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Prostate cancer metastases alter bone mineral and matrix composition independent of effects on bone architecture in mice — A quantitative study using microCT and Raman spectroscopy

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ABSTRACT

Prostate cancer is the most common primary tumor and the second leading cause of cancer-related deaths in men in the United States. Prostate cancer bone metastases are characterized by abnormal bone remodeling processes and result in a variety of skeletal morbidities. Prevention of skeletal complications is a crucial element in prostate cancer management. This study investigated prostate cancer-induced alterations in the molecular composition and morphological structure of metastasis-bearing bones in a mouse model of prostate cancer using Raman spectroscopy and micro-computed tomography (microCT). LNCaP C4-2B prostate cancer cells were injected into the right tibiae of 5-week old male SCID mice. Upon sacrifice at 8 weeks post tumor inoculation, two out of the ten tumor-bearing tibiae showed only osteoblastic lesions in the radiographs, 4 osteolytic lesions only and 4 mixed with osteoblastic and osteolytic lesions. Carbonate substitution was significantly increased while there was a marked reduction in the level of collagen mineralization, mineral crystallinity, and carbonate:matrix ratio in the cortex of the intact tumor-bearing tibiae compared to contralateral controls. MicroCT analysis revealed a significant reduction in bone volume/total volume, trabecular number and trabecular thickness, as well as significant increase in bone surface/volume ratio in tibiae with osteolytic lesions, suggesting active bone remodeling and bone loss. None of the changes in bone compositional properties were correlated with lesion area from radiographs or the changes in bone architecture from microCT. This study indicates that LNCaP C4-2B prostate cancer metastases alter bone tissue composition independent of changes in architecture, and altered bone quality may be an important contributor to fracture risk in these patients. Raman spectroscopy may provide a new avenue of investigation into interactions between tumor and bone microenvironment.

Introduction

Prostate cancer is the most common primary tumor and the second leading cause of cancer-related deaths in men in the United States [1]. More than 80% of patients who die from advanced prostate cancer have bone metastases that cause a variety of morbidities such as bone pain, pathological fracture, hypercalcemia, and spinal cord compression [2]. Such skeletal-related events significantly impact a patient's chance of survival and quality of life. Therefore, prevention of skeletal complications is a crucial element in cancer management.

Given the clinical significance of bone metastasis, research efforts have focused on understanding the mechanisms of metastasis progression and developing therapeutic interventions using animal models of human cancer and metastasis. Assessment of tumor development and bone degeneration is important to characterize the interaction between tumor cells and the bone microenvironment as well as to evaluate the response of tissue to treatments. A variety of imaging techniques such as magnetic resonance imaging (MRI) [3], micro-positron emission tomography (PET) [4,5], micro-computed tomography (microCT) [6], and optical imaging including bioluminescence and fluorescence imaging [7–11] have been developed to investigate cancer growth and metastasis in small animals for such purposes. Although each modality

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has its own specific advantages, most of the methods focus on monitoring the development and progression of tumor with limited or no information on cancer-associated changes in bone properties. Although microCT can measure the density and microarchitecture of bone tissue, it cannot examine the compositional quality of bone tissue, which may be a major determinant of bone strength and fracture risk. Hence, detailed characterization of cancer-associated alteration in bone composition, if they exist, is important to advance the understanding in tumor–bone interactions.

Vibrational spectroscopies (infrared and Raman) are ideal tools to characterize such biochemical changes because they are sensitive to molecular structure and composition in the tissue. Raman spectroscopy (RS) has additional advantages owing to its ability to analyze intact samples nondestructively and in a hydrated state. RS has previously been used in orthopedic research to assess predicted fracture risk by detecting alterations in the bone's material properties with aging or disease [12] Bone compositional properties derived from Raman spectral parameters have been correlated with tissue-level mechanical properties [13,14]. The decreased elastic deformation capability of aged bones associates with increasing collagen mineralization, crystallinity and carbonate substitution [15]. Fractured and unfractured osteoporotic bones showed differences in tissue composition detected by RS [16]. Specifically, iliac crest cortical bone from women with osteoporotic fractures had greater carbonate substitution in mineral structure than that from women without fractures [16]. These findings suggest that RS measurements of bone matrix composition could be important predictors of fracture risk and skeletal metabolism in other conditions including metastatic bone disease.

While numerous studies have addressed lytic and blastic architectural effects of bone metastases [17,18] it is currently unknown whether prostate cancer metastases to bone induce changes in the composition of the bone matrix. Based on the broad spectrum of interactions known to occur between cancer and bone cells in the tumor–bone microenvironment, the present study hypothesized that Raman spectroscopy can detect cancer-induced changes in the composition of metastatic bone. Furthermore, this study sought to determine whether metastatic effects on bone composition were independent of metastatic effects on bone architecture. A mouse model of prostate cancer was used to produce mixed osteoblastic and osteolytic lesions that mimic human prostate cancer bone metastases. Bone composition and architecture from the tumor-bearing tibiae and the contralateral controls were quantitatively characterized using RS and microCT, respectively.

