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TITLE: HOMEODOMAIN INTERACTING PROTEIN KINASES MODULATE HYPOXIC ADAPTATION AND CHEMORESISTANCE

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14. ABSTRACT

The goal of this project was to dissect the roles of Homeodomain Interacting Protein Kinases (HIPK) isoforms 2 and 3 in the development of castration resistant prostate cancer (PCa) following hypoxic reprogramming using established cell lines. Our objectives were to (1) ectopically express or silence HIPK2 and HIPK3 in PCa cell lines (LnCAP & LnCAP-abl), (2) define the impact of HIPK manipulation on PCa cell biology (proliferation, apoptosis, metabolism, etc.), (3) evaluate the interplay of HIPK2 and HIPK3 with the Hippo signaling pathway, and (4) delineate the roles of HIPK2 and HIPK3 in PCa chemo-resistance. Our studies were able to demonstrate that HIPK2 was androgen responsive and under direct control of the androgen receptor. We were also able to clone HIPK2 and HIPK3 into expressing vectors and obtain small RNAs for silencing. Technical difficulties prevented our group from evaluating the impact on HIPKs in PCa as ectopic expression produced a protein of incorrect size and silencing proved ineffective. We will resolve these issue and continue to examine HIPKs in PCa in future studies.

15. SUBJECT TERMS

Homeodomain Interaction Protein Kinases (HIPKs), Castration-resistant prostate cancer, hypoxia, chemoresistance.

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1. Introduction

The goal of Dr. Hodgson's project was to dissect the roles of Homeodomain Interacting Protein Kinases (HIPK) isoforms 2 and 3 during the development of castration-resistant prostate cancer (PCa) following hypoxic reprogramming using established, continuous cell lines. The objectives were to (1) ectopically express or silence HIPK2 and HIPK3 in PCa cell lines (LnCAP and LnCAP-abl), (2) define the impact of HIPK manipulation on PCa cell biology (proliferation, apoptosis, metabolism, etc.), (3) evaluate the interplay of HIPK2 and HIPK3 with the Hippo signaling pathway, and (4) delineate the roles of HIPK2 and HIPK3 in PCa chemo-resistance.

2. Keywords

Homeodomain Interacting Protein Kinases (HIPK), castration-resistant prostate cancer, hypoxia, chemo-resistance.

3. Accomplishments

Specific Aim 1: Determine the androgen regulation of HIPK2. Dr. Hodgson's team completed the major tasks of this aim, which were to clone HIPKs into the pCR3.1 plasmid and assess the regulation of HIPK2 by androgens. Firstly, the group cloned HIPK2 and HIPK3 open reading frames into the pCR3.1 vector, and this was confirmed by sequencing of the plasmids. With regards to androgen regulation, they found that HIPK2 activity was influenced by androgens. ChIP analysis (included as preliminary data in the program application) revealed that androgen receptor (AR) components were recruited to the HIPK2 locus in PCa cells.

Specific Aim 2: Evaluate AR-HIPK2-Hippo crosstalk. Dr. Hodgson's group was unable to accomplish the major task of this specific aim, wherein he proposed to define the interplay between HIPK regulation and Hippo signaling. While his team generated both transient and stable PCa cell lines silencing and expressing HIPK2 and HIPK3, they could not define significant changes in signal transduction, Hippo-related gene expression or metabolism.

Specific Aim 3: Determine the impact of HIPK proteins on PCa chemo-resistance and invasion. Dr. Hidgson's team was unable to complete the major task of this aim, which was to compare chemotherapy responses of PCa cells silencing or over-expressing HIPKs. Due to the lack of responsiveness in the other expression and silencing studies, they tried new plasmids and delivery systems to improve biological outcomes.

4. Impact

Dr. Hodgson's group was able to determine that HIPK2 was an androgen responsive gene under the control of the androgen receptor. However, technical difficulties involving the expression and silencing of HIPK2 and HIPK3 impeded their progress. Dr. Hodgson maintains that these proteins are crucial to the progression of PCa in humans and he will continue to dissect the mechanisms involving HIPKs in cancer cell biology.

