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Prostate Cancer

Patient-derived Hormone-naive Prostate Cancer Xenograft Models Reveal Growth Factor Receptor Bound Protein 10 as an Androgen Receptor-repressed Gene Driving the Development of Castration-resistant Prostate Cancer

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Abstract

Background: Although androgen deprivation therapy is initially effective in controlling growth of hormone-naive prostate cancers (HNPCs) in patients, currently incurable castration-resistant prostate cancer (CRPC) inevitably develops.
Objective: To identify CRPC driver genes that may provide new targets to enhance CRPC therapy.
Design, setting, and participants: Patient-derived xenografts (PDXs) of HNPCs that develop CRPC following host castration were examined for changes in expression of genes at various time points after castration using transcriptome profiling analysis; particular attention was given to pre-CRPC changes in expression indicative of genes acting as potential CRPC drivers.
Outcome measurements and statistical analysis: The functionality of a potential CRPC driver was validated via its knockdown in cultured prostate cancer cells; its clinical relevance was established using data from prostate cancer patient databases.
Results and limitations: Eighty genes were found to be significantly upregulated at the CRPC stage, while seven of them also showed elevated expression prior to CRPC development. Among the latter, growth factor receptor bound protein 10 (*GRB10*) was the most significantly and consistently upregulated gene. Moreover, elevated *GRB10* expression in clinical prostate cancer samples correlated with more aggressive tumor types and poorer patient treatment outcome. *GRB10* knockdown markedly reduced prostate cancer cell proliferation and activity of AKT, a well-established CRPC mediator. A positive correlation between AKT activity and *GRB10* expression was also found in clinical cohorts.
Conclusions: *GRB10* acts as a driver of CRPC and sensitizes androgen receptor pathway inhibitors, and hence *GRB10* targeting provides a novel therapeutic strategy for the disease.
Patient summary: Development of castration-resistant prostate cancer (CRPC) is a major problem in the management of the disease. Using state-of-the-art patient-derived hormone-naive prostate cancer xenograft models, we found and validated the growth factor receptor bound protein 10 gene as a driver of CRPC, indicating that it may be used as a new molecular target to enhance current CRPC therapy.

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1. Introduction

Prostate cancers (PCa's) are largely dependent on androgens for growth and survival [1], and androgen deprivation therapy (ADT) has become the standard treatment for locally advanced or metastatic PCa [2,3]. While most PCa patients initially respond positively to androgen ablation, castration-resistant prostate cancer (CRPC) inevitably develops. Current therapies for CRPC, for example, chemotherapy based on docetaxel or next-generation androgen receptor pathway inhibitors (ARPIs) such as enzalutamide (ENZ) and abiraterone (ABI), can extend patients' lives but are not curative as resistance to their use gradually emerges [4]. As such, there is a critical need for identification of hitherto unrecognized molecular mechanisms that drive the development of CRPC and may lead to novel targets that can be used in combination with ARPIs for more effective therapy.

The present study was aimed at identifying potential CRPC driver genes, in particular genes the elevated expression of which not only initiates but also sustains CRPC. Such genes would have a more important role in the development of CRPC, by inducing bypass pathways circumventing the androgen receptor (AR) pathway, than genes elevated only in CRPC that would mainly be involved in the aggressive growth of CRPCs. We postulated that CRPC driver genes should: (1) be upregulated in CRPC, (2) show elevated expression prior to, and during, the progression from hormone-naive prostate cancer (HNPC) to CRPC; and (3) be functionally essential for CRPC development. To this end, patient-derived xenograft (PDX) models of HNPC that develop into CRPC following ADT are powerful tools to identify such drivers [5,6]. In our laboratory, using the subrenal capsule (SRC) grafting technique, we have established over 45 transplantable PCa PDX lines (www.livingtumorlab.com) [6]. In this study, we applied host castration on 10 of our HNPC PDX lines. Following castration, seven of these lines developed recurrent CRPC tumors spontaneously. By longitudinally analyzing the gene expression profiles of these PDX lines before castration, at 12 wk following castration, and after CRPC development, we obtained evidence that the *GBR10* gene fulfills the above criteria for a CRPC driver. The human growth factor receptor bound protein 10 (*GRB10*) gene encodes an adaptor protein that modulates coupling of specific signaling pathways to cell surface receptor kinases [7]. Here, we provided evidence that GRB10 could be a driver of CRPC development and hence a potential therapeutic target for the disease.

2. Patients and methods

2.1. SRC grafting and development of transplantable HNPC lines

Patient HNPC tissues were cut into pieces ($1 \times 3 \times 3$ mm^3) and grafted into the SRC site of male nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice supplemented with testosterone as previously described [6]. The host mice were sacrificed in a CO_2 chamber, following growth for 3–6 mo (or earlier if reaching humane ending point). Tumors were then harvested and regrafted into another set of NOD/SCID mice at the SRC site. At each passage, the xenografts were harvested, measured, and fixed for histopathological analysis. The PDX lines used in this study were summarized in Supplementary Table 1.

The RNA microarray data of the PDX models used in this study can be accessed through Gene Expression Omnibus (GSE41193), and the Living Tumor Laboratory website (http://www.livingtumorlab.com/). Detailed information for other materials and methods is provided in the Supplementary material.

3. Results

3.1. Identification of genes as potential drivers of CRPC development: GRB10

To elucidate the molecular mechanisms underlying CRPC development, we applied castration to 10 HNPC PDX models developed in our laboratory (Fig. 1A). Castration of mice bearing such tumors leads, within a week, to a marked reduction in tumor volume accompanied by a substantial drop in host serum prostate-specific antigen (PSA) levels (Fig. 1B), that is, an effect mirroring the clinical response of PCa to ADT. Within a few months after castration, seven of these tumor lines, such as the LTL-313B line, gave rise to castration-resistant tumors and increased host serum PSA levels (Fig. 1B). On the histopathological level, we checked the expression of AR, PSA, and Ki67 in LTL-313B samples collected before and at various time points after host castration, and in recurrent tumors. After the 1st week of host castration, AR had diffused to the cytoplasm and PSA and Ki67 expressions had markedly decreased, indicative of reductions in AR transcriptional activity and cell proliferation (Fig. 1C and Supplementary Fig. 1A). After tumor recurrence (about 4 mo later), the scores of these markers had returned to preCx values, showing even higher expressions of AR and PSA (Fig. 1C and Supplementary Fig. 1A).

Among the 10 HNPC PDX lines, seven developed into CRPC after castration (Supplementary Table 1). To identify potential driver genes of CRPC development, we first analyzed gene expression differences using the seven pairs of PDX CRPC models by comparing each CRPC tumor line with its parental HNPC line. Consistently upregulated genes were then ranked based on their statistical significances between pooled HNPC lines and CRPC lines. Eighty genes were identified to be significantly upregulated in CRPC ($p < 0.05$; Fig. 1D and Supplementary Table 4). Next, we checked the expression of these 80 genes from all 10 HNPC PDX lines collected at 12 wk after castration before the appearance of developed CRPC, as indicated by the sustained inhibition of AR signaling suggested by Gene Set Enrichment Analysis (Supplementary Fig. 1B–E and Supplementary Table 2). Of these 80 genes, *GRB10* was the top-ranked gene based on the statistical significance between HNPCs and castrated samples (Fig. 1E and Supplementary Table 4). Thus, these data demonstrate that GRB10 is not only upregulated in CRPC tumors, but also increased before CRPC fully develops, suggesting that it is a potential early driver of CRPC development (Supplementary Fig. 1F).

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