Proteomics Analysis to Characterize Prostate Cancer Bone Metastases Subtypes

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Introduction and Objectives:

Prostate cancer (PC) bone metastases (BM) is a debilitating disease morbidity that primarily affects the axial skeleton. PCBMs cause severe pain, bone fragility and fractures, dramatically affecting the quality of life of patients. PCBM are associated to a variety of bone alterations from increased bone density (osteosclerotic), to reduced bone density (osteolytic). Structurally, PCBM present with irregular bone distribution, loss of collagen alignment and increased porosity that mimic features of woven bone that forms during development and repair. Different from reparative woven bone, PCBM is not replaced by normal trabecular bone, and the irregular woven bone structure remains permanently, affecting bone strength and predisposing to fractures. We hypothesize that PCBMs induce dysregulated repair-like bone remodeling activity leading to distinct protein signatures that correlate with the extent of osteosclerotic and osteolytic characteristics in different PCBM subtypes.

Methods:

We performed tandem mass spectrometry (TMT-MS) to measure the protein composition in lumbar vertebrae specimens from 3 age-matched controls and 32 cadaveric PCBM specimens: 17 defined as osteosclerotic and 15 as osteolytic based on microcomputed tomography bone volume vs total volume (BV/TV) measurements. We employed unsupervised hierarchical clustering and dimension reduction techniques to identify clusters with distinct protein composition profiles and performed differential expression analysis to identify protein signatures. We compared those observations with the histopathological description of PC found in the analyzed vertebrae.

Results:

Osteosclerotic PCBM specimens were uniquely enriched in bone sialoprotein 2, osteopontin, osteonectin, and the proteoglycans osteomodulin, lumican, biglycan and decorin. While osteolytic specimens had elevated levels of cathepsin G, myeloblastin, and neutrophil elastase compared to the osteosclerotic specimens. Unsupervised clustering segregated the 3 control samples and 17 osteosclerotic samples into distinct groups. The 15 osteolytic samples separated into three subgroups: one clustering with controls (n=3), one exhibiting features that overlap with osteosclerotic lesions (n=6), and one unique osteolytic group (n=6). The differences in protein distribution are also associated to the histological characterisation of the specimens since most of the osteosclerotic specimens also correspond with less anaplastic histopathology.

Conclussions

Proteomic profiling revealed distinct differences between predominantly osteosclerotic and osteolytic PCBM highlighting altered process of mineralization, suggesting potential biomarkers, and provide mechanistic insights. The osteosclerotic samples are distinctly characterized by proteins that promote bone production and mineralization. The osteolytic lesions displayed signatures of immune cell activity. The proteomic segregation of three molecular subtypes of osteolytic lesions was unexpected and demonstrates the sensitivity of MS-based analysis, highlighting its promise for further characterizing PCBM subtypes.

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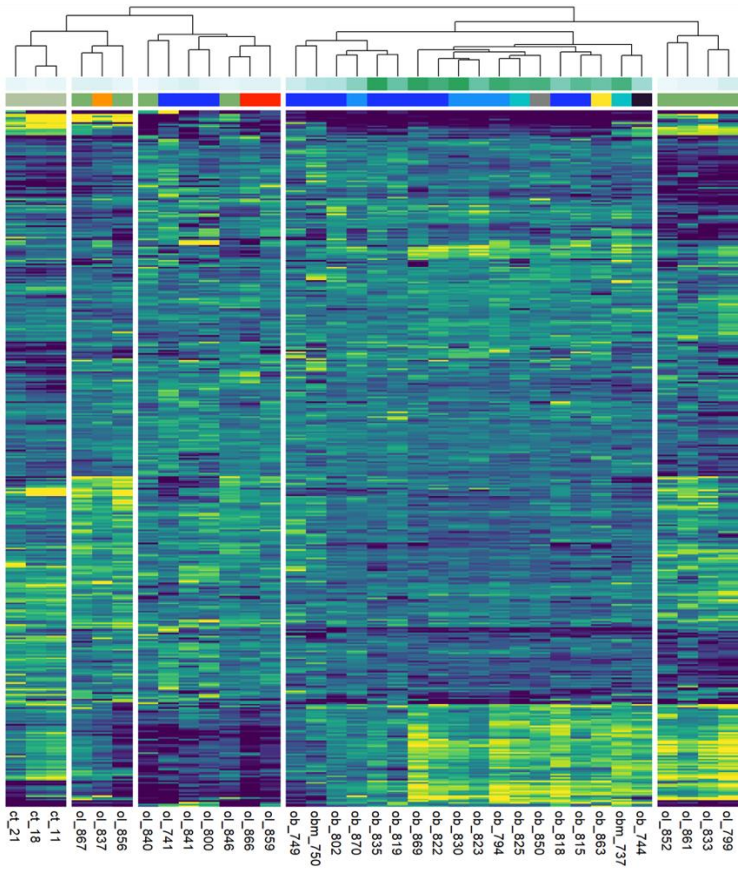

Fig 1 Unsupervised hierarchical clustering heatmap of relative protein expression. Controls (CT) segregate independently, while osteolytic (ol) and osteoblastic (ob) samples