5. Changes/Problems

Specific Aim 1: Determine the androgen regulation of HIPK2. Over-expression, which was confirmed by RT-PCR (data not shown), of HIPK2 and HIPK3 in these cells did not appear to affect proliferation (Figure 1A). Similarly, silencing either HIPK2 or HIPK3 isoforms (confirmed by RT-PCR – data not shown) did not alter the androgen responsiveness (as indicated by the lack of changes in related gene expression) in LnCAP cells (Figure 1B). Further, ectopic expression of HIPK2 or HIPK3 in PCa cell lines had no influence on apoptotic protein levels (Figure 1C). Evaluation of the proteins expressed by the pCR3.1 plasmids by western blot analysis suggests that HIPK3 was full length, while HIPK2 was produced as a smaller protein (nearly 20kDa) than expected (Figure 2). Re-sequencing the plasmids did not reveal any mutations that could explain the potential truncation of HIPK2. From these data, they surmised that HIPK2 is regulated by androgens and the androgen receptor; however, the roles of HIPKs on PCa cell biology remain an ongoing focus of their efforts.

Specific Aim 2: Evaluate AR-HIPK2-Hippo crosstalk. Briefly, PCa cell lines silencing HIPK2 and HIPK3 were produced using both transient and stable introduction of shRNAs. Silencing was confirmed by qPCR (Figure 3). Monitoring HIPK and Hippo signaling using qPCR, his group did not detect consistent changes in gene expression of any gene in the Hippo pathway either upstream or downstream of HIPKs or the Hippo kinase complex during the silencing of HIPK2 (Figure 4). Examination of cellular metabolism by the Seahorse XF-96 extracellular flux analyzer also did not reveal significant changes in glycolytic or respiratory metabolism (Figure 5). Ectopic expression of HIPK2 was problematic, as a full length protein does not appear to have been produced, as indicated above. However, altering HIPK3 expression in PCa cells did not result in a significant change in Hippo pathway gene expression or metabolism (Figures 4 and 5, respectively).

Dr. Hodgson's team also examined the effects of HIPK levels on hypoxic responses under normoxic and hypoxic (1% O2) conditions. While HIF1α levels were undetectable in normoxia, cells treated under hypoxic conditions had increased HIF1α (Figure 6). Silencing or expression of HIPK2 and HIPK3 had no impact on the HIF1α levels in PCa cells under normoxic or hypoxic conditions (Figure 6). They did not have time to test other stress responsive gene expression before the end of this project. Dr. Hodgson's group will continue to optimize the expression and silencing systems as they continue to dissect these crucial pathways in PCa.

Specific Aim 3: Determine the impact of HIPK proteins on PCa chemo-resistance and invasion. While they were able to produce EC50 values for chemotherapeutic drugs in PCa cell lines comparable to values presented in the existing scientific literature, his group was unable to do so in PCa cells with altered HIPK2 and HIPK3 expression. Unfortunately, Dr. Hodgson's group ran out of time before they could use the new PCa cell lines to conduct these studies.

6. Products

None to report

7. Participants & Other Collaborating Organizations

Myles C. Hodgson (PI)
Aymun Ahmed (undergraduate student)
Francesca Lantana (undergraduate student)
Victoria Sanchex (undergraduate student)

