


2015-01-01

Development Of A Yeast-Based Assay For The Functional Characterization Of Fkbp52/Î²-Catenin Interactions With The Androgen Receptor

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DEVELOPMENT OF A YEAST-BASED ASSAY FOR THE FUNCTIONAL
CHARACTERIZATION OF FKBP52/ β -CATENIN INTERACTIONS WITH
THE ANDROGEN RECEPTOR

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CHARACTERIZATION OF FKBP52/ β -CATENIN INTERACTIONS WITH THE
ANDROGEN RECEPTOR

by

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THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

August 2015

Acknowledgements

My first thank you goes to my mentor Dr. Marc Cox. I cannot express how grateful I am for all the support you have shown me in the pursuit of my goals. Never once did I feel judgment on why or what I wanted to do, instead you listened and supported my own personal growth. The time spent working in your laboratory, albeit short-lived, resulted in a confidence and motivation that I never would have been able to achieve on my own.

Next, I would like to say thank you to my committee members; Dr. Sid Das, Dr. Manuel Miranda and Dr. Wen-Yee Lee. The encouragement and guidance you gave to me was invaluable.

I cannot continue without a special thanks to my family. I am grateful for the love, appreciation, and help you have always given me. All my hard work is always driven by the want to make all of you proud. Thank you for always joining in my celebrations and comforting me in my defeats. Thank you especially to my mother and grandmother for showing me what a strong woman is capable of achieving and for selflessly putting me before all others. I love you all very much.

Another big thank you to all the members of the Cox laboratory. Thank you Yenni, Nai, Jeff, Nina, and Ashley for all of your support and for always taking time to help me. Thank you also to the undergraduates, Rosio, Omar, Paola, Luis, and Rodrigo. I also need to give recognition to my former graduate mentor Katie Meraz. Thank you Katie for the patience you've shown me and the encouragement you gave when I needed it the most. Also, a special thanks to Lorellie Frydenlund for all of the "therapy sessions" and coffee times we shared. You've been a close friend and confidant during my time here and will always value your friendship.

Special Thanks to the Louis Stokes Alliance for Minority Participation Bridge to the

Doctorate Program, Grant Number HRD-1301858 (BD 2013-2015). Thank you Dr. Knaust and Ari for all the help and guidance you provided during the BD seminar course. This research was funded through Cancer Prevention and Research Institute of Texas (CPRIT) (RP110444-P2). UTEP core facilities are funded by the NIH BBRC grant 5G12RR008124. This research was also supported in part by the National Institutes on Minority Health and Health Disparities (NIMHD), a component of the National Institutes of Health (NIH) under award number 2G12MD007592.

Lastly, my biggest thank you goes to my best friend, my therapist, the one who challenges me the most, as well as provides me with never ending support. Thank you Larbear for all the love you've shown to me these past 6 years. None of what I've accomplished would have been possible without you by my side. You will always be the Lennon to my McCartney.

Abstract

Prostate cancer development is uniquely dependent on the androgen receptor's (AR's) transcriptional regulation in response to hormone binding. Current therapies directly target AR acting as an antagonist at androgen binding sites. However, once androgen-dependence is lost, meaning the patient has progressed into a late-stage hormone resistant phenotype, all current treatments are essentially ineffective. Utilizing the yeast strain *Saccharomyces cerevisiae* we are capable of creating a model system that allows for the exogenous expression of AR while still retaining the chaperone components needed for this steroid receptor complex (Skena et al.,1988). The 52kDa FK506 binding protein (FKBP52) has been shown to be an important positive regulator of AR receptor function (Cheung-Flynn et al.,2005). Additional analysis has also identified a synergistic interaction occurring between FKBP52 and the cadherin-associated protein known as β -catenin (Storer., unpublished data).

Data gleaned from yeast-reporter assays determined that within *S. cerevisiae* this synergism is essentially lost. Previous research shows that the Inhibitor of β -catenin and T-cell factor (ICAT) protein is capable of modulating AR activity within mammalian cells (Zhou et al., 2011). Introduction of this protein within yeast, interestingly, resulted in a significant increase of AR activity while in the presence of only FKBP52. His tagged pull-down assays determined that there is no direct protein-protein interaction between the two. Instead, it is likely that FKBP52 and ICAT are capable of modulating AR activity directly on the receptor surface or can induce a positive conformational change. This information can help us understand the multi-protein complex that regulates AR activity for novel CRPC therapeutic sites.

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Chapter 1: Introduction

Androgens are essential for the normal differentiation of male reproductive organs as well as the development and progression of prostate cancer (PCa) (Jenster., 1999). A common treatment for patients with advanced PCa involves androgen depletion which typically results in apoptosis of primary tumor cells, demonstrating the disease's dependence on AR activation (Kyprianou and Issacs., 1988). However, despite the initial response to treatment, tumor development is likely to reoccur as castration-resistant prostate cancer (CRPC). During this disease developmental phase current clinical therapeutic options are unsuccessful.

1.1 Steroid Hormone Receptors

1.1.1 Receptor Assembly

Steroid hormone receptors (SHRs) are transcription factors that are responsible for the expression of hormone dependent genes. There are five main receptors in the SHR family, those being the androgen (AR), glucocorticoid (GR), progesterone (PR), mineralocorticoid (MR), and estrogen (ER) receptors (Pratt and Toft.,1997). Proper folding and activation of each SHR is dependent upon the dynamic assembly of the chaperone and cochaperone machinery (Edwards., 2000).

This assembly begins with the free cytosolic receptor binding to the cochaperone Heat shock protein 40 (Hsp40) and chaperone Hsp70 (refer to figure 1.1) (Young et al., 2004). Hsp70 is responsible for the fate of the receptor, either allowing it to continue to the intermediate complex or sending it for proteasomal degradation. If the receptor is damaged or missfolded, the proteins Bcl-2 associated athanogene (BAG) and carboxy terminus of Hsc70 interacting protein (CHIP) bind to Hsp70 and degrade the SHR via the ubiquitin-proteasome pathway (Connell et al., 2001). However, if there are no significant issues with the early complex it is then capable of proceeding to its next stage of maturation. This next phase allows for the Hsp 70-interacting protein (HIP) to displace Hsp40 and stabilize Hsp70, increasing its affinity for substrates (Whitesell and Lindquist., 2005). Following this, Hsp-organizing protein (HOP) in association

with Hsp90 is bound to the complex and results in the subsequent release of HIP, HOP and Hsp70. The Hsp90 cochaperone p23 then stabilizes the Hsp90 conformation and proteins known as immunophilins are recruited. These immunophilins are characterized by their Hsp-90 binding tetratricopeptide repeat (TPR) domain, examples of which include Cyclophilin (CyP40), Protein Phosphatase 5 (PP5) and the immunophilins our lab primarily study FKBP51 or 52 kDa. The final mature complex allows for high affinity hormone binding. In the presence of hormone the mature receptor dissociates from heat shock proteins, forms a homodimer, undergoes phosphorylation, and subsequently translocates into the nucleus (Galigniana et al., 2001; Claessens et al., 2008). Here, it binds to hormone response elements (HRE) and can begin initiating gene transcription.

1.1.2 Androgen Receptor Structure and Function

It is also important to take into account the structure of AR as this will provide us with information regarding its regulation and various protein interactions. There are four principal domains that make up the receptor; the N-terminal domain, the ligand binding domain (located at the C-terminus), the DNA binding domain, and the hinge region (figure 2) (Tsai et al., 1994; White et al., 1998).

The N-terminal domain (NTD) contains activation function 1, one of the two activation function (AF-1 or AF-2) sites found in AR. The activation domain's possesses the ability to activate transcription, although the mechanisms by which it is capable of doing so is still not fully understood (Jenster et al., 1995). The AF-1 region is the least conserved site amongst all SHR's, sharing only 15% homology (Lavery and McEwan., 2005). Coactivators are recruited to this region in a ligand-independent manner. In addition, there are a varying number of polyglutamine repeats within the (NTD) ranging from 8 to 31, the average being 20 (Quigley et al., 1995; Heemers and Tindall., 2007). Lengthening these repeats can give rise to mild androgen

