Am J Clin Exp Urol 2021;9(4):287-291
www.ajceu.us /ISSN:2330-1910/AJCEU0138240

Review Article

The classical and updated models of androgen receptor nucleocytoplasmic trafficking

Ryan Cole[1], Laura E Pascal[1,2], Zhou Wang[1,2,3]

[1]Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15232, USA; [2]UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA 15232, USA; [3]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15232, USA

Received August 12, 2021; Accepted August 25, 2021; Epub August 25, 2021; Published August 30, 2021

Abstract: This mini-review covers the classical model of androgen receptor (AR) nucleocytoplasmic trafficking and provides an overview of new data that updates the existing paradigm. The classical model of androgen receptor trafficking involves AR translocating to the nucleus in the presence of androgens and subsequently being exported back to the cytoplasm following the withdrawal of androgens. New data challenges and updates the fate of nuclear AR. In the updated model, the AR can be imported into the nucleus in the absence of androgens and nuclear AR is degraded, not exported. Further, androgens can enhance AR nuclear import and inhibit AR degradation in the nucleus; androgen withdrawal causes nuclear AR degradation, but not export. Enhanced androgen-independent AR nuclear localization and AR nuclear stability may be a hallmark of castration-resistant prostate cancer (CRPC). Further characterization of AR trafficking may aid in the development of new therapies for patients with CRPC.

Keywords: Androgen receptor, castration-resistant prostate cancer, nucleocytoplasmic trafficking, C4-2

Introduction

This mini-review is in dedication to the contributions Dr. Leland Chung has made to prostate cancer research. One of many contributions from Dr. Chung was the development of the castration-resistant prostate cancer (CRPC) cell line C4-2 [1], which was instrumental in studying and updating the model of androgen receptor (AR) nucleocytoplasmic trafficking and will continue to be an important model to developing therapies that treat CRPC. This article will review the classical model of AR trafficking and explain how new data challenges and updates the classical paradigm.

Defining the mechanisms of AR nucleocytoplasmic trafficking is fundamentally important and clinically relevant. AR functions as a transcription factor to regulate the development and growth of the prostate [2] as well as the growth and progression of prostate cancer [3]. Since transcription only occurs in the nucleus, one mechanism to control AR activity would be to regulate AR levels in the nucleus. For example, nucleocytoplasmic shuttling of the AR could influence AR level and activity in the nucleus and therefore may be a promising target to inhibit AR.

The classical model of AR trafficking

As a member of the nuclear hormone receptor family, the AR has several major functional domains, the N-terminal domain (NTD), DNA binding domain (DBD), hinge region (H), and the ligand binding domain (LBD) [4, 5] (**Figure 1**). Similar to other steroid receptors, AR signaling and trafficking is primarily regulated by the highly selective binding of its ligands (**Figure 2**, Left panel) [6]. Unliganded AR is largely localized in the cytoplasm, while liganded AR is localized in the nucleus [7, 8]. AR in the cytoplasm interacts with chaperone proteins such as heat shock proteins HSP40, HSP70, and HSP90 to maintain a conformation that allows for high affinity ligand binding [9]. Ligand binding induces a conformation change of the LBD causing the dissociation of these heat shock proteins and initiation of AR nuclear translocation [10].

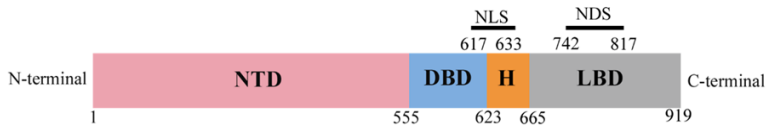

Figure 1. The N-terminal domain (NTD), DNA binding domain (DBD), hinge region (H), and ligand binding domain (LBD) are the four major functional domains of the androgen receptor (AR). The nuclear localization signal (NLS) and the nuclear degradation signal (NDS) regulate AR nuclear import and degradation and are localized in the DBDH region and the LBD region respectively.

