original magnification $\times 10$; calibration bars = 20 μm . **B:** Illustrations of the engrafting procedure in the L-5 vertebral body with subcutaneous tumor tissue from donor rats. **C:** After 540 days from tumor engrafting, chordomas invaded the vertebral body in the UCH1 and JHC7 rats. Bone tissue with tumor invasion showed the typical physaliferous cells, with intracytoplasmic vacuolization in the H & E sections (original magnification $\times 20$; calibration bars = 0.02 μm). Figure is available in color online only.

mor–engrafted ($n = 11$), 3) sham surgery ($n = 6$), and 4) naïve control ($n = 6$) rats. We performed an abdominal surgical approach to the L-5 vertebral body as previously described.²⁶ Briefly, while the rat was under complete anesthesia, the L-5 vertebral body was drilled 0.5 mm superior to the intervertebral disc to create a cavity for tumor implantation. Tumor samples (0.1 cm^3), obtained from the subcutaneous chordoma xenografts, were deposited in the cavity and then sealed with Geristore dual-cure resin ionomer polymer (DenMat) (Fig. 1B). The sham surgery group underwent an identical surgical approach with the exception that no tumor was implanted. The abdominal cavity was closed, and animals were maintained in vivarium conditions. Interestingly, when a chordoma cell suspension in Matrigel/serum-free media solution was injected directly into the vertebral body, no tumor growth was detected 8 months after implantation (data not shown).

Behavioral Tests

Motor and nociceptive functions were evaluated at Days 530 and 535, respectively, after tumor implantation to determine the effects of the chordoma growth at

a functional level. For all behavioral evaluations, the rats were gently handled for 5 minutes during a 5-day period to minimize stress. All of the behavioral tests were performed in a quiet, red-illuminated room and were done between 1200 and 1700 hours by a blinded researcher to avoid bias in the results.

Kinematic Analysis of Gait Locomotion

Rat locomotion was video recorded and evaluated as previously described.^{24,38} Briefly, each rat was trained to walk in a narrow Plexiglas runway to ensure locomotion in a straight line. Rats' hip, knee, and ankle (that is, lateral malleolus) joints were marked. Unrestrained gait of non-tumor and tumor-engrafted rats was recorded at 30 frames per second. We recorded 3- to 4-stride cycles per rat. Video analysis was performed using ImageJ software (<http://rsb.info.nih.gov/ij/>; National Institutes of Health) followed by our proprietary Walking Rats Software (developed by the IJ-E Laboratory).

Joint angles, stride length, duration, and flexion-extension of each articulation were determined. Swing and stance phases, as well as the hip-knee-ankle pendulum-like movement of each step cycle, were identified as described previously.³⁶ Briefly, the marked points in the rat's hindlimb were tracked frame by frame, obtaining 2D coordinates (x and y) using ImageJ software. Next, data were imported into Microsoft Office Excel and further analyzed with preassembled Excel sheets to model body segments as rigid straight lines between the marked points.

The kinematics of gait was then reconstructed from changes in the marked points located between consecutive frames, facilitating the generation of stick diagrams (superimposing modeled body segments of every frame) and spatial displacement plots. Angles and distances could be calculated directly by the software. Optical deformation of the image produced by the camera lens was determined and corrected by using an acrylic square (5 $\times$ 5 cm), which served as a bidimensional scale.³⁴

Open Field Test for Motor Behavior

To evaluate free rat movement, we used an OF-3C (Bioseb) with a previously published method of open field analysis.³⁴ The open field test was performed in a square (length 1000 mm/width 1000 mm/height 400 mm) arena with gray acrylic walls. Rats began the test at the center of the arena and were given 5 minutes to freely explore. The total distance traveled (cm) and the mean speed (cm/sec) in the arena were automatically registered.

Mechanical Paw Withdrawal Threshold Test

Nociceptive response was evaluated using the rodent pincher analgesia meter (Part # 2450, IITC Life Science, Inc.) to apply pressure on the left hind paw. The system measures and displays the amount of force (in grams) that has been applied to the rat's paw.⁴⁷ Response to the pressure applied (in grams) was manually registered upon paw withdrawal from stimulus. Pressure was increased by 5 gram/sec increments and measurements were performed in triplicates. Researchers performing the test in all groups were blinded to avoid experimenter bias.

