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Mitochondrial localization, import and mitochondrial function of the androgen receptor

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Running title: Mitochondrial import and function of androgen receptor

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Abstract

Nuclear localization of androgen receptor (AR) directs transcriptional regulation of a host of genes, referred to as genomic signaling. Additionally, non-nuclear or non-genomic activities of the AR have long been described, but the understanding of these activities remains elusive. Here, we report that AR is imported into and localizes to mitochondria and has a novel role in regulating multiple mitochondrial processes. Employing complementary experimental approaches of AR knockdown in AR-expressing cells and ectopic AR expression in AR-deficient cells, we demonstrate an inverse relationship between AR expression and mtDNA content and transcription factor A, mitochondrial (TFAM), a regulator of mtDNA content. We show that AR localizes to mitochondria in prostate tissues and cell lines and is imported into mitochondria *in vitro*. We also found that AR contains a 36-amino-acids-long mitochondrial localization sequence (MLS) capable of targeting a passenger protein (GFP) to the mitochondria and that deletion of the MLS abolishes the import of AR into the mitochondria. Ectopic AR expression reduced the expression of oxidative phosphorylation (OXPHOS) subunits. Interestingly, AR also controlled translation of mtDNA-encoded genes by regulating expression of multiple nuclear DNA–encoded mitochondrial ribosomal proteins. Consistent with these observations, OXPHOS supercomplexes were destabilized and OXPHOS enzymatic activities were reduced in AR-expressing cells and restored upon AR knockdown. Moreover, mitochondrial impairment induced AR expression and increased its translocation into mitochondria. We conclude that AR localizes to mitochondria where it controls multiple mitochondrial functions and mitonuclear communication. Our studies also suggest that mitochondria are novel players in non-genomic activities of AR.

INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer deaths in men in the United States (1-3). In spite of recent advances in prostate cancer diagnosis and treatment, mortality from PCa remains very high. Androgen deprivation therapy (ADT) is the standard treatment regimen, but patients initially responding well to ADT develop resistance to the treatment and progression to androgen-independent or castration-resistant prostate cancer (CRPC) occurs. Metastatic CRPC remains the biggest challenge to treat, as androgen independence leaves no straightforward way to its management (4). Numerous hypotheses have been put forward to explain the development of CRPC; over-expression of AR and constitutive AR activity as a result of AR amplification and/or mutations remain the most acceptable (5). Recent studies have identified AR mutations in prostate cancer that lead to ligand-free translocation of the receptor into the nucleus, resulting in constitutively active AR (6). A number of studies have also reported cytoplasmic functions of AR not only in prostate cancer cells (7-11) but also in non-prostate cells, where androgen dependent AR translocation in the nucleus is not observed (12). Another study demonstrated that

the role of AR in CRPC is not to direct the androgen dependent gene expression, but to execute a different program, resulting in androgen independent growth (13). Likewise, the ability of AR to enhance the invasion of prostate cancer cell lines independent of its nuclear localization has also been shown (14). These studies suggest that besides nuclear genomic signaling, AR also participates in non-genomic (outside the nucleus) signaling that may play an important role in prostate carcinogenesis (15). Unfortunately, the origin and nature of non-genomic AR signaling remain elusive.

Mitochondria regulate cell growth, survival, and death by an intricate and as yet poorly understood the mitochondrial-nuclear cross talk. This crosstalk is mediated by anterograde (nucleus to mitochondria) and retrograde (mitochondria to the nucleus) signaling, which continuously monitor and fine-tune signaling and cellular metabolism as a part of adaptation to a changing cellular environment. MtDNA depletion has been associated with multiple cancers (16-18) including prostate cancer (19,20). It has been demonstrated that apoptosis is inhibited in mtDNA depleted cells, favoring cancer progression (21-23). mtDNA depleted cells also show Warburg effect, metabolic reprogramming and cancer stem cell properties (24,25). We have provided evidence that, in African-Americans, a low mtDNA content is a risk factor for poor prognosis and aggressive prostate cancer (26). An interesting study demonstrated that the depletion of mtDNA from androgen-dependent LNCaP results in the loss of androgen dependence and restoration of mtDNA restores androgen dependence (27), suggesting mitochondria to be central to advanced stages of prostate cancer. These results indicate that mtDNA may play an important role in androgen dependence of prostate cancer cells. These studies establish an undisputed role of mitochondria in prostate carcinogenesis, and in cancer disparities in ethnically diverse populations.

The above pieces of evidence suggest that androgen receptor and mitochondrial functions are interlinked and are two important determinants of prostate cancer, which strongly affect risk and may play critical roles in the progression from androgen dependence to independence. However, very little is known about the cellular significance of the connection between these two. In the present study, we demonstrate that AR besides being nuclear also localizes into mitochondria, thereby regulating the mitochondrial function and retrograde signaling. We demonstrate the existence of an authentic mitochondrial localization sequence in the N-terminus of AR protein. We established a distinct AR function regulating mitochondrial processes directly and indirectly. Our study provides insights into a previously unrecognized non-genomic role of AR in modulating cellular functions.

RESULTS

We utilized two complementary approaches in our studies. First, a CRISPR-cas9 approach was employed to knockout AR in LNCaP cells. Western blot analyses showed about 80% decrease in AR expression in AR knockout cells

compared to mock control (Fig 1A). We also used harmalol hydrochloride (HH) in a pharmacological approach to inhibit AR expression (Fig 1B). In a complementary approach, we ectopically expressed AR in PC-3 cells lacking AR expression. Expression of AR in PC-3 cells was confirmed by western blot (Fig 1C). It is noteworthy that AR re-expression in PC3 cells has been shown to result in constitutively active nuclear AR, which does not require ligand for activation and transcription of its target genes (10,28,29).

AR maintains mtDNA homeostasis

To elucidate an association between AR and mtDNA, we measured mtDNA content, in AR knockdown LNCaP cells and in PC-3 cells expressing AR ectopically. An eighteen percent increase in mtDNA content was observed after AR knockdown in LNCaP while about twenty-five percent decrease was observed in PC-3 upon AR expression (Fig 2A).These studies inversely correlate mtDNA content with AR expression. The changes in the mtDNA content led us to investigate if AR modulates mtDNA via regulation of TFAM, a protein known to regulate mtDNA content. It has been previously shown that fibroblast derived from TFAM -/+ mice contain reduced mtDNA content (30). Interestingly AR knockdown in LNCaP cells increased TFAM expression (Fig 2B-i), while ectopic AR expression in PC3 cells led to a decrease in TFAM expression (Fig 2B-ii). To validate the semi-quantitative changes in TFAM expression, we performed real-time quantitative PCR analysis and observed a 2.3 fold increase and 73% decrease in TFAM expression in AR knockout LNCaP and AR expressing PC-3 cells, respectively (Fig2C-i and ii). These results suggest that AR maintains mtDNA homeostasis via regulation of TFAM.

Mitochondrial stress induces expression of AR

To determine if mitochondrial stress impacts the expression of AR, we employed two different approaches: Firstly, we used rho^0 cells which were devoid of mtDNA. Secondly, we utilized rotenone to inhibit OXPHOS complex I. Interestingly, we observed about a two-fold increase in AR expression in rho^0 cells (Fig 3A). An alternative approach was also used to introduce mitochondrial stress by inactivating mtDNA polymerase ϒ (POLG1) by CRISPR-cas9 (Fig 3B) and inhibiting complex I by rotenone (Fig 3C). POLG1 inactivation is known to deplete mtDNA and hence reduce mitochondrial OXPHOS function (31,32). Our experiments shows increased AR expression with a parallel increase in PSA expression due to defects in mitochondrial function (Fig 3B-C). These studies suggest that AR is a retrograde responsive protein regulating nuclear genes.

