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Subgroups of Castration-resistant Prostate Cancer Bone Metastases Defined Through an Inverse Relationship Between Androgen Receptor Activity and Immune Response

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Abstract

Background: Novel therapies for men with castration-resistant prostate cancer (CRPC) are needed, particularly for cancers not driven by androgen receptor (AR) activation.
Objectives: To identify molecular subgroups of PC bone metastases of relevance for therapy.
Design, setting, and participants: Fresh-frozen bone metastasis samples from men with CRPC ($n = 40$), treatment-naïve PC ($n = 8$), or other malignancies ($n = 12$) were characterized using whole-genome expression profiling, multivariate principal component analysis (PCA), and functional enrichment analysis. Expression profiles were verified by reverse transcription–polymerase chain reaction (RT-PCR) in an extended set of bone metastases ($n = 77$) and compared to levels in malignant and adjacent benign prostate tissue from patients with localized disease ($n = 12$). Selected proteins were evaluated using immunohistochemistry. A cohort of PC patients ($n = 284$) diagnosed at transurethral resection with long follow-up was used for prognostic evaluation.
Results and limitations: The majority of CRPC bone metastases (80%) was defined as AR-driven based on PCA analysis and high expression of the AR, AR co-regulators (FOXA1, HOXB13), and AR-regulated genes (*KLK2*, *KLK3*, *NKX3.1*, *STEAP2*, *TMPRSS2*); 20% were non–AR-driven. Functional enrichment analysis indicated high metabolic activity and low immune responses in AR-driven metastases. Accordingly, infiltration of $CD3^+$ and $CD68^+$ cells was lower in AR-driven than in non–AR-driven metastases, and tumor cell HLA class I ABC immunoreactivity was inversely correlated with nuclear AR immunoreactivity. RT-PCR analysis showed low MHC class I expression (*HLA-A*, *TAP1*, and *PSMB9* mRNA) in PC bone metastases compared to benign and malignant prostate tissue and bone metastases of other origins. In primary PC, low HLA class I ABC immunoreactivity was associated with high Gleason score, bone metastasis, and short cancer-specific survival. Limitations include the limited number of patients studied and the single metastasis sample studied per patient.
Conclusions: Most CRPC bone metastases show high AR and metabolic activities and low immune responses. A subgroup instead shows low AR and metabolic activities, but high immune responses. Targeted therapy for these groups should be explored.

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Patient summary: We studied heterogeneities at a molecular level in bone metastasis samples obtained from men with castration-resistant prostate cancer. We found differences of possible importance for therapy selection in individual patients.

1. Introduction

The growth of normal and malignant prostate tissue is regulated by androgens through activation of the androgen receptor (AR) in both epithelial and stromal cells, and androgen deprivation therapy (ADT) is standard treatment for patients with advanced prostate cancer (PC). However, after an initial period of reduced symptoms and tumor growth, relapse occurs and the PC becomes castration resistant (CRPC). Several mechanisms behind CRPC have been described, including AR amplification, AR mutations, expression of constitutively active AR variants, and intracrine steroid synthesis, as well as AR bypass mechanisms [1]. It has been shown that several new drugs prolong survival and increase quality of life for men with CRPC, including novel AR antagonists, cytostatic drugs, radioisotopes, steroidogenesis inhibitors, immunotherapies, and therapies targeting the tumor microenvironment [2]. Thus, there is a need for biomarkers that can guide CRPC therapy selection. Moreover, the fatal outcome for patients with CRPC highlights the necessity for further therapeutic developments, particularly for patients characterized by low AR activity and for whom no targeting therapy currently exists.

We previously identified heterogeneous gene expression patterns of clinical relevance in metastatic CRPC samples, and found that high levels of the constitutively active AR variant 7 (AR-V7) were associated with particularly poor prognosis [3]. Antonarakis and co-workers showed that detectable levels of AR-V7 in circulating tumors cells are predictive of poor response to AR-targeted therapies [4]. We also found a heterogeneous expression pattern for the steroidogenic enzyme AKR1C3 in clinical samples of CRPC metastases [5], and the relevance of AKR1C3 as a predictive marker for therapy response to the steroidogenesis inhibitor abiraterone is currently under evaluation.