Thermal Paw Withdrawal Latency Test

To evaluate the magnitude of thermal hyperalgesia, the thermal plantar (Hargreaves method) testing device was used to measure hind paw withdrawal latency in response to radiant heat (Plantar Test Instrument, Ugo Basile), as described elsewhere.¹⁷ Briefly, each rat was placed in a transparent Plexiglas chamber and given 5 minutes to habituate. An infrared heat source was positioned under the glass floor and directly inferior to the plantar surface of the hind paw. The infrared heat source (50°C) was subsequently activated with an automatic timer for rat reaction. Rat paw withdrawal deactivated the infrared source and the duration of stimulus was automatically recorded. A predetermined cutoff was established at 20 seconds to prevent tissue damage. The average withdrawal latencies for the left hind paw were determined from the average of 3 trials separated by a 10-minute interval to prevent thermal sensitization.

In Vivo Imaging of Spine Chordoma

Rats engrafted with chordoma into the spines were imaged by cone beam computed tomography (CBCT) at Day 450 after tumor implantation, using a small animal research radiation platform (Xstrahl Life Sciences) as previously described.^{49,52} Images were acquired at 65 kVp and 0.7 mA using a 20 × 20-cm beam. CBCT images were used to identify bone lesions in the UCH1 and JHC7 rats after tumor engraftment. Contrast-enhanced MRI was performed on Day 540. Live rats in the experimental groups (UCH1 and JHC7) were administered gadopentetate dimeglumine (0.1 mmol/kg intramuscular, Magnevist). Using a 9.4-T MRI animal scanner (BioSpec Bruker Series, Bruker Corporation), T1- and T2-weighted sequences were obtained before and after contrast administration. On Day 545, tumor-engrafted rats (1 rat per experimental group) underwent nanoScan PET/CT within a sealed biocontainment device (Minerve) as described previously³² and then were imaged using a small animal imager (Mediso). Images were reconstructed and visualized using VivoQuant 2.50 (inviCRO) and 3D images were obtained from contoured 2D images.

Histopathological Analysis

Rats engrafted with chordoma cells into the spine were killed on Day 550 after tumor engrafting. Rats were intracardially perfused with 4% paraformaldehyde; then spines were harvested and postfixed for 12 hours. After fixation, spines were decalcified with hydrochloric acid for 8 hours. A second dissection was made to localize the area where the tumor was engrafted. The segments were then processed for dehydration, clearing, and infiltration with paraffin. Axial sections of tissue (10 µm thick) were obtained and stained with H & E. An independent blinded pathologist evaluated the tissue characteristics.

Immunostaining Procedures

A separate group of tissue sections (10 µm thick) were immunostained for brachyury (Santa Cruz Biotechnology, C19, sc-17745, dilution 1:50), S100β (Abcam, ab11178, dilution 1:500), and cytokeratin (Keratin-Pan AE1/AE3,

Thermo Scientific, MS-343-P, dilution 1:100), as described previously.¹⁹ Biotinylated secondary antibodies and streptavidin-conjugated horseradish peroxidase were used to visualize the label. The chromogen 3′3-diaminobenzidine was used to visualize immunoreactivity. An independent blinded pathologist analyzed the stained sections.

Statistical Analysis

Locomotion and nociceptive parameters were evaluated using a nonparametric 1-way ANOVA, followed by a Dunnett post hoc test. Statistical significance was determined using GraphPad Prism version 6.0 software. The alpha value was set at 0.05. Results are reported as the mean ± SEM.