AR localizes into mitochondria

To analyze the subcellular localization of AR, we isolated nuclear, mitochondrial and cytosolic fractions from prostate PC-3 cells expressing AR (Fig4A-i), LNCaP endogenously expressing AR (mock), CRISPR-Cas9 AR knockout LNCaP cells (Fig 4A-ii) and normal mouse prostate tissues (Fig4A-iii). In addition to its expected

nuclear and cytoplasmic localizations, in each case, we observed a strong AR immunoreactive in the mitochondrial fraction (Fig 4A i-iii). Lamin A/C (nuclear), COXII (mitochondrial) and tubulin (Cytoplasmic) antibodies were as used controls.Since these experiments suggest AR presence in the mitochondria, we investigated if AR contained mitochondrial localization signal (MLS). To predict the probability of AR import, we analyzed AR protein sequence by using MitoProt database. The MitoProt software predicted MLS and a cleavage site after the first 14 amino acids at the N-terminus with a probability score of 0.25 (Fig 4B-ii). We cloned WT-AR in pHTC-Halo-Tag CMV-neo vector and using this template we generated an AR mutant (Δ36n-MLS-AR) lacking the first 36 amino acids long putative MLS present in N terminus of AR. We used the CRISPR-Cas9 system to knock out AR in LNCaP cells (Fig 4Aii right panel). LNCaP cells lacking AR expression were transfected with wild type (Fig 4B-i) and Δ36n-MLS-AR (Fig 4B-iii). We then isolated nuclear, mitochondrial and cytoplasmic fractions and conducted Western blot analyses. As expected WT-AR (containing the MLS) localized into mitochondrial compartment within the cell (Fig 4B-i) (Top panel), whereas Δ36n-MLS-AR (without MLS) lacked mitochondrial localization (Fig 4B-iii) (Top panel). A very faint immunoreactive band was detected in mitochondrial fractions of Δ36n-MLS-AR, which was sensitive to trypsin treatment (Fig 4B-iii) (bottom panel), indicating that AR lacking MLS was bound to the outer membrane and not targeted inside mitochondria. (Fig4B-iii). Interestingly, the mitochondrial fraction of WT-AR was resistant to trypsin treatment further confirming the intra-mitochondrial localization of WT-AR (Fig 4B-i)(Bottom panel). Additionally, mitochondrial fractions were lysed by treatment with 1% Triton X-100 (v/v) before trypsin treatment. The WT-AR mitochondrial protein became sensitive to trypsin following disruption of the mitochondrial membrane by treatment with Triton X-100 (TT), further confirming that it is localized in the mitochondrial matrix compartment (Fig 4B-i)(Bottom panel). Consistent with earlier observation wild type (and mutant) ARlocalized to nuclear and cytosolic fractions within the cell (Fig 4B-i and iii)(Top Panel).

We also performed in vitro mitochondrial import experiments (Fig 4B-iv, v). For these experiments (Fig 4B-iv and v), WT-AR, Δ36n-MLS-AR, Su9-DHFR(positive control) and DHFR (negative control) pHTC-Halo-Tag constructs were used. S^{35} radiolabeled methionine was used label the translated protein, (Supplementary figure 1). The constructs used in invitro experiments were transcribed and translated using rabbit reticulocyte lysate. The translated protein was used for import into isolated mouse brain mitochondria. Our results show that while WT-AR containing the MLS is imported into mitochondria (Fig 4B-v) (Supplementary Fig1-i), Δ36n-MLS-AR lacking the MLS is not imported into the isolated mitochondria (Fig 4B-v). Su-9 dihydrofolate reductase (Su9-DHFR), and dihydrofolate reductase (DHFR) were used as positive and negative controls respectively. Su9-DHFR

contains a classic mitochondrial targeting signal (MTS), a presequence of subunit 9 of Neurospora crassa F0F1-ATPase which has been fused to DHFR. Notably, Su9-DHFR trypsin untreated lane shows both MLS processed and unprocessed forms. Upon successful import, the MTS is cleaved. Thus only the cleaved protein is present and protected in the mitochondria after trypsin treatment (Fig 4B-v) (Supplementary Fig1-ii). Since DHFR, is a cytosolic protein, it was used as a negative control in the experiment (Fig 4B-v) (Supplementary Fig1-iii). While Δ36n-MLS-AR was not imported in the mitochondria, WT-AR was imported and protected from digestion by trypsin treatment, indicating that WT-AR is localized inside the mitochondria (Fig4 B-v). These studies suggest that first N terminal 36 amino acids in AR serve as MLS for import into the mitochondria.

Additionally, we took a complementary third approach utilizing the confocal microscopy. We cloned cDNA sequences containing MLS amino acids in frame with GFP. Cells expressing AR-MLS cloned in frame with GFP showed co-localization of GFP with mitotracker, which stains mitochondria in red (Fig 4Cbottom panel).We additionally transfected cells with GFP alone (lacking AR-MLS). These cells lack colocalization with mitotracker (Fig 4C top panel). These studies suggest that AR contains authentic MLS capable of transporting a passenger protein such as GFP into mitochondria.

We also asked if mitochondrial stress (induced by depletion of mtDNA encoded COXII by POLG1 knockout) and by inhibition of OXPHOS by rotenone (33) increased AR translocation into mitochondria. No change in AR translocation was observed in nuclear fractions after cellular stress. However, we found increased AR translocation in mitochondrial fractions after mitochondrial stress. There was a concomitant decreaseCOX-II expression (mtDNA encoded) upon POLG1 induced mtDNA depletion and after rotenone induced mitostress. These results demonstrate that mitochondrial stress either by genetic or metabolic stress induces increased translocation of AR into mitochondria (Fig 4D-i and ii).

Together, our studies suggest that AR i) localizes into the mitochondria of prostate tissue and prostate cell lines ii) contains an authentic MLS capable of transporting a passenger protein into mitochondria, iii) is transported into mitochondria in vitro iv) translocation is increased upon mitochondrial genetic and metabolic stresses, and v) is an authentic mitochondria localized protein.

AR regulates expression of mtDNA encoded OXPHOS subunit

AR is a well-known transcriptional regulator of a large number of nuclear genes (34). To analyze the effect of AR on mtDNA encoded OXPHOS subunits, we conducted OXPHOS blot analyses of proteins isolated from AR CRISPR-cas9 knockdown LNCaP cells. We observed increased expression of NDUFB8 (complex I), SDHB (complex II), and UQCRC2 (complex III) subunits (Fig 5A). In PC3 cells ectopically expressing AR mtDNA encoded OXPHOS

subunit COXII (complex IV) was markedly reduced. Interestingly, expression of nuclear DNA encoded OXPHOS subunits, ATP5A (complex V), UQCRC2 (complex III), SDHB (complex II), and NDUFB8 (complex I), was also significantly down-regulated (Fig 5B). These studies suggest that AR controls the expression of mitochondrial genes as well as nuclear-encoded subunits involved in OXPHOS function.

AR regulates expression of nuclear DNA encoded OXPHOS subunits

Based on the above observations, we expanded our studies to undertake detailed expression analyses of other nuclear DNA encoded OXPHOS subunits. Increased expression of all four Complex II subunits was observed upon AR knockdown in LNCaP (Fig 6A-i); conversely, all four subunits of complex II were down-regulated in AR-expressing PC-3 cells (Fig 6A-ii). Likewise, subunits of complex III (Fig 6B-i) and complex IV (Fig 6B-ii) were up-regulated after AR knockdown. Complex IV subunits were downregulated upon AR expression in PC-3 cells (Fig 6B-iii). Together these studies suggest that AR regulates a large number of nuclear genes encoding mitochondrial OXPHOS subunits.

AR regulates expression of OXPHOS assembly factors

Various OXPHOS subunits encoded by nuclear DNA are synthesized in the cytoplasm and transported into mitochondria, which are then assembled into functional units by OXPHOS assembly factors (35,36). Except for SDHAF1, expressions of SDHAF2 and SDHAF3 (Complex II) were increased upon AR knockdown (Fig 7A). Interestingly a marked increase in the expression of other known complex III assembly factors UQCC1, UQCC2, UQCC3 and TTC19 (Fig 7A), and complex IV assembly factors SCO1, COX10 and COX15 (Fig 7A), was observed. The mRNA quantification in PC-3 cells expressing AR showed a robust decrease in the expression of complex II assembly factors (Fig 7B), complex III assembly factors (Fig 7B), and complex IV assembly factors (Fig 7B). These results further confirm that the androgen receptor is a negative regulator of OXPHOS assembly factors.

AR impacts the stability of OXPHOS supercomplexes

OXPHOS complexes are organized as super complexes (37,38). Since our studies suggest that AR regulates nuclear DNA and mtDNA encoded OXPHOS subunits and OXPHOS assembly factors, we further analyzed the impact of AR on the stability of OXPHOS supercomplexes. A unique pattern for OXPHOS supercomplexes was revealed by Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) analysis in LNCaP mitochondrial fractions of both CRISPR knockdown and HH treated samples. The OXPHOS Super Complex I was more stabilized in AR knockdown cells as compared to the parental counterparts (Fig 8A-i). Minor changes were observed in the stability of complex III_2/complex IV and complex III_2 supercomplexes. Interestingly,

mitochondrial fractions from HH treated LNCaP cells showed more stabilized super Complex IV and super Complex II (Fig 8A ii). Furthermore, to validate the impact of AR on OXPHOS super complexes, we conducted BN-PAGE analysis in mitochondrial fractions of PC-3 cells ectopically expressing AR. We found destabilized super Complex I, super cComplex II, and super Complex IV. Super-complex comprising complex III_2 /complex IV and super Complex III_2 also appeared destabilized in these cells. (Fig 8B). We conclude that AR negatively impacts the stability of OXPHOS supercomplexes.