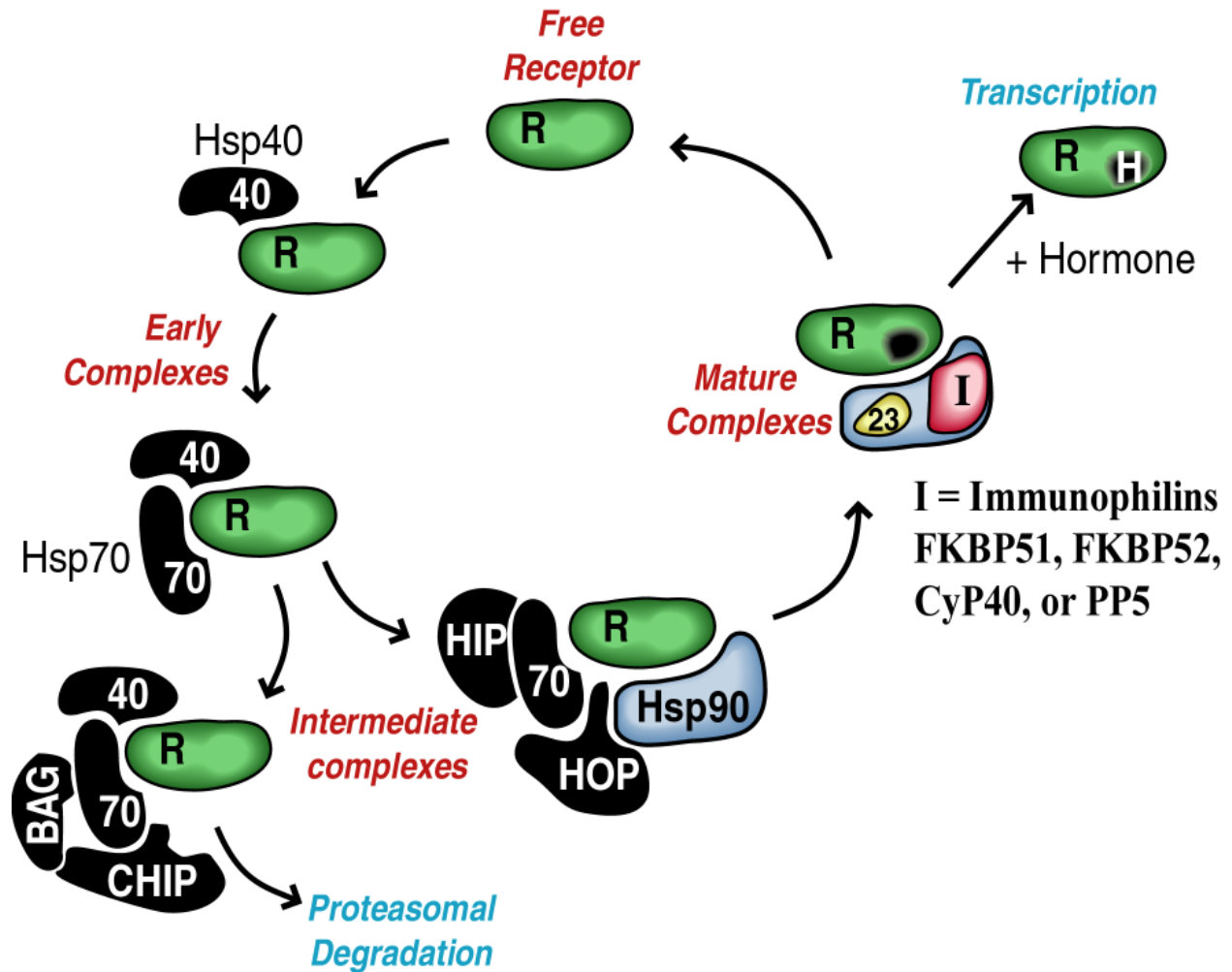


Figure 1.1: Steroid Hormone Receptor Maturation

Cytosolic SHR (R) initially binds to Hsp40 and Hsp70 forming an early complex. Upon binding Hsp70 the receptor is either targeted for proteosomal degradation or can continue towards an intermediate complex. HIP and Hop are recruited allowing for Hsp90 binding. At the last stage of the pathway the cochaperone p23 and an immunophilin (I) are recruited creating the final mature high affinity hormone binding complex. The receptor is then capable of translocating into the nucleus to begin gene transcription. Figure created by Marc B. Cox, Ph.D.

insensitivity, while shortening them has been shown to increase AR transcriptional activity. The N-terminal domain also contains a FxxLF and WxxLF motif that is responsible for its interaction with the C-terminal domain stabilizing AR/ligand interactions (He et al., 2002). This N and C terminal interaction was determined to be preferable over co-activator recruitment and is distinct for AR, which could possibly act as a novel drug regulatory site.

AF-2 is found within the ligand binding domain (LBD) and, opposite from AF-1, is highly conserved and its coactivator binding is ligand-dependent. The crystal structure of AR's ligand binding domain shows it contains 11 α -helices (labeled 1-12 in consistence with other SHR's, however no second helix exists) and two short β -turns (Matias et al., 2000). The ligand binding pocket, also known as Binding Factor 1 (BF-1), consists of helices 3, 5, 10, and 11. Upon hormone binding there is a conformational change and helix 12 repositions itself acting as a "lid" stabilizing the binding pocket/ligand interaction. This shift also allows for the activation of the hydrophobic AF-2 site. Co-activators containing an LxxLL motif within their nuclear receptor-interacting domain (NID) are recruited for binding to this surface (Heery et al., 1997). The FxxLF and WxxLF motifs in the NTD can compete with co-activators for binding at the AF-2 site, which as previously stated is a preferable interaction (He et al., 2002). Removal of the ligand binding domain gives rise to a constitutively active AR. Also located next to the AF-2 coactivator binding site within the LBD, is the newly characterized Binding Factor 3 (BF3) surface (Estébanez-Perpiñá et al., 2007; Buzón et al., 2011). This surface is similarly hydrophobic in nature. Screening of compounds that targeted this area resulted in a down regulation of AR mediated transcription and prevented certain co-activators from binding. Based on this information our lab previously developed the compound MJC13 which, through *in-silico* docking simulations, is predicted to bind to AR's BF3 surface (De Leon et al., 2011).

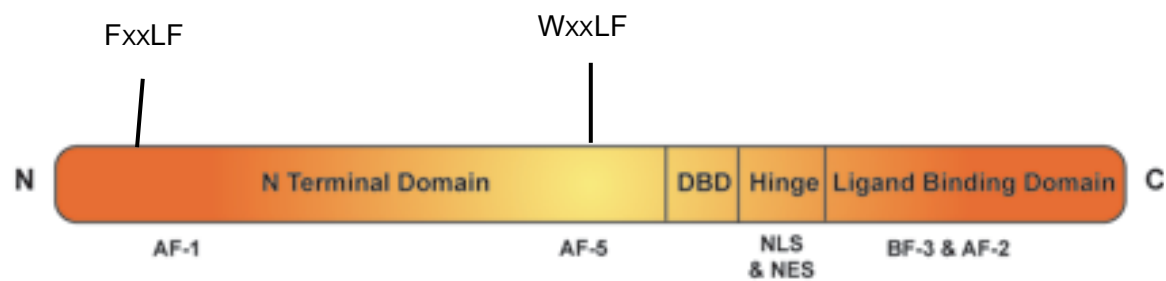


Figure 1.2: Schematic of Human AR

Representation of AR domains and the exons that encode for them. AR is composed of an N-terminal domain, which has the AF1 site and FxxLF/WxxLF motifs, and a Ligand binding domain, containing the AF2 site and BF3 surface. There also is a short hinge region and a highly conserved DNA binding domain. (Cox et al., 2005)

AR's DNA binding domain (DBD) is well conserved amongst SHR's demonstrating their common need to bind DNA, however their sequential differences highlight their selectiveness of particular genes. The mature AR homodimer binds to the androgen response element site through the first of its two zinc fingers while the second is involved in AR dimerization (Heemers and Tindall., 2007). There also is a nuclear localization signal (NLS) that spans the DBD and hinge region (Jenster et al., 1993; Zhou et al., 1994).

1.2 FKBP52

1.2.1 FKBP51 and FKBP52

The Immunophilin known as the 52 kDa FK506 binding protein (FKBP52) has been found to be a positive regulator of AR (Cheung-Flynn et al., 2005), PR (Tranguch et al., 2005), and GR (Riggs et al., 2003) in both cellular and whole animal models. FKBP52 belongs to the FKBP family of co-chaperones through its interaction with the immunosuppressive drug FK506 (also known as Tacrolimus). The immunosuppressive effects caused by FK506 only occur through its interaction with FKBP12's peptidyl-prolyl cis-trans isomerase (PPIase) pocket, creating a high affinity calcineurin interaction surface. The clinical success of Tacrolimus demonstrates that the hydrophobic PPIase pocket within the FKBP family is a druggable interaction surface having insignificant off-target effects.

FKBP51 is another member of the FKBP family that shares a significant amount of similarity to FKBP52. They both modulate SHR activity through the binding of Hsp90 as well as share a similar conformation. In fact, there is 70% homology in amino acid sequence conserved between the two (Figure 1.3). Both contain three functional domains and a short linker region. These include the FKBP12-like domain 1 and 2 (FK1 and FK2) as well as a Tetratricopeptide repeat (TPR) domain. The FK1 domain of both FKBP51 and FKBP52 has functional PPIase

activity while the FK2 domain in both immunophilins lack this. The TPR domain remains highly conserved and allows for interaction with Hsp90 by way of the C-terminal EEVD motif. The FK linker (also known as the FK loop) connects the FK1 and FK2 domains and is also capable of regulation. This region contains the 143-TEED sequence within FKBP52 and a 143-FED sequence in FKBP51. The 143-TEED sequence is phosphorylated by casein kinase 2 resulting in the reorientation and subsequent loss of function of the FK1 domain. Alternatively, the 143-FED sequence in FKBP51 lacks the Threonine phosphorylation site demonstrating a possible functional difference between the two proteins. The FKBP52 mutant, T143E, resulted in an abrogation of FKBP52's functional effects on the androgen receptor demonstrating that the FK-loop is another potential therapeutic site. The N-terminal FK1 domain in FKBP52 was discovered to be essential for AR regulation; however, the PPIase enzymatic activity was not responsible for the resulting potentiation. Instead, domain mapping studies have reported that the proline-rich loop that overhangs the PPIase catalytic pocket is critical for receptor regulation and may be a potential interaction surface (Riggs et al., 2007). FKBP51 is not capable of potentiating AR activity, and instead acts as a negative regulator on the receptor. However, random mutagenesis determined that the mutations A116V and L119P converted FKBP51 to a fully functional FKBP52-like state, capable of AR potentiation. Again, highlighting the importance of the FK1 proline-rich loop.

SHR's are found to reside within the nucleus and cytoplasm in the absence of ligand with their main method of transport believed to be simple diffusion. Recently, it was discovered that FKBP52 was co-immunoprecipitated in complex with Hsp90 and the dynein/dynactin complex, suggesting the use of retrograde transport. FKBP52's PPIase domain binds the dynein/dynactin motor complex independently of its PPIase activity. Instead the FK1 domain acts as a protein-

protein interaction domain. Treatment with geldanamycin (a Hsp90 inhibitor) increased the transport time of the steroid hormone receptors, MR and GR, from the cytoplasm into the nucleus by roughly 11-fold.

Murine studies have also demonstrated that FKBP52 plays a significant role in male sexual development and reproduction. Fkbp52 knock-out (52KO) mice were developed by removing exons that coded for the 52 protein. The resulting male mice displayed a phenotype similar to that of an androgen insensitivity syndrome (Cheung-Flynn et al., 2005; Yong et al., 2007). Morphological traits of 52KO mice included ambiguous external genitalia, dysgenic seminal vesicles, and a retention of nipples into adulthood. Female mice also showed a loss of fertility and development of endometriosis, while displaying no phenotypic differences (Hirota et al., 2008; Tranguch et al., 2005). Loss of FKBP51 expression within mice did not result in any morphological differences, however the loss of FKBP51 and FKBP52 proved to be embryonically lethal. This suggests some possible redundancy in the roles of FKBP51/52.