Results

Xenograft Characteristics in the Flank

Six months after the subcutaneous injection of UCH1 and JHC7 chordoma cells in the flank, rats developed small oval-shaped tumors that reached 5.69 cm³ and 2.86 cm³, respectively. Upon gross macroscopic evaluation, these tumors showed a lobular growth pattern with a peripheral pseudocapsule. Cross-sectional examination showed pale fibrous or fleshy areas mixed with mucinous matrix. Histologically, H & E-stained tumor sections showed clusters of small round nuclei with large, partly vacuolated—appearing physaliferous cytoplasm, consistent with the classically described histology of chordoma and phenotypically similar to the histopathology of the original patient tumor (Fig. 1A).

Growth of Human-Derived Chordoma in the Vertebral Body of Rats

Examination of all rats that underwent tumor implantation showed tumor growth in 54.54% of UCH1 (recurrent sacral chordoma) and 81% of JHC7 (primary sacral chordoma) at 550 days postimplantation. Macroscopically, UCH1-engrafted rats developed large tumor masses emerging from the vertebral body into the abdominal cavity, whereas JHC7 tumors were comparatively smaller with similar distribution. Tumors did not invade other surrounding soft tissues or organs. Bladder and bowel functions were conserved until the animals were euthanized. After gross sectioning, we observed tumor anatomy similar to that described for the subcutaneous tumors, with a pseudocapsulated lobular pattern and mixed fibrous and fleshy areas. In the vertebral body, tumor was localized to the site of implantation (Fig. 2A and B). The adjacent bone was noted to be fragile and soft. We observed that a single vertebral body was affected by the chordoma in both groups.

Histopathological analysis revealed cords and strands of tumor cells in an abundant myxoid matrix with mild atypia. Small areas of the vertebral body were composed of sheets of adipocyte-like cells, without a nodular growth pattern or myxoid background. Analysis of the vertebral bodies demonstrated proliferation of adipocyte-like vacuolated cells between thickened trabecular frameworks (Fig. 2C) with intercellular myxoid matrix, nuclear atypia, and proliferation of physaliferous cells with a prominent

FIG. 2. Histopathology of UCH1 and JHC7 spine chordomas. **A:** Macroscopic morphology of 1 representative rat each from the UCH1, JHC7, and control groups. Gross morphology examination of the L-5 vertebra showed a pink-yellow tissue encapsulating the L-5 vertebral body in the tumor-implanted UCH1 (blue solid line) and JHC7 (yellow solid line) rats. The UCH1 rat had a big mass emerging from the vertebral body, and the JHC7 rat presented with a small bone overgrowth in the vertebral body. **B:** Histological examination (H & E sections, original magnification $\times 2.5$) of the vertebrae axial sections confirmed chordoma (red square) emerging from the vertebral bodies without spinal cord involvement in the UCH1 tumor-engrafted rats (blue square). JHC7 rats presented with a small extradural chordoma (red square) and a tumor emerging from the vertebral body (blue square). No histopathological changes were observed in the control rats. **C:** H & E stain of the chordoma samples showed physaliferous cells (white arrows) with intracytoplasmic vacuolization in the spine chordomas formed by UCH1 and JHC7 cell lines, strong nuclear immunoreactivity for brachyury, and positive immunoreactivity for S100 β . Cytokeratins were observed in the UCH1 and JHC7 tumor-implanted groups. Original magnification $\times 63$; calibration bars = 0.01 mm. Figure is available in color online only.

intracellular eosinophilic matrix, consistent with tumor infiltration. Tumor cells were positive for brachyury, cytokeratin, and S100 β , as demonstrated by immunohistochemistry. Prominent nuclear immunoreactivity of brachyury, as well as cytoplasmic expression of cytokeratin, was observed.

Imaging Features of Orthotopic Human Chordoma in Rats

CBCT for UCH1- and JHC7-bearing rats demonstrated the presence of chordomas in the spine. All rats that developed intraspinal tumors also developed a kyphotic deformity in the thoracolumbar spine (Fig. 3A and B). Lesions were identified in the L-5 vertebral body of tumor-

engrafted rats. Trabecular sclerosis and osteolysis, which are 2 radiological findings commonly observed in human patients suffering from chordoma, were present in the adjacent vertebral bodies (Fig. 3B and G, black asterisks). No imaging alterations were observed in the naïve control and sham groups.