AR regulates nuclear-encoded mitochondrial ribosomal genes and mitochondrial translational machinery

Our study suggested that AR regulates the expression of nuclear genes controlling mtDNA content (Fig 2) and COXII gene expression (Fig 5 A and B). We analyzed selective AR gene targets involved in mtDNA metabolism (GFM1, GFM2) and mtDNA translation (MRPL-14, MRPL-27, MRPL-33, MRPL-39, MRPS-6, MRPS-29, and MRPS-33). The expressions of most of these gene targets were increased in AR knockdown LNCaP cells (Fig 9A, C). Interestingly, increased expressions of mitoribosomal genes were observed after AR knockdown (Fig 9C). The expressions of these nuclear-encoded mitochondrial protein synthesis genes decreased upon ectopic expression of AR in PC-3 cells (Fig 9B, D). Using ^{35}S labeling, we carried out mtDNA encoded protein translation in PC-3 cells expressing AR and observed a decrease in all 13 mitochondrial encoded proteins, compared to its mock control, with a pronounced difference in ND5 expression (Fig 9E). These results suggest that AR controls mitochondrial protein translation by controlling the expression of several nuclear-encoded mitochondrial ribomsomal genes.

AR regulates OXPHOS enzymatic activity

Since the expression of OXPHOS subunits and the stability of OXPHOS supercomplexes were altered, we analyzed the effect of AR on mitochondrial OXPHOS complex activities. Results indicate that AR knockdown affects complex I activity. A statistically significant increase of 16% and 6% was observed by genetic knockdown and pharmacological inhibition of AR, respectively (Fig 10A-i). Similarly, a statistically significant increase in Complex II activity of 28% and 30% by genetic knockdown and pharmacological inhibition, respectively, was observed (Fig 10A-ii). AR knockdown by genetic and pharmacological means showed an increase of 22% and 10% of Complex III activity, respectively (Fig 10A-iii). We also observed a statistically significant increase in complex IV activity by 27% and 13% upon genetic knockdown and pharmacological inhibition, respectively. (Fig 10A-iv). Conversely, PC-3 cells expressing AR consistently showed a decrease in Complex I, Complex II and Complex III activities (Fig 10B i-iii). However, Complex IV activity in PC-3 cells expressing AR remained unaltered (Fig 10B iv). Together these studies show that AR

modulates mitochondrial OXPHOS complex activities.

DISCUSSION

Mitochondria communicate with the nucleus to appraise the metabolic health of the cell. This involves signaling that alters expressions of many nuclear genes that are brought into action to modulate mitochondrial functions. Androgen receptor and mitochondria have been independently linked to prostate cancer risk, aggressiveness, and outcome (26,39). We discovered that AR knockdown increases mtDNA content and ectopic AR expression decreases the same, establishing an inverse correlation between AR expression and mtDNA content. In a complementary approach, we used rho^0 (devoid of mtDNA) cells, inactivated mtDNA polymerase ϒ (POLG1) by CRISPR-cas9, and inhibited OXPHOS complex I by rotenone, in order to induce mitochondrial stress and found increased AR expression. Interestingly, mitochondrial stress has been strongly correlated with prostate cancer (40,41). TFAM (transcription factor A, mitochondrial) encodes a protein that participates in mtDNA replication and transcription and is thus crucial for the maintenance of mtDNA content and mitochondrial function (42). We found that ectopic AR expression decreased TFAM expression and AR down-regulation increased TFAM expression. A TFAM dependent mechanism appears to underlie the inverse correlation between AR expression and mtDNA level.

It is well established that mitochondria import and export a number of proteins and metabolites to communicate with the nucleus in anterograde and retrograde manners to modulate metabolism and signaling depending upon mitochondrial health (43,44). We provide compelling evidence for AR localization into mitochondria. With ectopic AR expression in PC3 cells, we demonstrate a strong immunoreactive band in the mitochondrial fraction in addition to its presence in the cytoplasmic and nuclear fractions. To further substantiate these findings, we identified a putative mitochondrial localization sequence (MLS) and confirmed by GFP tagging that AR indeed contains a genuine 36 amino acid long MLS at the N-terminus, which helps localize AR into mitochondria. Sub cellular fractions as analyzed by Western blot and in vitro import results with WT-AR and Δ36n-MLS-AR, confirm that MLS resides in first 36 amino acids from N terminal of AR protein, and is prerequisite for AR to be imported into mitochondria. Consistent with our studies, a previous study has reported the presence of AR in human sperm mitochondria (45). We found that AR impedes the expression of a number of mtDNA-encoded proteins along with many nDNA-encoded mitochondrial proteins. This suggests that AR is a retrograde protein that plays a crucial role in mitochondrial-nuclear cross-talk, which may play a significant role in carcinogenesis. The list of nuclear proteins participating in such non-canonical signaling is rapidly growing. Other nuclear hormone receptors such as estrogen receptor (46), glucocorticoid receptor (47), thyroid hormone receptor (48) have also been reported

to migrate to mitochondria to fine-tune mitochondrial metabolism (49-51).

We have convincingly demonstrated that AR reduces the mtDNA content, and expression, assembly, stability, and activity of OXPHOS resulting in the loss of mitochondrial function. A strong negative impact on mitochondrial ribosomal genes and translational machinery highlights diminished protein synthesis activity in the mitochondria, upon AR expression. Mitochondrial function has been described as a tumor suppressor (16,18), (41). Notably, the mitochondrial dysfunction has been shown to result in a pro-oncogenic state, and shift to glycolysis (41). An energy-demanding process, such as cell division, is intricately wired to mitochondrial health and cellular signaling (52). The mitochondrial translocation of AR suggests a novel function impacting mitochondrial function, probably generating a pro-oncogenic environment. The rewired metabolism as a result of altered mitochondrial function may confer aggressive traits of metastatic competency and drug resistance, resulting in worse outcome in prostate cancer (41).

Nuclear localization of AR is well known to direct transcriptional regulation of a host of genes, which is referred to as genomic signaling. Non-genomic signaling or cytoplasmic functions of AR have long been emphasized (7-11), but the mechanisms remain elusive. AR and other steroid receptors activate many signaling molecules such as Src family kinases, Ras, MAPK, Akt, PKC, PLC, EGFR, and other secondary messengers. An interesting recent study demonstrated the ability of AR to enhance the invasion of prostate cancer cell lines via src that was independent of its nuclear localization (10). Wang et al., (13) in an extensive study demonstrated that AR in androgen independent prostate cancer regulates an entirely different signaling program than it does in an androgen dependent manner (13). Signaling via cell membrane localization is the only mode of non-nuclear signaling that steroid receptors including AR are known to participate in prostate cancer (53). Our discovery of AR localization into mitochondria may be central to non-genomic signaling in prostate cancer as the depletion of mtDNA has been shown to induce epigenetic modulation of miRNAs (54), the activation of CREB (55), NF-kB (56) and other pathways (57) affecting cell survival and proliferation (11). This non-genomic mode may also explain the androgen independent nature of prostate cancer. Since AR has both NLS and MLS, it is plausible that the shunting of AR between nucleus and mitochondria plays a major role in prostate tumorigenesis. Interestingly, a number of mutations in prostate cancer are reported within the NLS (58,59). Notably, mutations in the NLS have been shown to result in the retention of AR in the cytoplasm, but localization of the mutant protein in mitochondria has not been explored (60). Consistent with AR dual localization, other proteins containing dual localization signals have been reported. Interestingly, defects in one localization signal are known to result in preferential localization of the protein at an alternate location (61). It is plausible that the NLS mutations in AR propel mutant protein into the mitochondria, invoking distinct retrograde

signaling when compared to the AR in the nucleus (Fig 11). However, further investigation is needed to establish a role for such signaling in prostate cancer.

Ligand-free translocation of AR into the nucleus has been reported and suggested to occur as a result of constitutive AR expression in PC3 cells and due to alternative splicing, mutations and/or truncated versions of the protein. A previous study demonstrated that mitochondrial function played a crucial role in androgen dependence, the loss of which resulted in androgen independence (27). Our findings of AR localization into mitochondria suggest that mitochondrial dual localization may underlie androgen responsiveness. We found that AR translocation into mitochondria was independent of ligand presence. AR mutations have been reported in 30% of advanced prostate cancer patients; these mutations produce various truncated, alternate and mutated forms of AR that are more promiscuous concerning localization (5,6,62)(63). Under normal conditions, nuclear transcription is the primary activity of AR; however, as suggested earlier (13), it appears that in advanced stages of prostate cancer AR drives different programming in which mitochondria may play a key role in conferring aggressiveness to prostate cancer. Since androgens biosynthesis and translocation of AR to the nucleus are prime targets of prostate cancer drugs, ligand independent constitutive activation and transportation to nucleus and mitochondria pose a significant challenge. Identification of signaling originating from mitochondrial localization of AR adds another dimension that needs attention to prostate cancer treatment. Mitochondrial-AR axis to prostate cancer not only contributes to the complex biology of androgen signaling in prostate cancer but also brings new avenues for improving the success of treatment.