Regulation of AR activity requires the binding of FKBP52 to Hsp90 however, it is still unknown if FKBP52 is capable of directly interacting with the receptor. The use of chimeric receptor proteins has established that the FK1 site, specifically the proline-rich loop, of FKBP52 is a likely regulator at the receptor's LBD (Riggs., et al. 2003). Yeast studies also demonstrated that mutations on the AR BF3 surface increased its dependency of FKBP52. A compound library yeast screen determined that the small molecule inhibitor, MJC13, was capable of inhibiting AR function by preventing the hormone-induced release of the Hsp90-AR complex (De Leon et al., 2011). Available evidence suggests that the compound MJC13 binds to the BF3 surface of AR at the possible FKBP52 interaction site.

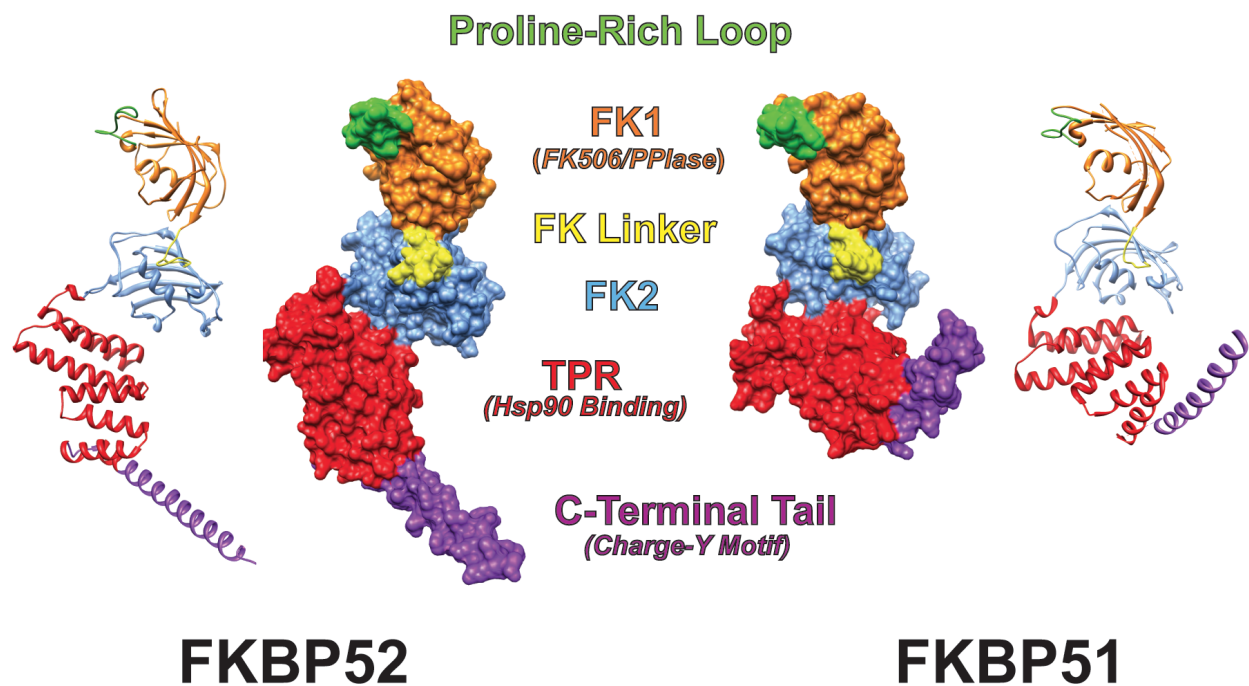


Figure 1.3: Structure of FKBP51 and FKBP52

FKBP51 and FKBP52 are composed of four domains. The FK1 domain, which confers PPIase activity and contains the Proline rich loop. The FK linker which connects the FK1 domain to FK2 and regulates FKBP52 functionality through phosphorylation from casein kinase 2 (CK2). FK2, which is structurally similar to FK1 however, it lacks PPIase activity. Lastly, the TPR region is responsible for binding to the EEVD motif of Hsp90 through its tetratricopeptide repeats (TPR) (Harris et al., 2014).

1.3 Beta-Catenin's Synergistic Effect

β -Catenin is a multifunctional protein that is involved in several signaling pathways and has implications in cell-cell adhesions (McCrea and Gumbiner., 1991; Pandur et al., 2002). In the latter role, β -Catenin is key in the linking of cadherin's cytoplasmic tail to α -catenin and the actin cytoskeleton. It is also capable of acting as a transcription factor within the canonical Wnt signaling pathway through its interaction with the LEF1/TCF (lymphoid enhancer factor/T Cell factor) family of proteins. More importantly, β -Catenin has recently been established as a well known regulator of AR mediated transcription (Yang et al., 2002).

It has a relative size of 92kDa and is composed of 12 armadillo repeats, a 140 amino acid N-terminus regulatory site, and a roughly 100 amino acid C-terminus trans-activation domain (McCrea et al., 1991). One armadillo repeat is composed of 42 amino acids arranged in three α -helices, designated H1, H2, and H3 (except for repeat 7 which lacks H1). The H3 helices allow for the formation of a positively charged groove extending from repeat 1 to 10 and a hydrophobic surface at repeats 11 and 12. Structural analysis has determined that the β -Catenin ligands human Tcf-4, E-cadherin, and APC all interact through similar mechanisms at the positive groove region between repeats 5 and 10 at two lysine residues called charge buttons (Huber et al., 1997).

Immunoprecipitation assays have demonstrated that AR complexes with β -Catenin in LNCaP cell lines, and even more so in the presence of hormone. Yet, there is no crystal structure demonstrating β -Catenin's direct interaction with AR or FKBP52. However, its interaction with the liver receptor homolog-1 (LRH-1), a member of the nuclear receptor subfamily, ligand binding domain was solved and superposed over analogous sites in AR (Figure 1.4) (Fletterick., 2009). This demonstrated that the armadillo repeats 3-7 of β -Catenin bound to a region similar to

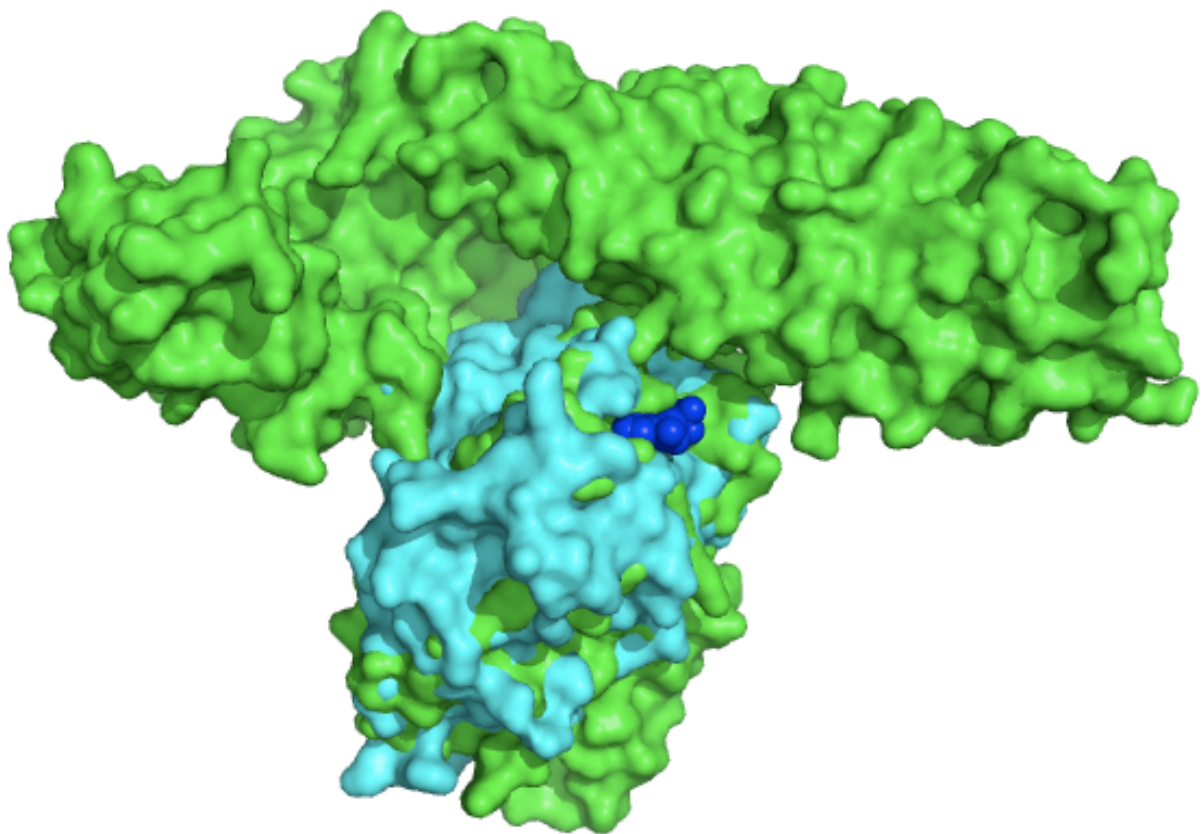


Figure 1.4: Model of β -Catenin Interaction With AR

Predicted model of AR interaction with β -Catenin shows binding site overlaps with the predicted FKBP52 regulatory site (BF3 surface). This interaction occurs through the Flufeamic acid (purple), β -Catenin (green), LBD (teal) (Fletterick., 2009)

the AF-2 and BF3 surface of AR. Following small molecule binding at the AR BF3 surface the AF-2 site endures a conformational change, possibly allowing for the formation of a β -Catenin interaction surface. This suggests that since the BF3 surface is the hypothesized FKBP52 interaction site, there may be a cooperative multi-protein complex directly regulating the androgen receptor.

Previous research within our lab investigated the effect of β -Catenin and FKBP52 overexpression on AR transcriptional activity within a fkbp52 knock-out mouse embryonic fibroblast cell line (52KO MEF) (Storer., unpublished data). This determined there to be a synergistic up regulation of said activity. The data revealed that FKBP52 and β -Catenin worked in concert with one another, losing this synergism if the other protein was not expressed (figure 1.5). Preliminary research on β -Catenin had indicated it acting alone on its synergism of AR however, these studies did not utilize cell lines that lacked FKBP52.