Imaging of soft tissues with contrast-enhanced MRI showed contrast enhancement in both the UCH1 and JHC7 groups. There was a significant extension of the tumor mass into the paravertebral space (Fig. 3C and H) and compression of the spinal cord. In the UCH1 group, the scan showed a heterogeneous enhancement of the tumor mass, with high signal intensity emerging from the verte-

FIG. 3. Imaging of UCH1 and JHC7 spine chordomas. **A and F:** Sagittal views of the CBCT scans acquired 450 days after engraftment show tumor growth at the L-5 level in rats implanted with UCH1 and JHC7. Lesions were identified in the L-5 vertebral body (black arrows) of tumor-engrafted rats. **B and G:** Axial scans show osteolytic lesions in the vertebral body (black asterisks) in both groups. The polymer used to seal the bone cavity after tumor implantation is visible below the vertebral body as a big white solid mass. **C and H:** Axial T2-weighted MR images show a large lumbar UCH1 chordoma (C) arising from the L-5 vertebral body (T) and compressing the nerve roots without spinal cord compression in the UCH1 group. For the JHC7 chordoma (H) arising from the L-5 vertebral body, the image demonstrates that the mass extends toward the lateral spinous process (T); there is a significant extension of the mass that is deforming the spinal cord and compressing the dorsal nerve roots. **D and E, I and J:** 3D reconstructions of the nanoScan PET/CT imaging performed 545 days after engraftment. Anterior (D and I) and lateral (E and J) views of the affected L-5 lateral spinous process and vertebral bodies show bone damage due to tumor osteolysis in the UCH1 vertebral body (white asterisks) and bone overgrowth in the JHC7 vertebral body (white asterisks). Sc = spinal cord; Sp = spinous process; T = tumor.

bral body of L-5 in a honeycomb pattern (Fig. 3B, black asterisk). In the JHC7 tumor-engrafted rats, nanoScan PET/CT (Fig. 3D, E, I, and J) showed an increase in bone formation on the periphery of the L-5 vertebral body (Fig. 3I and J, white asterisks). The UCH1 tumor-engrafted rats showed lytic lesions in the vertebral body and spinous processes (Fig. 3D and E, white asterisks).

Human Orthotopic Chordoma Growth Affected Locomotion, Allodynia, and Thermal Sensation in Rats

After confirmation of the presence of tumors in the vertebral body, motor function was evaluated on Day 530 in tumor-engrafted rats. Kinematic analysis of unrestrained gait at the end of the study showed gait disturbances in the hindlimbs in both groups of tumor-engrafted rats compared with rats in the sham and naïve groups (Fig. 4A–F).

The step length and duration were increased in UCH1 tumor-engrafted rats by approximately 327.27% and 190%, respectively. JHC7 tumor-engrafted rats showed similar significant increases of 381.81% and 153.33% ($p < 0.05$) in step length and duration, respectively (Fig. 4C and D). In addition, a significant difference ($p = 0.0012$) was observed in the ankle angle of UCH1 rats during unrestrained gait locomotion (Supplementary Fig. 1). The pendulum-like leg movements during gait strides decreased significantly ($p < 0.001$) during the flexion (69.81%) and extension (92.45%) phases of UCH1 tumor-engrafted rats,

but no significant changes were observed in JHC7 tumor-engrafted rats (Fig. 4E). Additionally, tumor-engrafted rats in both groups had a significant increase ($p < 0.001$) in the raising (UCH1 357.14% and JHC7 410.71%) and return (UCH1 440% and JHC7 460%) phases of the hindlimb (Fig. 4F).

In the open field test, it was observed that during the 5-minute period of analysis, tumor-engrafted rats had a significant ($p < 0.05$) decrease in exploratory behavior. The total distance traveled by the UCH1 tumor-engrafted rats was decreased by 61% and 66% and the total distance traveled by the JHC7 group was decreased by 23% and 31% compared with the control and sham groups, respectively (Fig. 5A). Similar results were observed in the total mean speed for the UCH1 rats (decreased by 68% compared with the control group and 69% compared with the sham group); JHC7-engrafted rats showed similar reductions in the total mean speed of 69% and 72% compared with the control and sham groups, respectively (Fig. 5B).

