University of Texas at El Paso
ScholarWorks@UTEP

Open Access Theses & Dissertations

2023-12-01

Proteomic Analysis Reveals FKBP51 and FKBP52 Interactors Implicated In Androgen Receptor-Mediated Castration Resistant Prostate Cancer

Olga Soto
University of Texas at El Paso

Follow this and additional works at: https://scholarworks.utep.edu/open_etd

 Part of the Biology Commons

Recommended Citation

Soto, Olga, "Proteomic Analysis Reveals FKBP51 and FKBP52 Interactors Implicated In Androgen Receptor-Mediated Castration Resistant Prostate Cancer" (2023). *Open Access Theses & Dissertations*. 4022.
https://scholarworks.utep.edu/open_etd/4022

This is brought to you for free and open access by ScholarWorks@UTEP. It has been accepted for inclusion in Open Access Theses & Dissertations by an authorized administrator of ScholarWorks@UTEP. For more information, please contact lweber@utep.edu.

PROTEOMIC ANALYSIS REVEALS FKBP51 AND FKBP52 INTERACTORS IMPLICATED IN ANDROGEN RECEPTOR-MEDIATED CASTRATION RESISTANT PROSTATE CANCER

OLGA B. SOTO

Doctoral Program in Biosciences

APPROVED:

Marc B. Cox, Ph.D., Chair

Sourav Roy, Ph.D., Co-Chair

Wen-Yee Lee, Ph.D.

Igor C. Almeida, Ph.D.

Manuel Miranda, Ph.D.

Siddhartha Das, Ph.D.

Stephen L. Crites, Jr., Ph.D.
Dean of the Graduate School

Copyright 2023 Olga B. Soto

DEDICATION

To my life partner, Jose Carlos Cano, whose love, and patience have been an immense source of support and encouragement during the challenges of graduate school and life.

To my brother, Juan A. Soto, for his unconditional love and support as we navigated life through the unexpected.

To my mother, Olga Soto, who was not able to share this adventure.

This degree is not only mine, but theirs as well.

PROTEOMIC ANALYSIS REVEALS FKBP51 AND FKBP52 INTERACTORS IMPLICATED IN ANDROGEN RECEPTOR-MEDIATED CASTRATION RESISTANT PROSTATE CANCER

by

OLGA B. SOTO, B.S.

DOCTORAL DISSERTATION

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

Doctor of Philosophy

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

DECEMBER 2023

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Marc B. Cox, for his unwavering support from the day I entered his office in search of guidance. His immense patience, invaluable advice, and sense of humor have made my graduate studies an unforgettable experience. I am grateful to have had such a wonderful mentor. I would also like to thank my co-mentor, Dr. Sourav Roy, whose expertise and support were crucial in finalizing this journey. To the rest of my committee members: Dr. Wen-Yee Lee, Dr. Siddhartha Das, Dr. Manuel M. Miranda, and Dr. Igor C. Almeida, thank you for your support and advice throughout the years. Dr. Jeffrey Sivils, aka Jeff, thank you for your guidance during my time in H-AGEP and in the lab. I always enjoyed conversing with you during your impromptu visits to the lab. I would also like to thank Dr. Hugues Ouellet, my first research mentor. Thank you for your patience and taking the time to train me, I would not have pursued research as a career if I had not had a positive experience in your lab.

I would like to thank my former and current lab members, who have also become close friends and a source of continued support through the struggles of graduate school: Ashley Payan, Isela Rodriguez, Kathia Rodarte, Jesse Diremeyer, Rina Koyani, Anahi Sanchez, Karla Parra, Jeannette Orbegozo, and Diana Prospero. I will always cherish our long conversations and beautiful memories. You brought comfort, joy, and laughter to this journey. A special thanks goes to Violeta Amaya and Christian Ramirez, the best students anyone could ask for. Their sunny temper and hardworking nature made mentoring more gratifying. Their dedication to their studies and commitment to research were crucial to the advancement of this project.

To Carlos Cano, I could not have asked for a better partner in life. You have given me unconditional love and support through the toughest times when I doubted myself. Thank you for the scientific talks, the gossip, and the life-threatening adventures around the world.

To Juan A. Soto, having a brother like you is one of life's greatest treasures. Thank you for your encouragement, tech support, and the unforgettable, yet overconfident, endurance challenges that have kept me sane.

ABSTRACT

Aberrant signaling mechanisms by the Androgen Receptor (AR) are attributed as the main culprits for the initiation and progression of prostate cancer (PCa). Due to its dependence on androgens, research efforts have focused on developing strategies to directly target androgen-mediated receptor activity. However, given the recurrence and treatment resistance of PCa despite androgen targeted therapies, recent efforts have shifted to find novel targets against the disease. These efforts include further revealing the molecular components and their mechanisms underlying AR signaling in both normal and disease physiological settings. Hence, our lab's work is focused on characterizing and targeting molecular chaperones that are critical for folding, activation, and translocation of AR. In this study, we aimed to discover auxiliary proteins influencing AR activity through known co-chaperones, FKBP51 and FKBP52, in PCa cells. To address this goal, I implemented tandem affinity purifications and a proteomic analysis. Among the top protein interactors, our results revealed associations by FKBP51 and FKBP52 with peroxiredoxins, a family of antioxidant proteins. Peroxiredoxins have been linked to AR signaling under normal physiological conditions, as well as to the progression of various types of cancer. Based on these reports and peroxiredoxins' ability to protect cells from oxidative stress, we hypothesize there is a cooperative interaction between members of the peroxiredoxin family and FKBP51 or FKBP52 that promote survival of cells in a prostate cancer setting. Identified proteins were then validated for their direct interaction using purified protein pull-downs or or indirect interaction by Co-Immunoprecipitation. Although further analyses are necessary to understand the role these protein interactions play in oxidative stress response under normal and PCa signaling, this dataset contributes to the growing list of functionally diverse protein-protein interactions that modulate AR transcriptional activity in PCa. Given that our research efforts mainly focus on FKBP52 as a druggable target against PCa, this interactome will also

provide insight into the cellular functions impacted by FKBP52 inhibition. In a broader context of AR signaling, future studies are required to characterize the mechanisms by which FKBP51 and FKBP52 and their interactors revealed in this study differentially regulate hormone-dependent signaling pathways.

TABLE OF CONTENTS

DEDICATION iii

ACKNOWLEDGEMENTS v

ABSTRACT vii

TABLE OF CONTENTS ix

LIST OF TABLES xii

LIST OF FIGURES xiii

CHAPTER 1: INTRODUCTION 1

Abstract 3

1.1 Background 3

1.2 FKBP51 and FKBP52 Structural domains 5

1.3 FKBP51 and FKBP52 regulation of steroid hormone receptor signaling 13

1.4 Role in Nuclear Translocation 15

1.5 FKBP51 and FKBP52 in reproductive disorders 17

1.6 Neurodegenerative diseases 21

1.7 Cancer 24

1.8 Stress-related disorders 28

1.9 Therapeutic targeting 31

1.10 Concluding remarks 34

1.11 Androgen Receptor Structure and Function 37

1.11.1 N-Terminal Domain 40

1.11.2 DNA-Binding Domain 40

1.11.3 Ligand Binding Domain 41

1. 12 Steroid hormone Receptor maturation and regulation by Chaperone Complex 41

1.12.1 Hsp90 Interactors 42

1.12.2 Early Complex 43

1.12.3 Ubiquitin/ Proteasome Pathway 44

1.12.4 Intermediate Complex 45

1.12.5 Mature Complex 45
1.13 Androgen Deprivation Therapy and PCa Progression 48
1.14 Elucidating the role of FKBP51- and FKBP52-mediated AR signaling in PCa 49
1.15 Dissertation Research Focus and Hypothesis 50
CHAPTER 2: IDENTIFYING FKBP51 AND FKBP52 INTERACTING PROTEINS *IN VITRO* USING HUMAN PROSTATE CANCER CELLS 51
2.1 Rationale 51
2.2 Materials and Methods 53
2.2.1 Construct of FKBP52-6xHis-Gly-FLAG 53
2.2.2 Cell Culture 54
2.2.3 Transient Transfections 54
2.2.4 Tandem Affinity Purification in 22RV1 cells 55
2.2.5 Western Blot Analysis 56
2.2.6 Sample Preparation for LC-MS/MS 57
2.3 Results 59
2.3.1 Tandem Affinity purification of FKBP51 and FKBP52 Western Blots 59
2.3.2 FKBP51 and FKBP52 associated proteins visualized by Heat Map 60
2.3.3 Network analysis of FKBP51 top 100 interactors reveals oxidative pathways 62
CHAPTER 3: VALIDATING LIGAND-DEPENDENT FKBP51 AND FKBP52 PROTEIN INTERACTIONS 66
3.1 Rationale 66
3. 2 Materials and Methods 68
3.2.1 Cell Culture 68
3.2.2 Transient Transfections 69
3.2.3 FLAG Purified Protein Pull-downs 69
3.2.4 His-tag Purified Protein Pull-downs 70
3.2.6 FLAG Co-Immunoprecipitations 70
3.2.7 Western Blotting 71
3.2.8 Cell viability assays 72
3.2.9 Apoptotic marker array 72

3.2.10 Apoptotic marker expression quantification 73
3. 3 Results 74
3.3.1 Reciprocal Co-Immunoprecipitations validate Peroxiredoxin interactions. 74
3.3.2 Purified Protein Pull-downs suggest direct association for PRDX3. 76
3.3.3 FKBP52 differentially regulates apoptosis-related proteins under H_2O_2-induced oxidative stress 80
CHAPTER 4: QUANTITATIVE COMPARISON OF FKBP51 AND FKBP52 INTERACTOMES 85
4.1 Rationale 85
4.2 Methods 86
4.3 Results 86
4.3.1 FKBP51 and FKBP52 share 45 common interactors 86
4.3.2 NRF2-mediated oxidative stress response is differentially regulated by FKBP51 and FKBP52 protein networks 88
4.3.3 Neutrophil degranulation is differentially regulated by FKBP51 and FKBP52 protein networks 96
4.3.4 HIF-1α pathway is differentially regulated by FKBP51 and FKBP52 protein networks 99
CHAPTER 5: DISCUSSION 102
BIBLIOGRAPHY 112
VITA 133

LIST OF TABLES

Table 3.1. Mammalian expression plasmids for Co-Immunoprecipitations. 74
Table 3.2. P-values of the Proteome Profiler Human Apoptosis Array kit. 84

LIST OF FIGURES

Figure 1.1. FKBP51 and FKBP52 Structure and Sequence Comparisons. 35
Figure 1.2. Protein-Protein Interaction Network for Known Interactors of both FKBP5 and FKBP4. 36
Figure 1.3. Androgen Receptor Signaling. 39
Figure 1.4. Chaperone mediated receptor maturation. 47
Figure 2.1. Evaluation of construct for FKBP51 and FKBP52 expression. 54
Figure 2.2. Schematic Representation of Proteomic Workflow. 58
Figure 2.3. Western Blot to confirm transfection and pull down of target proteins. 60
Figure 2.4. Top 50 proteins associated in FKBP51 and FKBP52. 63
Figure 2.5. Pathway analysis of FKBP51 interactors reveals oxidative pathways. 64
Figure 2.6. Pathway analysis of FKBP52 interactors suggests protein folding as primary role.....65
Figure 3.1. Reciprocal Co-IP targeting FLAG-tagged Peroxiredoxins 1-6. 75
Figure 3.2. FLAG-tagged Recombinant purified protein pull-down. 76
Figure 3.3. His-tagged Recombinant purified protein pull-down. 78
Figure 3.4. His-tagged Recombinant purified protein pull-down. 79
Figure 3.5. Effects of FKBP52 expression in H_2O_2 -induced oxidative stress. 81
Figure 3.6. Quantification of relative levels of 35 Human Apoptosis related proteins. ...82
Figure 3.7. FKBP52 differentially regulates several apoptosis-related proteins in H_2O_2-induced oxidative stress. 83
Figure 4.1. Venn diagram of common vs unique FKBP51 and FKBP52 interactors. 87
Figure 4.2. Top 50 pathways from unique FKBP51 interactors. 90
Figure 4.3. Top 50 pathways from unique FKBP52 interactors. 91
Figure 4.4 Top 50 pathways from common FKBP51 and FKBP52 interactors. 92
Figure 4.5. NRF2-mediated oxidative stress response regulated by FKBP51 network. 93
Figure 4.6. NRF2-mediated oxidative stress response regulated by FKBP52 network. 94
Figure 4.7. NRF2-mediated oxidative stress response regulated by common FKBP51 and FKBP52 network. 95
Figure 4.8. Neutrophil granules differentially regulated by FKBP51 and FKBP52 protein networks 98

Figure 4.9. HIF-1α signaling pathway downstream regulation by FKBP51 100
Figure 4.10. HIF-1α signaling pathway downstream regulation by FKBP52 101

CHAPTER 1: INTRODUCTION

The literature review presented in this chapter was submitted and published in Journal of Cellular Biochemistry on April 23, 2023. This literature review summarizes the current state of research on FKBP51 and FKBP52 under physiological conditions, as well as in multiple disorders.

Acknowledgements

The authors' laboratories are partially supported by NIH grant 2G12MD007592 to the Border Biomedical Research Center (BBRC), from the National Institutes on Minority Health and Health Disparities (NIMHD). M.B.C was also supported by the Lizanell and Colbert Coldwell Foundation and the Department of Defense (DOD) Prostate Cancer Research Program (PCRP) through grant number W81XWH-17-1-0435. S.R. is supported by the cancer Prevention and Research Institute of Texas (CPRIT) Texas Regional Excellence in Cancer Research (TREC) program through grant number RP210153. C.R. is supported by U-RISE through grant number T34GM145529 from the National Institute of General Sciences (NIGMS). J.D. is supported by G-RISE through NIGMS grant number T32GM144919.

Structure and Function of the TPR-Domain Immunophilins FKBP51 and FKBP52 in Normal Physiology and Disease

Olga B. Soto[1], Christian S. Ramirez[1], Rina Koyani[1], Isela A. Rodriguez-Palomares[1], Jessica R. Dirmeyer[1], Brian Grajeda[1], Sourav Roy[1], and Marc B. Cox[1,2]

[1]Border Biomedical Research Center and Department of Biological Sciences, University of Texas at El Paso, El Paso, TX 79968; [2]Department of Pharmaceutical Sciences, School of Pharmacy, University of Texas at El Paso, El Paso, TX 79968

Corresponding Author:

Marc B. Cox

Department of Biological Sciences

Biosciences Research Building Room 3.218

University of Texas at El Paso

500 W. University Ave.

El Paso, TX 79968

e-mail: mbcox@utep.edu

Running Title: FKBP51 and FKBP52 structure and function

ABSTRACT

Coordinated cochaperone interactions with Hsp90 and associated client proteins are crucial for a multitude of signaling pathways in normal physiology, as well as in disease settings. Research on the molecular mechanisms regulated by the Hsp90 multi-protein complexes has demonstrated increasingly diverse roles for cochaperones throughout Hsp90-regulated signaling pathways. Thus, the Hsp90-associated cochaperones have emerged as attractive therapeutic targets in a wide variety of disease settings. The TPR-domain immunophilins FKBP51 and FKBP52 are of special interest among the Hsp90-associated cochaperones given their Hsp90 client protein specificity, ubiquitous expression across tissues, and their increasingly important roles in neuronal signaling, intracellular calcium release, peptide bond isomerization, viral replication, steroid hormone receptor function, and cell proliferation to name a few. This review summarizes the current knowledge of the structure and molecular functions of TPR-domain immunophilins FKBP51 and FKBP52, recent findings implicating these immunophilins in disease, and the therapeutic potential of targeting FKBP51 and FKBP52 for the treatment of disease.

1.1 BACKGROUND

Immunophilins comprise two families of proteins that exhibit peptidyl/prolyl cis-trans isomerase (PPIase) activity that can also bind immunosuppressive drugs. In addition, their distinct binding affinity to specific immunosuppressive drugs is used to further classify immunophilins. For example, cyclophilin proteins have binding affinity to cyclosporin, whereas the FKBPs (FK506 binding proteins) bind FK506 or rapamycin with

high affinity (Galat 1993). To date, 24 cyclophilins, and 18 FKBPs have been identified as part of this superfamily in humans (He, Li et al. 2004, Somarelli, Lee et al. 2008). In the case of FKBPs, their calculated molecular weight is used to assign a name to each of the 18 members of this family(Dunyak and Gestwicki 2016).

The first description of an FKBP was documented by Harding in 1989. With a calculated molecular weight of 12kDa, it was termed FKBP12 and is currently the smallest FKBP family member (Harding, Galat et al. 1989, Kolos, Voll et al. 2018). With the use of an FK506 affinity matrix, Peattie and colleagues isolated an immunophilin with an approximate molecular mass of 55kDa that was later named FKBP52. The same studies revealed a consensus sequence with FKBP12, the archetypal member of immunophilins, and other FKBPs at the N-terminus (Peattie, Harding et al. 1992). Their role in basic cellular processes involving protein folding, receptor signaling, and protein trafficking has also been highlighted in a number of previous reviews (Baker, Ozsan et al. 2018, Zgajnar, De Leo et al. 2019) (Kang, Hong et al. 2008). Due to this involvement in an array of physiological processes, FKBPs have emerged as promising therapeutic targets for pathways associated with reproduction, lipid metabolism, regulation of stress response, hormone-dependent cancers including prostate and breast cancer, infertility, and stress-related psychiatric disorders.