Cellular β -Catenin levels have been found to be increased in not only prostate cancer, but also in a variety of human cancers, such as hepatocellular carcinoma (Miyoshi et al., 1998), medulloblastoma (Zurawel et al., 1998), endometrial cancer (Fukuchi et al., 1998), and ovarian cancer (Palacios et al., 1998). Therapeutically targeting this protein is an unlikely clinical treatment due to its involvement in cell-cell adhesions. Instead, it is of particular interest to study its interactions with the Androgen receptor and FKBP52 for potential target sites in prostate cancer.

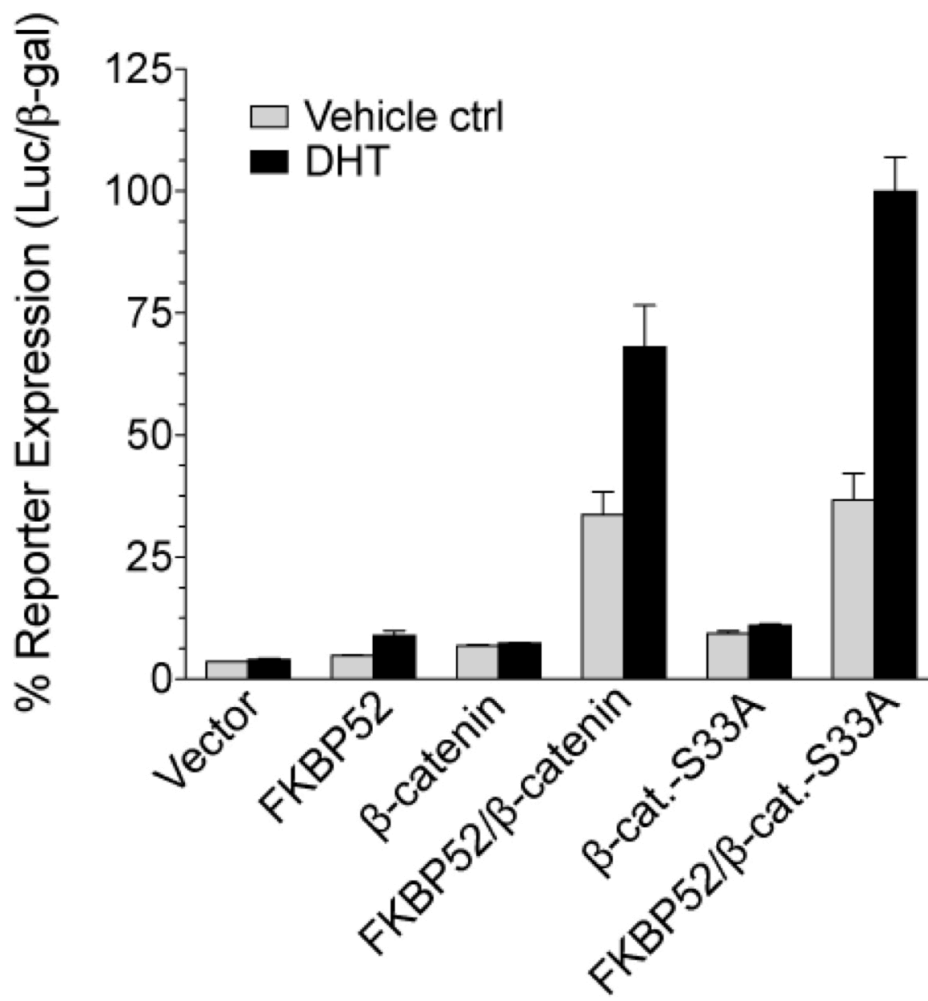


Figure 1.5: β -Catenin Overexpression Results in a Synergistic Effect Within 52KO MEFs.

β -catenin acts in synergy with FKBP52 in order to promote AR signaling within 52KO MEFs.

The β -cat S33A mutant is resistant to GSK3 β phosphorylation and subsequent degradation.

1.4 Prostate Cancer Development

1.4.1 Facts and Statistics

While cancer has no definitive origin, the fundamental abnormality that results in disease development is the continual unregulated growth of cancerous cells. Causes of cancer can include genetic irregularities, environmental exposure, infection with a pathogen, or lifestyle factors. Within the United States alone, there was an estimated 14 million citizens that reported a history of cancer (American Cancer Society., 2014). In addition, approximately 600,000 American's are expected to die as a result of cancer, making it the second most common cause of death in the U.S., behind heart disease.

Prostate cancer is the second most frequently diagnosed cancer in men, as well as the second leading cause of cancer death. There was an estimated 233,000 new cases in the U.S. during 2014 with a higher incidence rate was seen in African Americans males than in non-Hispanic whites. In addition, the National Cancer Institute asserts that PCa is more common in older men with a family history of the disease. It is most frequently diagnosed in males aged 65-74, which roughly accounts for 36.8% of all new PCa cases (American Cancer Society., 2014).

1.4.2 Clinical Diagnosis

Prostate cancer symptoms are generally mild and include frequent or difficult urination, erectile dysfunction, and/or blood in the urine or semen. Symptoms are similar to that of the enlargement of the urethral prostate tissue, known as benign prostatic hyperplasia (BPH). However, outcomes of both diseases are vastly different as BPH is non-cancerous, ordinarily treatable and can result in urinary tract and kidney problems. Prostatic intraepithelial neoplasia (PIN) and proliferative inflammatory atrophy (PIA) are two other conditions that display abnormal prostate gland cells within a biopsy (American Cancer Society., 2014). Research

suggests these may be a pre-cancerous state of the prostate. However, no definitive data has stated a direct correlation between these conditions and PCa development.

Diagnosis of prostate cancer occurs through the use of a prostate specific antigen (PSA) blood test. This involves the use of a blood sample to determine PSA levels within the individual. An increase of PSA levels is correlated with an increase in risk of tumor development, with majority of healthy men having PSA levels under 4 ng/mL of blood. Patients with PSA levels greater than 10 ng/mL typically have a 50% chance of having prostate cancer.

The most widely used staging system for prostate cancer is the American Joint Committee on Cancer (AJCC) TNM System. This looks at the extent of the primary tumor (T), whether it has spread to the lymph nodes (N), the absence or presence of distant metastasis (M), the PSA level at the time of diagnosis, as well as the Gleason score, which determines the extent to which the prostate tissue has altered to a cancerous state. Following the assembly of data the overall stage of the individuals PCa is scored in Roman numerals ranging from I to IV, with I being the least advanced and IV being the most. This helps in determining the patient's prognosis and expected survival.

Late-stage castrate resistant prostate cancer is developed when the tumor is no longer responsive to low-dose treatment of testosterone within the body. Currently, CRPC is an incurable disease and has a relatively low survival rate.

1.4.3 Genomic Aberrations

Inherited changes in DNA account for roughly 5 to 10% of total prostate cancer cases in the U.S. (American Cancer Society., 2014). Out of these changes, several of the cell's tumor suppressor genes have been found to be mutated. These include BRCA1 and BRCA2, which are responsible for repairing double-stranded DNA breaks. Carriers of BRCA1/2 mutations had a

relative risk of prostate cancer development of 4.65 by age 70 as well as an increased risk for other cancers such as pancreatic cancer, leukemia and lymphoma, as well as ovarian and breast cancer in women (Levy-Lahad and Friedman., 2007; Friedenson., 2007). However, only 1-2% of prostate cancers are believed to be caused by BRCA1 or BRCA2 mutations (Levy-Lahad and Friedman., 2007).

The ubiquitin ligase speckle-type POZ protein (SPOP) is mutated in 6-15% of prostate cancer cases. A few pro-oncogenic proteins targeted by SPOP include DEK (Theurillat et al., 2014), the P160 steroid receptor co-activators (SRCs) NCOA1, 2 and 3 (Gregory et al., 2001; Genge et al., 2013) as well as the androgen receptor itself (An et al., 2014). SPOP mutations prevent P160 SRC family proteins from being targeted for degradation and instead results in their accumulation. Overexpression of these proteins is linked to prostate cancer cell survival and can increase AR transactivation. The transcription factor GATA2 enhances the recruitment of NCOAs to the AR complex and is also found to co-localize with the forkhead protein (FOXA1) (He et al., 2014). FOXA1 was also determined to be genetically mutated in a small subset of prostate cancers as well as amplified or even deleted (Taylor et al, 2010; Grasso et al, 2012).

In addition, RNASEL which encodes for ribonuclease L (RNASEL), is a member of the interferon-mediated immune response and is found to mutated in a small percentage of CRPC cases (Liang., 2006). Infectious agents can result in intraprostatic inflammation leading to the damage of local tissue, which may in turn drive cancer progression. RNASEL is responsible for cleavage of viral RNA as well as induction of apoptosis. Advanced stages of disease were associated with the missense mutations R462Q and D541E within RNASEL.

AR mutations are also detectable in some CRPC patients with majority of these being in the LBD or co-factor binding regions (Gottlieb et al, 2012; Grasso et al, 2012). These mutations

have the capability of activating AR through other steroid metabolites. For example, the mutation L702H is able to repurpose glucocorticoids as AR ligands. Androgen receptor variants have also been found to be able to convert AR antagonists into AR agonists.

Following severe ligand depletion, a common issue CRPC patients face is the increase of AR gene transcription due to the cells innate feedback response (Wolf et al, 1993; Cai et al, 2011; Knuuttila et al, 2014; Wyatt et al, 2014). This overexpression of AR is driven by rearrangement of the X chromosome and leads to a hypersensitivity of castration levels of DHT. Increase of AR transcription pressure can also lead to hormone independent splice variants. These AR splice variants (AR-Vs) lack the LBD leaving them constitutively active. Those positive for the AR-V7 truncations are associated with primary resistance to the drugs abiraterone and enzalutamide (Dehm et al., 2011).

1.5 Current Treatment Options

1.5.1 Surgery and Radiation Therapy

The initial treatment response for low-grade prostate cancer involves either the use of radiation therapy or surgery. It is performed at an early disease development phase, typically stage I, which identifies the cancer as being localized to the prostate gland. Both surgery and radiation have a similar success rate and are routinely used in conjunction with one another to strengthen the possibility of total cancer eradication . (American Cancer Society., 2014).