Furthermore, the level of algesia in response to mechanical and thermal stimuli was evaluated in the tumor-engrafted, control, and sham rats. During mechanical stimulation of paw withdrawal, a significant increase in the force needed to induce a response from the tumor-engrafted rats compared with control rats was observed (UCH1 198% and JHC7 203%; $p < 0.001$). These results could indicate that tumor-engrafted rats have mechanical

FIG. 4. Effects of human orthotopic chordoma in rats on hindlimb gait locomotion. **A:** Anatomical reference points (1 = iliac crest; 2 = great trochanter; 3 = knee; 4 = lateral malleolus; 5 = metatarsal-phalanx joint) used in the left hindlimb joints to evaluate kinematic parameters and pendulum-like movements (P). **B:** The sequences of the hindlimb movements are shown in stick figures. Each panel represents 3 consecutive stride cycles in a graphic representation from the control, sham, UCH1, and JHC7 groups. The groups suffered from extended periods of stance (white) and swing (black) phases. **C and D:** Step length and step duration were evaluated on Day 530 in UCH1 and JHC7 tumor-engrafted rats. Data are presented as the mean $\pm$ SEM; * $p < 0.05$. Changes in the pendulum-like movement (**E**) and in the raising (Rs) and return (Rt) of the left hindlimb (**F**) occurred during flexion and extension in the unrestrained gait of rats. Data are presented as the mean $\pm$ SEM; * $p < 0.0001$. Figure is available in color online only.

hypoalgesia compared with control rats (Fig. 5C). In contrast, tumor-engrafted rats showed a faster response (paw withdrawal) to a constant radiant heat stimulus (50°C) than control rats (UCH1 144% and JHC7 152%), indicating thermal hyperalgesia (Fig. 5D). Sham rats showed no significant differences compared with naïve controls.

Discussion

The devastating nature of chordomas and our poor understanding of the biology necessitate the establishment of a reliable and clinically suitable animal model. The only orthotopic animal model of chordoma that has been reported uses zebrafish;^{6,12} however, the use of a nonmammalian system is not ideal for representing the complexity of

human disease. Other subcutaneous xenograft chordoma models derived from human tissues have been used to produce rodent tumors; however, they do not replicate the human disease in terms of neurological function, largely due to the ectopic location of tumors (hence a lack of influence on nociception and locomotion of the gait).^{9,19,21,23,40} In this study, we sought to develop and evaluate an orthotopic animal model involving human-derived chordoma growing in the L-5 vertebral body of immunocompromised rats. With progressive growth, these tumors affected the locomotion and nociceptive behavior of rats as well as the bone anatomy, based on imaging evidence. Thus, this model clinically and radiographically represents the human condition of spinal chordoma.

FIG. 5. Effects of intraspinal human chordoma on locomotion and nociceptive response in tumor-engrafted rats. Traveled distance (cm) (A) and mean speed (cm/sec) (B) were reduced in rats engrafted with chordoma. Changes in the nociceptive response due to a mechanical (C) and thermal (D) stimulus showed opposite effects of intraspinal chordoma. Data are presented as the mean $\pm$ SEM; * $p < 0.05$. Figure is available in color online only.

Tumor Growth

Chordomas are thought to arise from remnants of the primitive notochord⁴⁶ and are known to be locally aggressive and relatively unresponsive to chemotherapy and radiation.^{4,11,13,33} They exhibit highly infiltrative growth and are often lobulated by septa of connective tissue. In our preclinical model, an overall 68% of tumor-engrafted rats developed chordoma in the L-5 vertebral body within approximately 1.5 years, mimicking the slow-growth tumor pattern observed in patients with chordoma. The vertebral body and the lateral spinous processes in some of the UCH1 and JHC7 rats showed lytic lesions, as demonstrated by CBCT and the reconstructed nanoScan PET/CT images (Fig. 3). No other vertebral bodies were found to be affected. This is consistent with clinical reports where chordomas invade and destroy a single vertebral body without affecting the surrounding ones.¹⁰ Two rats in the UCH1 group presented a very large tumor with soft-tissue involvement. This is consistent with the description of the pathology of recurrent chordomas, in which these malignancies infiltrate muscle and soft tissue, especially in cases of tumors that have received radiotherapy.⁴⁴

