A COMPARATIVE APPROACH TO ASSESSING THE FUNCTIONAL AND STRUCTURAL CHARACTERISTICS OF HUMAN FKBP52 IN THE REGULATION OF STEROID HORMONE RECEPTOR SIGNALING PATHWAY

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PREVIEW

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DEDICATION

I want to dedicate this project to my everlasting love, Jesus Christ. In Him alone is the source of all knowledge and wisdom in this universe. Thank you for allowing me to scratch the surface of you magnificent handy work that continues to confound the minds of men. I am in awe that you would use me. I pray that you will use this work to advance science and improve the quality of life for those afflicted with diseases. Thank you for trusting me with this amazing opportunity. All glory, praise, and honor to you, my Savior and King!

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DISSERTATION

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ABSTRACT

The 52 kDa FK506 binding protein (h52) is a key positive regulator of Androgen Receptor (AR) in cellular and animal models and is an attractive target for the treatment of prostate cancer. Human FKBP52 is a known regulatory protein and co-chaperone that has been shown to play an important role in the regulation of the AR signaling pathway, and in the development of the male sexual phenotype. Cellular studies in mammalian and yeast cells reveal that FKBP52 is a positive regulator of AR, glucocorticoid receptor (GR), and progesterone receptor (PR), potentiating receptor-mediated gene expression up to 60-fold in some systems.

In targeting FKBP52, a thorough investigation and understanding of the structural elements that underlay its function is necessary. This permits a logical approach in targeting specific interaction motifs, such as those that exist between the AR and FKBP52. Here we use a cross-species comparative approach to analyze the mechanisms of potentiation and the functional difference between FKBP52 and *Danio rerio* (Zebra fish) FKBP52 (DrFKBP52). In this study we have taken advantage of this by comparing their differences to identify additional important domains and residues. Through this study we have identified the FK2 domain, a previously uncharacterized, non-functional domain, as playing a role FKBP52 activity. This observation dispels the notion that the FK1 domain is the sole regulatory domain, specifically the proline-rich loop. Though both have the proline-rich loop, its presence is not indicative of potentiation activity. A genetic selection screen generated in *Saccharomyces cerevisiae* for gain of potentiation activity, in a library of randomly mutated *DrFKBP*52 genes, identified two residues: position 111 in the FK1 domain and 157 in the FK2 domain as

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being the critical residues for activation of receptor potentiation by DrFKBP52. In both the yeast model and mammalian cells, the *DrFKBP*52 mutation A111V, which is an adjacent residues downstream of the proline-rich loop, confer significant potentiation activity, whereas the same mutation introduced to FKBP52 only slightly elevates activity. Three dimensional crystal structure homology modeling by I-TASSER indicate that when alanine is replaced by valine at position 111 this change affect both the surface charge (to more neutral) and the hydrophobicity (to more hydrophobic) in vicinity. We believe this change induces an open conformation of the proline rich loop notch, allowing for sufficient surface area for AR interaction. A second residue in the FK2 region, T157R, also greatly influences potentiation. Moreover, the DFKBPr52: A111V _T157R double mutant potentiated hormone signaling as well as wild-type hFKBP52. Collectively these results suggest that specific residues in both FK1 and FK2 domain are critical for full activity and are involved in receptor interactions, which potentiates steroid hormone receptor activity. These newly identified domains and residues could possibly become targets for inhibitors as they could be key residues to specifically disrupt AR-FKBP52 association.

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GLOSSARY OF KEY TERMS

17AAG – 17-N-Allylamino-17-demethoxygeldanamycin

52 KD 22RV1 – FKBP52 knock down 22RV1 cells

52 KO – FKBP52 knockout mice

52 KO MEF – FKBP52 knockout mouse embryonic fibroblasts

AAG – androstanediol glucuronide

ACS – American Cancer Society

ACTH- Adrenorcorticotrypic hormone

AD2 – autonomous activation domain 2

ADP-Adenosine diphosphate

ADT– androgen deprivation therapy

AF-2– Activation Function 2

AR – androgen receptor

ATCC – American Type Culture Collection

BAG-1 – BCL-2 athanogene-1

BCL-6 – B Cell lymphoma 6 protein

BF-1 – Binding Function 1

BF-3 – Binding Function 3

BPH – benign prostatic hyperplasia

ChIP – chromatin immunoprecipitation

CHIP – COOH terminus of the Hsp70-interacting protein

Co-IP – coimmunprecipitation

COX-2 – cyclooxygenase 2

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CRPC – castration-resistant prostate cancer

