

Osteoblastic and angiogenic reactions in prostate cancer bone metastasis models studied by macromolecular DCE-MRI and μ CT

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Introduction: Osteoblastic bone metastasis is the main cause of morbidity and mortality from androgen-independent prostate cancer. Currently there are very few models that reflect the osteoblastic phenotype of prostate cancer. Recently however, a clinically relevant xenograft model of osteoblastic bone metastases was established from an androgen-independent prostate cancer patient. This model demonstrated robust osteoblastic reaction (by recruitment of host cells) in the absence of androgen receptor and was used to identify some of the involved paracrine factors [1]. In this study we aim to further characterize the osteoblastic and angiogenic properties of this xenograft model by in vivo macromolecular DCE-MRI and ex vivo μ CT, and to compare it to an established osteolytic model.

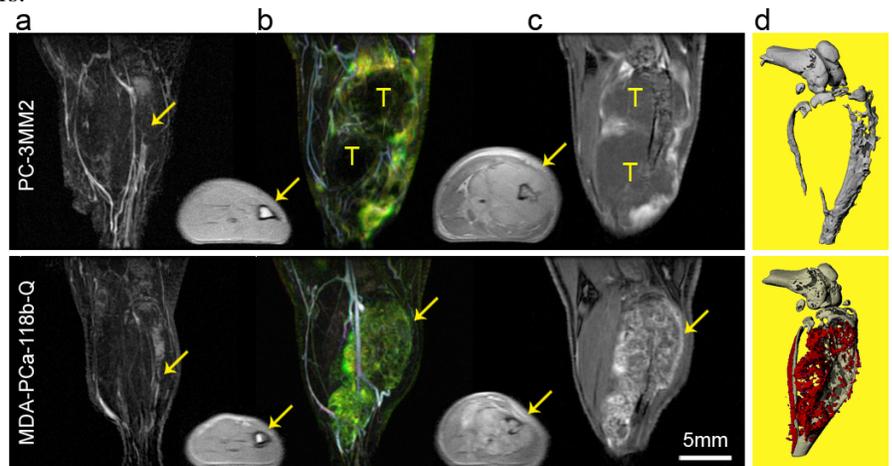
Methods: Osteolytic bone metastases were initiated by intratibial injection of human prostate cancer cells, PC-3MM2, in male CD-1 nude mice (0.2×10^6 cells/tibia) [2]. Osteoblastic MDA-PCa-118b-Q xenografts were propagated subcutaneously [1], dissected, mechanically dispersed and filtered to obtain a single cell suspension, prior to intratibial injection as above (0.5×10^6 cells/tibia).

MRI was performed weekly on a 3T GE scanner (GE Healthcare, Waukesha, WI) using a custom-built knee coil. T2-weighted FSE images: TR/TE 4500/15.6 ms, 2 NEX, slice thickness 1 mm, in-plane resolution $104 \times 104 \mu\text{m}$. Macromolecular DCE-MRI: 3D-fSPGR sequence, TR/TE 24.7/3.4 ms, flip angle 35° , 2 NEX, slice thickness $600 \mu\text{m}$, in-plane resolution $156 \times 156 \mu\text{m}$, acquisition time 3.4 min; acquired pre and 1.7, 16.7 and 31.7 min post injection of albumin-GdDTPA ([2]; ~ 85000 Da; 350 mg/kg iv).

Ex vivo micro-tomography (μ CT-40, Scanco Medical, Brüttisellen, Switzerland) was performed on excised, fixed, tumor-bearing tibiae: 250 projections, 55kV source potential, 75 ms integration time, $32 \mu\text{m}$ isotropic nominal resolution, total acquisition time 156 min. For visualization purposes, a dual threshold approach was used to segment extant (surviving, normally mineralized) osseous tissue from nascent tumor-induced mineralization.

Results: Tumors were hyperintense in the T2-weighted images especially at early stages (Fig 1, inserts). Macromolecular contrast enhancement highlighted blood vessels including in the intact bone marrow, but enhancement was diminished in regions occupied by the growing tumor masses (Fig 1 a). By week 5-6, substantial tumor growth was detected beyond the original bone either as a result of osteolysis and tumor growth outside the bone (PC-3MM2) or as a result of osteoblastic tumor growth (MDA-PCa-118b-Q). Both tumor types demonstrated high extravasation and accumulation of the macromolecular contrast agent, indication leaky angiogenic (newly-formed) blood vessels (Fig 1 b, c). However, PC-3MM2 tumors showed contrast accumulation only at the tumor periphery, whereas in MDA-PCa-118b-Q tumors the enhancement pattern resembled small nodules. Significant osteolysis of tibial cortical bone was revealed by μ CT in both tumor models (Fig 1 d). Additionally, irregular patterns of partially mineralized tissue were observed only at the periphery of MDA-PCa-118b-Q tumors.

Figure 1: Representative PC-3MM2 (upper) and MDA-PCa-118b-Q (lower) tumors at 2-3 (a) and 5-6 (b-d) weeks post tumor inoculation. 3D DCE-MRI images are presented as maximal intensity projections (MIP; a) or MIP overlay of 1.7 (blue), 16.7 (red) and 31.7 (green) minutes post contrast injection (b) or selected slice, approximately through the middle of the tumor (c). Inserts to the bottom right of each MIP are the corresponding T2-weighted images. Tumors are indicated by arrows or 'T'. μ CT 3D reconstruction shows the extant (normal; black & white) and tumor-induced (red) mineralization (d).



Discussion: These results suggest that the osteolytic model generates tightly packed tumors with high interstitial fluid pressure that allows extravasation of macromolecules only from peripheral blood vessels, where the pressure is lower, whereas the osteoblastic model has more stromal and structural support that allows maintenance of leaky blood vessels throughout the tumor. Importantly, T2 weighted images may not be useful for detection of osteoblastic lesions since bone components have low signal. CT can indicate bone formation and resorption but provides only a limited understanding of the molecular mechanism of osteogenesis. This study highlights the value of macromolecular DCE-MRI as a method that can provide structural information and monitor tumor interaction with stromal cells including recruitment of neovasculature and monitoring of response to antivascular treatment as demonstrated previously for the PC-3MM2 model [2].

References: [1] Li, Z. G., et al. *J Clin Invest*, 118: 2697-2710, 2008. [2] Dafni, H., et al. *Magn Reson Med*, 60: 822-833, 2008.

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