In this review we will focus on two larger members of the FKBP family of proteins, FKBP51 and FKBP52. FKBP51 and FKBP52 are encoded by FKBP5 and FKBP4 genes respectively and regulate steroid hormone receptor (SHR) activity via the heat shock protein 90 (Hsp90) heterocomplex (Kolos, Voll et al. 2018). Since their identification, the structural features of FKBPs have been well studied and crystal structures reveal several

conserved regions. FKBP51 and FKBP52 are well-established as closely related homologs that share 60% amino acid sequence identity and 70% amino acid sequence similarity with a similar tertiary structure (**Figure 1**) [4]. The conserved domains consist of an N-terminal FK1 domain with a peptidyl/prolyl cis-trans isomerase (PPIase) active site to which the immunosuppressant drug FK506 binds, a middle FK2 domain that lacks PPIase activity despite structural similarities with FK1, and a C-terminal Hsp90-binding tetratricopeptide repeat (TPR) domain (Storer, Dickey et al. 2011). Although highly similar, three-dimensional crystallographic structures suggest similar domain conformations with slight variations in domain orientations (Sinars, Cheung-Flynn et al. 2003, Wu, Li et al. 2004), although it is important to point out that the differences in domain orientation could be an artifact given that the full length crystallographic structure of FKBP52 that is typically shown is made up of two partial FKBP52 structures. The full-length structure of FKBP52 has not been solved to-date.

1.2 FKBP51 AND FKBP52 STRUCTURAL DOMAINS

1.2.1 FK1 domain

Both, FKBP51 and FKBP52, contain a N-terminal FK1 domain which exhibits PPIase activity, characteristic of FKBP family members. PPIases are ubiquitously distributed enzymes that are phylogenetically highly conserved and that accelerate the otherwise slow steps of refolding denatured proteins (Fischer, Bang et al. 1984). Hence, PPIases have been referred to as molecular switches that impact downstream signaling events. While the smallest members of FKBPs are composed almost entirely of a PPIase

motif in a single FK domain, the larger FKBPs, such as FKBP51 and FKBP52, possess functionally independent domains (Ghartey-Kwansah, Li et al. 2018).

Being PPIases, FKBP51 and FKBP52 have the enzymatic ability to catalyze conformational interconversions of their client proteins. More specifically, they catalyze a form of isomerization known as cis/trans isomerization. This conformational change consists of altering the spatial arrangement of functional groups across a prolyl bond, a peptide bond preceding proline, from the same side ('Cis') to opposite sides ('trans') (Rostam, Piva et al. 2015). Therefore, PPIases reduce the rotation barriers, alter the orientation of functional groups, and produce stereoisomers with functional variability. PPIase functions extend beyond their ability to catalyze protein folding. Studies *in vitro* demonstrated multiple effects by PPIases on client proteins in processes such as protein folding and trafficking(Ghartey-Kwansah, Li et al. 2018), and regulation of the cell cycle (Laplante and Sabatini 2012).

It is important to mention that, while PPIases play crucial roles in multiple cellular processes, their effects are not necessarily dependent on the enzymatic activity, but rather on the domain itself as an interaction and regulatory surface. Riggs et. al. initially suggested a role for FKBP52's PPIase activity in glucocorticoid receptor (GR) potentiation (Riggs, Roberts et al. 2003). This was concluded after GR potentiation was abrogated as a result of point mutations to the FK1 domain, and FK506 binding to PPIase pocket, both of which inhibit PPIase activity. Interestingly, it was previously established that the large FK506 drug can extend beyond the PPIase pocket, which may physically interfere with other interactions or allosterically inhibit them (Van Duyne, Standaert et al. 1991). Taking these findings into consideration, an important question arose; is enzymatic activity

responsible for receptor potentiation or is this effect independent of PPIase activity? Through mutation of additional residues important for PPIase activity, Riggs et. al. later demonstrated that enzymatic activity was, in fact, not necessary for steroid hormone receptor potentiation by FKBP52 (Riggs, Cox et al. 2007).

PPIase FKBP51 has become a promising target for androgen-dependent prostate cancer, since prostate cancer cell proliferation is increased in response to its interaction with the androgen receptor (AR) (Maeda, Habara et al. 2022). Given that AR is a client protein of FKBP51, and that this positive regulation of AR is inhibited by immunosuppressor FK506, it has been suggested that the mechanism of AR upregulation by FKBP51 is the PPIase effect on AR conformation alteration and isomerization of the peptide backbone (Periyasamy, Hinds et al. 2010).

Recently, the ability of FKBP51 to act on Cdk4 via the Hsp90-heterocomplex was identified. In this study, authors propose a possible role for FKBP51 PPIase activity for Cdk4 inhibition. By sequestering the Cdk4 in the Hsp90 complex, FKBP51 prevents the formation of the Cdk4-cyclin D1 complex resulting in proliferation inhibition and myogenesis activation (Ruiz-Estevez, Staats et al. 2018). In terms of PPIase regulation of Cdk4, FKBP51 PPIase promotes cis-trans isomerization of the Thr172-Pro173 peptide bond, which is required for Cdk4 activation. Conversely, FKBP52 does not show isomerization induction of Cdk4 (Ruiz-Estevez, Staats et al. 2018). This observation further highlights the different roles of FKBP51 and FKBP52 despite their highly conserved structural organization and PPIase domain similarities.

The ability of PPIases to exert functionally relevant conformational alterations at a single bond may offer a promising target for cell regulatory networks involved in disease.

However, given the structural similarities amongst PPIase family members, inhibitor specificity continues to be a challenge. Due to the structural similarities within the PPIase pocket of FKBP51 and FKBP52, drugs targeting the FKBP52 PPIase pocket will likely target, not only FKBP52, but also the closely related FKBP51 protein simultaneously.

To investigate the mechanism of selectivity against protein subtypes and its potential, Sattler and colleagues analyzed the structural conformation of FKBP51 bound to a class of recently discovered FKBP51 inhibitors called SAFit (Selective Antagonists of FKBP51 by induced fit) (Gaali, Kirschner et al. 2015). Combining NMR spectroscopy, molecular dynamics, and thermodynamics with mutational analysis, they identified minor variations in residue sequences that established selectivity of SAFit molecules against FKBP51. From their data, the authors conclude that SAFit molecules preferentially bind to FKBP51 at a transient pocket that is unavailable in its closely related paralogue FKBP52. However, despite the seemingly successful selectivity for FKBP51 over FKBP52, SAFit molecules were still not able to discriminate against FKBP12 and its isoform FKBP12.6 (Jagtap, Asami et al. 2019).

Hausch and colleagues were able to enhance SAFit molecules by macrocyclization and provide the first ligands able to selectively target FKBP51, but not FKBP12 and FKBP12.6. Macrocycles of SAFit analogs were synthesized and screened for affinity towards FKBP12, FKBP12.6, FKBP51, and FKBP52. In a cell system, the newly synthesized macrocycles were able to engage selectively with FKBP51 and interfere with its cellular functions (Voll, Meyners et al. 2021). It can be hypothesized that the lack of selectivity for FKBP52 when compared to FKBP51, is due to the fact that SAFit molecules target and inhibit PPIase activity of FKBP52 but may not affect the

conformation of the proline-rich loop that overhangs the PPIase pocket (discussed below). All things considered, conformational selection is now considered a contributing factor in drug binding mechanisms and specificity.

The FK1 domain also contains a proline-rich loop suspended above the PPIase pocket, which has been characterized as an important surface for SHR potentiation. Given that FKBP51 and FKBP52 are structurally similar, but FKBP51 is unable to potentiate AR like FKBP52 in the cellular systems being used, the goal was to determine if potentiation could be interchanged between both FKBPs through mutations. Riggs et. al. performed gain-of-function random mutagenesis in which two single point mutations (A116V and L119P) in the FKBP51 proline-rich loop resulted in AR potentiation similar to that of FKBP52 (Riggs, Cox et al. 2007). The functional difference that can be attributed to both immunophilins due to these residues suggests a critical role of the proline rich loop for FKBP-mediated regulation of receptor activity. This observation demonstrated that FKBP52-mediated receptor potentiation was, in fact, due to the FK1 domain itself rather than the PPIase activity exerted by this domain. The importance of the proline-rich loop surface was further highlighted by a more recent study in which gain-of-function random mutagenesis was used to identify a single point mutation (A111V) in the zebra fish FKBP52 proline-rich loop that confers full receptor potentiating ability; wild type zebra fish FKBP52 does not potentiate SHR activity (Harris, Garcia et al. 2019). Thus, it is likely that FKBP51 and FKBP52 functionally diverged sometime after the boney fishes in evolution through minor changes in the proline rich loop sequence.

1.2.2 FK linker

Joining the active FK1 domain to the FK2 domain is a flexible hinge region known as the FK linker. Structural differences between FKBP51 and FKBP52 can be seen in the 8 residues linking the two domains with FKBP52 containing an acidic, two residue-region at the first loop of FK2 domain(Sinars, Cheung-Flynn et al. 2003, Bracher, Kozany et al. 2013). The FKBP52 linker sequence also contains a TEEED phosphorylation sequence whose modification can inhibit FKBP52-mediated steroid regulation by interfering with FKBP52-Hsp90 interaction (Miyata, Chambraud et al. 1997, Zgajnar, De Leo et al. 2019). On the other hand, the FK linker in FKBP51 contains an FED sequence not subject to phosphorylation. Structural comparison of the conformational isomers of FKBP52 revealed the FK1 and FK2 domains to be fixed structural domains amongst all isomers, while the linker region demonstrated variations between isomers. Based on these structural analyses, the authors suggest the linker region as a flexible region that allows for alternate organization of the FK1 and FK2 domains in response to different interactions with client proteins (Bracher, Kozany et al. 2013). It has been proposed that modulation of this short region by phosphorylation may offer an explanation for the differential regulatory roles that FKBP51 and FKBP52 have on SHRs.

1.2.3 FK2 domain

Both FKBP51 and FKBP52 contain an FKBP-like domain (FK2) that is similar to FK1 but lacks PPIase activity and is unable to bind immunosuppressive drugs, such as FK506 (Chambraud, Rouviere-Fourmy et al. 1993) (Rouviere, Vincent et al. 1997). Hence, a crucial role for this domain has not been elucidated. However, point mutations in this domain did impair the proper intergration of FKBP51, but not FKBP52, into the

Hsp90 heterocomplex with PR, as demonstrated by coimmunoprecipitation (Sinars, Cheung-Flynn et al. 2003). The plasticity offered by the FK-linker may influence the orientation of the, seemingly trivial, FK2 domain that may play a role for FKBP51 interaction with some of the SHRs.

1.2.4 TPR domain

FKBP51 and FKBP52 also contain a C-terminal TPR domain consisting of 34 amino acids each arranged in tandem to form sets of alpha-helices. The TPR confers the ability to form protein associations with the unstructured C-terminal tail of Hsp90 and Hsp70 (Riggs, Cox et al. 2007, Jaaskelainen, Makkonen et al. 2011) (Smith 2004). Because the last four residues of the Hsp70 and Hsp90 peptides are identical (EEVD), specificity is achieved by electrostatic contacts between TPR domains and the EEVD motif, and by hydrophobic contacts with residues N-terminal to this EEVD motif (Scheufler, Brinker et al. 2000). It has been demonstrated via isothermal titration calorimetry studies comparing several PPIases, Cyp40, FKBP51, and FKBP52, that FKBP52 interacts with Hsp90 with the highest affinity of the three related proteins (Pirkl and Buchner 2001). In summary, it is through these interactions with Hsp90 that FKBP51 and FKBP52 are linked to steroid hormone receptor maturation.

At the C-terminus, FKBP51 and FKBP52 also contain a calmodulin binding consensus, like other members of the FKBP family. For example, the TPR-containing homolog FKBP38, which is otherwise PPIase inactive, can be activated allosterically by binding of calmodulin.

While FKBP38, and perhaps other FKBPs, can be dependent on calmodulin binding to this site, dependence of FKBP51 and FKBP52 has not been established (Callebaut, Renoir et al. 1992) (Shirane-Kitsuji and Nakayama 2014). Since its identification as a protein interaction module for cell division proteins in yeast, the TPR domain has been demonstrated to be ubiquitous (Sikorski, Boguski et al. 1990). Its presence in a variety of unrelated proteins has illustrated the involvement of the TPR domain as a mediator of protein-protein interactions. Thus, the TPR motif is not exclusive to cochaperones, but is rather present in a multitude of proteins with different functions.

1.2.5 Charge-Y domain

Lastly, downstream of the TPR domain in the C-terminal tail, an 11-amino acid motif has been identified as the charge-Y motif and is considered important for Hsp90 binding. Both, FKBP51 and FKBP52 contain a residues that match the consensus sequence of the charge-Y motif (Cheung-Flynn, Roberts et al. 2003). From the 7 helices (H1-H7) composing the TPR domain, the charge-Y motif lies at H7, which protrudes beyond the central portion of the TPR domain. Considering this location, several theories have been postulated on the possible mechanism by which the charge-Y motif mediates Hsp90 binding. One possibility for Hsp90 binding is that the charge-Y motif at H7 can directly contact Hsp90 in its extended site beyond the core TPR domain. A second possibility for binding is that H7 may be re-structured to form an eighth helix (H8) closer to the core TPR domain, and that would enhance TPR interaction with Hsp90. (Cheung-Flynn, Roberts et al. 2003)

Despite the growing knowledge of FKBP structure and their prevalence in disease, their specialized functions in a multitude of pathways remains poorly understood. Perhaps a relatively simple way of gaining insight into these specialized functions is through comparative studies using WT and KO models. Analysis of KO phenotypes of FKBP51 and FKBP52 can help elucidate how cell type specific signaling pathways are regulated in the presence or absence of these immunophilins and what role they play in cooperative protein networks.

1.3 FKBP51 AND FKBP52 REGULATION OF STEROID HORMONE RECEPTOR SIGNALING

FKBP51 and FKBP52 serve as modulators of nuclear receptor function, including the mineralocorticoid, glucocorticoid, androgen, and progesterone receptors. FKBP52 is a known positive regulator of AR, GR and PR activity (Cheung-Flynn, Prapapanich et al. 2005, Tranguch, Cheung-Flynn et al. 2005, Yang, Wolf et al. 2006, Ni, Yang et al. 2010, Cluning, Ward et al. 2013, Maeda, Habara et al. 2022), whereas FKBP51 has been characterized as a negative regulator of GR (Hubler, Denny et al. 2003, Tranguch, Cheung-Flynn et al. 2005, Ni, Yang et al. 2010, Storer, Dickey et al. 2011, Fries, Gassen et al. 2017, Zgajnar, De Leo et al. 2019) and PR (Hubler, Denny et al. 2003, Tranguch, Cheung-Flynn et al. 2005, Storer, Dickey et al. 2011, Maeda, Habara et al. 2022) activity, while positively regulating AR signaling (Ni, Yang et al. 2010, Zgajnar, De Leo et al. 2019, Maeda, Habara et al. 2022). It is important to note that the receptor specificity, as well as the nature of the regulation, displayed by FKBP51 and FKBP52 has been a point of confusion. Our group has reported for years that FKBP52 is a positive regulator of AR (Cheung-Flynn, Prapapanich et al. 2005), GR (Riggs, Roberts et al. 2003), and PR

(Tranguch, Cheung-Flynn et al. 2005), while FKBP51 does not regulate these receptors to any appreciable degree, as this is what we have observed in the cell lines and systems in which we have worked. That being said, others have reported divergent evidence that typically centers around FKBP51 including positive regulation of AR in prostate cancer cells as discussed above. Thus, it is likely that the FKBPs display cell and organ-specific regulatory mechanisms.

AR signaling is regulated via the orchestrated assembly of a mature receptor heterocomplex that involves the immunophilins FKBP51 and FKBP52. Recent studies where FKBP51 or FKBP52 were depleted in prostate cancer cells, showed reduced AR dimer formation, chromatin binding, and phosphorylation, suggesting that both proteins are necessary for dimer formation and chromatin binding of AR (Maeda, Habara et al. 2022). Similarly, it has been reported that both FKBP51 and FKBP52 enhance the biological activities of AR by increasing hormone affinity (Ni, Yang et al. 2010, Zgajnar, De Leo et al. 2019, Maeda, Habara et al. 2022).

Evidence suggests that FKBP52 potentiates the function of GR through hormone binding (Riggs, Roberts et al. 2003). In *Saccharomyces cerevisiae* models for GR activity, FKBP52 can potentiate hormone-dependent GR activity by as much as 20-fold at limiting hormone concentrations, while the co-expression of FKBP51 in the same model blocked FKBP52 mediated potentiation. In accordance with these findings, FKBP51 has been documented as a potent inhibitor of the GR signaling pathway leading to GR resistance (Denny, Valentine et al. 2000, Criado-Marrero, Rein et al. 2018), which has been associated with disorders such as reduced stress coping behavior (Criado-Marrero, Rein et al. 2018) and adipogenesis (Smedlund, Sanchez et al. 2021).