8. Special Reporting Requirements

None to report

9. Appedices (Figures)

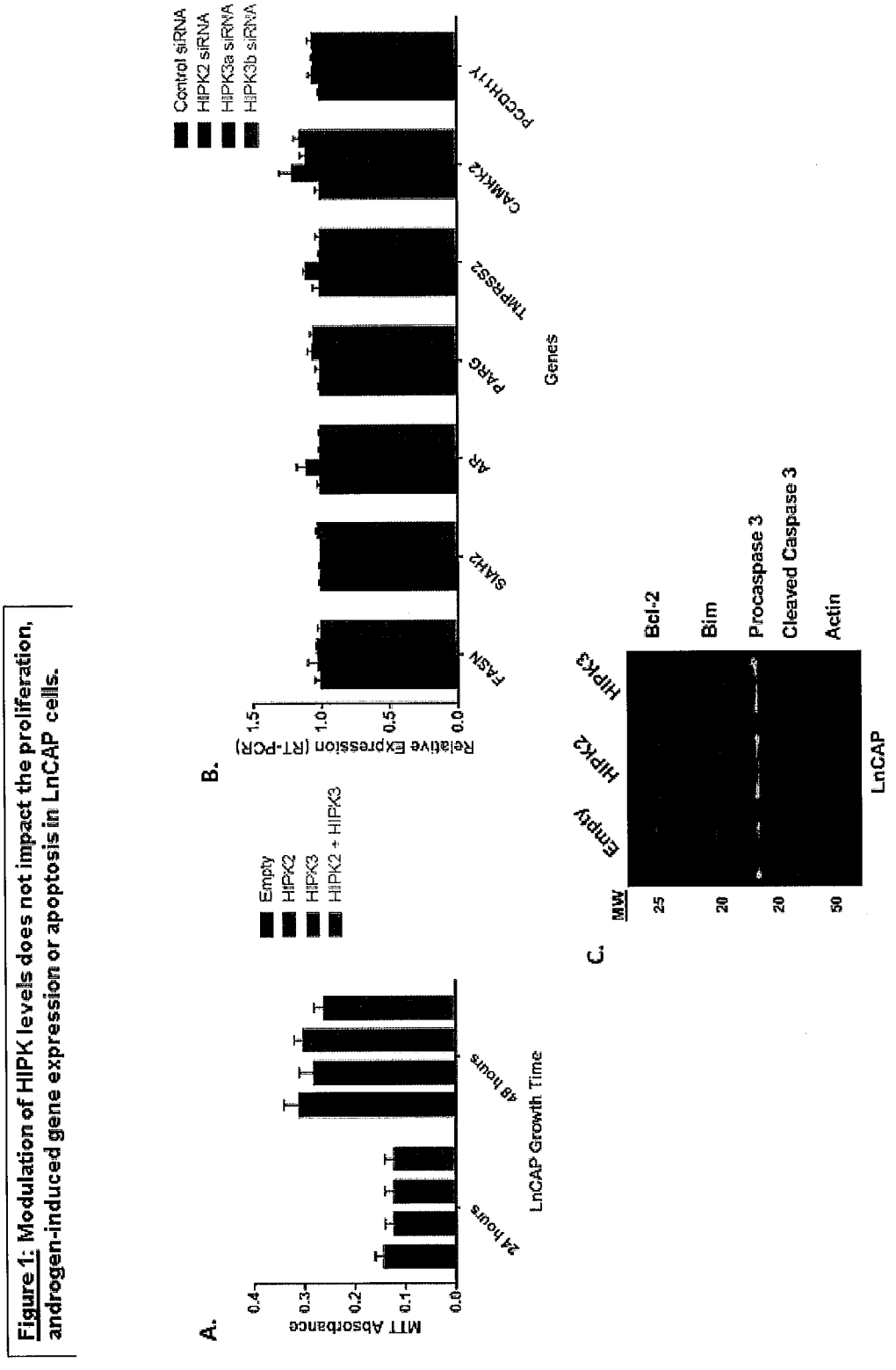


Figure 1: Modulation of HIPK levels does not impact the proliferation, androgen-induced gene expression or apoptosis in LnCAP cells.

Figure 2: Expression of HIPK2 in LnCAP cells produces a truncated variant.