Two types of radiation treatments are currently available to treat prostate cancer, these being external beam radiation therapy (EBRT) and brachytherapy. EBRT involves the use of a machine that focuses radiation beams on the prostate gland and is performed outside of the body. Brachytherapy (also known as seed implantation radiation therapy) utilizes small radioactively labeled pellets that are placed directly into the prostate. These “seed” sized pellets are inserted

through the use of a needle and either give off low doses of radiation for several weeks, or release high doses in a very short time period (typically two days). Both external and internal radiation run the risk of causing bowel and urinary problems, however, serious long-term issues occur in less than 5% of patients.

Surgery is a common treatment option and the predominant operation performed is known as a radical prostatectomy. This involves the removal of the entire prostate gland, as well as the surrounding tissue, seminal vesicles, and if necessary, nearby lymph nodes. Several surgical approaches are available, examples of which include a radical retropubic and radical perineal prostatectomy. Both of which involve invasive surgical incisions and are used less often than the third surgical approach, known as laparoscopic radical prostatectomy (LRP). LRP instead uses small incisions in which surgical instruments are inserted into and are controlled either manually or robotically by the surgeon. Side effects are similar to those of radiation therapy, and also include an increased risk of the loss of fertility, lymphedema, and inguinal hernia development.

1.5.2 Androgen Deprivation Therapies

If the disease develops into a late-stage metastatic form, surgery and radiation are no longer considered viable treatment options. Instead, therapy then focuses on targeting the ligands responsible for androgen receptor activity or a direct target of the receptor itself. Research has demonstrated that majority of all prostate cancers exhibit a response to the depletion of circulating androgens within the body. Because of this, androgen deprivation therapy (ADT) has been a staple in late stage prostate cancer treatment for nearly three-quarters of a century (Huggins et al., 1941).

The production of DHT can be halted through a variety of different means. Obstruction

of Testosterone synthesis can be achieved through the blockage of members of the cytochrome P450 (CYP) family of enzymes. These enzymes are responsible for the catalytic conversion of cholesterol into testosterone (Figure 1.6). The drug known as abiraterone acetate targets the CYP family enzyme CYP17A1, and has demonstrated significant improvement in median overall survival during phase III clinical trials (Fizazi et al., 2012; Ryan et al., 2013). Due to the relative success of abiraterone other CYP17A1 inhibitors, examples of which include Orteronel, VT-464, and Galeterone, were developed and are currently in phase I and II clinical trials. In addition, other members of the steroid hormone pathway have the potential of being therapeutically targeted. Dutasteride (brand name Avodart) inhibits the type 1 and type 2 5-alpha-reductase catalysis of testosterone to DHT (Steers et al., 2001). Following initial ADT the minor testosterone synthesis pathway that utilizes circulating DHEA-S derived from the adrenal glands remains capable of androgen synthesis through the aldo-keto reductase family 1, member C3 (AKR1C3) (Lin et al., 1997). The oral AKR1C3 inhibitor ASP9521 was terminated during phase I/II clinical trials due to ineffectiveness however, it is likely required to be used in combinational therapies to block one of the multiple pathways involved in DHT synthesis.

DHT synthesis can also be blocked through the use of luteinizing hormone releasing hormone (LHRH) agonists and antagonists, which interfere with the hypothalamic-pituitary-testis axis. The agonistic drug leuprolide works by stimulating a sizable release of lutenizing hormone (LH) from the pituitary gland. This increases testosterone production in the testis and adrenal glands. Through negative feedback inhibition the production of LH is dramatically reduced, subsequently down regulating testosterone production. Unfortunately, LHRH agonists result in an initial surge of testosterone synthesis lasting several weeks. This could result in spinal cord compression, increased pain at metastatic sites, as well as possible

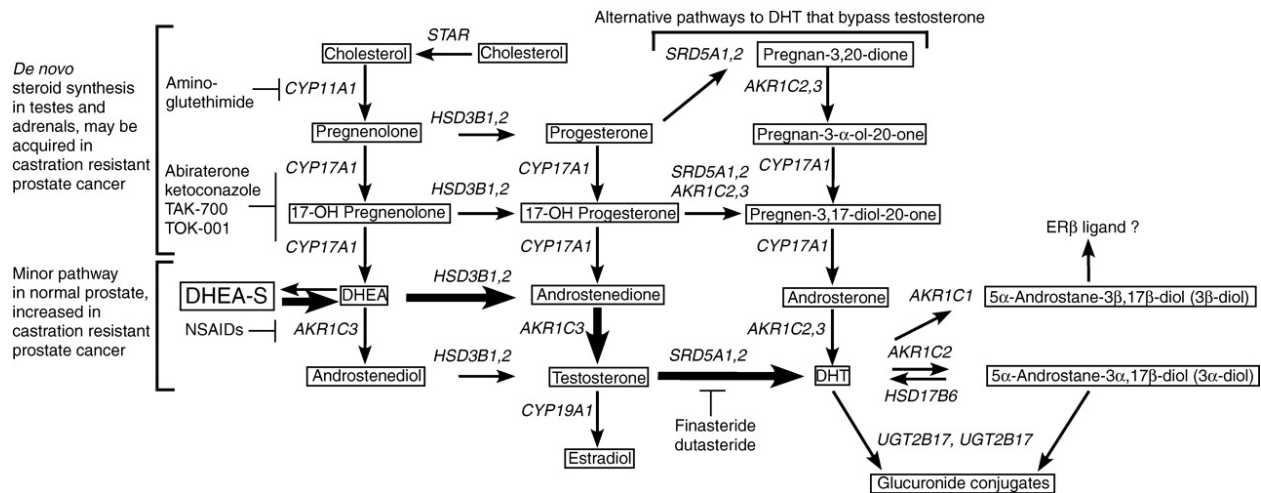


Figure 1.6: Alternative DHT Production Pathways

Testosterone synthesis relies on the P450 (CYP) family of enzymes. CYP proteins are targeted by the drug Abiraterone.acetate. Various other testosterone producing pathways, and the respective inhibitory drugs, are depicted above. (Cai and Balk.,2011)

sudden death (Brawer., 2004) Conversely, the LHRH antagonist known as degarelix obstructs LHRH-receptor binding within the pituitary gland preventing the release of LH. Treatment with LHRH antagonists does not surge testosterone levels as with agonists but alternatively, results in a rapid suppression of testosterone levels. The LHRH antagonist does not come without its share of side-effects as it is capable of inducing possible life-threatening histamine-mediated allergic reactions.

Directly targeting the androgen receptor is another therapeutic option in treating late-stage CRPC, and is typically utilized following the failure of a LHRH analogue and/or orchiectomy. These non-steroidal anti-androgens work by competing with hormone for AR binding at its LBD. This prevents the receptor from becoming activated and in turn prevents AR mediated transcription. Examples of AR antagonist include Bicalutamide, Flutamide, and Enzalutamide. Bicalutamide is the more widely used anti-androgen as it has less hepatotoxicity as well as a longer half life allowing for low-dose administration. AR-V expression has been detected in CRPC patients that have garnered a resistance to abiraterone and enzalutamide (Antonarakis et al, 2014). These truncations can be left unaffected by conventional AR therapeutics thus, highlighting the necessity of new target sites..

1.5.3 Alternative Treatment Options

Following resistance to hormone therapy, the next treatment option typically involves the use of the chemotherapeutic agent, docetaxel (Taxotere). This acts as an antimicrotubule agent preventing its disassembly, consequently resulting in the inhibition of mitotic cell division. Another possible treatment option is the use of the cancer vaccine known as sipuleucel-T (Provenge). Treatment involves the removal of dendritic cells from the patient. These are subsequently incubated with the prostatic acid phosphatase (PAP) antigen, as well as the

granulocyte-macrophage colony stimulating factor (GM-CSF), which promotes dendritic cell activation. The activated antigen presenting cell's are then re-infused into the patient and elicit an immune response against cancer cells expressing the PAP antigen.

Following the invasive procedure of surgical castration, the body undergoes a stress response that can induce heat-shock protein activity, preventing AR degradation. An example of which includes overexpression of Hsp27. Phosphorylation activates this chaperone which in turn enhances AR stability as well as promotes its nuclear transport and transcriptional activity. Prevention of S78 and S82 residues from being phosphorylated or directly targeting Hsp27 through the use of the drug OGX-427 promotes AR association with the E3 ubiquitin ligase MDM2 stimulating AR proteosomal degradation (Zoubeidi et al., 2007). OGX-427 is currently under phase II trials and has demonstrated, when in conjunction with prednisone treatment, there was a greater decline in PSA levels in addition to a higher percentage of progression free patients in comparison of treatment with prednisone alone. Targeting the Hsp90 is of clinical importance also as a majority of cancers accrue a high volume of missfolded proteins due to rapid expression. Hsp90 helps the progression of prostate cancer into a castrate-resistant state by refolding and promoting protein functionality. The small molecule inhibitor known as ganetespib (STA-9090) targets the N-terminal ATP pocket within Hsp90 (Heath et al., 2013). Despite promising preclinical studies, ganetespib did not demonstrate any advantage during phase II clinical trials in CRPC patients (Heath et al., 2013). It is highly likely that targeting only one chaperone is ineffective in the treatment of CRPC, instead a multi protein attack is needed to overwhelm the cellular system and to prevent the development of drug resistance.

