

Mechanisms Behind Growth Of Castration-Resistant Prostate Cancer Bone Metastases

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Akademisk avhandling

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Abstract

Background: The first-line treatment for patients with advanced prostate cancer (PC) is androgen deprivation therapy. This therapy is initially effective, but after some time tumors relapse, predominantly within the bone, and are then termed castration-resistant prostate cancer (CRPC). The majority of CRPC tumors show androgen receptor (AR) activity despite castrate levels of circulating testosterone. AR activity could be caused by several mechanisms including: intratumoral androgen synthesis, AR amplification, AR mutations and expression of AR splice variants. The mechanisms controlling CRPC growth in the clinically most relevant metastatic site, the bone, are not fully identified. The purpose of this thesis was therefore to explore AR expression and possible mechanisms behind CRPC growth in PC bone metastases in order to find mechanisms that could be targeted for treatment and/or predict response to certain therapies.

Materials and Methods: We have examined hormone-naïve and CRPC bone metastases samples obtained from patients at metastasis surgery, non-malignant and malignant prostate samples obtained from patients at radical prostatectomy, and PC cell lines cultured *in vitro*. Analysis has been performed using RT-PCR, whole-genome expression arrays, immunohistochemistry, western blotting, FISH, copy number assays and gene ontology analysis. Functional studies have been made by protein overexpression and knock-down in PC cells *in vitro* and effects studied by evaluation of cell viability, migration, and invasion.

Results: We found that high nuclear AR immunostaining (presumed to reflect high AR activity) in bone metastases from CRPC patients was associated with a particularly poor prognosis, while no difference in AR staining was observed between hormone-naïve and CRPC metastases. Further, expression of AR splice variants (*AR-V7*, *AR-V567es*) was associated with a high nuclear AR immunostaining score and shown to be increased in CRPC compared to hormone-naïve bone metastases. High levels (levels in the upper quartile) of AR splice variants in CRPC bone metastases was related to disturbed cell cycle regulation and short patients survival. No differences in steroidogenic enzyme levels were detected between CRPC and hormone-naïve bone metastases. Higher levels of enzymes involved in late steps of androgen synthesis (adrenal gland steroid conversion) were observed in bone metastases than in non-malignant and/or malignant prostate tissue, while the enzyme levels in earlier steps (*de novo* steroidogenesis) were lower in bone metastases. A subgroup of metastases expressed very high levels of AKR1C3, indicating that this group may have an induced capacity of converting adrenal-gland derived steroids into more potent androgens. This was not associated to CRPC but merely with the advanced stage of metastasis. High protein levels of AR splice variants were found in bone metastases with low AKR1C3 levels, while metastases with high AKR1C3 levels primarily contained low AR variant levels. Furthermore, about half of the CRPC bone metastases showed androgen receptor gene amplification which was associated with co-amplification of *YIPF6*, and a gene expression pattern that pointed at decreased osteoclast activity, and consequently decreased bone resorption.

Conclusions: The majority of CRPC bone metastases show high nuclear AR immunostaining that seems to be associated with a particularly unfavorable outcome after metastasis surgery. Subgroups of CRPC bone metastases could be identified according to presence of AR amplification and expression levels of AKR1C3 or AR splice variants, which might have clinical relevance for treatment of PC patients.

Keywords: Prostate cancer, castration-resistance, bone metastases, androgen receptor, intratumoral steroidogenesis, androgen receptor splice variants, androgen receptor amplification

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