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 dihydrotosterone (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) enable 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:

- 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).

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.

PLK1 phosphorylates AR in vitro:

- 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.

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.

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 nucleolar 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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