Chapter 2: Materials And Methods

2.1 Plasmid Development

For the reporter assays examining FKBP52 and β -Catenin synergy DNA cloning of respective cDNA into a yeast expression vector was necessary. FKBP52 was previously made by D. Riggs and is under a GPD promoter within a p424 plasmid. This vector is a 2u replicon and contains a Tryptophan screening marker, allowing for growth within SC-W yeast dropout media, as well as ampicillin resistance, for growth within *Escherichia coli*. The β -Catenin vector was made using the β -Catenin cDNA sequence derived from the pSPORT6 vector purchased through Open Biosystems. This was then inserted into the multiple cloning site of p413GPD at the BamHI and ClaI sites. The p413GPD vector is a CEN6/ARSH4 replicon and contains a Histidine 3 marker and Ampicillin resistance. Primers hBeta_CateninF (5'-CGGATCCATGGCTACTCAAGCTGATTTGATGG-3') and hBeta_CateninClaI.Rv (5'-ATATCGATTTACAGGTCAGTATCAAA-3') were used at 60 °C for the polymerase chain reaction. The insert and vector were then digested with the BamHI and ClaI restriction enzymes for 2 hours at 37°C. This was then ligated using (New England Biolab) T4 DNA Ligase at room temperature overnight. The ligation product was transformed into DH5 α competent cells and resulting colonies were screened through restriction digest, then confirmed by sequencing. Due to the number of plasmids being transformed into *S. cerevisiae* the cDNA encoding for ICAT was inserted into a pBEVY-A vector. This vector contains bi-directional GPD and ADH promoters allowing for the concurrent expression of two separate genes. ICAT was amplified from the pBS-CMV-FLAG-ICAT plasmid (provided by Dr. Zijie Sun's laboratory) using the primers ICAT_BamHI.Forward (5'-TATAGGATCCATGAACCGCGAGGGAGCTCCCGG-3') and ICAT_Sall.Reverse (5'-TATAGTCGACCTACTGCCTCCGGTCTTCCGTCTCCGA-3').

The pBEVY-A vector and ICAT insert region were then digested using the appropriate enzymes, ligated, and transformed in a similar manner as the p413GPD_ β -Catenin plasmid.

2.2 Yeast Reporter Assay

β -Catenin and FKBP52 were individually as well as jointly expressed in a yeast-based AR-mediated β -galactosidase reporter system. The yeast utilized was based on a W303a genetic background (MATa leu2-112 ura3-1 trp1-1 his3-11,15 ade2-1 can1-100 GAL SUC2) containing a URA3-marked β -galactosidase reporter plasmid and a LEU2-marked Androgen receptor plasmid. FKBP52 (TRP3- marked) and β -catenin (HIS3-marked) or their respective vectors were then transformed and grown on SC-LUHW plates for 3 days at 30°C. Three yeast isolates from each plate were chosen for each triplicate and grown in 5ml of SC-LUHW shaking overnight. An initial curve was created to determine the optimal concentration of DHT for the reporter assay. This demonstrated that the EC₅₀ of the curve, around 10-30nM, should be utilized. Yeast were then grown overnight in 5ml of the appropriate media and subsequently diluted to an OD₆₀₀ of 0.08. This dilution ensures equal logarithmic growth and was monitored fifteen minutes later using spectrophotometry. Following this, the appropriate concentration of dihydrotestosterone (DHT) or 100% ethanol was added to the diluted samples and allowed to shake at 30°C for two hours. In order to measure the resulting β -galactosidase expression, 100ul of the culture grown for two hours and 100ul of Gal-Screen TM substrate (Applied Biosystems) were combined in a 96-well plate and incubated at room temperature for another two hours. After this incubation the plate was read in a luminometer (BioTek). Relative light units (RLU) were measured against the resulting OD₆₀₀ of each culture and the normalized data was plotted in GraphPad Software. 2.2

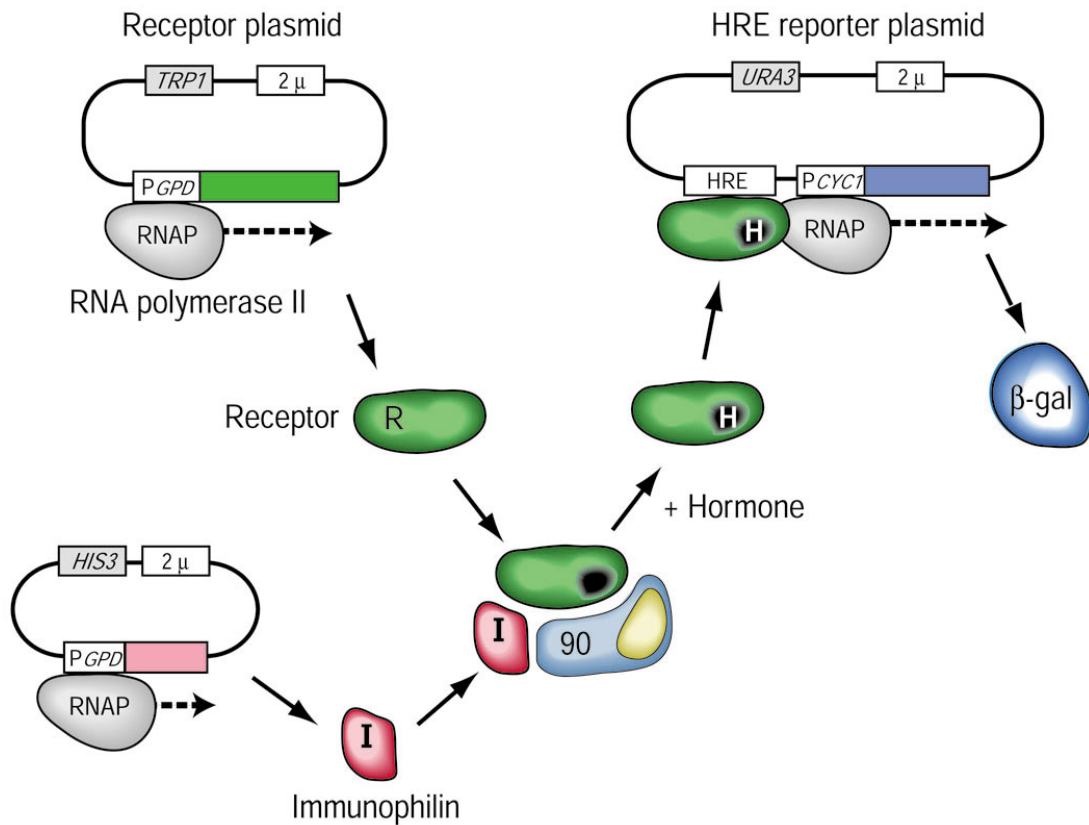


Figure 2.1: Steroid Hormone Yeast Assay

β -catenin and FKBP52 are individually as well as jointly expressed in a yeast-based AR-mediated β -galactosidase reporter system. The activated AR then binds to the hormone response element and results in transcription of the β -galactosidase reporter. The resulting β -gal expression levels allow us to take a direct quantitative measurement of AR activity through the use of a luminometer. Figure created by Marc B. Cox, Ph.D.

2.3 Western Blot of Yeast Lysates

To ensure that the changes we are seeing with reporter expression is not due to variations in protein expression, yeast lysates were prepared from the previous assay. Protein concentrations were determined through the use of a Bradford assay and a total of 35 μ g was loaded into each well. The proteins were then separated through electrophoresis and subsequently transferred onto an Immobilon membrane (Millipore, Bedford, ME). The primary antibodies used were β -Catenin (Santa Cruz, Santa Cruz, CA), anti-FKBP52 Hi52D (AbCam, Cambridge, MA), anti-AR (Santa Cruz, Santa Cruz, CA) and anti-L3.

2.4 In Vitro Pull-Down of 6xHis-Tagged FKBP52

500ng of 6xHis tagged FKBP52 and 500ng of Myc-FLAG tagged ICAT (purchased through Origene) recombinant proteins were utilized for an in-vitro pulldown to determine if they are capable of direct binding. 300 μ l of Qiagen Ni-NTA agarose beads were spun down at 800g's for 30 seconds. These were then washed three times with 1mL of cold buffer solution (1% Tween 20, 5mM Imidazole, 1x PBS). The beads were resuspended in 1mL of buffer and 100 μ l was aliquoted into each prelabelled microcentrifuge tube. 500ng of the required protein was then added into correct tubes. In addition, 50mM of MgCl₂ was added and the total volume was brought up to 500 μ l using cold Buffer. This was then incubated for 1 hour rotating in the 4°C walk in fridge. Following incubation the beads were subsequently spun down and washed in 1ml of buffer. This wash was repeated four more times then the beads were resuspended in 25 μ l of 4x SDS-PAGE Gel loading buffer + 10% β -mercaptoethanol. Tubes were then incubated at 90°C for 5 minutes and then the sample was loaded onto a GenScript ExpressPlus™ PAGE Gel (4-20%). Following electrophoresis the proteins were transferred onto a Immobilon membrane (Millipore, Bedford, ME). Primary antibodies anti-ICAT (FL-81)(Santa Cruz, Santa Cruz, CA),

and anti-FKBP52 Hi52D (AbCam, Cambridge, MA) were used.

Chapter 3: Results And Discussion

3.1 FKBP52 and Beta-Catenin's Effect on AR Synergy Within a Yeast Model System

Based on previous data it can be inferred that FKBP52 works in cooperation with β -Catenin to promote AR activity, even in yeast (Storer., Unpublished data). However, the results gleaned from this study not only indicate that FKBP52 and β -Catenin are unable to have a synergistic reaction when co-expressed, but also that the expression of β -Catenin inhibits FKBP52 potentiation on the androgen receptor. β -Catenin expressed alone shows a slight increase in AR activity however, when expressed in conjunction with FKBP52 the total reporter expression appears to have become an average of the two.

Protein Expression levels within the western blot indicate that β -Catenin and AR are stabilized when co-expressed. This could possibly be due to them forming a complex preventing their degradation or dissociation of AR co-factors.

3.1.1 Identification of Other Factors Necessary for Beta-Catenin Induced Synergism

The previous yeast reporter assay demonstrated that the transformation of FKBP52 and β -Catenin into *S. cerevisiae* containing AR and the β -gal reporter was not sufficient for the reported synergism seen previously within mammalian cell lines. The next step is to now determine what is missing from the original synergistic complex seen beforehand.

There still remains a variety of other factors that are capable of causing an up-regulation of AR activity. Glucocorticoid receptor interacting protein 1 (GRIP1), a member of the p160 family of steroid receptor coactivators, also synergistically enhances AR function (Li et al., 2004).

