Kinase Joins the Chaperone Club: Androgen-Regulated Kinome Reveals Choline Kinase Alpha as Potential Drug Target in Prostate Cancer

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In order to identify clinically relevant downstream effectors of androgen signalling, the androgen-regulated kinome was defined in prostate cancer (PCa). Within this study, Choline kinase alpha (CHKA) was identified as an androgen receptor chaperone that is both a biomarker of progression and a potential therapeutic target for PCa.

The androgen receptor (AR) is an oncogenic transcription factor implicated in prostate cancer (PCa) progression. Resistance to androgen deprivation therapy occurs in progression to castration-resistant prostate cancer[1] (CRPC) and is accompanied by resurgence of AR signalling. Multiple mechanisms can stimulate AR signalling in CRPC including gain-of-function mutations in the AR signalling pathway[2], up-regulation of heat shock proteins (HSPs) that act as chaperones for AR and kinases that can potentiate AR function[3]. Consistent with this concept, targeting of HSPs and kinases in pre-clinical models inhibits AR function and PCa tumour growth[4,5]. While, these diverse resistance mechanisms highlight the reliance of PCa on AR signalling, there is a critical need to identify therapeutic targets that regulate AR function as well as key downstream effector pathways.

We profiled the AR-regulated kinome in PCa with a view to demonstrating functionally and clinically important signalling events downstream of the AR. We showed that choline kinase alpha (CHKA) is a chaperone for the AR, promoting its stability and function[6]. This is the first report demonstrating that kinases can act as chaperones. During our studies we also identified 49 androgen-regulated kinases, of which 25 were up- and 24 down-regulated. We confirmed the *in vivo* androgen regulation of a subset of these genes in tumour tissue from chemically castrated PCa patients (18/25 androgen up-regulated kinase genes).

CHKA was overexpressed in localised (primary) and metastatic PCa, and decreased in tissues from castrated men. Protein levels of CHKA were correlated with Gleason Grade and Stage and CHKA was an independent predictor of biochemical recurrence. CHKA can exist as two isoforms CHKA2 and CHKA1. Interestingly, the interaction of CHKA2 with the AR decreased within 5-15 min of androgen treatment, consistent with the timing of AR release from cytoplasmic chaperones and its translocation to the nucleus. AR protein levels were reduced by CHKA knockdown indicating a reciprocal role of CHKA in regulating AR protein levels. A direct physical interaction was mapped between CHKA2 and the LBD of AR (AR^{LBD}), the AR domain that interacts with HSPs. In a protease protection assay, both DHT and CHKA2 were required to stabilise AR. In line with this, CHKA knockdown did not decrease the levels of an AR variant (ARv567es) lacking the LBD. Taken together, our data show for the first time that kinases can function as protein chaperones - a feature never previously attributed to any kinase.

CHKA inhibition decreased the activity of full length AR, but failed to inhibit AR variants lacking the LBD, indicating that CHKA regulates AR transcriptional activity by binding to AR^{LBD}. Transcriptome sequencing from CHKA knockdown in C4-2 cells found a highly significant bi-directional overlap between siCHKA and androgen-regulated genes, consistent with a global role for CHKA in AR regulation. Pathway analysis identified enrichment for pathways regulating protein folding and cellular protein localisation, consistent with the chaperone function of CHKA. These analyses highlight a functional link between AR and CHKA signalling, indicating a cooperative function of CHKA in driving the AR-regulated transcriptome by stabilising AR protein levels.

CHKA inhibition induced apoptosis in many CRPC cell line models in a manner similar to AR inhibition. The growth-promoting role of CHKA did not depend on the catalytic activity of CHKA since the addition of exogenous phosphocholine or phosphatidylcholine did not rescue growth inhibition by a CHKA inhibitor that reduced AR activity, AR levels and CHKA levels. In an *ex vivo* culture assay, treatment of hormone-naïve primary PCa tissue with CHKA inhibitor decreased AR expression in tumour epithelia, while increasing levels of cleaved caspase-3, a marker of apoptosis. Migration and invasion phenotypes were also inhibited by CHKA knockdown. Finally, PCa xenograft growth was inhibited using an inducible CHKA knockdown system. Collectively, these experiments indicate that CHKA inhibition opposes androgen action and that inhibition of CHKA reduces malignant phenotypes and triggers apoptosis in PCa cells and tissue. The data demonstrate the importance of CHKA in PCa growth and invasion, highlighting the potential future benefits of inhibitors of this kinase in the clinical management of PCa.