In conclusion, we have demonstrated that AR in addition to nuclear localization is imported into the mitochondria. The identification of MLS along with a proof of mitochondrial import is an interesting finding of this study. We found that mitochondrial stress increases not only the expression of AR but also its translocation to the mitochondria, suggesting an intricate relationship between the two. The localization of AR in mitochondria makes mitochondrion a significant contributor to non-genomic signaling described so often in the literature (7-11). AR negatively regulates the expression, assembly, integrity, and functions of the mitochondrial complexes, thus impacting mitochondrial overall functions. The regulation and translocation of AR into mitochondria suggests that AR is a retrograde signaling protein. The establishment of mitochondrial AR suggests complex mito-nuclear signaling in prostate cancer, which may offer new avenues for PC treatment.

Experimental procedures

Cell culture

LNCaP cells were grown in RPMI 1640 media containing penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. PC-3 cells were grown in Dulbecco's modified Eagle's

medium (DMEM) (Invitrogen) supplemented with 10% FBS (v/v) and penicillin (100 units/ml), streptomycin (100 µg/ml).

Transfection of AR cDNA and generation of stable cell lines

Transfection of PC-3 cells with wild-type AR (WT-AR) cDNA construct (a gift from Dr. Koocheckpour, Roswell Park Cancer Institute, Buffalo, NY), was carried out using FuGENE HD transfection reagent (Promega, Madison, WI). Cells were harvested 48 hours post-transfection and used for preparing subcellular fractions. Cell lysates and mitochondria were prepared to analyze AR expression. For a generation of stable cell lines, cells were selected with G418 (100 µg/ml) antibiotic and single colonies were selected and propagated.

Preparation of cell lysate and subcellular fractions, and immunoblotting

LNCaP and PC-3 cell lysates were made in M-PER mammalian protein extraction reagent (Pierce, Rockford, IL) to analyze androgen receptor expression. For isolation of mitochondria, cells were trypsinized, and the cell pellet was collected by centrifugation at 800 *g* for 5 min at 4ºC. The cell pellets were suspended in H medium (20mM potassium HEPES buffer (pH 7.5), 70mM sucrose, 220mM mannitol, 2mM EDTA) and mitochondria were prepared as described previously (64). For isolation of nuclear and cytosolic fractions, cells were washed twice with PBS and suspended in 0.5ml of Buffer A (10mM HEPES, pH7.9, 10mM KCl, 01.mM EDTA, 10% IGEPAL with protease inhibitor cocktail) and incubated at room temperature for 10 minutes. Cells were collected in Eppendorf tubes and placed on ice and later centrifuged at 4°C at 15000g for 5 minutes. The cytosolic supernatant was saved separately, and to the pellet 150 µl of Buffer B (20mM HEPES, pH7.9, 0.4mM NaCl, 1mM EDTA, 10% Glycerol with protease inhibitor cocktail) was added. This was vortexed vigorously for 15 sec and returned to the ice for 15 sec every 10 min, for a total of 40 min and later centrifuged at 4°C at 15000g for 5 minutes. Protein was measured using Pierce™ 660nm Protein Assay Reagent (Thermo Scientific, Waltham, MA). Proteins were solubilized in Laemmli sample buffer and incubated at 95 °C for 5 min. Protein preparation was resolved by electrophoresis on SDS-polyacrylamide (10%, w/v) for analyzing AR expression and on 14% (w/v) for analyzing OXPHOS subunits expression. SDS-gels were subjected to immunoblot analysis using primary antibodies for AR (1:500 dilution), Lamin A/C, β-Actin and Tubulin (1:1500 dilution) (Santa Cruz, Dallas, TX). For OXPHOS subunits (MitoProfile Total OXPHOS Rodent WB Antibody Cocktail, MitoScience, Eugene, OR) and COX-II (Life Technologies, Carlsbad, CA) 1:2,000, dilutions of primary antibody and 1:50,000 dilutions of IR Dye-conjugated secondary antibodies were used. Blots were imaged using an Odyssey scanner (LI-COR Biosciences, Bad Homburg, Germany) and Image Studio software.

Genetic knockout and pharmacological inhibition of androgen receptor

AR knockdown was achieved by using gene-specific CRISPR. The CRISPRs were designed using the online tool available at http://crispr.mit.edu/. Primers for gene-specific guide RNAs were designed and annealed into the pSpCas9(BB)-2A-GFP vector. The transformation was performed in DH5-alpha *E. coli*, and transformants were screened by PCR. The plasmid was isolated and transfected into cells using the Fugene HD according to the manufacturer's specifications. After 24 hours of transfection, cells were FACS sorted for GFP. Single colonies were selected and grown for screening. Pharmacological inhibition was achieved using 300nM of harmalol hydrochloride for 24hrs (65).

Mitochondrial OXPHOS complex activities

Mitochondrial OXPHOS complex activities were assayed with minor modifications as described previously (66), using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA). Briefly, 30-50µg of mitochondrial samples were used to measure complex I activity by monitoring the oxidation of NADH at 340nm for 3 min, indicating the extent of NADH oxidation (slope 1). Rotenone (5µg/ml) was added to each reaction, and the absorbance was measured at 340 nm for another 2 min (slope 2). The rotenone sensitive activity (slope 1--slope 2) was used to calculate the final complex I activity for each sample. Complex II activity was measured by analyzing the reduction of 2'6 Dichlorophenolindophenol (DCPIP). 30-50 µg of mitochondria were preincubated with 20mM succinate at 30^0C for 10 min in assay medium (25mM potassium phosphate pH 7.2, 5mM magnesium chloride), after incubation 2µg/mL Antimycin A, 2mM KCN, 2µg/mL Rotenone and 50 µM DCPIP was added and a baseline absorbance was recorded for two minutes at 600nm. Subsequently, 65µM Ubiquinone was added and the absorbance recorded for three minutes. The baseline slope was subtracted from the Ubiquinone slope, and the activity was calculated. Mitochondrial complex III activity was measured by monitoring the reduction of cytochrome-c by ubiquinol at 550 nm. 10-30µg of mitochondria was added in assay medium (25mM potassium phosphate pH 7.2, 5mM magnesium chloride, 2.5mg/mL BSA, 2mM potassium cyanide, 15µM cytochrome C, 0.6mM lauryl maltoside, 1µg/mL Rotenone and 35µM Ubiquinol) and the increase in absorbance was measured at 550nm for one minute. Further, complex IV activity was assessed by measuring the oxidation of Cytochrome C at 550nm for one minute after addition 10µg mitochondria to complex IV assay buffer (20mM potassium phosphate pH 7.0, 15µM reduced Cytochrome C and 0.45 lauryl maltoside).

Blue-Native polyacrylamide gel electrophoresis

Impact of AR knockdown (in LNCaP cells) and its expression (in PC-3 cells) on mitochondrial supercomplexes was analyzed by BN-PAGE as described previously with minor modifications (67). Gradient gel of (4 – 13%) [w/v] was used for the analysis. Mitochondrial samples were

solubilized with 1% Triton (v/v) and with 1% [w/v] digitonin in sample loading buffer (10% [v/v] glycerol, 50mM NaCl and 20mM Bis-Tris pH 7.4). After a brief incubation on ice for 15 min, insoluble material was removed by spinning the samples at 16000 g for 5 min at 4ºC. Electrophoresis was done for 4 hours at 600 V with anode buffer (50mM Bis-Tris, pH 7.0) and cathode buffer (50mM tricine, 15mM Bis-Tris pH 7.0, 0.02% [w/v] Coomassie blue G 250). After the samples had moved from stacking gel, the cathode buffer was replaced with a fresh cathode buffer lacking dye.

^{35}S methionine pulse labeling of mitochondrial translation products in vivo

[^{35}S]-Methionine pulse labeling of mitochondrial translation products in vivo Pulse labeling experiments were performed in minimum essential DMEM medium without methionine, glutamine, or cysteine, and dialyzed serum (25mM Tris–HCl, pH 7.4, 137mM NaCl, and 10mM KCl) following previous protocol (68,69). 0.2mCi/mL of [35S]-methionine-containing medium (Perkin Elmer) was added to the cells to label the mitochondrial- encoded proteins. Two hours post incubation, cells were lysed in lysis buffer (50mM Tris–HCl, pH 7.6, 150mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, and 0.5% NP-40) supplemented with 1mM PMSF and protease inhibitor cocktail (Sigma-Aldrich). To separate the products formed, electrophoresis of whole cell lysates (40µg) was performed through 12% SDS-PAGE. The gels were dried on 3 mm chromatography paper, and the total intensities of the signals were quantified by phosphor imaging analysis.

Cloning in pHTC-Halo-Tag vector

To conduct another set of in vitro mitochondrial import assays, we cloned WT-AR (with MLS), Δ36n-MLS-AR (lacking 36 amino acids from N terminal containing the MLS), Su9-DHFR (positive control) and DHFR (negative control) were cloned in pHTC-Halo-Tag vector (Promega, Madison, WI) between NheI and XhoI restriction sites.