Due to the large size of the AR (~110 KDa), AR requires active transport to cross the nuclear pore complex [11]. Prior work has suggested that the AR and other steroid receptors such as the glucocorticoid receptor (GR) contain two nuclear localization signals, a bipartite nuclear localization signal in the DBDH region (NLS) (**Figure 1**), and a signal in the LBD region (NLS2) [12-14]. When AR is unliganded, a nuclear import receptor known as importin 7 shields the NLS and prevents nuclear localization. Conversely, ligand binding promotes the exchange of importin 7 for karyopherin alpha which permits nuclear localization [15]. Although prior literature suggests that the presence of ligand and the NLS is a prerequisite for nuclear localization of AR, AR in CRPC cells localizes in the nucleus in the absence of androgens [16, 17]. In CRPC cells this androgen-independent nuclear localization appears to require HSP90, although the mechanism is not well understood [18, 19].

While the nuclear import of AR is relatively well understood, the fate of AR after nuclear translocation is not. Studies show that, in the presence of protein synthesis inhibitor cycloheximide, ligand withdrawal caused a shift from AR being predominantly in the nucleus to the cytoplasm [20, 21]. Since cycloheximide inhibited the production of new cytoplasmic AR [22], this finding was interpreted as the export of nuclear AR back to the cytoplasm upon ligand withdrawal. AR export was thought to be either directly or indirectly impacted by ligand and this hypothesis led to the discovery of a potential nuclear export signal (NES^{AR}) in the LBD of AR [23]. The NES^{AR} is active in the absence of ligand and appears to be a necessary region for AR cytoplasmic localization [23]. Although the NES^{AR} was identified as a potential nuclear export signal, the mechanism for this export was not well understood and does not appear to involve known cellular export machinery [24].

The updated model of AR trafficking

New evidence regarding AR trafficking has challenged the existing paradigm of nuclear export of the AR. Investigation into the mechanisms of NES^{AR} action showed that NES^{AR} significantly enhanced AR degradation and polyubiquitination [25]. Proteasome inhibitor MG132 enhanced the level of GFP-NES^{AR}, indicating that GFP-NES^{AR} is rapidly degraded via proteasomal pathway. Treatment with MG132 also increased the level of GFP-LBD but did not affect the level of GFP-ΔNES^{AR}-LBD [26]. These results suggested that the NES^{AR} is an important region influencing AR degradation. Since NES^{AR} regulates both subcellular localization and degradation of AR, it was hypothesized that NES^{AR} promotes cytoplasmic localization through enhancing AR degradation in the nucleus. This hypothesis was supported by the observation that proteasomal inhibition did not affect the localization of AR constructs lacking the NES^{AR} but caused a shift toward the nucleus in constructs containing NES^{AR} [26]. These observations suggested that the apparent AR cytoplasmic localization following androgen withdrawal was due to rapid AR nuclear degradation instead of nuclear export, calling into question the paradigm of NES^{AR} acting as an export signal. As such, we propose renaming the NES^{AR} as a nuclear degradation signal (NDS^{AR}) and we will use NDS^{AR} in the subsequent text (**Figure 1**).

Since the NDS^{AR} is in the LBD of AR and is regulated by androgen [23], this led to exploration of the fate of nuclear AR following androgen withdrawal. A pulse-chase method [27] demonstrated pulse-labeled nuclear GFP-AR was not detectable in the cytoplasm in the absence or presence of proteosome inhibition following androgen withdrawal. MG132 treatment prevented degradation of the pulse labeled GFP-AR but caused no export of the pulse-labeled GFP-AR to the cytoplasm [26]. Taken together, these results indicated that pulse-labeled GFP-AR was degraded in the nucleus, not exported, and that MG132 was sufficient to stop nuclear