Similar to GR regulation, the antagonistic effects induced by FKBP51 and FKBP52 have been recorded for PR. Studies with 52KO female mice, failed to complete embryo implantation in the uterus due to compromised uterine receptivity (Tranguch, Cheung-Flynn et al. 2005). Similarly, FKBP52 deficient female mice, presented complete sterility due to in utero implantation failure (Cox, Riggs et al. 2007). These results established FKBP52 as an essential regulator of PR activity in vivo. Contrary to FKBP52, FKBP51 is a negative regulator of PR function. Recent studies found that an enhanced FKBP51-PR interaction play a significant role in preterm birth, while making 51 a novel therapeutic target to prevent this disease (Guzeloglu-Kayisli, Semerci et al. 2021). Overall, FKBP51 has been reported to act as a negative regulator of SHR activity, as opposed to FKBP52 that positively regulates AR, GR, and PR.

1.4 Role in Nuclear Translocation

Several characteristics of the large immunophilins have been demonstrated to be essential for SHR function, such as Hsp90 interaction, interaction with dynein, and PPIase enzyme activity. Based on the classic model of steroid receptor nuclear translocation, hormone binding to SHR in the Hsp90-heterocomplex was deemed a crucial trigger for receptor dissociation, thus, facilitating nuclear translocation of the un-complexed receptor. This proposed model was partly due to the idea that the nuclear localization signal (NLS) in cytoplasmic steroid receptors remains hidden while bound to the Hsp90 heterocomplex (Stewart 2007, Sivils, Storer et al. 2011). However, studies on the GR- and MR-Hsp90 heterocomplexes demonstrated that cross-linked complexes can pass through the nuclear pore intact and accumulate in the nucleus. The fact that Hsp90

hetercomplexes can exist in the nucleus, may suggest that the entire complex can pass intact through the nuclear pore. The findings that the larger immunophilins, as part of these complexes, can travel across the nuclear membrane suggest that transformation and dissociation of the heterocomplex occurs at the nucleus, instead of the cytoplasm (Echeverria, Mazaira et al. 2009, Galigniana, Erlejman et al. 2010). It has been reported that GR hormone binding and recruitment of the transport protein dynein is increased in the presence of FKBP52, while FKBP51 is known to inhibit hormone binding to GR and decrease nuclear transport (discussed below). These findings led to the idea that hormone binding results, not in the dissociation of the receptor from the heterocomplex, but rather in a switch from FKBP51 to FKBP52 in the heterocomplex (Ebong, Beilsten-Edmands et al. 2016).

The switching of both immunophilins prior to nuclear translocation is coherent with the fact that FKBP51 and FKBP52 compete for binding to the Hsp90 heterocomplex (Nair, Rimerman et al. 1997). Furthermore, FKBP51 and FKBP52 have regulatory roles in steroid hormone receptor interaction with the dynactin complex involved in nuclear translocation. Dynactin is an essential cofactor for dynein, and a multi-subunit protein complex that is required for dynein-driven retrograde transport of vesicles along cytoskeletal microtubules (Schroer 2004). In the context of the Hsp90-SHR heterocomplex, the question was raised regarding the mechanism for the continuous transport of SHR to and from the nucleus (Pratt, Silverstein et al. 1999, Galigniana, Harrell et al. 2002). Using purified proteins, it was demonstrated that FKBP52 and an independent PPIase fragment can bind directly to cytoplasmic dynein and plays a role in tethering SHRs to the retrograde transport machinery (Galigniana, Harrell et al. 2002,

Wochnik, Ruegg et al. 2005). On the contrary, FKBP51 displayed the opposite effect on nuclear translocation of GR. The inhibitory effects of GR were demonstrated in mammalian cells when efficient nuclear translocation of GR was delayed in the presence of FKBP51, which makes sense given the limited interaction of FKBP51 with dynein, as opposed to FKBP52 (Davies, Ning et al. 2002). Interestingly, swapping the N-terminal PPIase domains of FKBP51 and FKBP52 reverses their activity in a way that FKBP52 exhibits GR inhibitory effects with reduced dynein association, as is expected for FKBP51. The opposite occurred with FKBP51 whose inhibitory effect on GR was abolished and was able to form an association with dynein (Wochnik, Ruegg et al. 2005). Based on these data, it is clear that FKBP51 and FKBP52 have more diverse roles in the SHR signaling pathways than was originally thought, including continued regulation after hormone binding.

1.5 FKBP51 AND FKBP52 IN REPRODUCTIVE DISORDERS

A role for FKBP51 and FKBP52 in a variety of disorders of the reproductive system have been identified. Female reproductive disorders, such as endometriosis and polycystic ovarian syndrome (PCOS), are associated with opposite hormonal profiles involving steroid hormone receptors (Dinsdale and Crespi 2021). Given that FKBP52 is crucial for progesterone receptor (PR) function, and that the anti-inflammatory effects of PR's signaling axis are suppressed in endometriosis, FKBP52 has been studied as a potential contributing factor (Bulun, Cheng et al. 2006, Lousse, Van Langendonckt et al. 2012). To study the effects of FKBP52 deficiency on endometriosis, Hirota and colleagues transplanted endometrial tissue into the peritoneum of mice with different expression

levels of FKBP52. They found that 52KO mice developed significantly higher number of endometriotic lesions when compared to their WT counterparts. In the context of PR, deletion of FKBP52 reduced PR mediated signaling, which led to increased cell proliferation and inflammation (Hirota, Tranguch et al. 2008). In women and non-human primate models with endometriosis, there is diminished levels of FKBP52 in the ectopic endometrium, which leads to dysregulated progesterone response and upregulation of MicroRNA-29c (miR-29c) expression (Joshi, Miyadahira et al. 2017). MiR are single stranded RNA molecules that function to repress gene expression through messenger RNA (mRNA) and have been implicated in reproductive disease, such as endometriosis. The results of this study suggest that absence or decrease in levels of FKBP52 directly affect levels of miR-29c by causing levels to increase, resulting in poor progesterone signaling. This suggests that FKBP52 may be involved in progesterone resistance often seen in endometriosis. Recently, an endometrial stromal cell (ESC) model was developed to evaluate the relationship between FKBP52 and progesterone receptor (PR) levels. These findings revealed that when downregulation of FKBP52 occurs, PR expression decreases, leading to proliferation of ESC and development of endometriosis. These data are consistent with clinical data for women with endometriosis in that there was a direct correlation between FKBP52 and PR levels (Liu, Cheng et al. 2021). FKBP52 is also implicated in infertility as human endometrial stromal cells treated with FKBP52 and progesterone revealed that FKBP52 expression mediated through the HOXA10 pathway is diminished in endometriosis and leads to disrupted decidualization, progesterone resistance, and infertility in women (Yang, Zhou et al. 2012).

Along these lines, FKBP52 has been shown to impact the development and progression of polycystic ovarian syndrome (PCOS) as an analysis of the influence of multiple genes on PCOS revealed that FKBP52 influences development risk. Two single nucleotide polymorphisms (SNPs) of FKBP52, rs4409904 and rs2968909, were associated with reduced risk of PCOS development. Rs4409904 was associated with lowered odds of PCOS and rs2968909 was associated with lower body mass index (BMI) and diminished adiposity, which are traits known to amplify PCOS symptoms (Ketefian, Jones et al. 2016). In another instance, a recent discovery revealed that rats with PCOS display higher levels of FKBP52 expression in all cell types of the ovary when compared to expression levels observed in control group rats (Song and Tan 2019).

Finally, FKBP52 may be implicated in preeclampsia (PE) and intrauterine growth restriction (IUGR) in pregnant women. A case study revealed that FKBP52 was downregulated in the placentas of PE patients when compared with the control group with normal placentas. Additionally, FKBP52 was upregulated in the placentas of IURG patients (Acar and Ustunel 2015).

Similar to FKBP52, FKBP51 has also been linked to female reproductive disorders relating to the establishment and maintenance of pregnancy. More specifically, a role for FKBP51 in decidualization has been described (Gellersen and Brosens 2014, Wei, Gao et al. 2018). Decidualization refers to the process by which endometrial stromal fibroblasts specialize into secretory decidual cells, which is a crucial step for embryo implantation and placental development (Gellersen, Brosens et al. 2007, Gellersen and Brosens 2014). This transformative event is indispensable for the initiation and maintenance of pregnancy because it provides maternal immunological tolerance against fetal antigens

and protects the conceptus against stressors (Leitao, Jones et al. 2010). Given that FKBP51 plays a role in steroid hormone response and in the AKT pathway, and that AKT pathway is closely related to the decidualization progress (Hirota, Acar et al. 2010), Wei et al sought to examine a potential mechanism for FKBP51 in the regulation of decidualization in endometrial stromal cells (ESCs). Interestingly, knockdown of FKBP51-shRNA in ESCs *in vitro*, resulted in decidualization inhibition, while reintroduction of FKBP51-cDNA was able to rescue this inhibition. These results supported that FKBP51 can promote decidualization perhaps by reducing AKT phosphorylation levels, as suggested by the authors (Wei, Gao et al. 2018).

The role of FKBPs does is not limited to reproductive disorders in females. FKBP52 KO mouse lines have highlighted a role for FKBP52 in the development of the male reproductive system as well. Adult FKBP52-deficient male mice display phenotypes corresponding to partial androgen insensitivity, such as development of hypospadias, and prostate dysgenesis (Yong, Yang et al. 2007), likely due to the role of Fkbp52 as a co-chaperone of the androgen receptor (AR). In these same studies, Yong et al. reported that FKBP51 showed no defects in AR-mediated reproductive function and no hypospadias. Complementing the data that demonstrates FKBP52's role in male reproductive health, FKBP52 KO animal models have also been reported to have reduced sperm motility and reduced fertilizing capacity (Hong, Kim et al. 2007). All things considered, FKBP51 and FKBP52 seem to play critical roles in normal reproductive health of males and females, while FKBP52 has demonstrated a greater effect on male reproductive health and sexual differentiation.

1.6 NEURODEGENERATIVE DISEASES

In recent years, studies have revealed that FKBP51 and FKBP52 are important for neurological function and may be a key factor in the development of neurodegenerative diseases. FKBP52 is widely expressed throughout the nervous system and is critical for signaling, transport of protein, neurite outgrowth and differentiation of neurons (Quintá, Maschi et al. 2010, Giustiniani, Sineus et al. 2012). Given that FKBP52 is important for several neurological functions, we can assume that it also plays a role in disease progression in the brain.

FKBP52 has been implicated in the development of Alzheimer's disease (AD) through its roles on the regulation and translocation of steroid hormone receptors, including glucocorticoid receptors (GR), into the nucleus of neurons (Chambraud, Byrne et al. 2022). Since the GR is known to regulate tau protein pathology, this potentially provides a mechanism by which AD is influenced by FKBP52 (Blair, Baker et al. 2015). In addition, FKBP52 is thought to exacerbate tau pathology through its direct interaction with Tau-P301L, a Tau mutant known to induce significant tauopathy in humans (Meduri, Guillemeau et al. 2016). In this instance, FKBP52 not only has the ability to interact with the mutant, but it also contributes to protein conformational changes, leading to the assembly of filaments (Giustiniani, Chambraud et al. 2014). In fact, FKBP52 has been proposed as a biomarker for AD since patients with AD exhibit abnormally low expression levels of FKBP52 in the frontal cortex. This observation correlated with pathological levels of tau in the cerebral cortex, which were not attributed to neuronal loss (Giustiniani, Sineus et al. 2012). Interestingly, FKBP52 has also been shown to induce tau proteins that display prion like behavior in vitro. These tau proteins have the capacity to penetrate

neurons as well as propagate and migrate to other neurons (Giustiniani, Guillemeau et al. 2015).

Given that FKBP52 is known to play a role in tau accumulation, naturally, it also has been associated to memory deficits. In mice expressing a Tau variant (rTg4510), viral overexpression of FKBP52 resulted in neuronal dysfunction mediated by tau via a caspase-dependent pathway. In turn, this resulted in impaired spatial learning and neuronal loss in the hippocampus at 6-months old when compared to control wild type mice. This suggests that FKBP52 itself is not responsible for memory and learning deficits but that these deficits are the result of tau accumulation in combination with FKBP52. It is still unknown what contribution FKBP52 has at different points of tau accumulation and further studies are needed to better understand this mechanism (Criado-Marrero, Gebru et al. 2021). Regarding the role of FKBP51 in AD, an initial study overexpressing FKBP51 in HeLa cells resulted in a dramatic increase in tau levels. It was proposed that this increase is the result of FKBP51 disabling the ubiquitination of tau, and thereby, preserving its tau levels. FKBP51 may also utilize tau to alter the dynamics of microtubules as shown in western blots by the increase of microtubule complexity when FKBP51 is present as opposed to when it is absent. This suggests that FKBP51 has an impact in both tau levels and microtubule formation and function (Jinwal, Koren et al. 2010). To complement these findings, 51KO mice present low levels of tau in the brain, while human AD patients have increased levels of FKBP52 associated with tau accumulation. This increase in FKBP51 accumulation was found to be correlated with age, as higher levels of FKBP51 are observed in old patients diagnosed with AD (Blair, Nordhues et al. 2013). In this manner, it is possible that some individuals are predisposed

to high levels of FKBP51, and therefore, have a higher risk of AD development. Also, AD pathogenesis may drive the increased FKBP51 expression. Both hypotheses account for the high levels of FKBP51 seen in old individuals with AD in comparison to age-matched healthy individuals. Overall, this study showed that the increase of FKBP51 in aged AD brains likely promoted an environment where tau accumulation could occur. It was also shown that FKBP51 synchronizes with Hsp90 to not only preserve tau structure but also promote its formation (Blair, Nordhues et al. 2013). Hsp90 works as a scaffolding protein to bring FKBP51 into proximity to Tau. More specifically, Hsp90 can join FKBP51's PPIase pocket with Tau's proline rich region, allowing FKBP51 to perform co-chaperone regulatory action. These dynamics, combined, work to amplify tau oligomerization (Oroz, Chang et al. 2018).

FKBP51 and FKBP52 have also recently been identified as potential therapeutic targets for Huntington disease (HD) and Parkison's disease (PD). By lowering the levels of FKBP52, Bailus et al, demonstrated that levels of mutant huntingtin (mHtt) were reduced in vitro and in vivo HD models. This is significant as reduced levels of mHtt correlated with reduced HD pathology suggesting that FKBP52 is implicated in HD and offering support for its potential development as a therapeutic target (Bailus, Scheeler et al. 2021). In the case of PD, FKBP51 has been shown to interact with PTEN-induced putative kinase 1 (Pink1), which plays a major role in the development of PD. Furthermore, recent data indicates that FKBP51 negatively regulates PARK2 resulting in a volumetric reduction of hippocampi in Fkbp51 KO mice (Qiu, Zhong et al. 2022). This observation suggests a regulatory mechanism of FKBP51 on PARK2 expression, and potential target to regulate PARK2 expression in the onset of PD. In the same context, a

mitochondrial serine/threonine-protein kinase, encoded by the PINK1 gene and associated with the onset of autosomal recessive from of Parkinson's disease (PD), has been demonstrated to promote neuronal survival via direct inhibition of FKBP51-PHLPP (PH domain leucine-rich repeat protein phosphatase) interaction and subsequent activation of the AKT pathway (Boonying, Joselin et al. 2019). More studies are needed to further explore this interaction and how these FKBPs may be involved in HD and PD (Boonying, Joselin et al. 2019).

1.7 CANCER

Over the past decade, significant information about the role of FKBP51 and FKBP52 in human malignancy has highlighted the dysregulated expression of these proteins. Functional attribution of these immunophilins in regulation of different signaling pathways such as steroid receptor signaling, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Fries, Gassen et al. 2017)and Protein kinase B- leucine-rich repeat protein phosphatase (AKT – PHLPP) (Pei, Li et al. 2009), determines their role in tumorigenesis and chemoresistance of several cancers. As regulatory components of AR signaling pathways, FKBP51 and FKBP52 play an important role in the etiology of prostate neoplasia. Protein expression analysis from 500 PCa samples revealed that overexpression of FKBP52 was correlated with poor prognosis in hormone naïve PCa patients (Federer-Gsponer, Quintavalle et al. 2018). Furthermore, patient samples with hormone CRPC presented elevated levels of FKBP52 when compared to their hormone naïve counterparts, which authors propose as a potential mechanism for tumor evolution and CRPC development. Considering the integral role of chaperones regulating the

multiple steps in AR signaling leading up to CRPC, Maeda et al. sought to investigate the role of FKBP51 and FKBP52 in AR dimer formation (Maeda, Habara et al. 2022). Given that AR dimerization is believed to play a crucial role in ligand-dependent activation of the receptor, the authors analyzed AR dimer formation after the addition of DHT, and in the presence or absence of FKBP51 and FKBP52. It was demonstrated that dimer formation in response to DHT was reduced in cells deficient of FKBP51 or FKBP52 by 0.54-fold and 0.30-fold, respectively. These results suggest that both immunophilins may influence AR signaling by targeting similar receptor functions, and hence both are promising targets for prostate cancer treatment. Interestingly, a separate study of PCa tissues measuring mRNA and protein expression of PPIases Cyp40, FKBP51, and FKBP52, showed upregulation of FKBP51. On the contrary, FKBP52 levels remained unaltered when comparing PCa samples with normal tissue (Periyasamy, Hinds et al. 2010). The opposing roles of both immunophilins has also been recently documented in breast cancer cells. FKBP52 has been suggested to stabilize ER, thus promoting breast cancer cell proliferation, while FKBP51 was shown to reduce stability of ER (Habara, Sato et al. 2022). FKBP52 overexpression is also suggested to be involved in breast cancer progression and invasion considering its significant association with advanced Tumor-Node-Metastasis (TNM) stage and lymph node metastasis (Hong, Li et al. 2017).