Materials and methods

Animal study

All procedures were performed in compliance with the Vanderbilt University Institutional Animal Care and Use Committee and the National Institutes of Health guidelines. The LNCaP C4-2B prostate cancer cells (100,000 cells in 10 μl) were injected into the right tibiae of 5-week-old male, severe combined immunodeficiency (SCID) mice (Harlan Sprague–Dawley, n = 10), while the left tibiae were injected with phosphate buffered saline (PBS) as contralateral control (n = 10). Another parallel control group of SCID mice received vehicle injection in the right tibiae and no treatment in the left side (n = 10). The injection procedure was performed under anesthesia using previously reported methods [19,20]. Briefly, the hind limbs were treated with depilatory cream for hair removal. After disinfection with Betadine and alcohol wipes at the knee area, a 27-gauge needle was inserted ~3 mm into the proximal end of the tibiae by twisting through the cortical bone in a drilling motion, and the cancer cells or PBS were then injected. The procedure was performed on 5-week-old mice with an open growth plate to reduce the osseous blockage during injection. After tumor induction, animals were treated with one dose of analgesic Buprenex subcutaneously at 2.5 mg/kg body weight, and were monitored daily for discomfort and pain in the first 3 days after injection. All mice were sacrificed at 8 weeks post tumor induction when bone lesions were evident on the radiographs. All the tumorous, contralateral and control tibiae were harvested, cleaned of excess soft tissue, and stored in 70% alcohol at 4 °C before microCT and Raman measurements.

Digital radiographs were acquired weekly in vivo from the tumor-bearing mice starting 4 weeks after injection to monitor lesion development. With an exposure energy of 35 kVp for 8 s, plane radiographs were acquired while the mice were lying in a prone position under anesthesia using a XR-60 digital radiography system (Faxitron). All radiographs were evaluated in a blinded fashion. The number and area of osteolytic and osteoblastic bone metastases were calculated on radiographs using MetaMorph, a computerized image analysis system (Molecular Devices, Inc.) [21,22].

MicroCT

The types of tumor-induced lesions (osteolytic, osteoblastic, or both) present in tibial metaphysis and the effect of the tumor on mineralization, cortical and trabecular architecture were determined using micro-computed tomography. Cross-sectional images of the proximal tibia were acquired ex vivo using a μCT40 (Scanco Medical, Brüttisellen Switzerland). The region of interest including the metaphysis and mid-diaphysis of tibia was identified from a 'scout' scan of each bone prior to image acquisition. The region was scanned at an isotropic voxel size of 12 μm with X-ray source settings at 70 kVp and 145 mA, 250 projections per 180°, and an integration time of 300 ms. Bone was segmented from soft tissue using a threshold of 411 mg HA/ccm, Sigma 0.2 and Support of 1. The cortical bone volume and mean cortical thickness were quantified from the mid-diaphysis using Scanco evaluation software. Trabecular bone volume and architecture in the proximal metaphysis were calculated as previously described [23], including bone volume fraction (BV/TV), trabecular number (Tb.N), and trabecular thickness (Tb.Th). Bone tissue mineral density (TMD) and bone surface to volume ratio (BS/BV) were also determined from the tumor-bearing tibiae and the contralateral tibiae without tumor.

Raman spectroscopy measurement

Following microCT analysis, Raman measurements of the periosteal surface of intact host cortical bone at the tibial metaphysis were carried out using a confocal Raman microscope (Renishaw, Ramascope Mark III), which couples a Raman spectrometer to a confocal microscope, a CCD detector, and a diode laser emitting at 785 nm. The intact tibiae were mounted on a microscope slide using mold polymer clay, with the surface of the proximal metaphysis leveled horizontally. The 785 nm laser light was focused to the metaphysis of tibiae through the Leica 50× N Plan (NA = 0.75) lens with a laser power of 30 mw on sample and laser spot size ~3–4 μm in diameter. Three spectra were collected from the host cortical bone of proximal tibia metaphysis (schemed in Fig. 1). Each spectrum consisted of three accumulations with an exposure time of 10 s, a binning of 3 and spectral resolution of 3 cm^{-1}.

Custom scripts written in Matlab were used to correct the baseline of each spectrum and to calculate the compositional properties. Peak intensities of Raman signatures from mineral and collagen were determined, including phosphate v1 (960 cm^{-1}), carbonate (1070 cm^{-1}), proline (856 cm^{-1}), hydroxyproline (876 cm^{-1}) and amide 1 (1665 cm^{-1}) [14,24].

Statistical analysis

Statistical analysis was performed using SigmaPlot 12 software (Systat Software, Chicago, IL, USA). The statistical significance of tumor-associated changes in microCT and Raman spectral measures