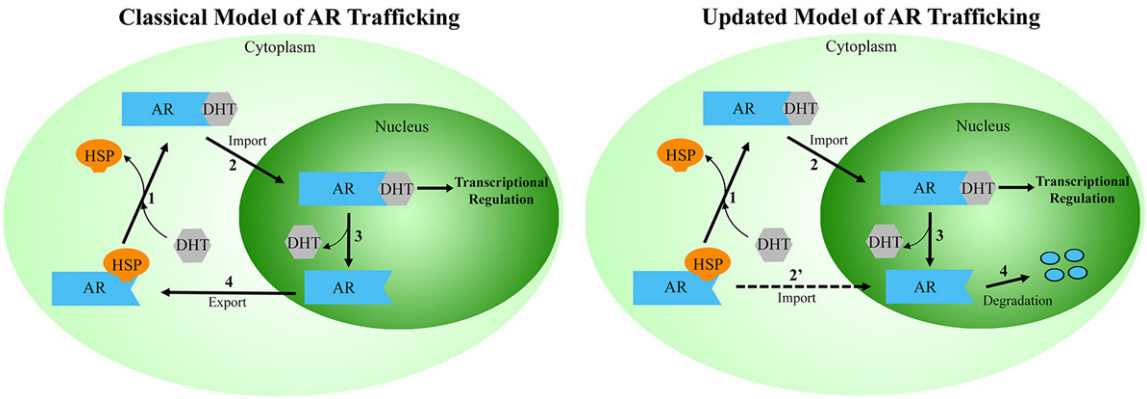

Figure 2. The classical model (left) and the updated model (right) of AR trafficking. In the classical model, newly synthesized AR in the cytoplasm is complexed with heat shock proteins (HSP) and undergoes the following steps: (1) DHT binding dissociates HSP from AR, (2) liganded AR undergoes nuclear import, (3) androgen depletion causes DHT dissociation from AR, and (4) ligand-free AR is exported. In the updated model, steps 1-3 are identical to the steps in the classical model. However, in step 4, ligand-free AR in the nucleus is degraded, instead of being exported. Ligand-free AR can also be imported, in step 2', possibly via its nuclear import signal NL1, and then quickly degraded in the nucleus.

degradation. Moreover, pulse-labeled GFP-AR was more resistant to degradation in CRPC cell line C4-2 [26] indicating increased stability of nuclear AR in CRPC cells in the absence of androgens. These results were further confirmed by testing endogenous AR. These findings are not compatible with the existing paradigm of AR nuclear export and further suggest that nuclear AR is instead degraded.

Consistent with nuclear AR degradation, western blot analysis of nuclear and cytoplasmic fractions revealed that AR polyubiquitination occurred in the nucleus of AR, and that CRPC AR polyubiquitination occurred less than hormone sensitive prostate cancer [26]. MDM2 is an important E3 ligase that catalyzes AR polyubiquitination [28], so we first investigated the role of MDM2 in AR polyubiquitination in the nucleus. The E3 ligase MDM2 was predominantly localized in the cytoplasm but could be imported to the nucleus in the presence of DHT. Furthermore, co-immunoprecipitation showed that MDM2 was bound to AR in the nucleus, but not the cytoplasm [26]. Also, MDM2 siRNA transfection increased the amount of nuclear AR and inhibited polyubiquitination. Taken together, these results indicate that MDM2 is an important E3 ligase regulating nuclear AR polyubiquitination. Further study of MDM2 and other E3 ligases such as SKP2 [29] and CHIP [30] could aid in understanding the regulation of AR nuclear degradation.

Since unliganded AR was rapidly degraded in the nucleus, this revealed the possibility that androgen independent nuclear localization of AR occurs but is not normally detectable due to its rapid degradation. Androgen-insensitive GFP-AR^{L859F} is incapable of binding to androgen [31], and was utilized to explore androgen-independent nuclear localization. Androgens did not induce nuclear import of GFP-AR^{L859F}. However, in the presence of MG132, GFP-AR^{L859F} was observed in the nucleus [26]. This finding demonstrated that while androgens can promote nuclear localization of AR, AR can be imported into the nucleus independent of androgens. CRPC and non-CRPC cells appeared to have similar AR nuclear import rates, but CRPC cells exhibited slower nuclear degradation rates. This difference in degradation rates may be partially explained by the downregulated levels of AR E3 ligase MDM2 in CRPC cells [26]. MDM2 catalyzes AR polyubiquitination [32] that selectively occurs in the nucleus, so a downregulation in MDM2 could lead to slower nuclear degradation. More work is needed to understand the mechanism of import for unliganded AR and to understand the increased nuclear stability of AR in CRPC.