While very little work has been done to characterize a role for PR in PCa, data suggests that PR expression is elevated in metastatic disease, and that PR antagonist are also potential treatments for prostate cancer (Check, Dix et al. 2010) (Fischer 1994). Thus, FKBP51 and FKBP52 regulation of PR signaling may also be a relevant therapeutic target in this disease setting.

In several other types of cancer, the influence of FKBP51 on neoangiogenesis, cell proliferation, invasion, motility, and chemosensitivity is modulated through the NF-κB and AKT pathways. Overexpression of FKBP51 triggering NF-κB activation can result in sustained cell proliferation and chemoresistance in different cancer types. For instance, elevated FKBP51 has been reported in all kinds of glioblastoma cells, including GSCs (Glioma stem cells) and vascular endothelial cells (Rotoli, Diaz-Flores et al. 2022), and its expression is inversely correlated with overall glioblastoma patient survival rates (Jiang, Cazacu et al. 2008). In oral squamous cell carcinoma (OSCC), patients with high FKBP51 (>51% of FKBP51 positive tumor cells) have and 88% estimated probability of death within five years from the diagnosis (Russo, Merolla et al. 2017). Analogous outcomes were reported by Xie et al. in ulcerative colitis (UC) associated colorectal cancer (CRC) patients where elevated FKBP51 expression is associated with increased levels of TANs (tumor-associated neutrophils), which in turn regulates the inflammatory microenvironment and is associated with UC-CRC progression and poor prognosis (Xia, Zhang et al. 2021). Here, the regulation of inflammatory microenvironment mediated by FKBP51 may depend on NF-κB. Consistent with this notion, NF-κB constitutive activation by FKBP51 in aggressive malignant melanoma cells, elicits apoptosis resistance by escaping antitumor immune response via various mechanisms (Tufano, Cesaro et al. 2021). Upregulation of FKBP51 inducing NF-κB activation also promotes the progression of castration resistant prostate cancer (Yu, Sun et al. 2019). The underlying mechanism involves physical interaction of FKBP51 with IKK (IkappaB kinase) subunits to facilitate IKK complex assembly and, in this IKK regulatory role, both enzymatic (as an isomerase) as well as scaffold function of FKBP51 are essential (Romano, Xiao et al. 2015). In

addition, higher expression of FKBP52 also promotes transcriptional activation and nuclear translocation of NF-κB in lung cancer and causes significantly shorter survival with aggressive cell proliferation, invasion, and metastasis (Zong, Jiao et al. 2021).

On the contrary, it has also been demonstrated that FKBP51 overexpression induces inhibition of glioma cell proliferation, apoptosis ad chemosensitivity, which repudiates the involvement of only NF-κB because FKBP51 hyperexpression should promote the IKK activation and increase the cell proliferation (Li, Jiao et al. 2020). This contradictory action implies the engagement of another regulating mechanism that is cell context dependent. Pei et al. showed that AKT phosphorylation is the responsible mechanism for such response and mechanistically AKT is negatively regulated by FKBP51 (Pei, Li et al. 2009). The mechanism elucidated involves a functional role for FKBP51 as a scaffold protein enhancing PHLPP-AKT interaction and enabling PHLPP mediated dephosphorylation of AKT-Ser473 (Hou and Wang 2012). Increased AKT phosphorylation mediated by downregulation of FKBP51 reduces chemosensitivity, as observed in pancreatic and breast cancer (Li, Lou et al. 2011). Whereas a decrease in AKT phosphorylation mediated by FKBP51 hyperexpression increases chemosensitivity as reported in endometrial adenocarcinoma where high FKBP51 attenuates cell proliferation and progestin resistance by decreasing AKT signaling (Dong, Jiao et al. 2017). FKBP51 overexpression also promotes cellular apoptosis in lung cancer by enhancing p53 signaling pathway activity (Chen, Liu et al. 2020). Although it is known that p53 interacts with nuclear AKT and regulates its activation (Chen, Liu et al. 2020), and may promote cellular autophagy following inhibition of AKT activation (Cordani, Butera et al. 2017), comprehensive elucidation of the p53-AKT mechanism impeding lung

malignancy is yet to be determined. However, association between FKBP51 and p53 expression implies that lung cancer progression can be blocked by FKBP51 via this pathway (Chen, Liu et al. 2020). On the other hand, overexpression of FKBP52 in non-small-cell lung cancer, triggers tumorigenesis by potentiating Akt signaling (Meng, Meng et al. 2020), instead of suppressing malignant progress. Identical outcomes were also recorded with a triple negative breast cancer cell model and murine xenograft tumor models which offers an alternative treatment to hormonal therapy for non-responding triple negative breast cancer patients (Mange, Coyaud et al. 2019).

Conclusively, the findings to-date have established TPR-domain immunophilins FKBP51 and FKBP52 as prognosis biomarkers, therapeutic targets, and chemotherapy response indicators which can assist to tailor individualized anticancer treatments. Yet, the roles of FKBP51 and FKBP52 as tumor regulators remain controversial, delineating a complex scenario of various intersections of FKBP51-related molecular pathways among different tumors (Staibano, Mascolo et al. 2011). Hence, the comprehensive elucidation of these metabolic pathways and underlying mechanisms remains elusive and warrants further investigation.

1.8 STRESS-RELATED DISORDERS

The underlying mechanisms of stress-influenced health conditions involves the role of FKBP51 and FKBP52 as integral components of glucocorticoid receptor complex. FKBP51 and FKB52 are major regulators of glucocorticoid receptor activity and participate in restoring hypothalamic–pituitary–adrenal (HPA) homeostasis post stress induction, which is mediated by various feedback signaling loops. FKBP51 displays

negative feedback control of GR sensitivity by decreasing glucocorticoid receptor (GR) affinity for glucocorticoids and nuclear translocation of GR (Riggs, Roberts et al. 2003, Zannas, Wiechmann et al. 2016, Gan, Wang et al. 2022). Malfunction of GR activity owing to FKBP51 overexpression is one of the most prominent characteristics associated with stress-related illnesses and psychiatric disorders (Wang, Chai et al. 2010, Xie, Kranzler et al. 2010, Fani, Gutman et al. 2013). Since FKBP52 is known to positively regulate the glucocorticoid receptor activity, heterozygous *fkbp52*-deficient mice exhibited induced stress sensitivity mimicking the response to FKBP51, likely owing to reduced GR sensitivity (Hartmann, Wagner et al. 2012). The intricate combinations of reactions like deletion of FKBP52, and downregulation of FKBP51 may provide a possible explanation (Hartmann, Wagner et al. 2012). Furthermore, FKBP52 does not engage in all areas of GR signaling pathways. Instead, regulation of GR transcriptional activity by FKBP52 is gene specific, where FKBP52 may act as a modulatory factor (Wolf, Periyasamy et al. 2009). However, precise mechanisms involved here remain elusive.

Studies on the role of FKBP51 in PTSD and major depression showed that alterations in the epigenetic modifications of fkbp5 can lead to glucocorticoid resistance in the brain, which contributes to the onset of these neuropsychiatric disorders, among others. (Zannas, Wiechmann et al. 2016). Genes regulating HPA homeostasis are often associated with neuronal functions and stress related disorders, and interaction of these genes with stress factors have influence on the disease onset. Fkbp5 genetic variants (single nucleotide polymorphisms) expressing higher FKBP51 is frequently found in multiple stress related anxiety and depression disorders. For example, exposure of a stressor with FKBP5 single nucleotide polymorphisms (SNP) is correlated with increased

risk of post-traumatic stress disorder (PTSD) (Sabbagh, Cordova et al. 2018). In line with these findings, combination of FKBP5 SNPs with early life stressor such as childhood adversity can cause maladaptive GR activity and long term HPA homeostasis disequilibrium. Ultimately, this influences adult response to trauma, and increases the risk of PTSD (Binder, Bradley et al. 2008, Sabbagh, Cordova et al. 2018). These findings delineate a role for FKBP51 in neuronal plasticity and identify it as a hallmark for PTSD prediction. There is significant association between FKBP51 and hippocampal volume alterations in PTSD patients (Dannlowski, Grabe et al. 2015, Yun, Jin et al. 2022).

Direct correlation between FKBP51 and anxiety related behavior is supported by the observations of reduced corticosterone secretion with deletion of FKBP51 in mice (Hartmann, Wagner et al. 2012, Hartmann, Wagner et al. 2015). Lack of FKBP51 is found to change the brain structure and connectivity, revealing link among genetic status of FKBP5, stress sensitivity and psychiatric disorders (Engelhardt, Boulat et al. 2021). Site specific deletion of FKBP5 increases GR sensitivity and rescues the stress response, whereas overexpression of the same gene exhibits over-activation of HPA axis (Hausl, Brix et al. 2021). This suggests that FKBP51 is a potential target for advanced treatment of stress related disorders, and that FKBP51 hypoexpression could have a wider role in inflammatory stress-associated mental disturbance (Gan, Wang et al. 2022).

Evidence suggests that GR-FKBP51 complex or FKBP5 genes can be potential therapeutic targets or stress responsive markers for various central nervous system disorders. While new reports corroborating correlation of FKBP51 and FKBP52 with stress related disorders are emerging, there is a lot more to unveil to develop diagnostic tools and disease specific treatments.

1.9 THERAPEUTIC TARGETING

Given the many roles for FKBP51 and FKBP52 discussed above, the FKBPs have emerged as attractive therapeutic targets for a myriad of endocrine and cardiovascular diseases (Sivils, Storer et al. 2011, Guy, Garcia et al. 2015, Ghartey-Kwansah, Li et al. 2018) and are well-documented druggable targets due to their PPIase catalytic site, which is an ideal hydrophobic drug-binding pocket. While the FKBP51 and FKBP52 PPIase pockets have been selectively targeted with the SaFit class of drugs (Gaali, Kirschner et al. 2015, Jagtap, Asami et al. 2019, Bauder, Meyners et al. 2021), as previously discussed, this class of drugs are not likely to have utility in the treatment of hormone-dependent diseases given that FKBP regulation of the SHRs is independent of the PPIase catalytic activity. That being said, the FKBP51 and FKBP52 PPIase inhibitors will likely have therapeutic utility in a wide variety of disease settings in which the PPIase activity is critical; the stabilization of abnormal tau protein in AD is a prime example (Jinwal, Koren et al. 2010) .

Given the positive regulation of AR by FKBP52 in prostate cancer, FKBP52 and other cochaperones have emerged as potential therapeutic targets for the treatment of androgen-dependent prostate cancer. In line with this idea, we previously characterized a putative FKBP52 regulatory surface on AR termed binding function 3 (BF3) (De Leon, Iwai et al. 2011). In addition, we have demonstrated that FKBP52 interacts directly with β-catenin and promotes β-catenin interaction with AR, likely through the AR BF3 surface, to promote a synergistic upregulation of AR activity. Interestingly, the FKBP52 proline-rich loop, but not FKBP52 binding to Hsp90, is critical for this novel co-regulatory mechanism (Storer 2010). We have also developed a first-in-class drug, termed MJC13,

that specifically targets the FKBP52-dependent potentiation of AR activity, likely through binding the AR BF3 surface (De Leon, Iwai et al. 2011). Consequently, MJC13 was also shown to block FKBP52/β-catenin interaction with, and potentiation of, AR (Suh, Chattopadhyay et al. 2015). Thus, MJC13 represents the first drug that is capable of blocking the regulation of AR activity by both FKBP52 and β-catenin, and has displayed promising results in prostate cancer xenograft models (Liang, Bian et al. 2016). While the rationale for the use of MJC13 in the treatment of prostate cancer is obvious, recent findings strongly suggest that these drugs will also have utility in the treatment of subsets of breast cancer (D'Amato, Gordon et al. 2016). AR is expressed in 90% of ER positive breast cancer tumors, and by decreasing AR nuclear localization and genome binding using MJC13 and other antiandrogens, the authors suggest that AR supports ER activity in breast cancer. Additionally, it was demonstrated that inhibiting AR directly, also inhibits ER indirectly in breast cancer (D'Amato, Gordon et al. 2016). While MJC13 shows great promise for the treatment of prostate cancer and specific subtypes of breast cancer, it will not likely have utility outside of these disease settings given that it specifically targets an FKBP52 regulatory surface on the androgen receptor and is specific to FKBP52-regulated AR activity. Thus, targeting FKBP52 directly could potentially have broader utility across many relevant disease settings.

The inhibition of FKBP52 PPIase activity is not synonymous with the inhibition of FKBP52 regulation of receptor activity. As discussed above, the proline-rich loop overhanging the PPIase pocket is the more functionally relevant target. However, the proline-rich loop surface does not represent an ideal small molecule docking site. To overcome this barrier, we more recently used structure-based drug design and *in silico*

screening to identify an early hit molecule termed GMC1 that is not only predicted to target the FKBP52 PPIase pocket, but is also predicted to affect the conformation of the proline-rich loop surface (Ekpenyong, Cooper et al. 2020). Targeting the PPIase pocket with molecules that can simultaneously disrupt interactions on the proline-rich loop surface would be predicted to target a wide variety of factors simultaneously including AR, GR and PR activity, as well as PPIase-dependent functions of the FKBPs. Much work remains to be done to validate this targeting approach. However, combining this approach with the approach used to develop SaFit molecules with the ability to selectively target individual FKBP proteins holds promise for the development of FKBP targeting drugs with broad utility.

Much like FKBP52, FKBP51 has also been the focus of novel therapeutic interventions of diseases that have an aberrant expression of FKBP51. FKBP51 is a negative regulator of GR signaling that can dysregulate the HPA axis through alteration of GR. Thus, Sabbagh et. al sought to determine if disturbing FKBP51 could restore GR activity in Hela cells by treating cells with benztropine mesylate. Unequivocally, an in vitro protein interaction assay demonstrated the disruption of FKBP51 from the GR/Hsp90 complex in response to treatment, which was later verified by co-immunoprecipitation of GR heterocomplex. Furthermore, benztropine reversed FKBP51 inhibition of GR translocation and restored expression of GRE regulated genes (Sabbagh, Cordova et al. 2018).

1.10 CONCLUDING REMARKS

The functions of the TPR-domain immunophilins FKBP51 and FKBP52 in normal physiology and disease are diverse, and new roles, mechanisms, and interactors for these FKBPs continue to be identified. FKBP51 and FKBP52 are now known to interact, or at least associate, with a wide variety of other proteins (**Figure 1.2**). Among these 50 genes that were identified from the database, PINK1, PARK2, and AR have been discussed previously in this article as interactors of immunophilins and their relationship described. While many of these associated factors are shared among the two FKBPs, many are also unique to FKBP51 or FKBP52. While the *fkbp51*- and *fkbp52*-deficient mice display unique phenotypes (Yang, Wolf et al. 2006, Tranguch, Wang et al. 2007), the knockout of both FKBP51 and FKBP52 in mice results in embryonic lethality (Storer, Dickey et al. 2011). Thus, FKBP51 and FKBP52 not only have distinct functions, but they clearly have some redundant functions that are critical for embryonic development. Perhaps the most logical next step towards understanding the critical roles that FKBP51 and FKBP52 have in normal physiology and disease is to compare the known interactomes for FKBP51 and FKBP52 to begin to understand their distinct, as well, as shared functions. This would, in turn, better inform the therapeutic targeting strategies, given that FKBP51 and FKBP52 have emerged as attractive therapeutic targets in a wide variety of disease settings.