The aim of this study was to further characterize gene expression in bone metastases from men with CRPC to identify subgroups of relevance for therapy choice.

2. Patients and methods

2.1. Patients

Fresh-frozen bone metastasis samples were obtained from a series of men with PC ($n = 65$) or other malignancies ($n = 14$) who underwent surgery for metastatic spinal cord compression at Umeå University Hospital between 2003 and 2013. The PC patient series has been described before [3,5,6] and the clinical characteristics are summarized in Table 1. Formalin-fixed, paraffin-embedded (FFPE) metastasis samples were available for 41 of the 54 CRPC patients in Table 1 and matched diagnostic prostate biopsies were available in 29 cases, obtained at a median of 37 mo (interquartile range [IQR] 16–79) before the metastasis biopsy. The study also included 12 separate men who were treated with radical prostatectomy at Umeå University Hospital between 2005 and 2006; the median age for these men was 61 yr (IQR 57–67) and median prostate-specific antigen (PSA) was 11 ng/ml (IQR 5.3–18 ng/ml). Clinical local stage was T2 ($n = 3$) or T3 ($n = 9$) and Gleason score (GS) was 7 ($n = 10$) or 8 ($n = 2$).

Tissue microarrays (TMAs) were previously constructed from samples taken during transurethral resection of the prostate (TURP) performed between 1975 and 1991 as previously described [7]. Gleason score was reevaluated by one pathologist (L.E.) and TMAs were constructed containing five to eight samples of tumor tissue and four samples of nonmalignant tissue from each patient. For this study, TMAs from 284 patients had tissue available for analysis (Supplementary Table 1). The patients had not received cancer therapy before TURP and, according to the therapy traditions in Sweden at that time, the majority ($n = 202$) were managed via watchful waiting.

The study was approved by the local ethics review board of Umeå University (Dnr 03-185, 2010-240-32, and 02-283).

2.2. Tissue preparation

Bone metastasis samples were instantly fresh-frozen in liquid nitrogen or placed in 4% buffered formalin. Fixed samples were decalcified in formic acid before being embedded in paraffin. Fresh radical prostatectomy specimens were received at the pathology department immediately after surgery and cut in 0.5-cm-thick slices before fixation. From these slices, 20 samples were taken using a 0.5-cm skin punch and frozen in liquid nitrogen within 30 min after surgery. The prostate slices were formalin-fixed, embedded in paraffin, cut in 5 μm-thick sections, whole-mounted, and stained with hematoxylin-eosin. Tissue sample composition (nonmalignant or malignant) was determined according to location in the whole-mount sections.

2.3. RNA extraction

Representative areas of fresh-frozen bone metastasis samples and of malignant and nonmalignant prostate tissue (obtained in pairs from the same patient) were cryosectioned into extraction tubes and RNA was isolated using the Trizol (Invitrogen, Stockholm, Sweden) or AllPrep (Qiagen, Stockholm, Sweden) protocol. The percentage of tumor cells in the samples was determined by examination of parallel sections stained with hematoxylin-eosin, and varied between 50% and 90%. The RNA concentrations were quantified by absorbance measurements using a spectrophotometer (ND-1000; NanoDrop Technologies, Wilmington, DE, USA). The RNA quality was analyzed on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and verified to have a RNA integrity number $\geq$6.

2.4. Whole-genome expression profiling

For each sample, 300 ng of total RNA was amplified using an Illumina TotalPrep RNA amplification kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. A total of 750 ng of cRNA from each sample was hybridized to HumanHT-12 v4 Expression BeadChips, including more than 47 000 probes covering over 31 000 annotated genes,

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