Delank et al.¹⁰ reported that the clinical symptomatology of human spinal chordoma is determined by the location and expansion of the tumor as well as the tumor's relation to adjacent anatomical structures. The origin of the tumor is centrally based in the vertebral body, which is possibly explained by the embryological development of the notochord. The tumor location and characteristics are responsible for the fact that there often is a long latency between the development of the tumor and the diagnosis in spinal chordomas (an average of 1.5 years).^{10,35}

These findings are consistent and accurate with our chordoma model, where we engrafted the tumor in a central location. The tumor from the 2 chordoma cell lines grew and infiltrated the bony tissue in approximately 1.5 years. Spinal chordomas often spread through the bloodstream,⁵¹ with distant metastatic rates differing based on location. The most common sites of metastases are lung and lymph nodes. Liver and bone metastases are also frequent.^{28,37,45,51} In our UCH1 or JHC7 tumor-engrafted rats, there were no signs of metastasis in other organs at the completion of the study (unpublished data); this is consistent with the lack of metastasis observed in patients.^{2,16}

Tumor Histology

Gross consistency of chordoma is macroscopically described as semiliquid, soft, green, and transparent.^{19,29,41,44} Chordomas frequently contain focal calcifications, ossifications, hemorrhagic areas, necrosis, and cyst formations. Microscopic examination shows the pathognomonic histological characteristics of these tumors, which is the presence of physaliferous cells (Fig. 2C), described as vacuolated cells that contain intracytoplasmic mucus. These cells are separated by fibrous septa into lobules and surrounded by a basophilic extracellular matrix rich in mucin and glycogen.^{8,43} Our results showed that chordomas engrafted in immunocompromised rats retained the morphological and histological characteristics of human chordoma, indicative that a chordoma developed in the vertebral body (Figs. 1 and 2), as confirmed by a blinded pathologist. Immunostaining of UCH1 tumor for cytokeratins was positive in the tumor cells and negative in the connective tissue. In the

UCH1 and JHC7 groups, tumor was present in the trabeculae of the bone, showing the typical multinodular arrangement (Fig. 1C). Chordoma histology was better preserved in the JHC7 group; physaliferous cells were observed between the fibrous septa. Strong nuclear immunoreactivity for brachyury was confirmed in both tumors, with clearly negative immunoreactivity in the stroma (Fig. 2C).

Imaging Characteristics

The imaging characteristics of the chordomas in our model showed radiographic features that are similar to those observed in patients. The vertebrae appear with irregular anatomy because of the expansive destruction induced by a slow-growing soft-tissue lesion, remodeling and reactivating bone formation.⁴⁶ According to Sundaresan,⁴⁴ a soft-tissue mass anterior to the involved vertebrae is the most important radiological finding and shows that paravertebral tissues are more affected than osseous tissues, similar to what we observed in the UCH1 tumor-engrafted rats. The vertebral lesions are characterized primarily by destructive changes involving the body, often with a surrounding area of reactive sclerosis. Involvement of adjacent vertebral bodies is a frequent finding in chordomas, but in our model we found that only 1 vertebra was involved.

In our CBCT images from UCH1 and JHC7 tumor-engrafted rats, we observed some areas of calcifications, sclerotic changes, and bony destruction (Fig. 3A, B, F, and G). Contrast-enhanced MRI was used to confirm the spinal chordoma. It is well known that MRI is the gold standard for diagnostic purposes; chordoma is commonly described as iso- or hypointense on T1-weighted images and moderately hyperintense on T2-weighted images. T1 with contrast shows a characteristic heterogeneous enhancement that is classically described as honeycombing. In our UCH1 and JHC7 tumor-engrafted-rats, we observed on T1- and T2-weighted MR images an iso-intensity and high-intensity signal, respectively, as shown in Fig. 3C and H.