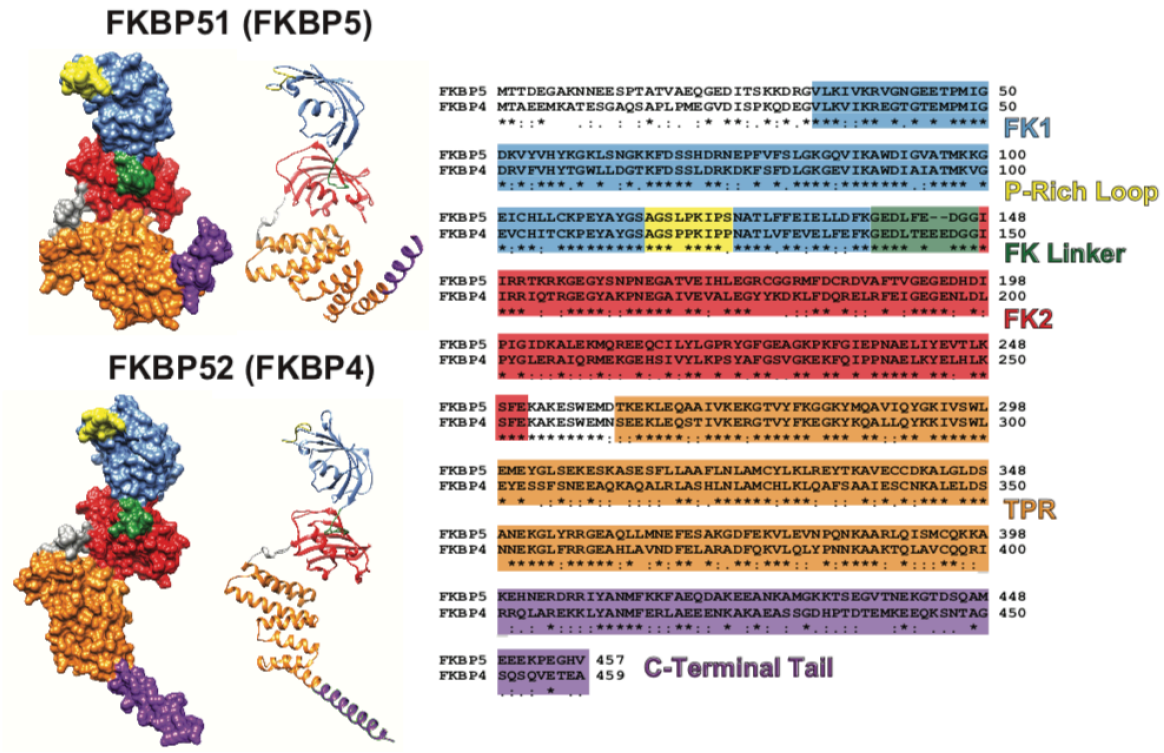

Figure 1.1. FKBP51 and FKBP52 Structure and Sequence Comparisons.
Both the ribbon and molecular surface models of the human FKBP51 crystal structure (FKBP5; PDB ID 1KT0) and overlaid images of two partial human FKBP52 crystal structures (FKBP4; PDB ID 1Q1C and 1P5Q) are shown on the left. The visible difference in the orientation of the TPR (orange) and C-Terminal Tail (purple) between FKBP51 and FKBP52 may be an artifact given that FKBP52 has been only partially crystalized. A multiple sequence alignment comparing the FKBP51 and FKBP52 amino acid sequence is shown on the right with known functional domains and regions colored according to their location on the crystal structures (* denotes identical amino acids, : denotes highly conserved amino acids, and . denotes partially conserved amino acids). Human FKBP51 and FKBP52 are approximately 60% identical and approximately 70% similar. The functional domains and regions highlighted include: the FK506 binding domain 1 (FK1) that contains a functional PPIase active site to which FK506 binds (blue); the proline-rich loop that overhangs the PPIase pocket and serves as a functionally relevant surface for the regulation of steroid hormone receptors (yellow); the linker region that links the two FK domains and contains a casein kinase 2 phosphorylation site (T143) in FKBP52 that may regulate Hsp90 binding (green); the FK2 domain that is structurally similar to FK1, but lacks a functional PPIase active site (red); the tetratricopeptide repeat (TPR) domain that mediates binding to the C-terminal EEVD motif on Hsp90 (orange); and the C-terminal Tail containing the Charge-Y motif that has also been shown to influence Hsp90 binding (purple). It is important to note that FKBP51 and FKBP52 were crystalized without the last 45 C-terminal amino acids and 32 C-terminal amino acids respectively. Thus, the structures shown on the left are truncated within the C-Terminal Tail. UCSF Chimera candidate version 1.12 (build 41600) was used to generate the ribbon and molecular surface images. The multiple sequence alignment was generated by CLUSTALW prior to illustration.

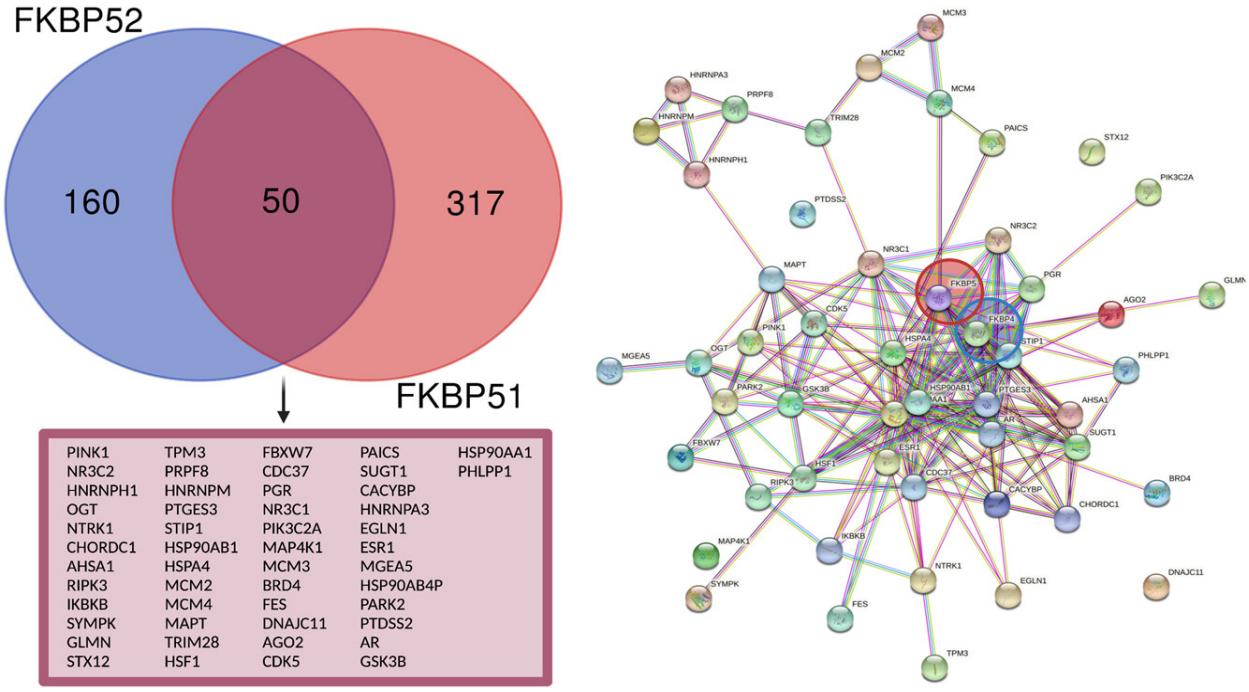

Figure 1.2. Protein-Protein Interaction Network for Known Interactors of both FKBP5 and FKBP4

The Venn diagram (top-left) collates interactors from the BioGRID protein interaction database for FKBP4 and FKBP5, respectively. TheBioGRID database was accessed 25 September 2022 at 2:32 AM UTC. Results were filtered by evidence type “Interactors with ONLY Physical Evidence (LTP or HTP)” and organism (H. sapiens). Interactors identified only in pre-publication datasets were excluded. Shared interactors (bottom left) were used to generate a PPI network using STRING v11.5 (1–α ≥ 0.5). While interactors were filtered based on methodology detecting physical interaction, not all of these methodologies distinguish between direct interaction versus association through protein complexes. Thus, the interactors shown here may be associated with the FKBPs through larger protein complexes in some cases, but the figure highlights the fact that FKBP51 and FKBP52 have shared interactors as well as a significant number of distinct interactors.

[End of publication]

1.11 Androgen Receptor Structure and Function

AR is a Steroid Hormone Receptor (SHR) part of the steroid receptor subfamily of nuclear receptors which also include the glucocorticoid receptor (GR/NR3C1), mineralocorticoid receptor (MR/NR3C2), progesterone receptor (PR/NR3C3) and estrogen receptors a and b (ERa/NR3A1; ERb/NR3A2). It is found on the X chromosome (Xq11-12) and spans approximately 180kb of DNA and 8 exons coding for a 110kDa protein (Gelmann 2002, Lonergan and Tindall 2011). The AR is a ligand-dependent nuclear transcription factor and is therefore arrested in the cytoplasm in the absence of ligand but can translocate to the nucleus in response to androgens (Lu, Feng et al. 2013). Upon ligand binding, AR initiates signaling cascades responsible for normal development and homeostasis of male reproductive organs during embryogenesis, as well as fertility and other aspects of adult sexual behavior (Chang, Lee et al. 2013).

The AR responds to two endogenous ligands, testosterone and 5α-dihydrotestosterone (DHT). Testosterone is the most abundant circulating androgen, while DHT is functionally the most important androgen in the prostate (Kinter and Anekar 2020). Despite their differences, testosterone serves as a precursor to synthesize DHT by action of 5α-reductase enzyme in the prostate (Agoulnik and Weigel 2008). Testosterone is mainly produced by Leydig cells in the testicles utilizing cholesterol as a precursor and is transported by sex hormone binding protein SHBP and released across the plasma membrane. Testosterone is then converted into DHT, the more potent ligand for AR. (Schmidt, Regan et al. 2009). DHT binds to AR with a 2-fold higher affinity and a 5-fold lower dissociation rate than testosterone (Wilson and French 1976, Grino, Griffin et al. 1990). In a similar manner of progesterone binding to the LBD of the PR, DHT binds

to the LBD of the AR by interacting with helices 3,5, and 11 (Sack, Kish et al. 2001). Upon hormone binding to the LBD, it is postulated that the receptor undergoes a conformational change resulting in dissociation from chaperone molecules and homodimerization prior to translocating to the nucleus (Solit, Scher et al. 2003). While in the nucleus, ligand-bound AR binds to cis-acting target genes known as androgen response elements (ARE) and recruits coregulators and cofactors to regulate transcription of ARE-driven androgen dependent genes (Figure 1.1) (Kumari, Senapati et al. 2017).

Extensive studies have emphasized the significant effect the structure of the AR has on its signaling. Mutational and functional studies have uncovered four major functional domains structurally consistent with other members of the steroid hormone receptor (SHR) family. These domains, which have demonstrated autonomous function, include an N-terminal domain (NTD)- followed by a DNA binding domain (DBD), and a C-terminal ligand binding domain (LBD) connected to the DBD by a flexible hinge region (Tan, Li et al. 2014).

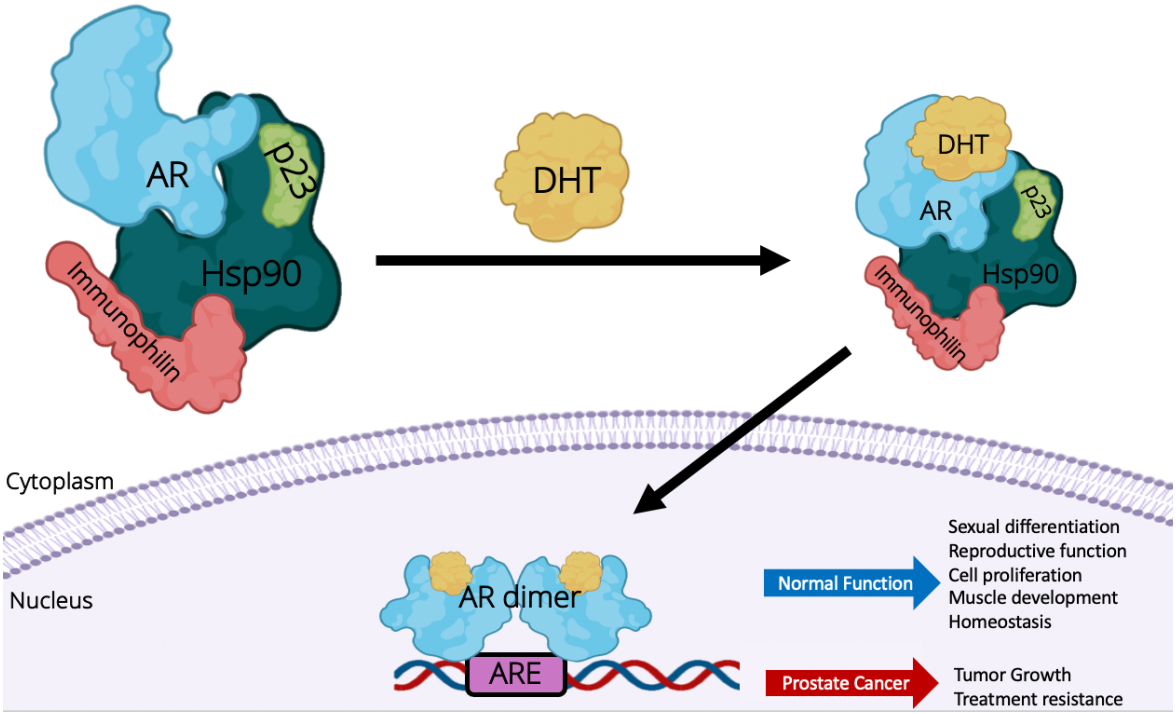

Image created with BioRender

Figure 1.3. Androgen Receptor Signaling.
In the absence of bound androgen, AR is maintained in the cytoplasm of the cell interacting with heat shock proteins, cytoskeletal proteins, chaperones, and co-chaperones. Following the maturation process, AR conformation is stabilized by the Hsp90 heterocomplex, which includes p23 and immunophilins, and allows AR to bind androgens with high affinity. Upon binding of androgen, AR undergoes conformational change that enables chaperone heterocomplex dissociation, AR dimerization, and nuclear translocation. In the nucleus, AR binds to androgen-responsive elements (ARE) within androgen target genes to initiate transcription.

1.11.1 N-Terminal Domain

The N-terminal domain (NTD) displays the greatest sequence variability across members of the steroid receptor family. In the AR, this domain comprises more than half of the entire receptor sequence, spanning residues 1-555 (Tan, Li et al. 2014). Through deletion mutagenesis of 338 amino acids in this region, transcriptional activation has been attributed to the N-terminal domain (Simental, Sar et al. 1991). In fact, the NTD contains the ligand-independent Transactivation Function (AF1) region known to be the primary effector and required for maximal transcriptional activity of the AR. AF-1 includes two transcription activation units (Tau-1 & Tau-5) required for full AR activity and stabilization of AR dimer by supporting ligand dependent interaction of NTD and LBD (Giovannelli, Di Donato et al. 2018).

1.11.2 DNA-Binding Domain

The centrally located DNA Binding Domain (DBD) spans residues 555-623 and its structure is highly conserved among NHRs. This region consists of two zinc-fingers and a C-terminal domain. The zinc finger closest to the NTD contains the “recognition helix” and is directly involved in determining DNA specificity, while the second zinc-finger mediates AR dimerization (Heemers and Tindall 2007, Koochekpour 2010). Furthermore, the DBD serves as an anchor for AR to directly interact with promoter and enhancer regions in DNA to allow the activation functions of the NTD and LBD to initiate transcription of AR-regulated genes (Tan, Li et al. 2014). The DBD domain is linked to the ligand binding domain (LBD) by a flexible linker known as the Hinge region. At the junction between the DBD and the hinge region, lies a nuclear localization signal (NLS) located

across the C-terminus of the DBD, suggesting the Hinge region is involved in translocating the receptor to the nucleus. (Zhou, Sar et al. 1994).

1.11.3 Ligand Binding Domain

The Ligand Binding Domain (LBD) is a 295 amino acid sequence coded by exons 4-8 (Koochekpour 2010). Unlike the NTD, several structures have been characterized by crystallography for monomeric AR-LBD bound to agonists or antagonist (Matias, Donner et al. 2000, Sack, Kish et al. 2001, Bohl, Gao et al. 2005, Estebanez-Perpina, Moore et al. 2005, Nadal, Prekovic et al. 2017). Closely resembling other nuclear receptors, the AR LBD is composed of 12 alpha helices organized in an antiparallel sandwich (Aranda and Pascual 2001). This domain interacts with the N-terminus of the AR to stabilize bound androgen and mediates interaction of the AR with heat-shock and chaperone proteins through activation function-2 (AF-2) and the BF3 surface (He, Kemppainen et al. 1999, Heinlein and Chang 2002).

Upon agonist binding, the LBD undergoes a conformational change which stabilizes helix-12 and results in the formation of activation function 2 (AF-2), a hydrophobic pocket that enables binding with LXXLL coactivator motifs or interdomain interaction with AR FXXLF motif (Bevan, Hoare et al. 1999, Heemers and Tindall 2007). Importantly, AR AF-2 preferentially binds this AR FXXLF motif in the NTD in a ligand-dependent manner, which mediates interaction between the NTD and LBD (Langley, Kemppainen et al. 1998).

1. 12 Steroid hormone Receptor maturation and regulation by Chaperone Complex

Molecular chaperones mediate protein folding, assembly, and disassembly of macromolecular complexes. In the cytosol, AR is never found alone but rather with

chaperones that regulate its conformation and localization. Prior to reaching its high-affinity ligand binding conformation, AR undergoes a maturation process dependent upon the sequential action of a network of chaperones, all of which have piqued interest as prospective therapeutic targets. At the center of many cellular processes and receptor maturation for steroid receptor function, lies Heat-shock protein 90 (Hsp90) for which AR is a 'client' protein. Together with Hsp70, Hsp90 collaborates with a multitude of accessory proteins called 'co-chaperones' in order to form dynamic multi-chaperone complexes (Figure 1.2) (Li, Soroka et al. 2012).

1.12.1 Hsp90 Interactors

Heat-shock protein 90 (Hsp90) is the most abundant among its family of heat-shock proteins, all of which play important roles in refolding of proteins in response to cellular stresses. Given that heat-shock proteins are upregulated in tumor cell environment and that cancer cells can survive extreme environmental stress, it has been suggested that heat-shock proteins are involved in this malignant transformation (Neckers 2007). Additionally, out of the more than 200 client proteins that Hsp90 has, many of these are potentially oncogenic proteins, such as AR and other steroid receptors (http://www.picard.ch/ downloads). Many of the physiological mechanisms that promote cellular homeostasis, including regulation of SHR activity, rely on Hsp90 as a central player. Hsp90 is known to associate with steroid hormone receptors in the absence of cognate ligands. In fact, Hsp90-SHR interaction is required for conformational maturation of these and other signaling proteins.