CTD- C-terminal domain

CTE- Carboxyl terminal extension

CyP17-Cytochrome P450 17A1

CyP – cyclophilin

DBD – DNA Binding Domain

DHEA-Dehydroepianorosterone

DHT – 5α-dihydrotestosterone

DM – double mutant

DMEM – Dulbecco's modified eagle medium

DMSO – dimethyl sulfoxide

DOC- deoxycorticosterone

DrFKBP52- *Danio rerio* FK506 Binding protein 52

EC_{50} – half maximal effective concentration

EC_{20} – 20% maximal effective concentration

EDTA – ethylenediaminetetraacetic acid

EGF – epidermal growth factor

ELISA – enzyme-linked immuno sorbent assays

ER – estrogen receptor

FBS – fetal bovine serum

FDA- Food and Drug Adminstration

FEN1-Flap endonuclease 1

FKBP51– FK506 Binding Protein 51

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FKBP52 – FK506 Binding Protein 52

FSH – follicle stimulating hormone

Foxo3a- Forkhead Box O3

GAPDH – glyceraldehyde-3-phosphate dehydrogenase

GR – glucocorticoid receptor

GST – glutathione-S-transferase

HDAC – histone deacetylase

HER2 – human epidermal growth factor receptor 2

HIP – Hsp interacting protein

HOP – Hsp organizing protein

HRE – hormone response element

HRPC – hormone refractory prostate cancer

HSF-1 – heat shock transcription factor 1

Hsp – heat shock protein

IC_{50} – half maximal inhibitory concentration

ICAT – inhibitor to β-catenin and TCF-4

IGF-1 – insulin growth factor 1

Imp1 – importin B1

IRF-1 – interferon regulatory factor 1

KLK 2,3- Kallikrein related peptidase 3

LBD – ligand binding domain

LEF-1 – lymphocyte enhancement factor 1

LH – leutenizing hormone

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LHRH – luteinizing hormone releasing hormone

LRH-1 – liver receptor homologue-1

MEF – mouse embryonic fibroblast

MEK-K – mitogen activated protein kinase

MEM/EBSS – minimal essential media/eagles essential salt solution

MPER – mammalian protein extraction reagent

MR – mineralocorticoid receptor

mTOR – mammalian target of rapamycin

NCoR- Nuclear receptor co-repressor 1

NCI – National Cancer Institute

NFKB- Nuclear factor Kappa light chain enhancer of activated B cells

NR – nuclear receptor

Nup62 – nucleoporin 62

PC- Prostate cancer

pCAF – p300/CBP-associated factor

PEST – proline, glutamic acid, serine, and threonine-rich

PI3K – phosphatidyl inositol-3'-kinase

PIH1 – protein interacting with Hsp90

PIN – prostatic intraepithelial neoplasia

PPAR- γ – peroxisome proliferator activated receptor γ

PPIase – peptidyl prolyl cis-trans isomerase

PP5 – serine/threonine protein phosphatase 5

PR – progesterone receptor

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PSA – prostate specific antigen

PTEN – phosphatase and tensin homolog

PVDF – polyvinylidene fluoride

RL – reticulocyte lysate

RLU – relative light units

RPMI – Roswell Park Memorial Institute

RT-PCR – real time PCR

SGT1 – small glutamine rich tetratricopeptide repeat protein 1

SHBG – sex hormone binding globulin

SHR – steroid hormone receptor

shRNA – short hairpin RNA

siRNA – small interfering RNA

Sp1-specificity protein 1

SRC 1, 2 – steroid receptor coactivator 1, 2

StAR- steroidogenic acute regulatory protein

SUMO – small ubiquitin like modifier

SWI/SNF – SWItch/sucrose non-fermentable

TAH1 – tetratricopeptide repeat-containing protein associated with Hsp90

TCF4 – ternary complex 4

TGF β – transforming growth factor β

TF- transcription factor

TIF-2 – transcriptional mediators/intermediary factor 2

Tip60 – HIV-Tat interacting protein

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TMPRSS2 – human transmembrane protease serine 2

TPR – tetratricopeptide repeat

VEGF-2 – vascular endothelial growth factor 2

Wif1 – Wnt inhibitory factor 1

Wnt – wingless/int

Wt- wild type

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CHAPTER 1: INTRODUCTION

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1.1 ANDROGEN RECEPTOR IN DISEASE

The Androgen receptor (AR) and androgens play a critical role in the regulation of male sexual development and physiological processes, specifically the development and maintenance of the male reproductive system. Given its crucial role in normal male physiology, deregulation of AR and androgen signaling pathways has been implemented in a variety of disorders and diseases such as: Androgen Insensitivity Syndrome (AIS), Spinal bulbar muscular atrophy (SBMA), and Prostate cancer (PCa). (38)