Clinical Evaluation

Patients with spinal chordomas more commonly present with paresthesias and pain; nevertheless, neurological symptoms are seen in later stages of the disease.¹⁵ The symptoms are closely related to localization and growth rate of the tumor, as well as the compression of nerve roots, spinal cord, paravertebral tissues, and surrounding anatomical structures by the tumor. Gallia et al.¹⁵ reported that a 52-year-old patient with a large sacral chordoma presented with a 15-year history of sacral pain and a 4-year history of constipation. On physical examination, they observed a mass protruding through the right side of the sacrum and the right sacroiliac joint region.

These findings are consistent with 1 of our rats with a UCH1 chordoma, which was found to have a mass protruding through the left side of the sacrum at the same level as that demonstrated clinically. Necropsy of the lower spine of the rat revealed a large tumor invading the abdominal cavity, similar to the patient described by Gallia et al. In their study, the patient's MR image revealed a large tumor originating from the S2–3 junction, extending posteriorly,

laterally involving the right sacroiliac joint, and anteriorly filling the entire pelvic cavity. The MR image of the UCH1-bearing rat revealed a large tumor originating from the L-5 vertebral body and extending posteriorly to the sacrococcygeal spine (Fig. 3C). The neurological evaluation of the patient with a large chordoma demonstrated isolated plantar flexor weakness bilaterally. The patient suffered no sensory loss in the lower extremities or perianal region. The patient in this study exhibited a normal gait according to the authors,¹⁵ but other patients with chordoma present with abnormal gait or gait difficulties.^{16,30}

Gait kinematic analysis is a useful tool to evaluate the motor function of the hindlimbs in tumor-engrafted rats.³⁸ The stride length of the left hindlimb was affected 530 days after tumor engraftment; the right hindlimb had a compensatory role by supporting more weight during locomotion. Interestingly, compared with the control and sham groups, the tumor-engrafted rats exhibited increased stride lengths in the left hindlimb due to the increased length in the stance and swing phases. In patients, the majority of abnormalities of pathological gait are observed during the stance phase, when all body weight is supported by 1 leg. It is during this phase that pain, muscle weakness, and joint abnormalities produce their predominant effects.

In our chordoma model, we observed abnormalities in the swing and stance phases in tumor-engrafted rats. In our control rats, during the stance phase, the dorsiflexion in the ankle reached its peak near the midstance phase, and then plantar flexion was observed until the end of this phase. In our UCH1 tumor-engrafted rats, the dorsiflexion in the ankle was significantly decreased ($p = 0.0012$) (Supplementary Fig. 1) and the plantar stepping was absent (data unpublished). Raising and return of the hindlimb was increased in all of the tumor-engrafted rats. This could be explained by the decrease in the dorsiflexion of the knee and ankle due to muscle weakness as a consequence of compression of the sciatic nerve by the chordoma.

Gait kinematics are well characterized in intact rats,^{18,25} but there is a lack of studies on pathologic gait due to chordomas in rodents. In this study, we analyzed sagittal walking of rats in a corridor. The UCH1 group showed an exaggerated hip and knee flexion and diminished flexion-extension of the ankle during gait. This could have been due to weakness of the dorsi-flexor muscles of the leg (caused by compression of the sciatic nerve by the tumor, resulting in motor neuron degeneration and consequent loss of innervation and strength). These abnormalities were observed during the swing phase (Fig. 4). The effects of compression by tumor in our model can be compared with the effects caused by direct mechanical compression of the sciatic nerve, where the muscles below the knee present weakness while walking.¹

Lesions in the spine due to chordoma frequently cause motor changes in patients due to spinal cord or root compression, but paraplegia rarely occurs as a complication. Gait locomotion analysis in the tumor-engrafted rats showed some joint disturbances, that is, the sacroiliac joint in the UCH1 and JHC7 tumor-engrafted rats showed a slight (nonsignificant) increase in displacement during the flexion-extension phases. The same results were observed in the knee and ankle joints (data not shown) except for the