In summary, CHKA can act as a chaperone that regulates AR signalling, elucidating a feed-forward AR-CHKA signalling loop that reinforces AR activity, allowing CHKA to maintain its own expression in PCa. The chaperone function of CHKA confers a growth advantage on PCa by stabilizing the AR in addition to its well-known function in fuelling membrane production through the Kennedy pathway [7] (Figure 1), suggesting a *de facto* role for CHKA as a rate-limiting factor in cancer cell growth.

This discovery should accelerate the screening of potential CHKA inhibitors that will not only inhibit the catalytic function but also decrease the CHKA protein level. We believe our work will direct efforts in the treatment of PCa in particular, but also for other cancers, by stimulating a systematic re-evaluation of CHKA as a therapeutic target in cancers where it is overexpressed and exhibits analogous effects on other oncogenic proteins.

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Figure 1. Classical and non-classical functions of CHKA. Classical model involves ubiquitination and activation of AR-dependent transcription that results in up-regulation of CHKA, a rate limiting enzyme in the Kennedy pathway (production of phosphatidylcholine, required for plasma membrane biogenesis). In addition to its role in Kennedy pathway, CHKA can also interact with the AR in cytoplasm and promotes its stability (non-classical). This could lead to AR overexpression and increased AR signalling which in turn

may allow more CHKA production. AR=androgen receptor; U=ubiquitin; CHKA=choline kinase alpha.

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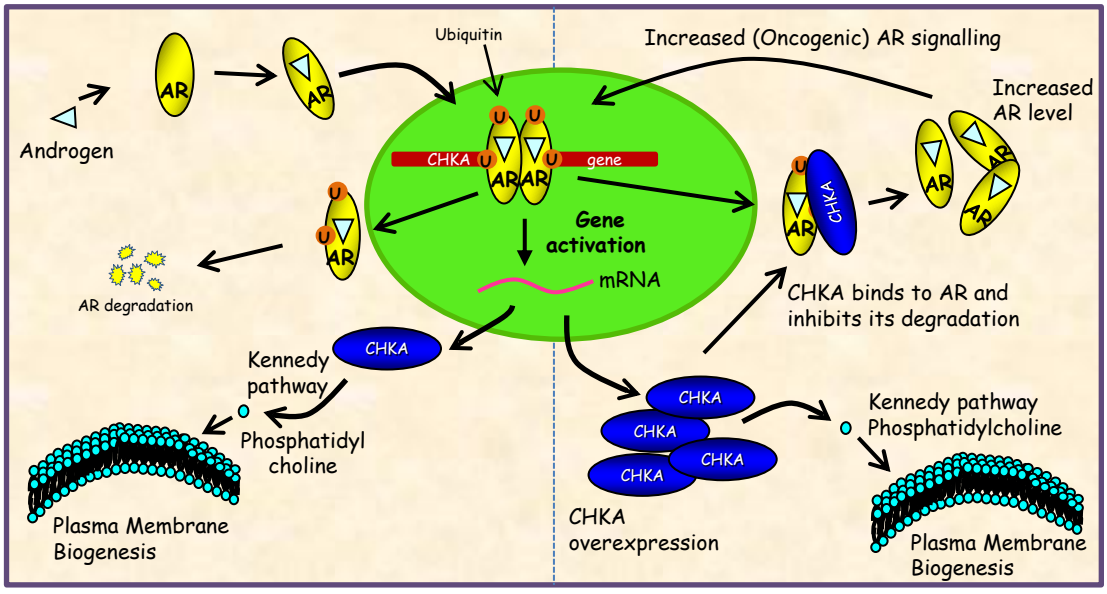
Figure 1



Classical CHKA function in Kennedy pathway

Dual function of CHKA in AR signalling