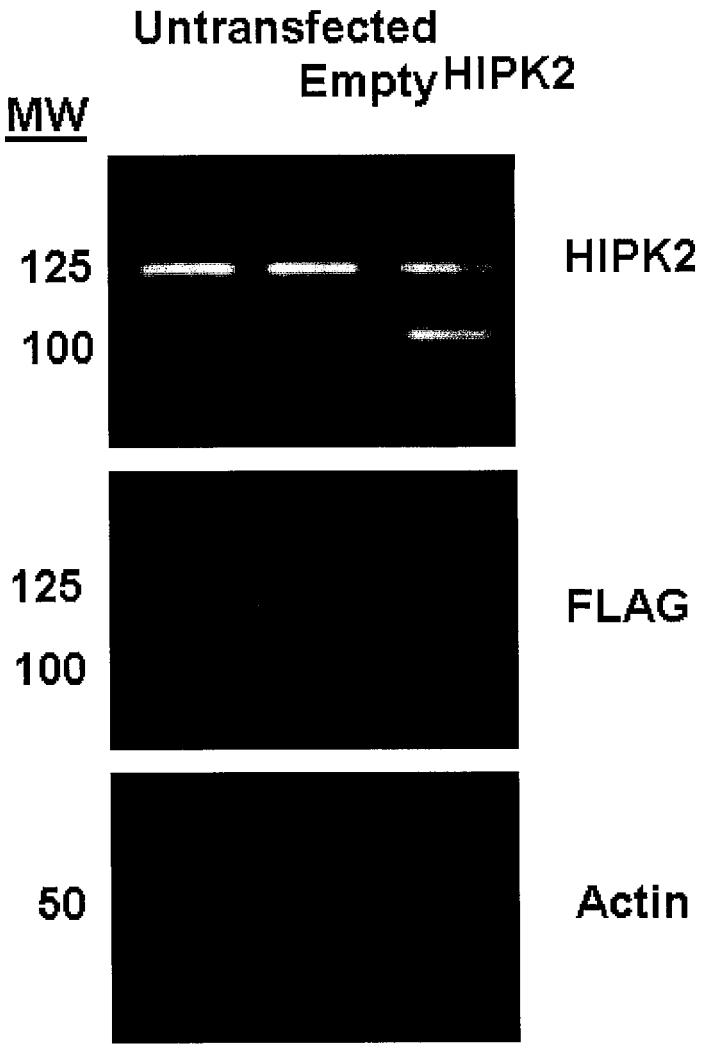


Figure 3: Silencing HIPK Expression with siRNAs in LnCAP cells.

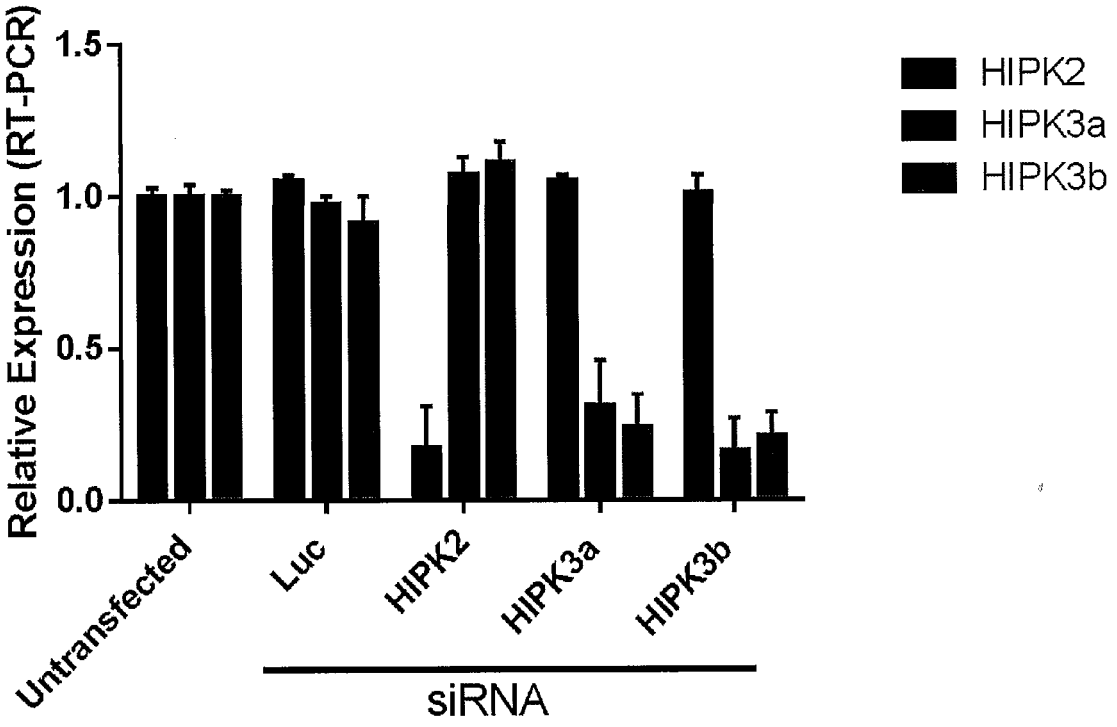


Figure 4: Silencing HIPKs does not impact the expression of Hippo pathway proteins in PCa cells.