1.1.2 Androgen Insensitivity Syndrome (AIS)

The essential role of AR and androgens in male sexual physiology has been established through androgen insensitivity syndrome (AIS). This genetic disorder is a condition that results in the partial or complete inability of the target cells to respond to androgens. It is primarily caused by missense and nonsense AR mutations, resulting in amino acid substitution in the ligand binding domain (LBD) and DNA binding domain (DBD). Defective AR proteins are unable to bind androgens or androgen response elements (AREs). AR's inability to bind AREs inhibits the expression of androgen target genes in the body, dramatically affecting male sexual phenotype. Depending on the severity of receptor mutations, individuals with AIS can fall into two categories, complete AIS (CAIS) or partial AIS. Affected individuals have a Y chromosome, but still have mild to severe defects in external virilization. Mutation of part, or deletion of the entire AR gene leads to CAIS, resulting in a dysfunctional receptor protein that cannot respond to circulating androgens. CAIS can also be caused by an absence of androgens in the blood. Patients with CAIS display feminized external genitalia, intra-abdominal testes

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and fail to develop secondary sex characteristics at puberty. Except for the absence of a uterus most CAIS individuals maintain all other female phenotype (44). The clinically relevant AR mutant, P723S, was identified in genital skin fibroblasts from a patient with complete androgen-insensitivity syndrome. This mutant was observed to have normal maximum androgen binding, but elevated equilibrium dissociation constants (45). Its activity was assessed in yeast and mammalian cells, and, as expected, the P723S mutant had minimal responsiveness to high amounts of dihydrotestosterone (DHT). Interestingly, in the presence of FKBP52, an Hsp90-associated co-chaperone, AR P723S matched the activity of wt-AR. Thus, the AR P723S mutant displays increased dependence on FKBP52 for function.

Partial AIS is also caused by AR mutations, which alter the structural conformation of AR and diminish AR responsiveness to androgens. The structural modification of the androgen receptor decreases the receptor's ability to bind hormones with high affinity, leading to reduced AR-mediated gene expression in target tissues. As a result, patients present with a number of physiological defects in male reproductive development (16). Interestingly, many of the defects seen in PAIS individuals correlate with those seen in *fkbp52*-deficient mice (6).

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1.1.3 Spinal Bulbar Muscular Atrophy (SBMA)

Spinal bulbar muscular atrophy (SBMA), or Kennedy's disease, is an X-linked hereditary neurodegerative disease caused by expansion of CAG codon repeats. These repeats code for a polyglutamine (polyQ) tract at the amino terminus of the androgen receptor. Polymorphic repeat ranges from 11-35 CAGs in normal males and 37-65 CAGs in the SBMS, causing a dramatic impact on AR transactivation. Like native AR,

this ligand-dependent neurotoxic AR elicits effects through the normal steroid hormone receptor maturation cycle. Heat shock protein 90 (Hsp90) plays a critical role in AR maturation, contributing to receptor stability and the high affinity hormone binding conformation of the receptor. Once hormone is bound, the polyQ tract of AR is exposed, allowing either the association with other AR polyQ regions or abnormal conformational changes in AR. This leads to aggregates that are resistant to proteosomal degradation (46). Although transactivation is compromised, this doesn't result in a loss of transcriptional activity. Therefore, gain-of-neurotoxic effects caused by aggregates form intranuclear inclusion in motor neurons of the brain stem and spinal cord result in transcriptional deregulation. However, the exact molecular mechanism is still unclear. The physiological manifestations are muscle cramps, arm and leg weakness, difficulty speaking and swallowing and increased incidence of AIS. Treatment using anti-androgen such as Leuprorelin, Flutamide (reduce testosterone levels) and Dutasteride (5-α-reductase inhibitor) showed little to no improvement in SBMA clinical trails. Interestingly, the dutasteride study showed that higher levels of testosterone are associated with increased muscle strength and function (51). Hsp90 inhibitors (17-AAG and 17-DMAG) developed for the treatment of cancer have been studied for treatment in an SBMA mouse model. Both Hsp90 inhibitors were shown to decrease intranuclear aggregate formation, leading to an improvement in motor performance. Unfortunately, clinical trials for these inhibitors have not lasted more than 6 months due to toxicity including temporary blindness and liver toxicity (51). The lack of efficacy in anti-androgens and toxic Hsp90 inhibitors demonstrates the need to develop other therapeutic targets for this disease.

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