Multimodality Characterization of a Bone-Metastasis Model

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Introduction: Bone metastases are established when cancer cells reach the bone marrow through blood vessels in Haversian canals, extravasate, multiply, and eventually neovascularize. Metastatic bone lesions can be classified as osteolytic, osteoblastic and mixed. In osteolytic lesions, which are the most common, bone destruction outstrips the laying down of new bone. Osteoblastic lesions result from new bone growth stimulated by the cancer cells. Microscopically, most lesions contain both characteristics. The application of noninvasive multi-modality molecular imaging to understand and characterize bone metastatic lesions will provide new insights that can be exploited for defining and attacking new targets under image-guidance, and for monitoring response to treatments. Here we present data to demonstrate the feasibility of performing CT, MRI and MRSI of a bone-metastasis model of renal cell carcinoma.

Methods: Initial studies were performed with renal carcinoma cells inoculated in the tibia of nude athymic mice in a well established bone-metastasis model. Approximately 2.5×10^5 cells were injected intra-tibially in 0.01 ml of Hanks balanced salt solution. Characterization of bone lesions was performed using micro CT to visualize bone damage, MRI of the intravascular agent albumin-GdDTPA to determine vascular uptake, and MRSI to detect total choline. Micro CT studies were performed using a Gamma Medica X-SPECT scanner that provided a 100 µm spatial resolution. MRI and MRSI studies were performed on a 9.4T Bruker spectrometer using a home built 15 mm resonator. 3D T_1 weighted images were acquired with a gradient echo FLASH sequence using parameters TE/TR= 1.5/10 ms with fat suppression and flip angle of 60-degree. The high molecular weight MR contrast agent, albumin-GdDTPA, was injected i.v. in the tail vein at a dose of ~ 0.1 mmole Gd/kg. MRI scans were performed before and for 30 min after administration of the contrast. MR spectroscopic imaging was performed with the same experimental setup using a standard 2D CSI sequence with parameters TE/TR= 60/1500 ms, water suppression, for an in-plane spatial resolution of 1×1 mm and 2 mm slice.

Results and Discussion: We are developing targeted theranostic agents for metastatic disease that require the delivery of nanoplexes containing a prodrug activating enzyme and multiple siRNAs to downregulate target genes including choline kinase. It was therefore important to establish the vascular delivery of macromolecules in bone lesions, and demonstrate the feasibility of detecting total choline in the lesion.
With micro CT we were able to detect destruction of the bone by the lesion (Figure 1, left panel). MRI studies of the macromolecular contrast agent albumin-GdDTPA confirmed adequate delivery of the agent in the lesion (compare precontrast to post-contrast images in Figure 1).

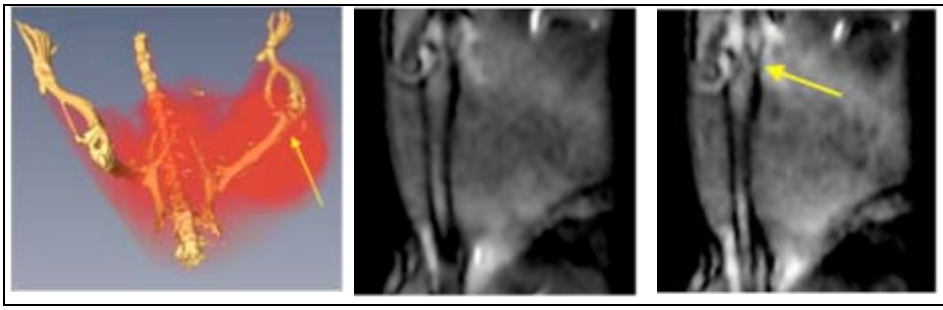

Figure 1. **Left:** Micro CT scan of mouse skeleton. Significant destruction of the right knee by an aggressive renal carcinoma inoculated in the tibia is shown by the arrow. Rendering of soft tissues (red) demonstrates the tumor growing in the leg. **Middle:** Pre-contrast T_1-weighted MR image is shown in the middle panel. **Right:** Uptake of macromolecular MR contrast agent albumin-GdDTPA in the tumor. Strong accumulation of the contrast agent in the tumor is evident in the post-contrast T_1 weighted MR image.

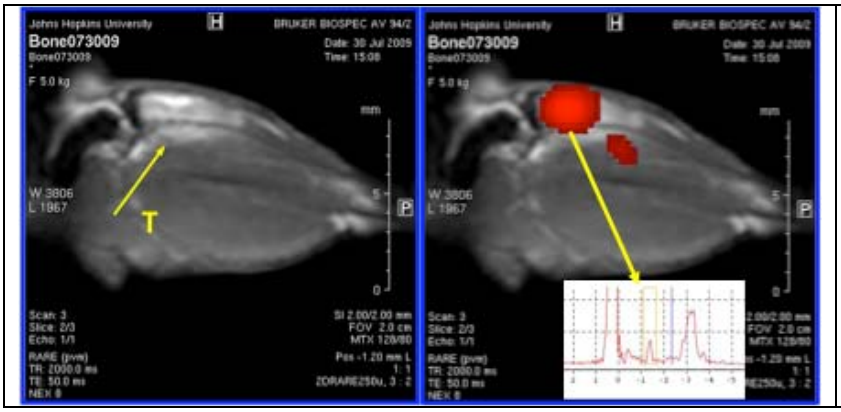

Figure 2. Total choline (right) detected in bone tumor nodule (left) from renal cell carcinoma cells. Spectroscopic imaging was performed using 2D CSI at 9.4T with an in-plane resolution of 1 × 1 mm from a 2 mm thick slice (zerofilled resolution 0.5 × 0.5 × 2 mm). Inset shows total choline signal from a representative voxel in the lesion.

With MRSI we detected elevated total choline in the bone lesion as shown in Figure 2. These data demonstrate the feasibility of detecting the delivery of macromolecular agents to bone lesions and characterizing the vasculature and total choline levels in these lesions. These capabilities will allow us to deliver molecular-targeted nanoplexes containing siRNA against choline kinase under image-guidance and detect the downregulation of total choline as a novel strategy to treat metastatic disease.

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