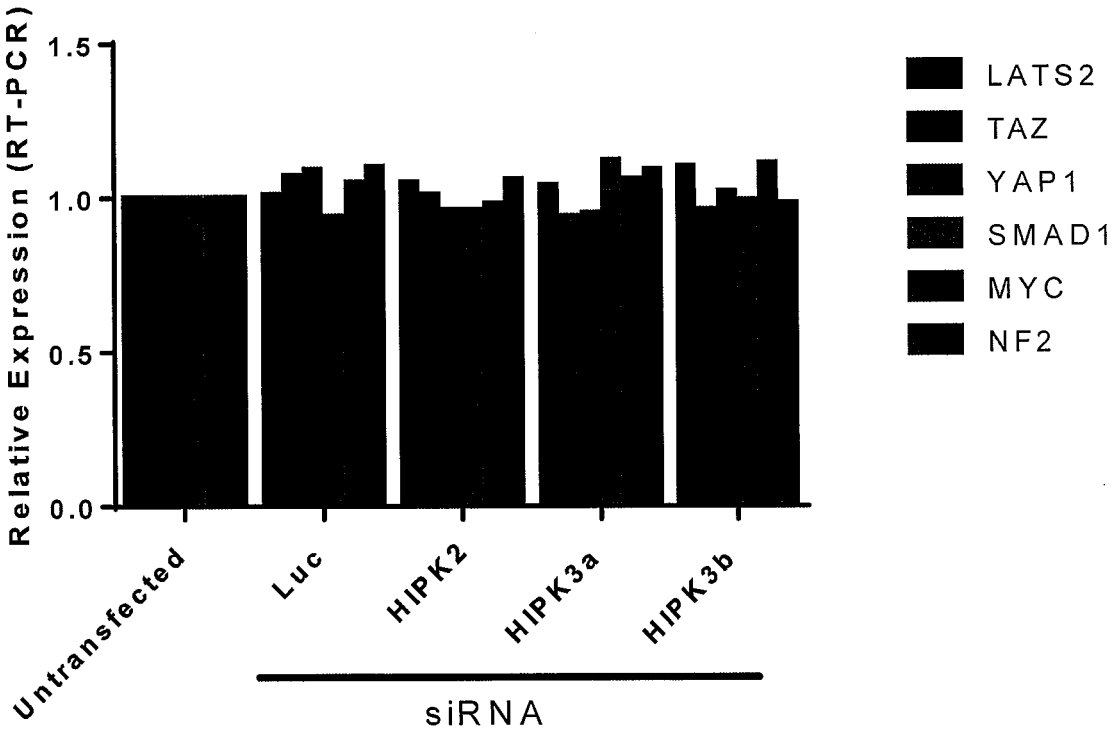
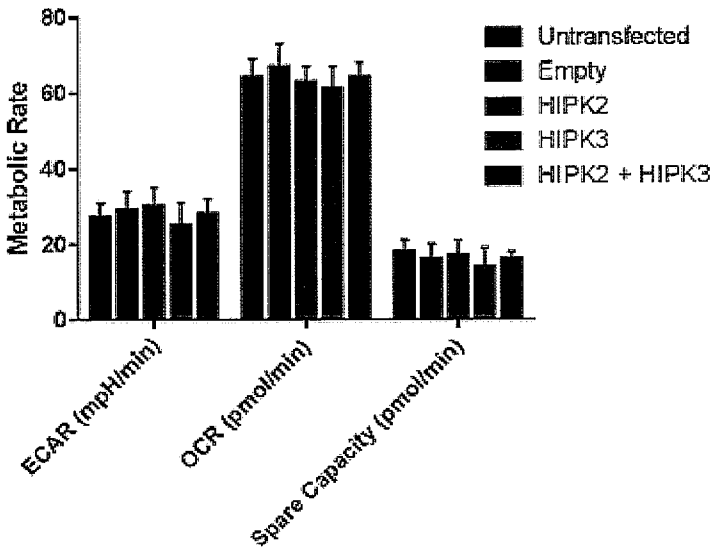


Figure 5: Altering HIPK expression does not impact glycolytic or respiratory metabolism in PCa cells.



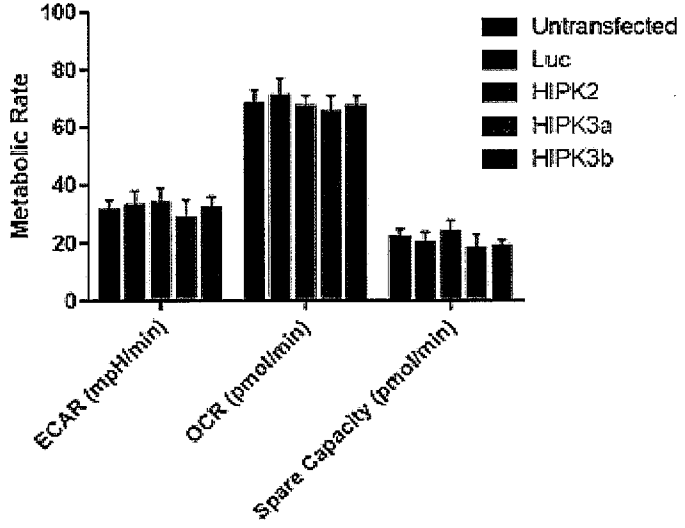


Figure 6: Altering HIPK expression does not impact HIF1a expression in PCa cells under normoxic or hypoxic conditions.