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Polo-Like Kinase 1 Mediated Regulation of Androgen Receptor: Potential Role in the Development of Castration Resistant Prostate Cancer

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Polo-Like Kinase 1 Mediated Regulation of Androgen Receptor: Potential Role in the Development of Castration-resistant Prostate Cancer

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Introduction

- Prostate cancer (PCa) is among the most common cancers and is the second highest cause of cancer-related deaths in American men.
- The androgen receptor (AR) is a nuclear receptor transcription factor, activated by dihydrotestosterone (DHT), that is required for normal prostate development and homeostasis.
- AR is subject to multiple post-translational modifications, including phosphorylation, that regulate protein stability, localization, and activity.
- Initially, PCa requires AR signaling for growth providing the clinical basis for androgen deprivation therapy (ADT), however, most patients relapse to castration-resistant prostate cancer (CRPC).
- Relapses have been attributed to receptor overexpression, intratumoral androgen biosynthesis, and aberrant regulation of AR-mediated signaling pathways.
- Polo-like kinase 1 (PLK1) is a serine/threonine kinase that is overexpressed in PCa and overexpression levels are positively correlated with cancer aggressiveness and poorer clinical outcome.
- PLK1 upregulation causes oncogenic transformation, epithelial-to-mesenchymal transition, and increased motility in prostate epithelial cells; and inhibition causes mitotic arrest and apoptosis.
- Gene expression profiling comparing androgen-independent and androgen-dependent prostate cancers shows an upregulation of PLK1 in androgen-independent cancers.
- PLK1 inhibitors in combination with androgen signaling inhibitors (ASI) enabled ASI resistance to be overcome in patient-derived tumor xenografts.
- In overexpression conditions, AR and PLK1 form a complex that can be immunoprecipitated (data not published).
- These data suggest a potential role of PLK1 in the transition to CRPC.

Significance and Objectives

- Current treatment options for CRPC are limited; androgen biosynthesis inhibitor abiraterone (Zytiga) and AR antagonist enzalutamide (Xtandi) offer more time for patients, but the prognosis remains terminal.
- Gaps in our understanding of the molecular changes associated with CRPC development hinder the development of mechanism-based therapies for use in this advanced disease stage.
- This project sought to investigate the role of PLK1 in the development of CRPC
- We hypothesized an endogenous interaction between PLK1 and AR resulting in a PLK1-mediated phosphorylation of AR

Results

PLK1 and AR interact endogenously:

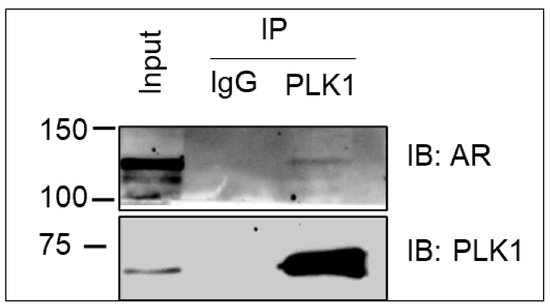

Figure 1. Mitotically arrested C4-2B cells were subjected to co-immunoprecipitation using anti-PLK1 antibodies. Immunoblotting was carried out using anti-AR and anti-PLK1 antibodies.

- We sought to investigate if an endogenous interaction between PLK1 and AR could be detected
- For this experiment, C4-2B cells were used as they are an androgen-independent cell line derived from the bone metastases of castrated nude mice implanted with LNCaP xenographs.
- Cells were arrested in mitosis to investigate the cell cycle dependency after being unable to obtain signal in asynchronous cells.
- Mitotically arrested C4-2B cells were lysed and subjected to co-immunoprecipitation, revealing that PLK1 and AR form an endogenous complex (Fig. 1).

Results

PLK1 phosphorylates AR *in vitro*:

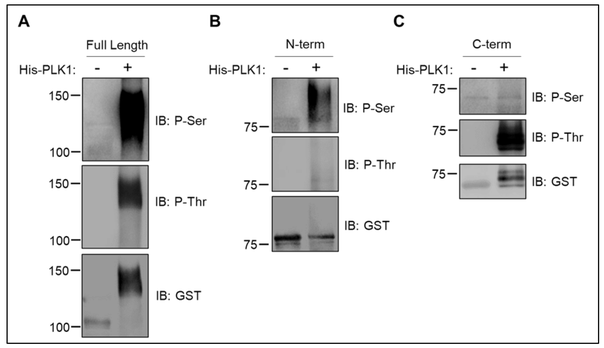

Figure 2. AR is phosphorylated by PLK1. (A) An in vitro kinase assay was performed using commercially obtained His-tagged PLK1 as the kinase and GST-tagged full-length AR purified from bacteria as substrate. For immunoblotting, the membrane was probed with phospho-serine and phospho-threonine antibodies. An anti-GST antibody was used as a loading control. (B) An in vitro kinase assay was performed with GST-tagged N-terminal AR as substrate. (C) GST-tagged C-terminal AR was used as substrate in an in vitro kinase assay.

- Due to PLK1 consensus sequences being present in the AR primary structure, we hypothesized that AR may be a phosphorylation substrate of PLK1.
- Full length AR was incubated with the kinase followed by immunoblotting with anti-phospho-serine and anti-phospho-threonine antibodies, which revealed strong phosphorylation at serine and threonine residues (Fig. 2A).
- The AR N-terminal domain (amino acids 1-504) produced a strong serine phosphorylation band and a faint threonine band (Fig. 2B).
- The reverse was observed for the C-terminal domain (amino acids 505-519), in which strong phosphorylation was observed for threonine but not serine (Fig. 2C).
- An anti-GST antibody was used as a loading control and revealed mobility shifts were observed for the full length and c-terminal (Figs. 2A and C).
- Collectively, these data suggest that AR is a phosphorylation substrate of PLK1.

Summary and Future Directions

- The data presented herein provides preliminary evidence to support an endogenous interaction that results in phosphorylation of AR by PLK1.
- A number of additional experiments must be conducted to strengthen these findings, these include reciprocal co-IPs, *in vitro* kinase assays with phosphomutant substrate, and *in vivo* phosphorylation experiments.
- Following these conformational studies, we will investigate the functional significance of this interaction. However, we hypothesize that phosphorylation of AR results in nuclear translocation and increased transcription factor activity, likely in a ligand-independent manner.
- Characterization of this interaction could elucidate a molecular driving force behind CRPC development potentially offering a novel therapeutic avenue for those suffering from this lethal disease.

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