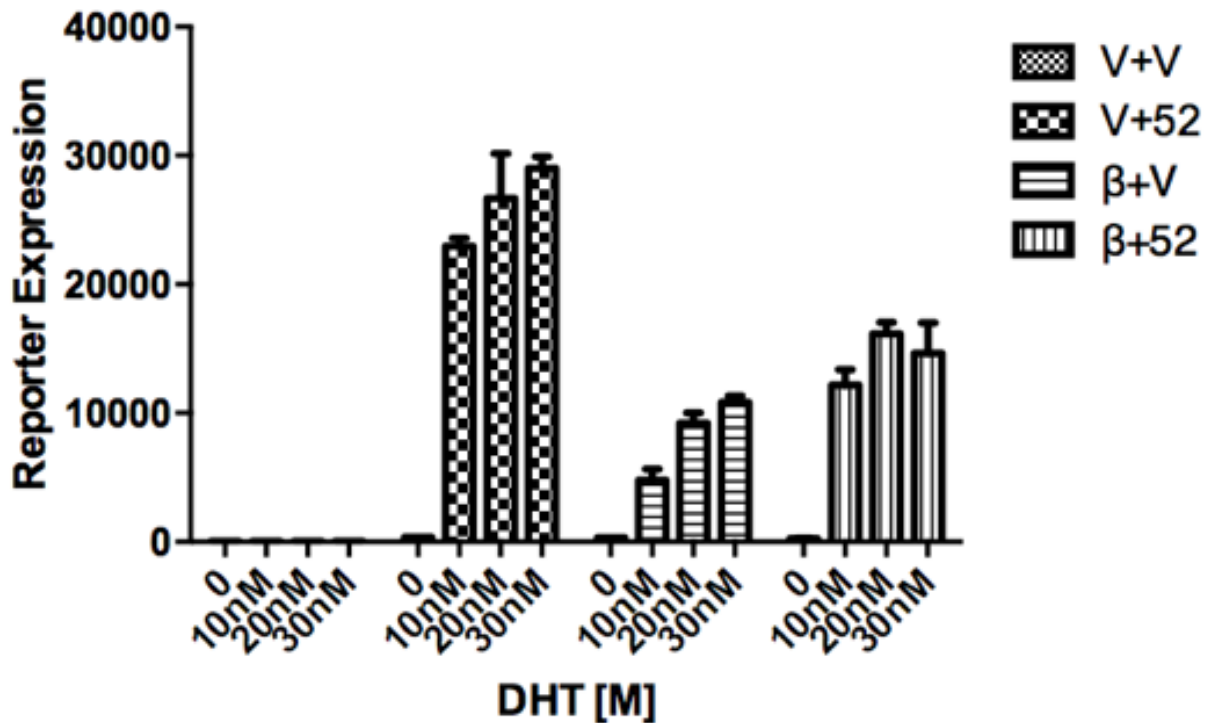


Figure 3.1: Yeast Reporter Assay Testing AR Reporter Expression in the Presence of β -Catenin and/or FKBP52.

Using either a 10, 20 or 30 nM concentration of Dihydrotestosterone (DHT) the resulting β -galactosidase expression levels act as a direct quantitative measurement of steroid hormone receptor function. Yeast cells were lysed and immunoblotted against β -Catenin, FKBP52, AR, and L3 (loading control)

GRIP1 possesses 3 LxxLL motifs that allow for interaction with the AF-2 site in AR's LBD. GRIP1 is capable of directly binding to β -Catenin through its AD2 domain and synergism is lost in truncated proteins missing this site, suggesting a requirement of the GRIP1/ β -Catenin complex. The secondary coactivator known as Coactivator-associated arginine methyltransferase 1 (CARM1), a histone modifying enzyme, is recruited to DNA bound AR through the AD2 domain of GRIP1 (Koh et al., 2002). Interestingly, it was also found to directly interact with β -Catenin and aid in its synergism of AR. In addition, the chaperone regulator BAG-1 isoform known as BAG1-L is capable of significantly increasing AR-induced transactivation of reporter genes (Froesch et al., 1998). AR mediated transcription was also found to be affected by the Wnt signaling pathway protein known as ICAT (Zhuo et al., 2011). Expression of ICAT enhanced AR function for both endogenous and exogenously expressed receptors. This enhancement of AR activity is believed to be mediated purely through β -Catenin, as it remains one of the few proteins ICAT is reported to bind to. ICAT is a somewhat newly characterized enhancer of AR activity and will be further explored.

Within the cell, ICAT's main role is the disruption of the canonical wnt signaling pathway. This pathway initiates with the expression of lipid-modified signaling glycoprotein known as wnts. During the cells "wnt-on" state, wnts form a complex with frizzled receptors and LRP5/LRP6 coreceptors (Nusse., 2005). Following receptor activation the β -Catenin destruction complex is inhibited preventing the phosphorylation, ubiquitination, and degradation of free cytoplasmic β -Catenin (Aberle et al., 1997; Hart et al., 1998; Polakis., 1999). This protein then begins to accumulate and translocate into the nucleus. Within the nucleus, β -Catenin forms a complex with LEF1/TCF and acts as a transcriptional co-activator of various genes, such as c-myc, and cyclin D1, both of which play pivotal roles in cell growth, proliferation, and

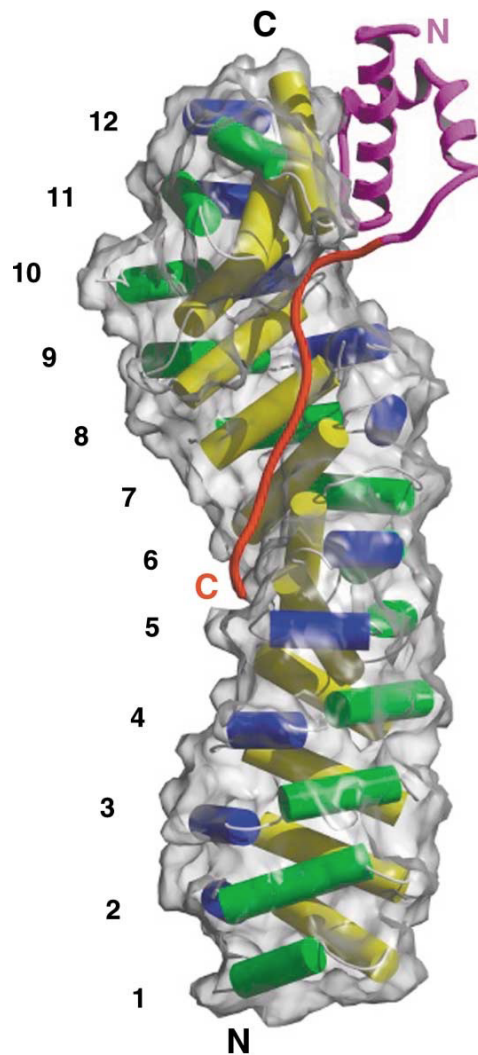


Figure 3.2: Structure of ICAT Interacting With β -Catenin.

The N-terminus 3-helix bundle (shown in fuchsia) of ICAT binds to armadillo repeats 11 and 12 of β -Catenin. The extended C-terminus region (shown in red) binds along the positively charged groove formed along repeats 5-9. Lys312 and Lys435, two important residues involved in the binding of TCF were found to form salt bridges with the C-terminus tail of ICAT. In vivo studies demonstrated that the C-terminus region was required for the blocking of TCF to β -Catenin however, cadherin was still capable of binding despite it having similar binding sites. When comparing the ICAT bound β -Catenin three-dimensional model with its predicted binding model in Figure 3 it appears that the ICAT C-terminal tail does not interfere with any protein-protein interactions required for this binding. Each repeat in β -Catenin consists of three helices colored in blue (H1), green (H2), and yellow (H3). (Graham et al., 2002).

differentiation (He et al., 1998; Shtutman et al., 1999; Tetsu and McCormick, 1999). Colon cancer can be developed if APC (a member of the β -Catenin destruction complex) or β -Catenin become mutated or dysregulated (Kinzler and Vogelstein 1996; Polakis 1997; Bienz 1999). ICAT is believed to be a colon cancer tumor suppressor protein due to its ability to prevent β -Catenin from activating transcription of wnt target genes. ICAT prevents the interaction of β -Catenin and TCF by binding its N-terminal helical domain to armadillo repeats 11 and 12 and its extended C-terminal tail to repeats 5-10 (Graham et al., 2002). These binding sites, known as charged lysine buttons, are similar to those that TCF and other β -Catenin ligands interact with, also preventing the binding of the transcriptional co-activator CBP/p300. While ICAT remains a possible gene therapy agent for the treatment of colon cancer, compiling evidence suggests there to be an opposite functional effect seen in prostate cancer cells.

Through the use of immunoprecipitation assays it was demonstrated that endogenous ICAT forms a complex with β -Catenin and AR (Zhuo et al., 2011). The expression of the N-terminal domain of ICAT specifically, demonstrated a more pronounced enhancement of β -Catenin/AR binding apposed to β -Catenin expressed alone. Deregulation of ICAT levels have been reported within a small percentage of prostate cancer tissues (Reifenberger et al., 2002). Increased levels were not only found to enhance AR mediated transcription in a dose dependent manner, but also enhance the growth of prostate cancer cells by roughly thirty percent (Zhuo et al., 2011). Previous data demonstrated the receptor's need for β -Catenin however, this was done in a cell line that expressed the ICAT protein. Due to the gathered information ICAT will be explored as a possible factor for β -Catenin mediated synergism.

3.2 Introduction of ICAT Into the FKBP52/Beta-Catenin Yeast Model System

It is understood that β -Catenin and FKBP52 are capable of synergizing androgen receptor activity within mammalian cells. However, in *S. cerevisiae* this synergism is seemingly lost. The small 9kDa protein known as ICAT could have possible implications in a multi-protein complex located on AR. Following sequence conformation of the newly developed pBEVY-A_ICAT plasmid this was then transformed into yeast already containing FKBP52 and β -Catenin or their respective vectors. Unfortunately, expression of the pBEVY-A_ICAT plasmid resulted in inconsistent reporter-assay data. This is likely due to promoter competition as the majority of the plasmids transformed into the yeast contain a GPD promoter. However, one notable trend observed was the increase of β -gal expression within yeast that contained the sequence for FKBP52 and ICAT.

