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TUMOR GROWTH ATTENUATION BY INHIBITION OF TOPK/PBK, A NOVEL REGULATOR OF THE TRANSITION TO ANDROGEN INDEPENDENT PROSTATE CANCER

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Introduction: Prostate cancer (PCa) is the most commonly diagnosed invasive malignancy and the second leading cause of cancer-related deaths among men in the United States. The introduction of the PSA test has led to a dramatic increase in the early detection of the disease. Yet, the 5-year survival rate of patients with metastatic PCa drops below 31%. Androgens, through the androgen receptor (AR) are crucial for the initiation and progression of PCa and thus, androgen deprivation therapy (ADT) has been the standard first line therapy for the early metastatic disease for over 40 years. Almost all PCa patients initially respond to different types of androgen ablation, however the majority of them develop resistance and progress to a castration-resistant PCa (CRPC) state, where the disease remains essentially untreatable.

Objectives: Several evidence suggest that androgen insensitive PCa cells have undergone a genetic reprogramming to selectively upregulate the expression of M-phase cell cycle genes. Therefore, the goal of this project is to gain novel molecular insights into the involvement of mitotic regulators in the acquisition of prostate tumors androgen independence.

Methodology: The LNCaP androgen dependent (AD) cell line, representing an early stage of the disease, and its androgen independent (LNCaP AI) counterpart, derived from the former and that represent the transition to a castration-resistant stage, were used in this study. Cells were arrested in mitosis and the proteome of both was compared using stable isotope labeling with amino acids (SILAC) in combination with LC-MS. Protein candidates were further analyzed in *vitro* and clinically relevant candidates were validated in human TMAs (tissue microarrays) by immunohistochemistry representing different stages of the disease. Their potential prognosis value was also evaluated on these samples. Finally, the therapeutic potential of some druggable candidates is currently under study using pre-clinical mouse models.

Results: In this study, the differential global protein expression between the two PCa cell lines arrested in mitosis was examined. Based on the H/L ratio of the three replicates, 2830 proteins were identified and quantified using the MaxQuant software. 198 proteins were found to be significantly (q-value < 0.05) differentially expressed in LNCaP AI cells compared to LNCaP AD cells. Among them, 60 proteins with FC > 2 resulted to be upregulated in the LNCAP androgen independent cell line. Gene Ontology (GO) analysis of the highly differentially expressed upregulated proteins in LNCaP AI compared with LNCaP AD showed that the most enriched GO biological process was "cell division", suggesting that higher expression of cell cycle genes, in particular M-phase genes, may contribute to the progression of PCa to androgen independence. The gene expression of the top candidates was further validated *in vitro* by means of Western Blot and mRNA expression levels were assessed *in silico* using public databases, being the serine/threonine protein kinase PBK one of the most outstanding mitotic candidates. Protein expression of the kinase was evaluated by immunohistochemistry in a cohort of PCa patient samples (including CRPC tissues). PBK had preferentially higher and nuclear staining patterns in aggressive PCa, indicating that it seems to be over-expressed in advanced PCa and therefore might play a role in the progression to a castration-resistant (CRPC) state. In addition, genetical and pharmacological inhibition of PBK resulted not only in reduced proliferation of PCa cells *in vitro,* but also in a reduction of the tumor growth *in vivo* in pre-clinical mouse models. Moreover,

the capacity of androgen-dependent cells overexpressing PBK of growing under conditions of hormone-deprivation is being evaluated.

Conclusions: Altogether, the results indicate that key mitotic regulators, and in particular TOPK/PBK, are upregulated in androgen independent PCa and could be considered promising therapeutic targets for the molecular intervention of CRPC patients.

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