Mitochondrial targeting sequence cloning in GFP expression vector

Androgen receptor protein sequence was analyzed by using MitoProt, which predicts the probability of protein to be imported into mitochondria. A probability score of 0.25 was noted, and the software predicted a cleavage site after the first 14 amino acids**.** To validate the prediction, the mitochondrial localization Sequence (MLS) containing (1-36 amino acids) was cloned in the pEGFP-N_2 vector by PCR amplification within the EcoR1 site and BamH1 site. Cells were transfected using Fugene (Promega, Madison, WI) according to the manufacturer's protocol. Immunocytochemical analysis was done to analyze the co-localization of GFP with MitoTracker dye signal and mounted with Prolong Gold antifade containing DAPI (ThermoFisher Scientific, Waltham, MA) 48 hours post-transfection. Images were obtained using fluorescence microscopy.

In the vitro mitochondrial import

To conduct new in-vitro mitochondrial import, WT-AR (with MLS), Δ36n-MLS-AR Su9-

DHFR and DHFR were cloned in pHTC-Halo-Tag vector (Promega, Madison, WI) between NheI and XhoI restriction sites. Using T7 polymerase-coupled rabbit reticulocyte lysate transcription-translation systems (Promega, Madison, WI) all above mentioned pHTC-Halo-Tag constructs were transcribed and translated and modified later (70,71). Freshly isolated 500µg mouse brain mitochondria were used for import of translated proteins. The import assays were carried out in a 200 µl final volume and contained 500 µg mitochondria, (from a 10 mg/ml suspension in sucrose mannitol buffer), 60µl energy mixture (10 mM ATP, 10 mM GTP, 2.5mM CDP, 2.5 mM UDP, 50 mM malate, 20 mM isocitrate), 70µl transport buffer (0.6 M mannitol, 20 mM Hepes, pH 7.4, 1 mM $MgCl_2$. The import reactions were incubated at 28°C for 60 min. After import, the reaction mixtures were cooled on ice for 5 min, and each mixture was divided into two equal portions (no trypsin and tryspin treatment). The trypsin digestion of mitochondria was performed for 20 min on ice (150µg of trypsin/mg of mitochondrial protein). Control mitochondria were incubated similarly without adding trypsin. Soybean trypsin inhibitor (1.5 mg/mg of protein) was added to all samples to terminate the reactions. Mitochondria from both trypsin-treated and untreated samples were re-isolated by pelleting through 0.8 M sucrose, and the proteins were subjected to SDS-PAGE followed Western blot, and analyzed by Odyssey imaging system.

Initially, the androgen receptor cDNA construct (in pCDNA3.1 vector) was used as template in T7 polymerase-coupled rabbit reticulocyte lysate transcription-translation systems (Promega, Madison, WI, Supplementary fig 1) in the presence of [^{35}S]Met as described previously (70) and modified later (70,71). Su9-DHFR and DHFR cDNA construct were used as template in Sp6 polymerase-coupled rabbit reticulocyte lysate transcription-translation systems (Promega, Madison, WI) in the presence of [^{35}S]Met as described previously (70). Freshly isolated rat liver mitochondria were used for import of ^{35}S-labeled translation products. Control experiments were carried out by preincubating mitochondria with carbonyl cyanide m-chlorophenylhydrazone (50µM; Sigma-Aldrich) or oligomycin (50µM; Sigma-Aldrich) at 25°C for 20 min before initiation of the import reaction. After import, trypsin digestion of mitochondria was performed as the above-mentioned procedure. Mitochondria from both trypsin-treated and untreated samples were re-isolated by pelleting through 0.8 M sucrose, and the proteins were subjected to SDS-PAGE followed by fluorography.

RNA Extraction and PCR Amplification

For mRNA quantification, total RNA was isolated from cells using TRI reagent per the manufacturer's instructions (Molecular Research Center, Cincinnati, OH). RNA was digested with RQ1 RNase-Free DNase (Promega, Madison, WI). Total RNA (2 µg) was reverse transcribed using iScript cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA). 25ng cDNA was used for quantification of mtDNA content with iQ SYBR Green Super Mix (BIORAD, Hercules, CA) on an ABI 7300 real-time PCR

machine and analyzed using Primer Express 3.0 (Applied Biosystems). Other gene targets were PCR amplified using BIO-RAD T100 thermal cycler and resolved on ethidium bromide stained 1.5% agarose gel. List of primer sequences is appended in the supplementary table 1.

Statistical Analysis

The means ± S.D. were calculated from three to five experimental values. P values were calculated using a one-tailed distribution and unequal variance to calculate significance (compared with vector control).

Conflict of Interest

The authors declare no conflicts of interest.

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Author Contributions

KKS conceived the project. KKS and GS designed the project. PB performed the experiments described in figure 1 to 8 and 10. EK conducted the mitochondrial translation studies (fig 9). PB, RS, and KKS wrote the manuscript.

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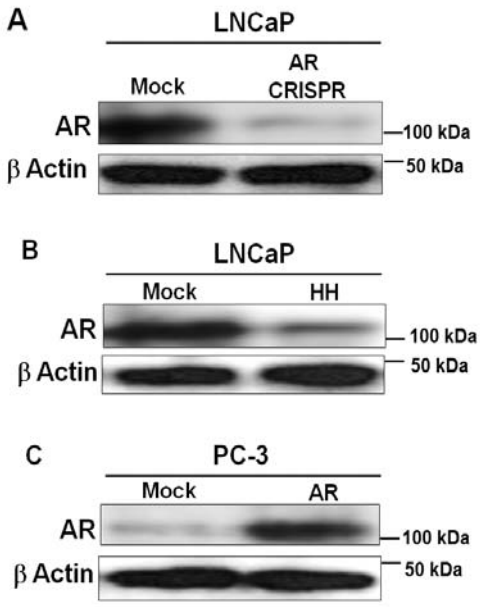

Figure 1. Cellular models employed in this study: (A) Genetic knockout of AR in LNCaP cells was achieved using CRISPR-cas9, and (B) pharmacological inhibition was achieved by Harmalol Hydrochloride (HH) treatment for 48 hrs. (C) AR over-expression in PC-3 cells was achieved by transfecting these cells with AR cDNA. Western blots show AR down-regulation in LNCaP cells (A and B) and upregulation in PC3 cells (C).

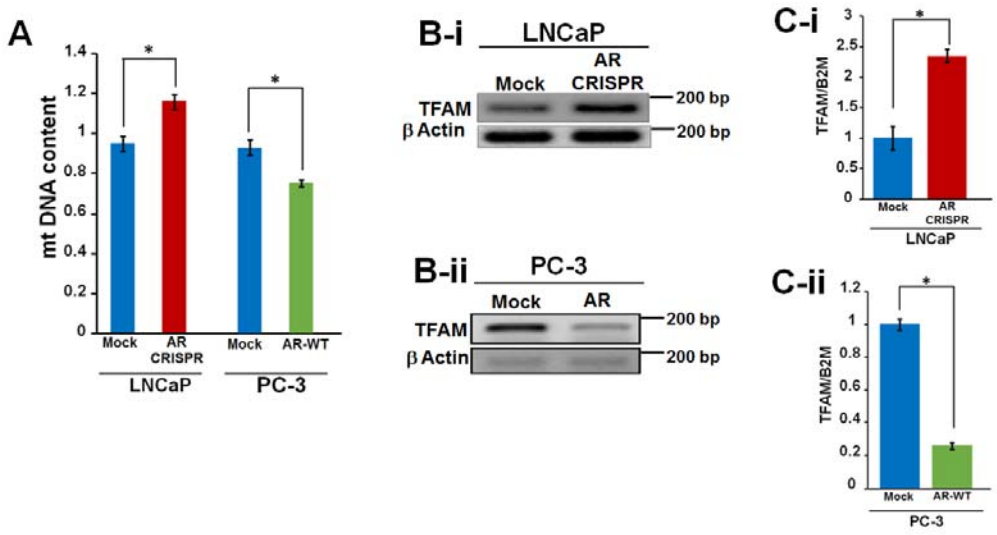

Figure 2. Androgen receptor expression regulates mtDNA content, and TFAM level: (A) AR inactivation in LNCaP cells increases mtDNA content, while ectopic AR expression in PC3 cells reduces mtDNA content. The ratio of mtDNA to nuclear DNA was used as an index for measuring the mtDNA content. (B-i) AR knockout in LNCaP cells increased TFAM expression (B-ii) and AR expression in PC-3 cells decreased TFAM expression. TFAM expression was analyzed by semi-quantitative PCR. (Ci-ii) Real-time quantitative PCR analysis of TFAM expression: (C-i) Validates semi quantitative PCR results showing increased TFAM expression in AR knockout LNCaP cells; (C-ii) and decreases TFAM expression in PC-3 cells ectopically expressing AR confirmed as observed from as observed from semi quantitative PCR. Statistical significance was calculated by student's t-test, and significant (p*0.05) are marked with asterisks. All experiments were done in triplicates.