Conclusions

In the updated model of AR trafficking (**Figure 2**, Right panel), AR is not exported from the nucleus and recycled as previously thought. Instead, AR is polyubiquitinated and degraded

in the nucleus in the absence of androgens [26] (**Figure 2**, Right panel). This degradation is regulated by the NDSAR, which was initially thought to function as an export signal [23, 25]. AR import occurs in the absence of androgens, but unliganded AR is rapidly degraded. This degradation occurs more slowly in CRPC cells than in hormone sensitive prostate cancer cells and provides a mechanism for the increased stability of AR in CRPC. The progression of CRPC is largely AR dependent [33], and nuclear AR stabilization is particularly important in CRPC cells that are hypersensitive to low levels of androgens [17]. Thus, understanding the mechanisms regulating AR nuclear level and stability is important for developing novel therapies targeting AR to treat CRPC. AR nuclear import and degradation are two potential mechanisms regulating AR level in the nucleus. Since NDS^{AR} regulates nuclear AR degradation, it may play an important role in regulating AR level and stability in the nucleus of prostate cancer cells, including CRPC cells. Future studies should continue to characterize the underlying mechanisms that regulate AR nuclear import and degradation.

Acknowledgements

This work was supported by the Department of Urology, University of Pittsburgh School of Medicine.

Disclosure of conflict of interest

None.

Address correspondence to: Zhou Wang, Department of Urology, University of Pittsburgh School of Medicine, 5200 Centre Avenue, Pittsburgh, PA 15232, USA. E-mail: wangz2@upmc.edu

References

[1] Thalmann GN, Anezinis PE, Chang SM, Zhau HE, Kim EE, Hopwood VL, Pathak S, von Eschenbach AC and Chung LW. Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. Cancer Res 1994; 54: 2577-81.

[2] Chang C, Saltzman A, Yeh S, Young W, Keller E, Lee HJ, Wang C and Mizokami A. Androgen receptor: an overview. Crit Rev Eukaryot Gene Expr 1995; 5: 97-125.

[3] Lonergan PE and Tindall DJ. Androgen receptor signaling in prostate cancer development and progression. J Carcinog 2011; 10: 20.

[4] Gao W, Bohl CE and Dalton JT. Chemistry and structural biology of androgen receptor. Chem Rev 2005; 105: 3352-70.

[5] Weikum ER, Liu X and Ortlund EA. The nuclear receptor superfamily: a structural perspective. Protein Sci 2018; 27: 1876-92.

[6] Tan MH, Li J, Xu HE, Melcher K and Yong EL. Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin 2015; 36: 3-23.

[7] Georget V, Lobaccaro JM, Terouanne B, Mangeat P, Nicolas JC and Sultan C. Trafficking of the androgen receptor in living cells with fused green fluorescent protein-androgen receptor. Mol Cell Endocrinol 1997; 129: 17-26.

[8] Roy AK, Tyagi RK, Song CS, Lavrovsky Y, Ahn SC, Oh TS and Chatterjee B. Androgen receptor: structural domains and functional dynamics after ligand-receptor interaction. Ann N Y Acad Sci 2001; 949: 44-57.

[9] Smith DF and Toft DO. Minireview: the intersection of steroid receptors with molecular chaperones: observations and questions. Mol Endocrinol 2008; 22: 2229-40.

[10] Prescott J and Coetzee GA. Molecular chaperones throughout the life cycle of the androgen receptor. Cancer Lett 2006; 231: 12-9.

[11] Marte B. Passage through the nuclear pore. Nat Cell Biol 2001; 3: E135.

[12] Zhou ZX, Sar M, Simental JA, Lane MV and Wilson EM. A ligand-dependent bipartite nuclear targeting signal in the human androgen receptor. Requirement for the DNA-binding domain and modulation by NH2-terminal and carboxyl-terminal sequences. J Biol Chem 1994; 269: 13115-23.

[13] Kaku N, Matsuda K, Tsujimura A and Kawata M. Characterization of nuclear import of the domain-specific androgen receptor in association with the importin alpha/beta and Ran-guanosine 5'-triphosphate systems. Endocrinology 2008; 149: 3960-9.

[14] Freedman ND and Yamamoto KR. Importin 7 and importin alpha/importin beta are nuclear import receptors for the glucocorticoid receptor. Mol Biol Cell 2004; 15: 2276-86.

[15] Ni L, Llewellyn R, Kesler CT, Kelley JB, Spencer A, Snow CJ, Shank L and Paschal BM. Androgen induces a switch from cytoplasmic retention to nuclear import of the androgen receptor. Mol Cell Biol 2013; 33: 4766-78.