Similar to steroid hormone receptors that require cooperation of proteins, proper function of chaperone Hsp90 also depends on co-chaperones, including TPR domain-

containing proteins, such as the immunophillins FKBPs. The first clues regarding SHR-chaperone relationship were acquired through many studies of steroid hormone receptors other than the AR. Receptor-Hsp90 heterocomplexes were initially identified for the Glucocorticoid receptor (GR), progesterone receptor (PR), mineralocorticoid receptor (MR), and estrogen receptor (ER) (Kumar and McEwan 2012). The observation that AR shares a highly conserved LBD with these nuclear receptors suggested that a similar AR-Hsp90 heterocomplex existed (Marivoet, Van Dijck et al. 1992, Veldscholte, Berrevoets et al. 1992). It is now well established that AR maturation and stabilization involves the regulated assembly of multiprotein complexes consisting of Hsp70, Hsp40, Hop, Hsp90, and p23 (Figure 2) (Guy, Garcia et al. 2015).

1.12.2 Early Complex

The initial interaction of a newly synthesized AR polypeptide occurs with Hsp40 along with Hsp70 (Langer, Lu et al. 1992, Hendrick, Langer et al. 1993). Initially, Hsp40 binds to the LBD of the non-native SHR peptide in order to facilitate transfer of the SHR to Hsp70. Hsp40 can also bind Hsp70 through its HPD motif, which interacts with the N-terminal ATPase domain of Hsp70 in order to transfer the client protein in a process that involves Hsp40-dependent hydrolysis of ATP by Hsp70 (Hartl and Hayer-Hartl 2002). This hydrolysis reaction is critical since the ATP bound form of Hsp70 has been suggested to release peptides rapidly as opposed to the ADP bound form which releases peptides slowly, thus giving this form of Hsp70 a higher affinity for its substrate protein (Hohfeld, Minami et al. 1995). The cooperation between Hsp40 with Hsp70 is also known to prevent protein aggregation and maintain receptor solubility (Cyr 1995). In vitro experiments in yeast *Saccharomyces cerevisiae* demonstrated that in the absence of Hsp40, the AR was

unable to bind hormone with high affinity when compared to its wild-type counterpart (Fliss, Rao et al. 1999). Through the use of Hsp40 point mutants, it was concluded that proper function of Hsp40 is necessary for Hsp70 to subsequently capture non-native proteins (Fan, Ren et al. 2005). In conclusion, the role of Hsp40 in the assembly-line of steroid receptor maturation is to prime the client proteins for the following steps.

1.12.3 Ubiquitin/ Proteasome Pathway

The cooperation between these molecular chaperones and the cell's degradation machinery is crucial for optimum protein and cellular function. Following the initial Hsp40 and Hsp70 interactions, the SHR-chaperone complex undergoes a quality control check to determine whether the SHR will be targeted for degradation by C-terminus of Hsc70-interacting protein (CHIP) or shuttled to reach the intermediate complex phase of receptor maturation. Protein quality control and degradation of steroid hormone receptors occurs via the Ubiquitin-proteasome pathway with CHIP being the quality control regulator (Prescott and Coetzee 2006). CHIP is highly conserved across species and consists of an N-Terminal TPR domain, a central a-helical domain and a U-box at the C-terminus with ubiquitin ligase activity. CHIP competes with Hop for Hsp70 binding in a rate-limiting step to determine the fate of substrates that will either be directed towards re-folding or degradation processes (Kundrat and Regan 2010). By associating with the C-terminal sequences of Hsp70 and Hsp90 via its TPR domains, the C-terminus of Hsp70-interacting protein CHIP ubiquitinates and primes the chaperones' client proteins to the proteasome for degradation (Kundrat and Regan 2010).

1.12.4 Intermediate Complex

Once the SHR-chaperone complex bypasses the quality control and following the initial binding of Hsp40 and Hsp70, Hsc interacting protein (Hip) binds to stabilize interactions in the intermediate chaperone complex. After Hsp40 dependent hydrolysis, Hip stabilizes the ADP state of Hsp70 which results in higher affinity for the SHR and a prolonged interaction (Hohfeld, Minami et al. 1995, Prescott and Coetzee 2006). Subsequently, Hsp organizer protein (Hop), recruits a homodimer of Hsp90 and serves in the transition from Hsp70 to Hsp90 chaperone system by bridging the two through its two separate TPR domains (Chen, Sullivan et al. 1998). Both Hsp70 and Hsp90 interact with TPR-domain containing co-chaperones through an EEVD sequence at their C-terminus (Tao and Zheng 2011). Once the SHR has complexed with Hsp90, a small acidic protein called p23, acts to stabilize the complex in its ATP-bound form (Morishima, Kanelakis et al. 2003). The affinity of Hsp90 for Hop is weakened by the presence of p23, which results in the release of Hip, Hop, and Hsp70 and allows for the recruitment of other TPR-containing proteins such as: FKBP51, FKBP52, Cyp40, or PP5. The dissociation of Hop is accelerated by BAG-1 (Bcl2-associated gene product 1). More recent findings, however, suggest that Hsp90 is capable of binding Hop and FKBP52 simultaneously (Prescott and Coetzee 2006, Hildenbrand, Molugu et al. 2011). If these findings were to hold true, the existence of an Hsp90-FKBP52-p23-Hop complex would reduce the number of intermediate steps necessary in the maturation pathway for SHRs.

1.12.5 Mature Complex

Finally, through the structural stabilization by the Hsp90 complex, the SHR and chaperones become the ‘mature complex’ in this molecular chaperone pathway. The

binding of p23 and immunophilins to the Hsp90-receptor complex gives rise to an SHR conformation that is highly responsive to hormones. Upon androgen binding, a conformational change is produced which results in dissociation of cytoplasmic chaperones, ultimately revealing the nuclear localization signal. The hormone-bound AR then dimerizes and translocates to the nucleus, where it interacts with a series of transcriptional coregulators to bind DNA and regulate target gene expression (Ai et al., 2009; Koochekpour, 2010). Generally, disruption of the SHR-Hsp90 complex is thought to occur upon hormone binding and prior to nuclear translocation. However, it has been postulated that in the case of the GR, this requires the cooperation and movement of the entire Hsp90 heterocomplex for nuclear transport after ligand binding (Galigniana, Radanyi et al. 2001). In the case of AR, it remains unclear whether hormone-bound AR can permeate through the nuclear pores with or without the Hsp90 heterocomplex.

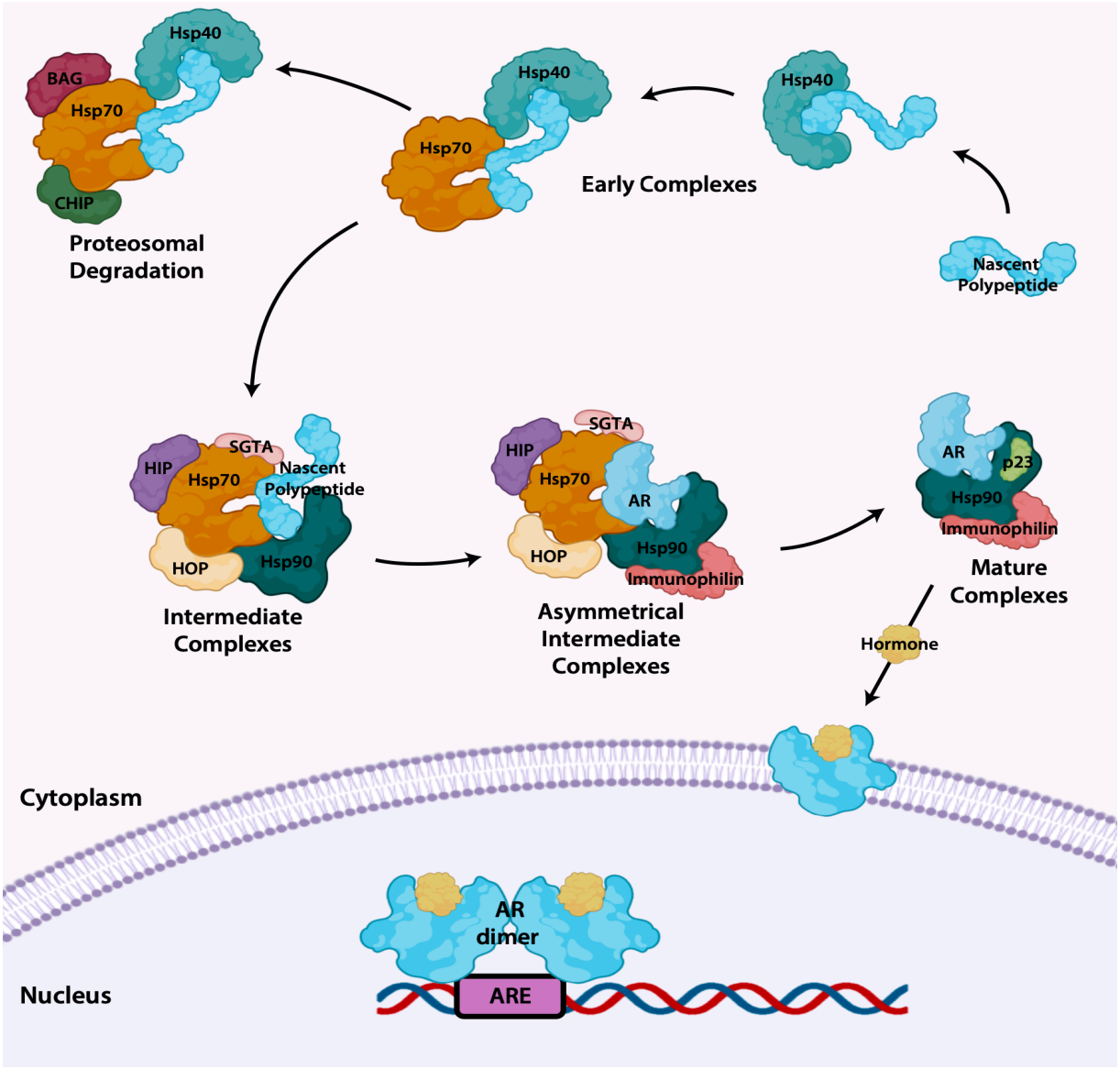

Figure 1.4. Chaperone mediated receptor maturation
The basic heterocomplex required for proper folding and stabilization of SHRs consists of Hsp70 (hsc70), Hsp40 (Ydj1), Hop (p60), Hsp90, and p23. Initial assembly of the SHR-chaperone complex occurs when Hsp40 interacts with the nascent polypeptide followed by recruitment of Hsp70. Proteins unable to fold properly are ubiquitinated by CHIP and with the help of BAG are targeted for degradation while properly folded proteins continue towards receptor maturation pathway. Chaperone-SHR complex is then bound by HIP which stabilizes interactions in the intermediate chaperone complex. Subsequently, HOP binds to the complex and serves as a bridge between Hsp70 and Hsp90 to transfer the client protein to the latter. Both Hsp70 and Hsp90 interact with two separate TPR-domains in HOP through their C-terminal EEVD sequence. Finally, p23 is recruited to stabilize the chaperone-SHR complex. Consequently, p23 weakens the affinity of Hsp90 for HOP and results in the release of Hip, Hop, and Hsp70 and allows for the recruitment of other TPR-containing proteins such as immunophilins. As a result, the receptor reaches high affinity hormone binding conformation.

1.13 Androgen Deprivation Therapy and PCa Progression

Androgen deprivation therapy (ADT) is given as the first line of defense or initial treatment. Huggins and Hodges first reported that androgen ablation resulted in remission of metastatic PCa, which was evident by the significant improvement in the clinical condition of patients, while re-administration of androgen resulted in aggravation of symptoms. Since these observations indicate that androgens can facilitate prostate tumor growth, the goal of many therapies is reducing synthesis of circulating androgens or inhibiting AR signaling. ADT can implement an orchiectomy (surgical castration) or the use of luteinizing hormone–releasing hormone (LHRH) agonist/antagonist (chemical castration) in order to lower levels of circulating testicular androgens (Ceder, Bjartell et al. 2016). Most tumors respond well to androgen deprivation therapy, but after an apparently successful initial response, tumors recur and become resistant to this type of treatment. Prostate cancer advances into a more aggressive form termed castration-resistant PCa (CRPC) for which there is no cure. Increasing evidence suggests that reactivation of AR signaling is the major biochemical driving force leading to the castration resistance phenotype. Cases that progress despite castrate levels of testosterone (50ng/mL), have poor prognosis and an expected survival time of 1-2 years from the time of progression (Huang, Chau et al. 2012). The phenotype exhibited in this recurring phase was originally believed to have bypassed the need for AR, and thus became known as hormone insensitive. Interestingly, we now know that the AR remains critical for signaling, and this type of cancer that has become resistant to hormone deprivation still remains ligand/AR-dependent.

1.14 ELUCIDATING THE ROLE OF FKBP51- AND FKBP52-MEDIATED AR SIGNALING IN PCA

Studies have demonstrated the importance of FKBP regulation of steroid receptor signaling for endocrine-related diseases and cancer progression (Solassol, Mange et al. 2011, Yao, Liang et al. 2011). High expression of FKBP51 and FKBP52 has been found in varying types of cancer, including prostate cancer. Using human prostate cancer cells *in vitro*, immunosuppressor FK506 demonstrated inhibition of androgen-induced proliferation of LNCaP cells. While these results complement the findings that both FKBP51 and FKBP52 can associate with LNCaP AR, whether FK506 is selective for one over the other remains elusive (Periyasamy, Warrier et al. 2007). Furthermore, it was demonstrated that the inhibitory effects of FK506 on FKBP52-mediated potentiation of SHR does not result in immunosuppression; although, it successfully inhibits LNCaP cell proliferation suggesting that targeting FKBP52 can be an alternative approach of targeting AR signaling indirectly. (De Leon, Iwai et al. 2011). Taken together, these observations highlight the importance of FKBP51 and FKBP52 in AR activity, under normal and prostate cancer circumstances, and offer an alternative to target the molecular complex required for AR signal transduction. Understanding the role of AR signaling axis in the progression of PCa is fundamental in the interpretation of the molecular mechanisms behind this malignancy. There are currently no drugs targeting immunophilins as it is not entirely understood how they regulate steroid hormone receptors in disease progression. The studies herein proposed will lead to FKBP51 and FKBP52 interaction models to expand on their molecular mechanisms under normal and PCa contexts, and eventually, help in the development of inhibitors for use in the treatment of PCa and other hormone-related diseases.

1.15 Dissertation Research Focus and Hypothesis

Specific Aims

To identify novel FKBP51- and FKBP52-receptor interactors and their potential as therapeutic targets for use in the treatment of hormone-related diseases, such as prostate cancer, we have designed the following specific aims using *in vitro* cellular models:

Specific Aim 1: Identify FKBP51 and FKBP52 interacting proteins in 22RV1 Human Prostate Cancer cells.

Specific Aim 2: Validate FKBP51 and FKBP52 interacting proteins in human Prostate Cancer cells and functionally characterize their role in FKBP-mediated signaling

Specific Aim 3: Comparative analysis of FKBP51 and FKBP52 pathways based on identified interactors.

CHAPTER 2: IDENTIFYING FKBP51 AND FKBP52 INTERACTING PROTEINS *IN VITRO* USING HUMAN PROSTATE CANCER CELLS

2.1 RATIONALE

Ongoing research on the role of molecular chaperones in AR function has shed some light on the intricate mechanisms underlying receptor signaling under healthy and disease progression states. Despite this increasing knowledge and the opportunities to develop novel therapeutics, the demand to further understand the crosstalk between signaling pathways and protein interactions persists, such is the case for AR. Immunophilins FKBP51 and FKBP52 have demonstrated crucial roles in AR potentiation and signaling, although, their interactions and underlying mechanisms remain unclear.

Despite regulating some of the same SHR client proteins, FKBP51 and FKBP52 differentially regulate these receptors. Yeast reporter assays for GR activity with single or co-expression of FKBP51 and/or FKBP52 demonstrated their opposing regulatory effects on GR activity in response to hormone (Cheung-Flynn, Roberts et al. 2003). Authors reported a 20-fold increase in receptor activity in the presence of FKBP52 that was significantly decreased when FKBP51 was co-expressed (Cheung-Flynn, Roberts et al. 2003). This observation of FKBP51's counteracting FKBP52's GR potentiation is consistent with the inhibitory effect of FKBP51 in mammalian models. In the case of another client protein for FKBP51, Progesterone Receptor, PR, maternal stress-induced FKBP51 inhibited uterine PR function in female mice, while the absence of FKBP51 in knockout mice resulted in prolonged gestation (Guzeloglu-Kayisli, Semerci et al. 2021), likely due to FKBP51's reducing PR's ligand binding ability (Hubler, Denny et al. 2003). On the other hand, FKBP52 appears to be critical for uterine PR activity and reproductive function since FKBP52-deficient mice are infertile (Tranguch, Cheung-Flynn et al. 2005).

Overall, these findings highlight the opposing and context-dependent roles for both immunophilins.

Although FKBP51 and FKBP52 can act as positive regulators of AR, there is contentious evidence on the regulatory mechanism and the degree of potentiation on AR function. Cheun-Flynn et al demonstrated the importance of FKBP52, but not FKBP51, in AR function. Similar to their findings with GR, yeast reporter assays showed enhanced hormone-induced AR activity mediated by FKBP52, but not FKBP51, while male FKBP52-KO mice exhibited impaired reproductive development (Cheung-Flynn, Prapapanich et al. 2005). Interestingly, a double knockout FKBP51/FKBP52 mouse model resulted in embryonic lethality suggesting a redundant role in embryonic development (Yong, Yang et al. 2007). The question of how these immunophilins can uniquely, yet sometimes interchangeably, regulate SHRs may be resolved by identifying the protein interactions that underlie these mechanisms.