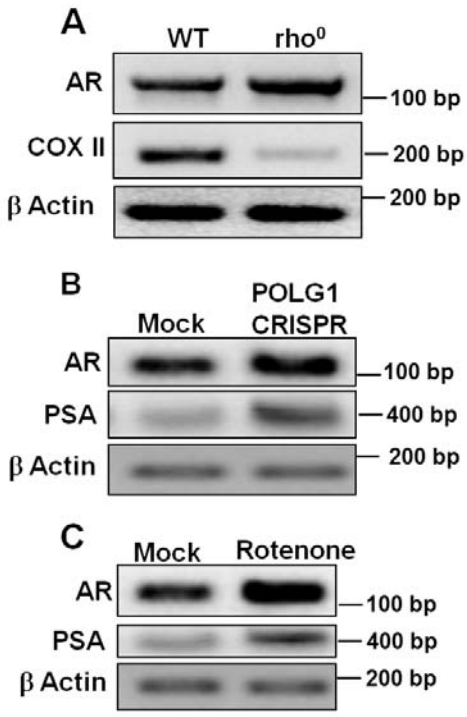

Figure 3. Mitochondrial dysfunction increases androgen receptor expression: (A) rho^0 (mtDNA-depleted) cell line showed increased AR expression when compared with parental cells. (B) CRISPR targeting POLG1 (C) and rotenone treatment (25nM for 24hrs) were used to induce mitochondrial stress in LNCaP cells, both of which showed increased expression of AR and PSA (B and C).

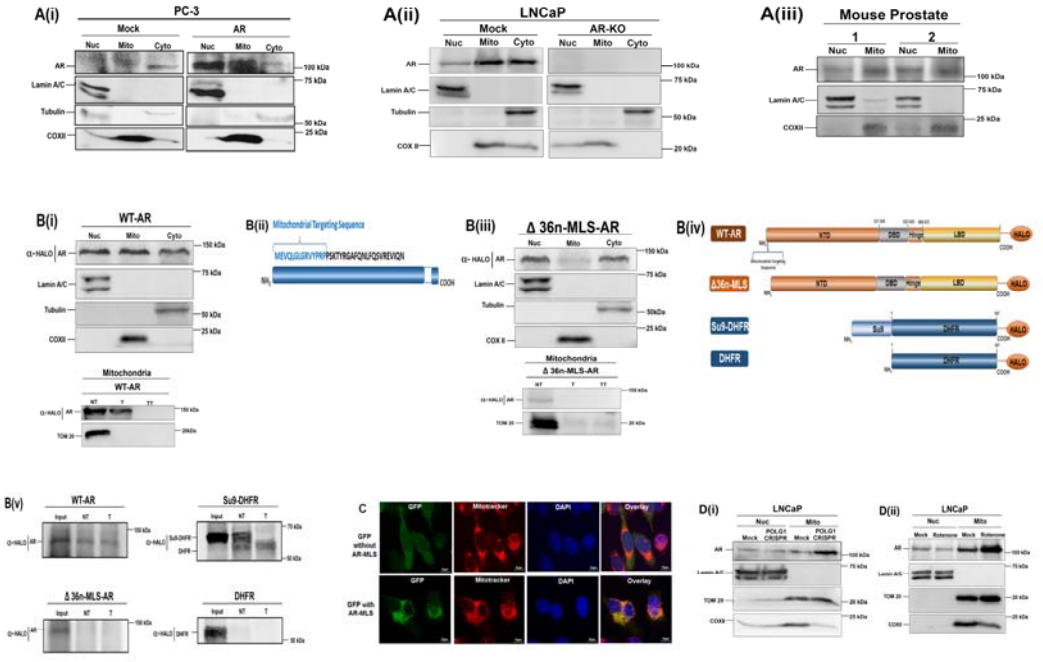

Figure 4. Mitochondrial Localization of the androgen receptor: (A-i) Exogenous expression of WT-AR in PC-3 cells. PC-3 cells transiently transfected with AR construct showed a prominent presence of AR in the nuclear and mitochondrial fractions in addition to its presence in the cytosolic fraction. (A-ii) Endogenous expression of AR in LNCaP cells and LNCaP CRISPR-Cas9 AR knockout (KO) cells. Western blot analysis of nuclear, mitochondrial and cytosolic fractions to analyze endogenous AR expression in LNCaP (mock) and AR knockout by CRISPR Cas9 in LNCaP cells (AR-KO) cells. 80µg of protein was resolved on 10% SDS gel. (A-iii) Endogenous expression of AR in mitochondrial fractions of mouse prostate tissue was analyzed by fractionation followed by Western blot. Blots were probed with antibodies against AR, Lamin A/C (nuclear control), Tubulin (cytosolic control) and COXII (mitochondrial control) antibodies to analyze cross-contamination with nuclear, cytosolic and mitochondrial fractions, respectively. (B-i) Wild-type AR expression in AR knockout cells: LNCaP CRISPR-Cas9 AR knockout cells (KO) cells were transfected with WT-AR cloned in p-HTC-Halo-Tag vector. Western blot with subcellular fractions of nuclear, cytosolic and mitochondrial were made to analyze WT-AR expression (Top Panel). To confirm intra-mitochondrial localization of WT-AR, mitochondrial fractions of WT-AR were treated with trypsin (T), additionally, one set was treated with 1% Triton X-100 (v/v) (bottom panel), before trypsin treatment. WT-AR containing MLS translocates to mitochondria and is resistant to trypsin (T). The WT-AR mitochondrial protein became sensitive to trypsin following disruption of the mitochondrial

membrane by treatment with Triton X-100 (TT), further confirming that it is localized in the mitochondrial matrix compartment. (B-ii) Schematic of predicted mitochondrial localization sequence (MLS) in the AR protein (B-iii) Mutant Δ-36n-MLS-AR expression in AR knockout cells. LNCaP CRISPR-Cas9 AR knockout cells (KO) cells were transfected with Δ36n-MLS-AR cloned in p-HTC-Halo-Tag vector. Western blot demonstrates AR expression in mitochondrial fractions was drastically reduced upon truncating 36 amino acids from N terminal (Δ36n-MLS-AR). This Δ36n-MLS-AR mutant lacked mitochondrial localization and was detected in nuclear and cytosolic fractions (Top panel). Blot was probed with Lamin A/C (nuclear control), COXII (mitochondrial control) and Tubulin (cytosolic control). Further, tryspsin treatment in mitochondrial fractions of Δ36n-MLS-AR, lacking MLS was sensitive to trypsin indicating that it was membrane bound, and did not translocate to mitochondria. NT: No trypsin; T: Trypsin treatment; TT: Trypsin treatment with 1% Triton X-100 (v/v). (B-iv) Schematic showing cDNA constructs cloned in pHTC-Halo-Tag vector used for in-vitro transcription and translation and western blot analysis as presented in (Fig 4 Bi and Bii). (B-v) In vitro mitochondrial import of wild type and Δ36n-MLS-AR mutant AR. We carried out an *in vitro* import experiment in a mouse brain mitochondrial system after translating AR cDNA in rabbit reticulocyte lysate (RRL). Limited trypsin treatment showed protection to trypsin in WT-AR, accounting for the intra mitochondrial localization of AR, 36n-MLS-AR mutant was sensitive to trypsin treatment, indicating that AR protein lacking 36 amino acids of MLS did not translocate to mitochondria. Su-9 dihydrofolate reductase (Su9-DHFR), and dihydrofolate reductase (DHFR) were used as positive and negative controls respectively. Su9-DHFR contains a classic mitochondrial targeting signal (MTS), a presequence of subunit 9 of Neurospora crassa F0F1-ATPase which has been fused to DHFR. Upon successful import this MTS is cleaved after entry into mitochondria, thus only the cleaved protein is present inside mitochondria after import and protected from trypsin treatment. Since DHFR, is a cytosolic protein, it was used as a negative control in the experiment. Western blots were probed with anti-Halo-Tag antibody. (C-i) Mitochondrial localization of GFP by AR-MLS: pEGFP-N2 containing MLS derived from AR shows strong GFP localization in mitochondria (bottom panel). pEGFP-N2 lacking MLS was used as a negative control (top panel) and lacked co-localization with Mitotracker signal. (D) Increased translocation of AR into mitochondria under stress conditions generated by POLG1 CSIRPR knockout and rotenone treatment (a complex I inhibitor) was observed. Western blot showing nuclear and mitochondrial fractions isolated from LNCaP exposed to mitochondrial stress. The mitochondrial stress was induced by (i) POLG1 CRISPR and (ii) rotenone (25nM for 24hrs) inhibition of OXPHOS complex I. Blot was probed with Lamin A/C (nuclear control) and TOM20 (mitochondrial control) to analyze cross contamination. COXII was used as a marker of mitochondrial dysfunction.