UCH1 tumor–engrafted rats, which presented a significant increase ($p < 0.05$) of 83.33% in the flexion and 82.45% in the extension of the ankle when compared with the control group. Loss of plantar flexion and dropped foot are the most common symptoms in patients with tumors affecting the L4–5 vertebrae, which is correlated with nerve root tumor involvement.⁵³

The methods used to evaluate pain behavior in our model are well established and reliable quantitative measures of pain hypersensitivity in models of neuropathic pain. Currently, there are no techniques available to evaluate pain derived from spinal tumors in rodents. We evaluated the nociceptive response to mechanical and thermal stimuli, because the major cutaneous receptor types are found in the paw skin.²² Our results are similar to the ones found in rats with neuropathic pain due to sciatic nerve constriction.³¹ The tumor-engrafted rats showed thermal and mechanical hyperalgesia. This could be explained by the compression produced by the tumor in the dorsal nerve roots, as observed in the MR images of the UCH1 and JHC7 tumor–engrafted rats (Fig. 3C and H).

Macroscopic analysis revealed that the tumor extended significantly into the paravertebral area, compressing the nerve roots at the L-5 vertebra. Patients with lumbar vertebra chordoma can suffer from neuropathic pain and radicular pain during the course of their illness, years before diagnosis of the tumor. Pain in the lower back or coccygeal region is the most frequent symptom^{3,5,8,15,16} of patients with tumors located in these areas. Thus, our model underscores the need to use orthotopic tumor models to emulate the functional deficits presented by patients with this disease. Through the use of clinically relevant tumor models, we can gain further insights into the biology of these rare malignancies.

With this model, we have characterized a human-derived chordoma in the spine of an immunocompromised rat. Thus, we have created a platform that may accurately represent the human condition with respect to tumor growth, histology, imaging, and clinical presentation. Given the challenges of treating chordoma in the human population, we hope to further refine this model and use it as a tool to study the biology of these rare but lethal tumors, as well as to evaluate the efficacy of various existing and novel treatment modalities directed at improved local control and possible cure for chordoma. Nevertheless, our group understands the limitations of this model, as well as the optimization required to make this a standardized model for the study of chordoma. The latency of symptom appearance after engraftment, although it recapitulates the clinical scenario, poses a challenge to making this a simple model for the study of chordoma biology and its therapeutic implications.

Our group and others have used patient-derived xenografts,^{9,21,40,41} which exhibit faster growth rates that could shorten the length of time before the appearance of symptoms. Previously we observed that subsequent implantation of subcutaneous grafts from JHC7 became more aggressive with each passage, increasing its growth rate substantially.³ The aforementioned techniques would help increase the tumor establishment rate, which we acknowledge is not optimal. There is a concern whether xenografts

established from cell lines derived from patients will have an altered biology compared with xenografts derived directly from intraoperative tissue. Nonetheless, the former confers the benefit of allowing for the performance of in vitro studies, which can lead to novel discoveries in chordoma biology, as well as large drug screening studies.

In summary, this chordoma model constitutes an advance for the study of this malignant disease, setting the basis for a broader study to improve our understating of the neurological deficits that occur when chordoma affects the spine. Understanding the entire pathophysiology of the disease will help tailor holistic therapeutic strategies that might lead to decreases in morbidity and mortality in patients with spine chordoma.

Conclusions

In this study, we developed an orthotopic rat model of spine chordoma using human cells. Tumor-engrafted rats were evaluated for neurological impairment and underwent histopathological analyses to determine the extent of disease. Tumor growth affected the locomotion and pain sensation of tumor-engrafted rodents in ways that mimic deficits present in humans afflicted with chordoma. Our intraspinal preclinical model represents a reliable method to evaluate experimental therapeutic approaches to human chordoma in rats.

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Supplemental Information

Online-Only Content

Supplemental material is available with the online version of the article.

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