In agreement with this idea, our lab found that FKBP52 promotes interaction of beta-catenin with AR leading to co-activation of AR-mediated transcription. This upregulation of AR was effectively blocked by MJC13, a small molecule characterized by our lab that targets the AR BF3 surface. The synergistic effect of FKBP52 and beta-catenin on AR signaling demonstrated by our lab highlights the intricacy of protein networks underlying AR regulation. Therefore, we directed our research efforts to investigate unique and distinct protein interactions of FKBP51 and FKBP52 that can ultimately serve as druggable targets.

We hypothesized that FKBP51 and FKBP52 cooperate with currently unknown interactors to promote PCa progression through AR signaling pathways. Therefore, to further understand the protein networks influencing the differences in FKBP-mediated

receptor function, the objective of the present investigation was to generate interactomes for FKBP51 and FKBP52 in prostate cancer cells. To address this objective, we performed a top-down proteomics approach using tandem affinity purification in 22RV1 PCa whole-cell lysates. Ultimately, this would help us identify potential mechanisms responsible for signaling differences mediated by each and both proteins. Given that our research efforts mainly focus on FKBP52 as a druggable target against PCa, this interactome would also provide insight into the cellular functions impacted by FKBP52 inhibition.

2.2 Materials and Methods

2.2.1 Construct of FKBP52-6xHis-Gly-FLAG

Using PCR recombinant techniques, a full-length FKBP52 cDNA was tagged at the C-terminus with a 6X His-tag followed by a Glycine linker and a FLAG (DYKKDDDDK) epitope. Using XbaI and XmaI restriction enzymes, the construct was cloned into a mammalian expression vector (pCI-Neo) with a CMV promoter and a Neomycin marker. Expression of tagged protein from both plasmids was verified by Western Blot before continuing with experimental procedures (Figure 2.1).

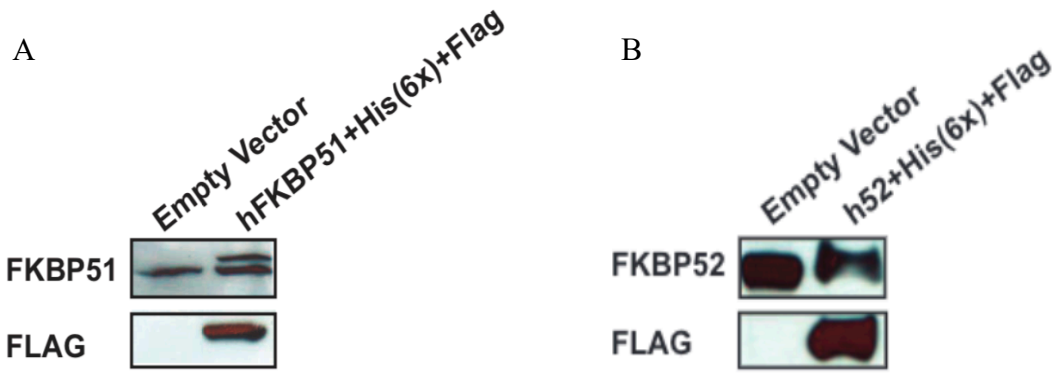

Figure 2.1. Evaluation of construct for FKBP51 and FKBP52 expression.
Expression of exogenous FLAG-tagged proteins was evaluated following transient transfection of LNCaP prostate cancer cell lines. Endogenous expression of FKBP51 **(A)** and FKBP52 **(B)** can be seen in both, empty vector, and FKBP-containing plasmid lanes. Exogenous expression is indicated by presence of FLAG tag expression present only in the FKBP-containing plasmid lanes.

2.2.2 Cell Culture

Human prostate carcinoma epithelial cell line, 22RV1, was obtained from American Type Culture Collection (ATCC CRL-2505™) and cultured in RPMI-1640 medium containing 25mM HEPES and 2mM L-Glutamine (HyClone ™ GE) supplemented with a final concentration of 10% fetal bovine serum (Corning). The 22RV1 cell line was derived from a xenograft that was "serially propagated in mice after castration-induced regression and relapse of the parental, androgen-dependent CWR22 xenograft" (Sramkoski, Pretlow et al. 1999). The 22RV1 FKBP52KO cell line was obtained from Synthego and maintained in Hyclone™ RPMI 1640 media containing 25mM HEPES and L-glutamine (GE Healthcare Life Sciences) supplemented with a final concentration of 10% FBS. Both cell lines were maintained at 37ºC in a humidified atmosphere of 5% CO_2.

2.2.3 Transient Transfections

To induce transient expression of FKBP51 or FKBP52, WT 22RV1 and 52KO 22RV1 cells were plated in 150mm^2 tissue culture petri dishes at a density of 12.0x10^6

cells/dish and incubated overnight at 37°C in a cell incubator with 5% CO_2. After 24 hours, when the cells had reached 60-80% confluence, cells were transfected using Lipofectamine® 2000 reagent (Invitrogen™, Thermo Scientific) according to manufacturer's instructions. The transfection was performed using 40ug of mammalian expression empty vector PDS-404 (pCI-neo) as a control, 40ug of PMC-249 (pCI-neo FKBP52-6xHis-Gly-FLAG), or 40ug of PMC-261 (pCI-neo FKBP51-6xHis-Gly-FLAG). DNA/lipofectamine complexes were prepared in serum-free RPMI 1640 (HyClone ^{TM} GE) with a DNA (µg):Lipofectamine2000 (µl) ratio of 1:3 and incubated for 10 minutes at room temperature. Prior to aliquoting transfection reactions to each 150mm^2 culture dish, cells were washed twice with PBS and media was replaced with 10% Charcoal-stripped FBS supplemented RPMI. Transfected cells were then incubated for 24hrs without removal of complexes, as suggested by manufacturer.

2.2.4 Tandem Affinity Purification in 22RV1 cells

Following 24hr of transfection incubations, the cells were dosed with 1nM of DHT or equal volume of ethanol vehicle as control for 3hrs and incubated at 37°C with 5% CO2. Cells were then washed with cold PBS three times and lysed with 2.5ml of ice-cold hypotonic lysis buffer (50 mM Tris-HCl pH 7.6, 10 mM NaCl, 0.5% NP-40, Protease Inhibitor Cocktail-EDTA Free (Thermo), 1mM NaF, and 1mM NA_3VO_4) and incubated for 20mins with rocking. The cells were scraped and transferred to a 10mL conical tubes and centrifuged at 150xg for 20mins at 4°C. The supernatant was used immediately for Bradford assay followed by nickel purification. The tandem affinity purification was performed in batch in duplicates. A visual representation of the experimental approach is depicted in Figure 2.2. Of total protein, 1mg was incubated with 100uL of His-Pur Ni-NTA

resin (Thermo) and a final concentration of 20mM of Imidazole was added to the reaction. To investigate the effect of incubation periods on protein interactions, one duplicate was incubated for 1hr at room temperature and the other was incubated overnight at 4°C. Following incubation periods, the resin-lysates were washed 5 times with cold wash buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% NP-40, 20 mM Imidazole) ensuring a pH of 7.0. The elution was performed with 300uL of elution buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 300 mM Imidazole; pH 8.0). Eluate was used immediately for FLAG co-immunoprecipitation following Krogan *et al.* protocol (197). The eluate was further analyzed using 40uL packed gel volume of Anti-FLAG M2 magnetic beads (Sigma) in a final volume of 500uL. The eluate from the 1hr incubation was then incubated for 1hr with FLAG magnetic beads, while eluate from overnight incubation was incubated overnight at 4°C. After FLAG pull down, the bead-cell lysates were washed 3 times with cold lysis buffer with low detergent concentration (50mM Tris pH7.5, 150mM NaCl, EDTA, 0.05% Nonidet P40, protease inhibitor) followed by one wash with lysis buffer without detergent (Krogan *et al.*). Samples were eluted with 150 µL of 100 µg/ml 1X FLAG peptide (Sigma F3290)

2.2.5 Western Blot Analysis

From each tandem affinity purification eluate, 30ul were set aside (1/5 of total final eluate volume) for Western blot analysis. Protein eluates were mixed with NuPage LDS sample buffer (Novex) and NuPage sample reducing agent (Invitrogen) at a final concentration of 1X. The protein samples were boiled for 5mins at 80°C, separated by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to Polyvinylidene Fluoride Membranes (PVDF, Thermo Scientific) membranes. A total of 3 membranes

containing the same set of samples were blocked with 5% skim milk in TBS-T (pH 7.4, PBS with 0.1% Tween-20) for 1hr and incubated with the following primary antibodies: mouse monoclonal anti-FKBP51 (1:5,000) and anti-FKBP52 Hi52D (1:5,000) both prepared in the David F. Smith Lab, mouse monoclonal anti-FLAG M2 (1:1,000; Sigma), and mouse monoclonal GAPDH loading control (1:3,000; Santa Cruz Biotechnology) overnight at 4°C with gentle rocking. The proteins of interest were then detected using AP-conjugated secondary antibodies (dilution ratio 1:10,000 Santa Cruz Biotechnology) for 1hr at room temperature. The membranes were then developed with Immuno-Star™ AP substrate (BioRad) and exposed on CL-X Posure™ film (Thermo Scientific).

2.2.6 Sample Preparation for LC-MS/MS

22RV1 eluate samples were prepared for LC-MS/MS proteome analysis using the filter aided sample preparation (FASP™) Protein Digestion Kit (Expedeon) and modified accordingly. Samples were digested using SIGMA Trypsin Proteomics Grade and incubated overnight at 37°C. Digested samples were eluted into a clean collection tube with 0.1 % formic acid. The final solution was concentrated in the vacufuge and frozen until LC-MS/MS analysis. During LC-MS/MS analysis, the peptides from the resulting protein digestions are fragmented and their mass-to-charge ratio is measured. A second MS analysis then determines each peptide's sequence. All data collected is then computationally matched to a database to identify the proteins that have been identified in the protein complexes. The complex peptide mixture from individual samples were analyzed by LC-MS/MS at UTEP's Biomolecule Analysis Core Facility.

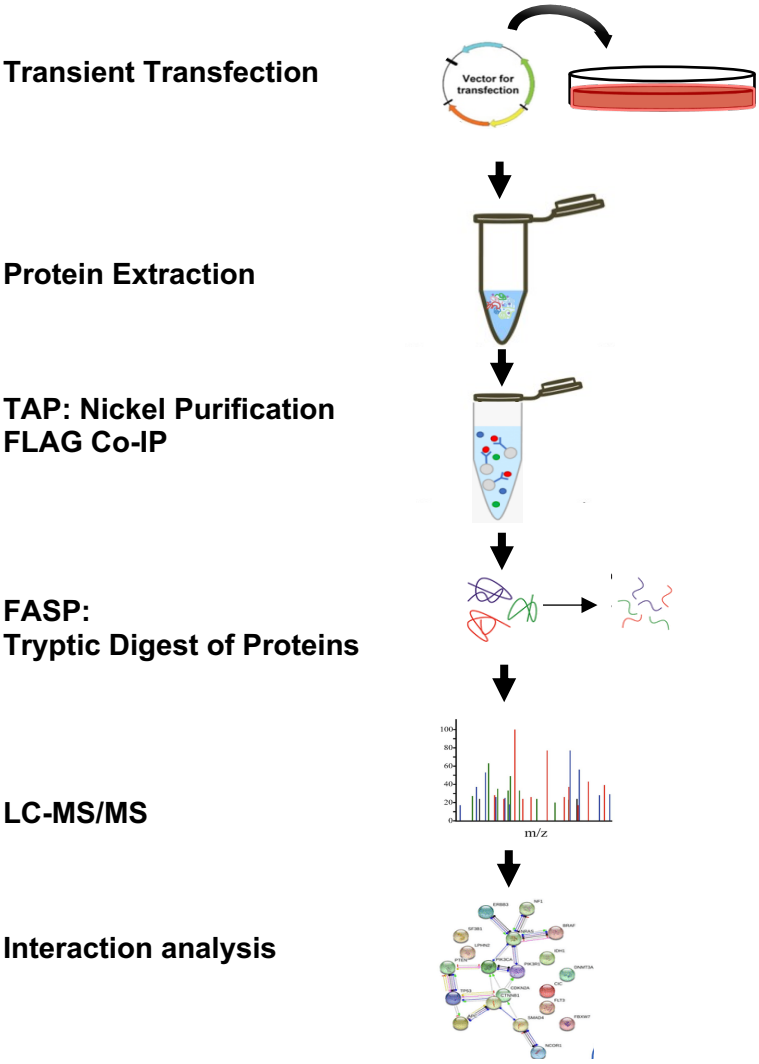

Figure 2.2. Schematic Representation of Proteomic Workflow.
22RV1 Cells were plated to reach 70-80% confluence for transfection with the empty vector control (pCI-Neo) and FKBP51-6XHIS-FLAG or FKBP51-6XHIS-FLAG using Lipofectamine2000. Cells were then lysed, and a nickel purification was performed followed by a FLAG-Co-IP with two different incubation periods (1hr vs O/N). The eluates were Trypsin-digested using a FASP Kit and analyzed by LC-MS/MS. Quantification of the spectral count was performed, and networks were generated and assessed. *B***.** FKBP51 and FKBP52 protein expression is verified using immunoblots for the input and eluate experiments in both conditions 1hr vs overnight.

2.3 RESULTS

2.3.1 Tandem Affinity purification of FKBP51 and FKBP52 Western Blots

To identify novel protein interactions for FKBP51 and FKBP52, we performed tandem affinity purification of both proteins in the presence or absence of hormone in a castration resistant cell model, 22RV1. Western blot analysis of cell lysates following initial transfection with His- and FLAG- tagged FKBP51 and FKBP52 plasmids, demonstrate successful expression of tagged protein in 22RV1 cells to be used for tandem affinity purifications (Figure 2.3A, B). Transfection did not alter the cells' endogenous protein expression, as seen in the empty vector lane indicating the presence of endogenous FKBP51 in 22RV1 cells when compared to the endogenous expression of transfected cells. A fraction of the final eluate was also analyzed by Western Blot to verify pull-down of target proteins, FKBP51 and FKBP52 (Figure 2.3C). No protein band is present in the empty vector eluate probing for FKBP51, but there appears to be some non-specific binding of FKBP52 in the same empty vector eluate. Taking these observations into consideration, we generated and compared the interactome for empty vector or FKBP transfected cells to exclude non-specific protein interactions that were found in the empty vector control.

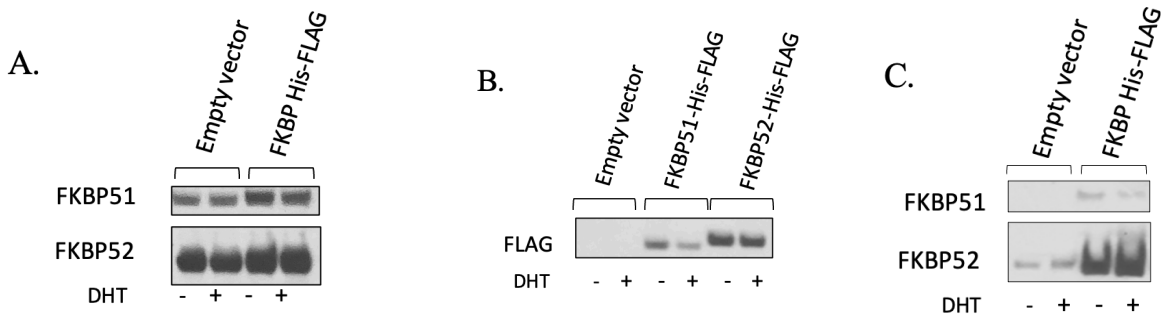

Figure 2.3. Western Blot to confirm transfection and pull down of target proteins. **A.** Western blot analysis showing FKBP51 and FKBP52 protein expression in 22RV1 whole-cell lysate following transient transection of His-Flag-tagged FKBP51 or FKBP52. **B.** Western blot analysis showing FLAG protein expression in 22RV1 whole-cell lysate following transient transfection of PDS-404 empty vector, His-Flag-tagged FKBP51 or His-Flag-tagged FKBP52. **C.** Western blot analysis showing tandem affinity purification of His-FLAG-tagged FKBP51 or His-FLAG-tagged FKBP52 following treatment with DHT or EtOH vehicle control in 22RV1 cells.

2.3.2 FKBP51 and FKBP52 associated proteins visualized by Heat Map

In collaboration with Dr. Sourav Roy (UTEP), the heatmaps of the total and top interactors were generated. The top 50 proteins associated with FKBP51 and FKBP52 with or without hormone were selected and shown in Figure 2.4. Tandem MS/MS spectra were searched against a protein database of *Homo sapiens*. The increased presence of proteins across all samples was annotated based on their high spectral counts and represented in red, while low spectral counts are represented in blue. The first step in analyzing this interactome was to survey all heatmaps and STRING networks (not all images are presented in this thesis) to find already established protein interactions with the goal of corroborating the validity of this dataset. The interaction between FKBP51 and FKBP52 with Hsp90 is well-established and deemed crucial for AR regulation (Buchner, Weikl et al. 1998, Pirkl and Buchner 2001). Therefore, the presence of Hsp90 across the four conditions presented (Figure 2.4 black arrows) is one example that demonstrates that the complexes isolated through tandem-affinity purifications are biologically relevant.