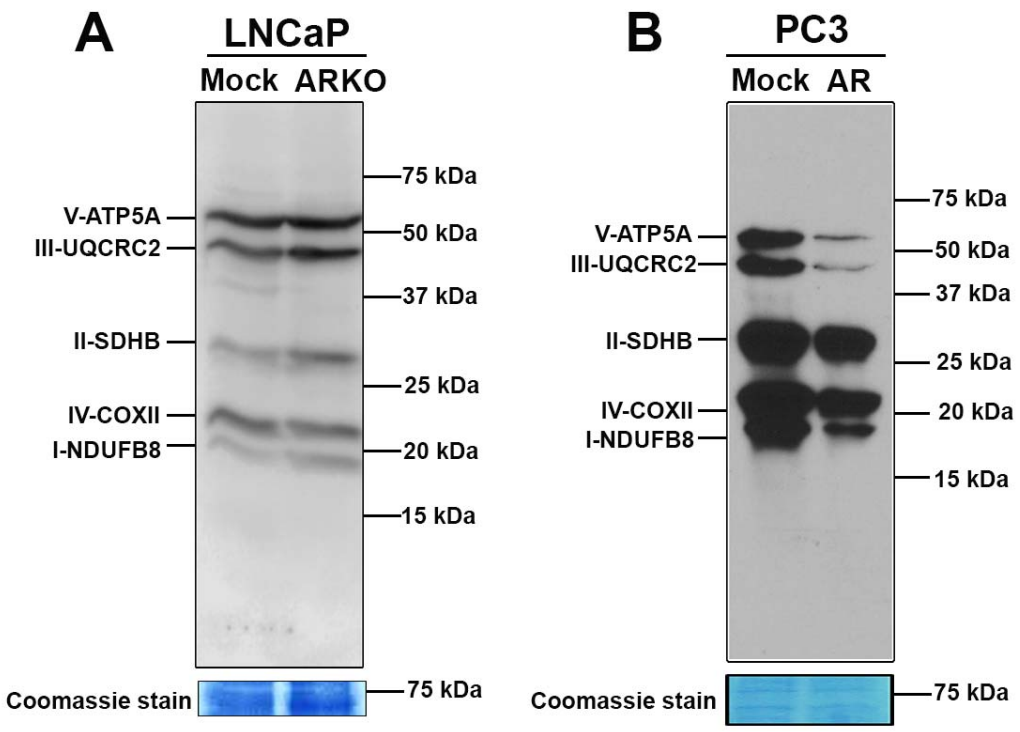

Figure 5. Androgen receptor regulation of OXPHOS subunits: Western blot showing the effect of AR on expression of OXPHOS subunits. (A) AR knockdown LNCaP cells showed an increase in OXPHOS subunits expression and (B) AR expression in PC3 cells led to a decrease in expression. Blots were probed with OXPHOS antibody cocktail. Coomassie staining shows equal loading of proteins.

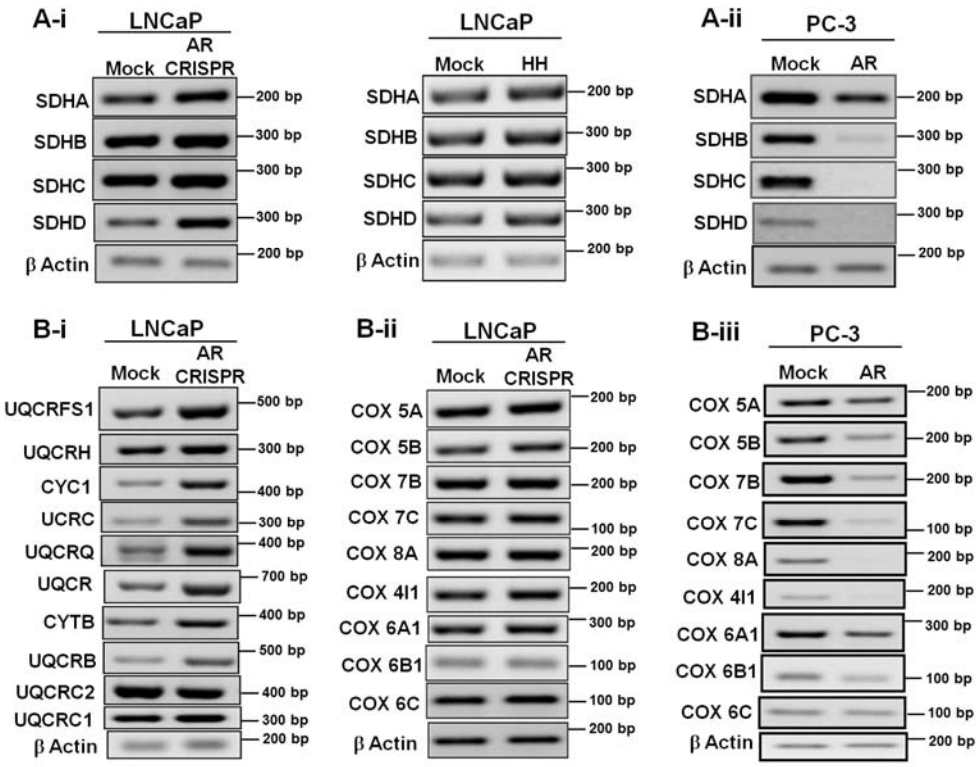

Figure 6. Expression of nuclear-encoded OXPHOS subunits modulated by AR: Expression analyses of OXPHOS subunits by PCR. (A i) Complex II subunits were up regulated in AR knockdown LNCaP cells and in HH treated LNCaP cells, and (A ii) down-regulated in AR-expressing PC-3 cells. (B i-ii) Expression of complex III and IV subunits was up-regulated in AR knockdown LNCaP cells and (B-iii) Complex IV subunits expression was down-regulated in AR-expressing PC-3 cells.

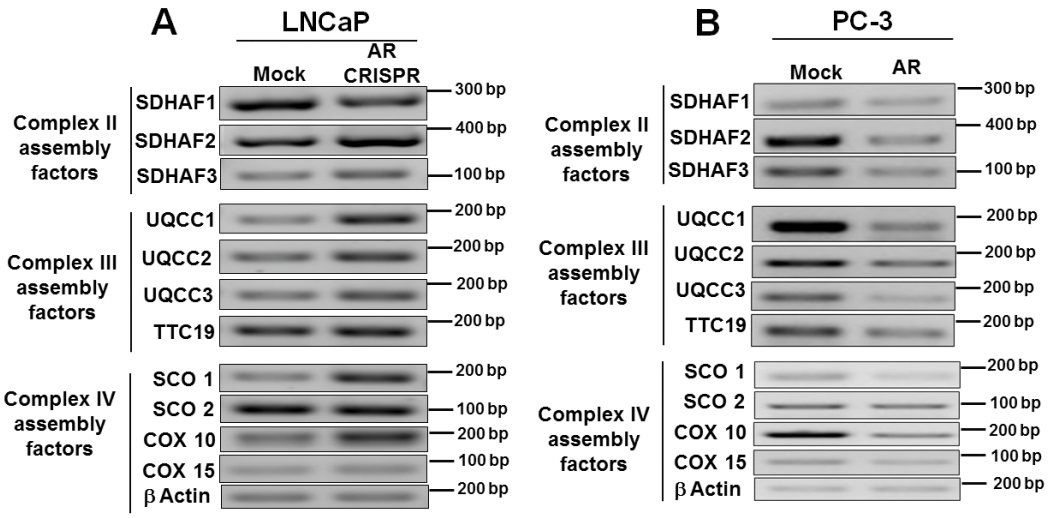

Figure 7. Regulation of OXPHOS assembly factors by AR: PCR was done for expression analysis. (A) Various OXPHOS assembly factors were up-regulated in AR knockout LNCaP cells and (B) down-regulated in AR-expressing PC-3 cells.