[16] Dar JA, Masoodi KZ, Eisermann K, Isharwal S, Ai J, Pascal LE, Nelson JB and Wang Z. The N-terminal domain of the androgen receptor drives its nuclear localization in castration-resistant prostate cancer cells. J Steroid Biochem Mol Biol 2014; 143: 473-80.

[17] Gregory CW, Johnson RT Jr, Mohler JL, French FS and Wilson EM. Androgen receptor stabilization in recurrent prostate cancer is associ-

ated with hypersensitivity to low androgen. Cancer Res 2001; 61: 2892-8.
[18] O'Malley KJ, Langmann G, Ai J, Ramos-Garcia R, Vessella RL and Wang Z. Hsp90 inhibitor 17-AAG inhibits progression of LuCaP35 xenograft prostate tumors to castration resistance. Prostate 2012; 72: 1117-23.
[19] Ai J, Wang Y, Dar JA, Liu J, Liu L, Nelson JB and Wang Z. HDAC6 regulates androgen receptor hypersensitivity and nuclear localization via modulating Hsp90 acetylation in castration-resistant prostate cancer. Mol Endocrinol 2009; 23: 1963-72.
[20] Tyagi RK, Lavrovsky Y, Ahn SC, Song CS, Chatterjee B and Roy AK. Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. Mol Endocrinol 2000; 14: 1162-74.
[21] Mora GR, Prins GS and Mahesh VB. Autoregulation of androgen receptor protein and messenger RNA in rat ventral prostate is protein synthesis dependent. J Steroid Biochem Mol Biol 1996; 58: 539-49.
[22] Zhao XY, Ly LH, Peehl DM and Feldman D. Induction of androgen receptor by 1alpha,25-dihydroxyvitamin D3 and 9-cis retinoic acid in LNCaP human prostate cancer cells. Endocrinology 1999; 140: 1205-12.
[23] Saporita AJ, Zhang Q, Navai N, Dincer Z, Hahn J, Cai X and Wang Z. Identification and characterization of a ligand-regulated nuclear export signal in androgen receptor. J Biol Chem 2003; 278: 41998-2005.
[24] Nguyen MM, Dincer Z, Wade JR, Alur M, Michalak M, Defranco DB and Wang Z. Cytoplasmic localization of the androgen receptor is independent of calreticulin. Mol Cell Endocrinol 2009; 302: 65-72.
[25] Gong Y, Wang D, Dar JA, Singh P, Graham L, Liu W, Ai J, Xin Z, Guo Y and Wang Z. Nuclear export signal of androgen receptor (NESAR) regulation of androgen receptor level in human prostate cell lines via ubiquitination and proteasome-dependent degradation. Endocrinology 2012; 153: 5716-25.
[26] Lv S, Song Q, Chen G, Cheng E, Chen W, Cole R, Wu Z, Pascal LE, Wang K, Wipf P, Nelson JB, Wei Q, Huang W and Wang Z. Regulation and targeting of androgen receptor nuclear localization in castration-resistant prostate cancer. J Clin Invest 2021; 131: e141335.
[27] Griffin BA, Adams SR and Tsien RY. Specific covalent labeling of recombinant protein molecules inside live cells. Science 1998; 281: 269-72.
[28] Lin HK, Wang L, Hu YC, Altuwaijri S and Chang C. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. EMBO J 2002; 21: 4037-48.
[29] Li B, Lu W, Yang Q, Yu X, Matusik RJ and Chen Z. Skp2 regulates androgen receptor through ubiquitin-mediated degradation independent of Akt/mTOR pathways in prostate cancer. Prostate 2014; 74: 421-32.
[30] Rees I, Lee S, Kim H and Tsai FT. The E3 ubiquitin ligase CHIP binds the androgen receptor in a phosphorylation-dependent manner. Biochim Biophys Acta 2006; 1764: 1073-9.
[31] Rajender S, Singh L and Thangaraj K. L859F mutation in androgen receptor gene results in complete loss of androgen binding to the receptor. J Androl 2007; 28: 772-6.
[32] Li B, Lu W and Chen Z. Regulation of androgen receptor by E3 ubiquitin ligases: for more or less. Receptors Clin Investig 2014; 1.
[33] Nelson PS. Molecular states underlying androgen receptor activation: a framework for therapeutics targeting androgen signaling in prostate cancer. J Clin Oncol 2012; 30: 644-6.