This increase in reporter expression was somewhat surprising, as previous research has shown that ICAT interacts with the androgen receptor through β -Catenin (Zhou et al., 2011). In fact, knock-down of β -Catenin expression resulted in the loss of ICAT mediated AR potentiation. However, this study took place within LNCap cell lines and the data we see in figure 3.3 is within, a much simpler model system.

S. cerevisiae contains a much smaller genome and with the help of the *Saccharomyces* Genome Database (SGD) we are able to look at specific protein interactions with little background interruption. It is likely that due to ICAT's very small size it is unable to properly bind with AR, and instead requires a shuttling protein (β -Catenin) to promote binding and proper orientation. Within yeast, β -Catenin could possibly bind AR with too high of an affinity or is missing other factors necessary for AR activation and instead, acts as a hindrance on FKBP52 synergism. As a great number of proteins are not being expressed in yeast a possible β -Catenin

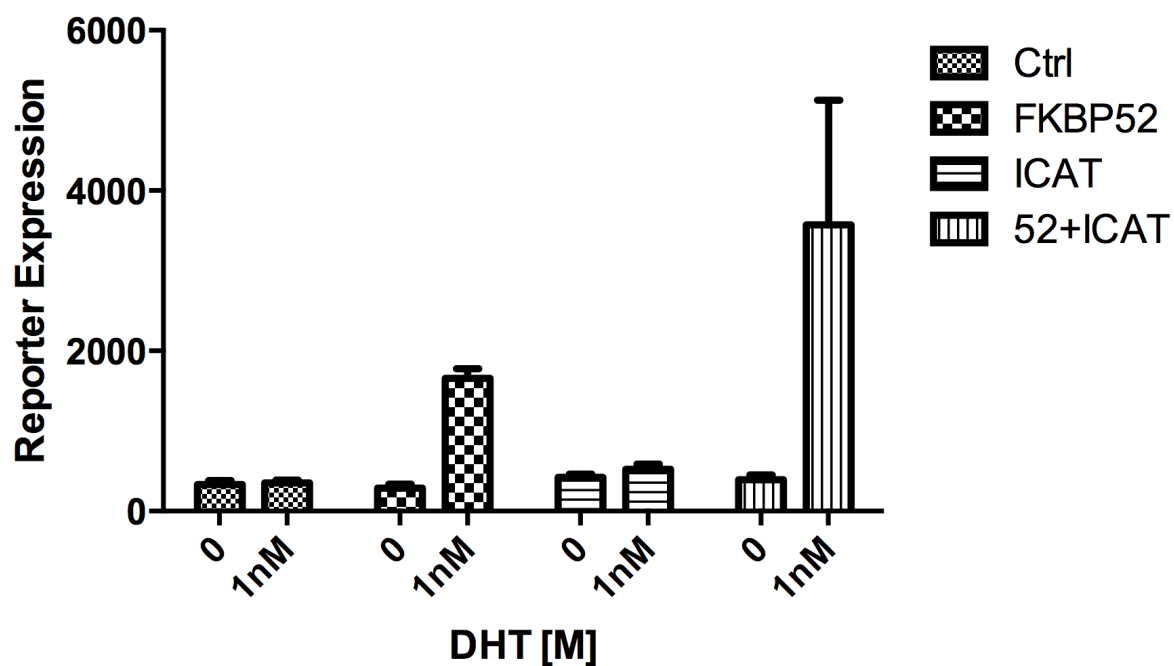


Figure 3.3 ICAT is Capable of Potentiating AR in the Presence of FKBP52

FKBP52 expressed in yeast alone is capable of potentiating the androgen receptor. In the presence of ICAT alone no significant changes occur with reporter expression. However, when FKBP52 is expressed in conjunction with ICAT we see substantial change in the levels of AR mediated reporter expression. This indicates that ICAT and FKBP52 may work in concert with one another to modulate AR transcription.

release factor could be missing from the AR complex.

3.3 In Vitro Pull-Down Using His-Tagged FKBP52

Due to the significant increase of reporter expression in yeast containing FKBP52 and ICAT, it is now of interest to determine if these proteins directly interact with one another *in vitro*. Myc-FLAG-ICAT was purchased through Origene and purified FKBP2 was utilized. Using equimolar amounts of FKBP52 and ICAT we do not see any protein being pulled down in complex with the immunophilin. Instead, FKBP52 is pulled down alone and not with ICAT. It is possible that FKBP52 and ICAT interact utilizing a third protein or interact on the androgen receptor surface. Previous studies within our lab determined that β -Catenin was pulled down in complex with FKBP52 in the absence of AR. It is likely that since β -Catenin is one of the few proteins known to complex with ICAT, it can possibly mediate FKBP52 and ICAT interactions.

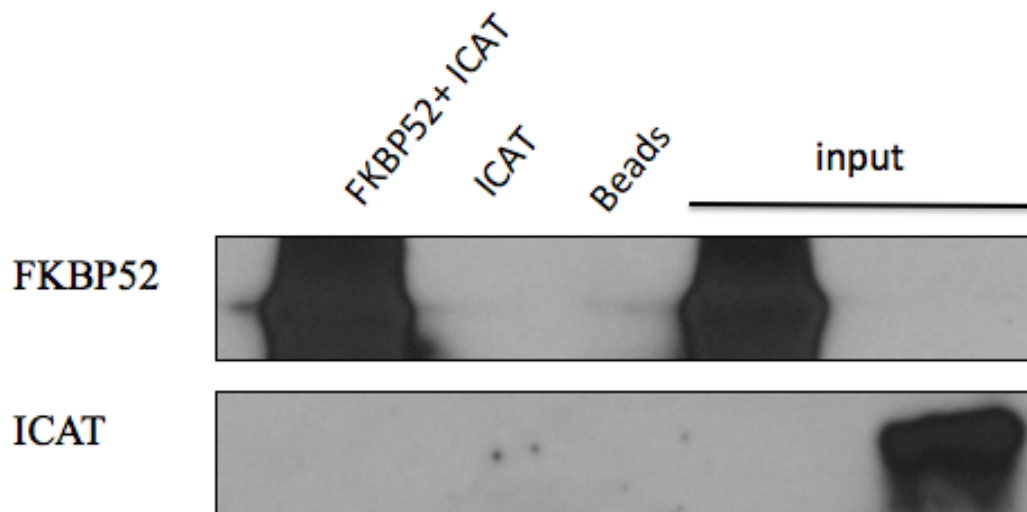


Figure 3.4 In-vitro Pull Down of 6xHis-Tagged FKBP52

FKBP52 Recombinant protein was incubated with FLAG-tagged ICAT protein in order to assess protein-protein interactions. It appears that FKBP52 does not pull-down ICAT in an *in vitro* model. This suggests that the increase in reporter expression within yeast assays is not likely due to the two proteins directly interacting with each other. Instead, it is probable these proteins interact either through the androgen receptor surface or through an uncharacterized multi-protein complex.

Chapter 4: Summary, Conclusions And Future Directions

4.1 Summary and Conclusions

β -Catenin and FKBP52 interaction with AR results in a drastic upregulation of AR mediated transcription within mammalian cell lines (Storer., Unpublished data). This synergism however, does not appear to occur within the *S. cerevisiae* model system. Other factors required for AR activation are likely not present within yeast. The β -Catenin binding protein known as ICAT, has been demonstrated to modulate AR activity, specifically in the presence of β -Catenin (Zhuo et al., 2011). No known homolog of ICAT exists within yeast as this remains a possible source of AR/ β -Catenin/FKBP52 synergism. ICAT binds to β -Catenin within armadillo repeats 5-12 and also is found to form a ternary complex with β -Catenin and AR in vivo (Graham et al., 2002). FKBP52 was previously demonstrated to directly bind to β -Catenin *in vitro*. It is likely that the FKBP52/ β -Catenin/AR complex also includes the binding of ICAT at a site near the AF-2 surface where β -Catenin is understood to interact. A conformational change within the receptor or the development of an interaction surface that will allow the binding of other effector proteins could be responsible for the potentiation caused by FKBP52's proline rich loop interaction with AR's BF3 surface.

4.2 Future Directions

Experiments looking at the interaction between FKBP52 and β -Catenin within a yeast model system resulted in the conclusion that there may be a much larger multi-protein complex involved in β -Catenin/AR synergism within mammalian cells. In fact, we were able to determine that the small wnt-signaling pathway protein, ICAT, has possible implications in this complex. Development of an ICAT knockout prostate cancer cell line would give us data as to how the

absence of this protein would effect β -Catenin's and FKBP52's potentiation on AR. Also, It would be interesting to determine if the addition of β -Catenin within the in vitro pull-down would result in the three proteins interacting with one another. β -Catenin is one of the few proteins known to bind to ICAT, so it would be surprising if these three would be unable to complex with one another. In addition, the use of tagged recombinant proteins could have an effect on their binding to one another. Co-immunoprecipitation from a prostate cancer cell line would be another important experiment in the future to determine if this is effect seen *in vivo*.

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Vita

Theresa Anne Rodriguez was born in El Paso, Texas on April 16, 1991. She graduated from J.M. Hanks High School in 2009 and in that same year entered the University of Texas at El Paso. She was a member of several honor societies such as Alpha Lambda Delta, Golden Key, Alpha Epsilon Delta, and Alpha Chi. Theresa also was the recipient of several scholarship awards such as the Bristol/Mayberry award, the Dan McKinney Scholarship and the UTEP International Fee Scholarship, which allowed her to study abroad in London during the summer of 2012. During her senior year as an undergraduate Ms. Rodriguez began working in the laboratory of Dr. German Rosas-Acosta studying the effect of the cellular SUMOylation on Influenza A viral infection. She graduated Cum laude with her bachelors degree in biological sciences and a minor in chemistry.

Following graduation, Theresa completed a summer accelerated biomedical research (SABR) internship at the Texas Tech University Health Sciences Center (TTUHSC) in El Paso, Tx. That fall she then joined the master's of science program at UTEP. In 2014 she began working in the laboratory of Dr. Marc Cox and focused on the beta-catenin/FKBP52 interactions on the androgen receptor within a yeast model system. Following graduation with her master's degree, Theresa will begin medical school at TTUHSC El Paso in the Fall of 2015.

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