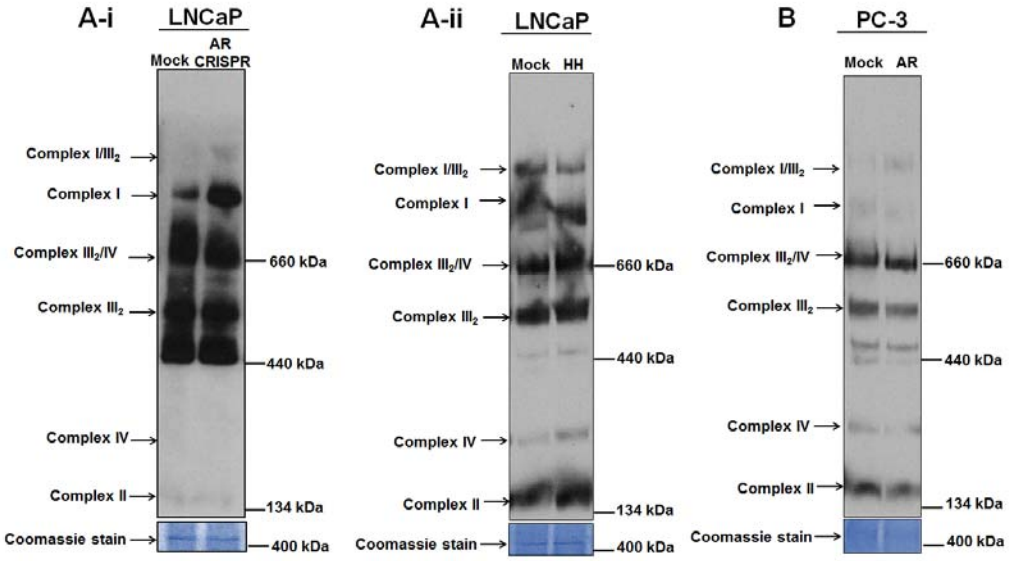

Figure 8. Androgen receptor affects stability of OXPHOS supercomplexes: BN-PAGE was performed with mitochondrial fractions from AR knockdown, HH treated LNCaP cells, and PC3 cells expressing AR ectopically were probed with OXPHOS antibody cocktail, (A-i-ii) More stabilized supercomplexes were observed upon AR knockdown in LNCaP, and after pharmalogical inhibition of AR by HH in treated LNCaP cells and (B) Destabilized super complexes were observed in AR-expressing PC3 cells.

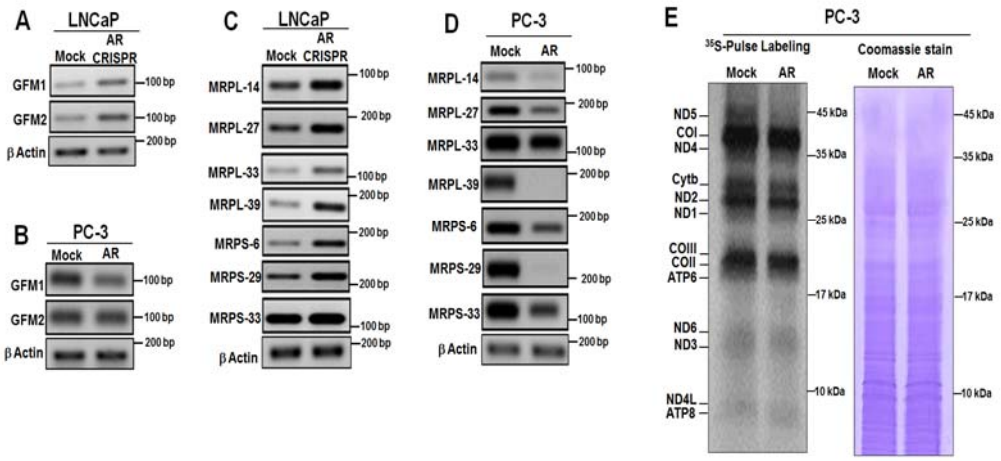

Figure 9. Androgen receptor regulates mitochondrial translation: Gene expression was analyzed by RT-PCR (A) AR knockout in LNCaP cells upregulated, while AR expression in PC3 cells down-regulated (B) for GFM1 and GFM2 genes. (C) AR knockout in LNCaP, while (D) AR ectopic expression in PC-3 cells negatively regulates the expression of mitoribosomal genes. (E) Pulse chase-labeling experiment showed electrophoretic pattern of the de novo synthesized translational products of Complex I (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6 subunits); Complex III (Cyt b subunit), Complex IV (COI, COII, and COIII subunits), and Complex V (ATP6 and ATP8 subunits). Coomassie blue staining of the same gel (total protein) shows equal loading of protein.

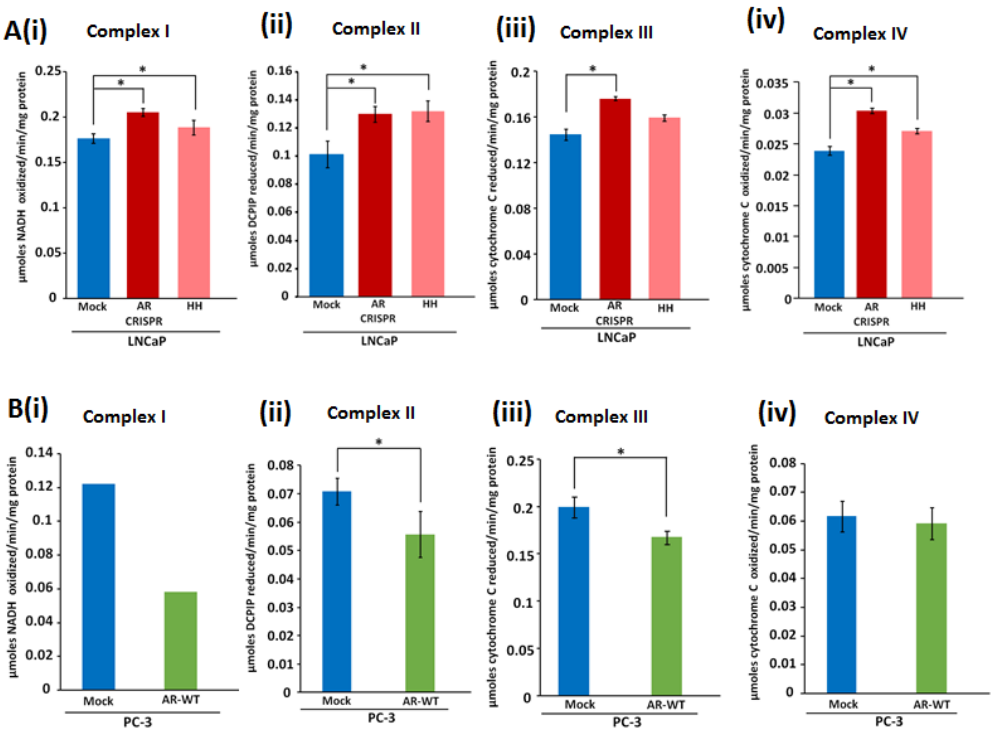

Figure 10. Androgen receptor regulates mitochondrial respiratory complexes activities: (A i-iv): Increased complex I to IV activities upon AR knockdown, and (B i-iv): decreased activities upon AR expression The statistical significance (p*0.05) is marked with asterisks.

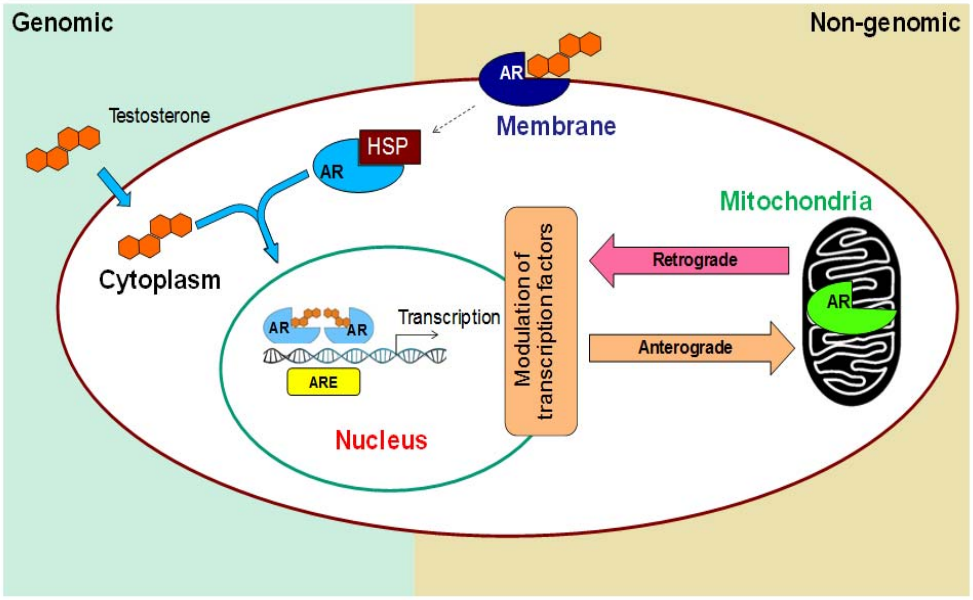

Figure 11. Schematic figure showing a novel role of mitochondria in non-genomic AR signaling: In the traditional genomic pathway, AR undergoes conformational change and dimerization upon ligand binding, followed by its migration to the nucleus. Subsequent binding to the androgen response elements induces target gene transcription. In the non-genomic pathway, membrane-bound AR signals in the cytoplasm, which via second messengers activates other transcription factors. Our studies suggest that mitochondria are novel players in the non-genomic action of AR. Signaling cascades triggered upon translocation of AR into mitochondria may in a retrograde manner, affect nuclear gene transcription that may contribute to aggressive prostate cancer.

Mitochondrial localization, import and mitochondrial function of the androgen receptor

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