From this interactome, we have identified several interaction partners whose role in the AR-Hsp90 heterocomplex is worth investigating. Among these candidate proteins, the peroxiredoxin family of proteins stood out for the following reasons. Our lab has previously conducted a genomic analysis on FKBP52-regulated hormone induced transcription (Ortiz 2021). ChIP and RNA-seq data illustrated the differences in gene expression between wild-type 22RV1 cells and 52KO 22RV1 cells in response to hormone. The results showed differences in AR DNA binding sites as well as changes in gene expression dysregulated in the absence of FKBP52. The network analysis of the pathways affected revealed mitochondrial disfunction and dysregulation of oxidative phosphorylation pathways, which complement the proteins found in the interactome. Additionally, there are multiple reports in the literature describing peroxiredoxin overexpression in various types of cancers (Shiota, Yokomizo et al. 2011, Whitaker, Patel et al. 2013, Rafiei, Tiedemann et al. 2015, Bajor, Zych et al. 2018, Thapa, Jiang et al. 2023).

The top 50 interactor heatmap for FKBP51 and FKBP52 identified PRDX3 as one of the top interacting proteins. Based on the spectral counts, FKBP2 with or without hormone appears to have a higher degree of association (red) with PRDX3 when compared to FKBP51 under the same conditions (blue). Despite the difference in the presence of PRDX3 across all samples, its presence is consistent with previous reports of PRDX3 upregulation in anti-androgen resistant PCa cell lines (Whitaker, Patel et al. 2013). Out of all six peroxiredoxins in the family, PRDX5 was not present in any of the samples across all conditions. This may not be an absolute indication that an association exists, but rather may be affected by the conditions of the experiment.

2.3.3 Network analysis of FKBP51 top 100 interactors reveals oxidative pathways

The network analysis shows the predicted associations for particular sets of proteins. The top 100 proteins for FKBP51 (not shown) were selected and further used to measure the interactions between them. The protein-protein interaction (PPI) networks shown in Figure 2.5 with the original network (A) depict 94 nodes and 424 edges. The original interaction network was further divided into significant clusters based on the Minimal Common Data Elements (MCODE) algorithm. Figure 2.5B shows cluster 1 which has 18 nodes and 69 edges. Cluster 2 (Figure 2.5 C) contains 8 nodes with 15 edges as shown in Figure 2.5 (B, C). Gene Ontology (GO) term enrichment analysis of FKBP51 was used to generate the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) network for interacting partners' graphic representation (Figure 2.4). The GO and KEGG pathway analysis for the top two clusters was performed using ClueGO. The biological processes in common between the genes depicted in cluster 2 and the percent distribution are presented in Figure 2.5D.

The pathway analysis of top FKBP51 interactors identified several genes involved in oxidative pathways. Depicted by its gene name, ANXA2 in cluster 2, Annexin A2 is suggested as a novel cellular redox regulatory protein in cancer progression by acting as a cellular redox regulatory protein (Figure 2.5C) (Madureira and Waisman 2013). Additionally, cluster 2 shows PRDX1-3, which are also involved in redox regulatory pathways and may be involved in cancer progression.

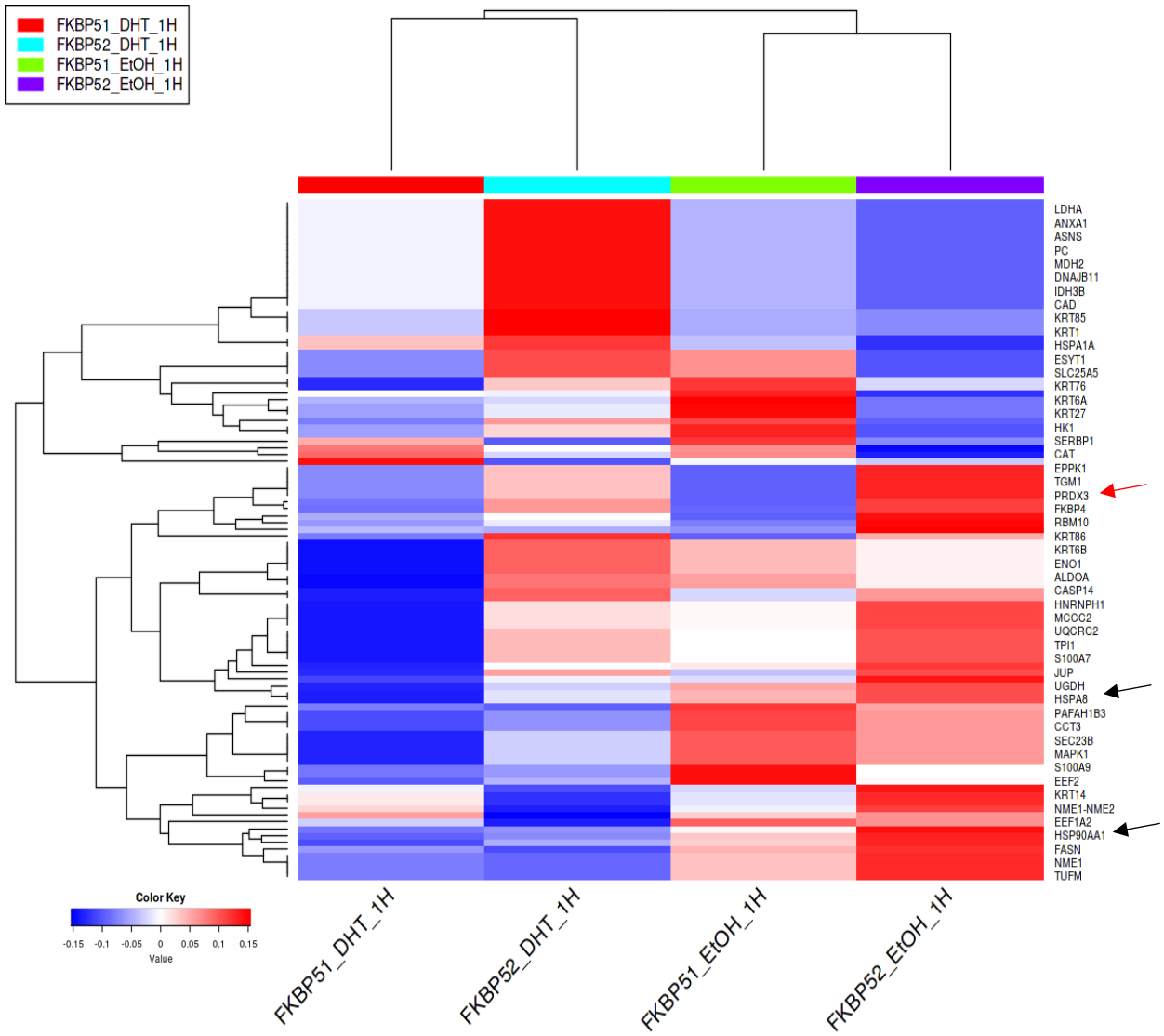

Figure 2.4. Top 50 proteins associated in FKBP51 and FKBP52.
Heat map representations show the top 50 interacting proteins across the four samples analyzed. The increased presence of proteins across all samples was annotated based on their high spectral counts (represented in red). White denotes no significant difference in protein pulled down from all samples and blue denotes a lower spectral count (decreased presence of protein pulled down). Our proteome dataset identified several Peroxiredoxins, such as Peroxiredoxin 3 (PRDX3), as one of the top 50 interactors for FKBP51 and FKBP52 (red arrow). Black arrows point to Hsp90 protein interactions, which are established interactions and indicate the physiological relevance of our findings.

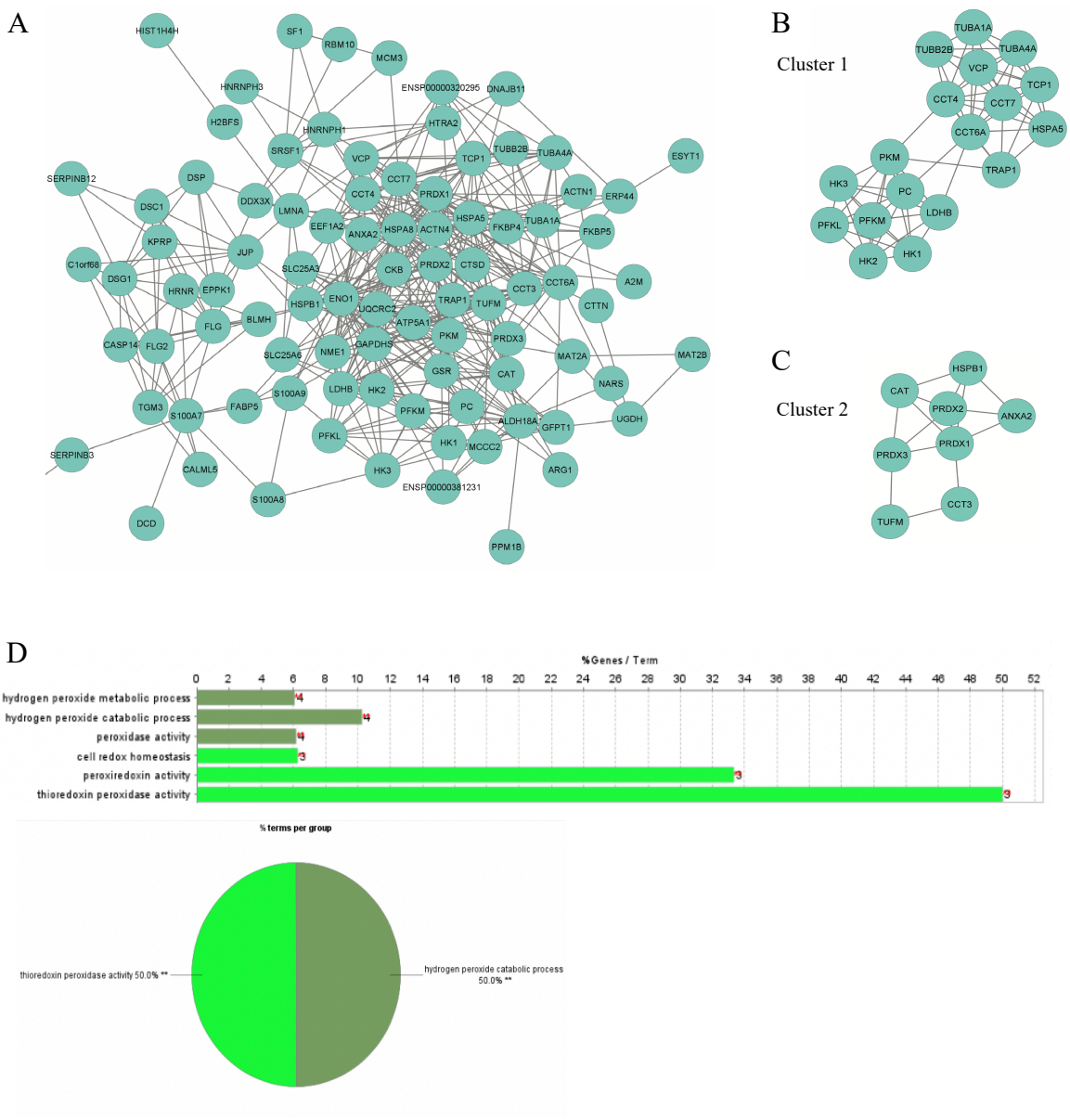

Figure 2.5. Pathway analysis of FKBP51 interactors reveals oxidative pathways. **A.** The original interaction network was generated by measuring interactions between the top 100 associated proteins for FKBP51. The network nodes represent proteins, while the edges represent the interaction between them. Proteins are represented by their respective NCBI gene names. **B, C.** The interaction network was further divided into two significant clusters based on the MCODE algorithm where cluster C shows a network of peroxiredoxins. **D.** GO_BP and KEGG pathways for cluster C demonstrate biological processes in common (top) between genes depicted in cluster C along with percent distribution (bottom).

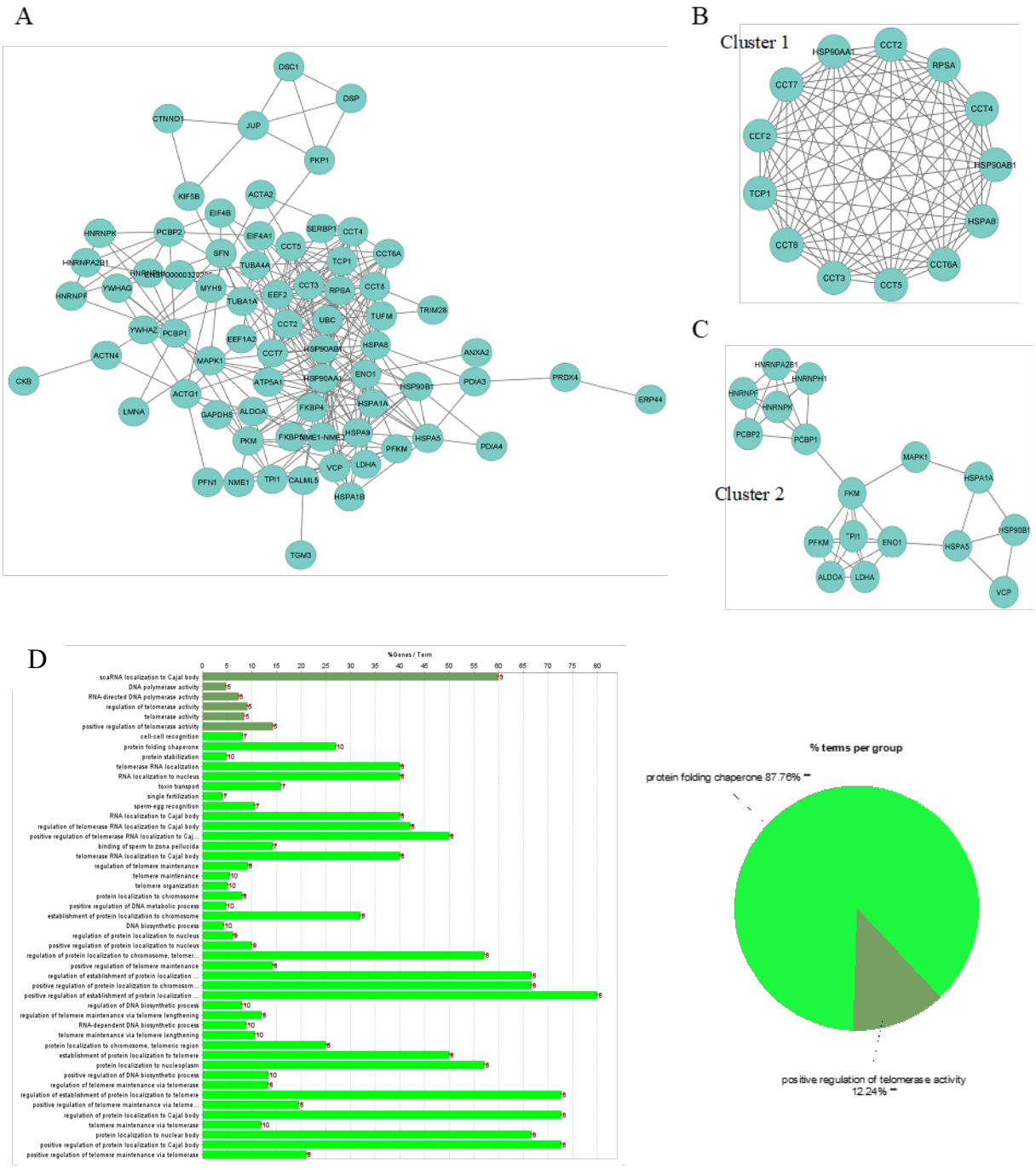

Figure 2.6. Pathway analysis of FKBP52 interactors suggests role in protein folding
The original interaction network was generated by measuring interactions between the top 100 associated proteins for FKBP52. The network nodes represent proteins, while the edges represent the interaction between them. Proteins are represented by their respective NCBI gene names. **B, C.** The interaction network was further divided into two significant clusters based on the MCODE algorithm where cluster 2 shows a network of protein folding chaperones and regulators of telomerase activity. **D.** GO_BP and KEGG pathways for cluster 2 demonstrate biological processes in common (left) between genes depicted in cluster 2 along with percent distribution (right).

CHAPTER 3: VALIDATING LIGAND-DEPENDENT FKBP51 AND FKBP52 PROTEIN INTERACTIONS

After FKBP51 and FKBP52 and their co-immunoprecipitated interacting partners were identified by mass spectrometry, subsequent experiments were conducted to validate these interactions and identify the putative binding site for FKBP52 interactors.

3.1 RATIONALE

The FKBP51 and FKBP52 proteomic analysis from Aim 1 identified 395 unique proteins in complex with both immunophilins in the presence or absence of hormone. Naturally, the next step was to streamline the multitude of interactors to validate those of interest. Among the entire dataset of interactors, we found a major family of antioxidant proteins, peroxiredoxins, which play a crucial role in regulating reactive oxygen species (ROS) and protecting cells from oxidative stress. This observation is consistent with previous studies from our lab in which changes in gene expression influenced by FKBP52 were characterized using 22RV1 PCa cells with or without FKBP52 and hormone. This previous pathway analysis identified dysregulation of oxidative phosphorylation as the major pathway affected in the absence of FKBP52 (Ortiz 2021). Considering the crucial role peroxiredoxins play in oxidative pathways, our genomic and proteomic data suggest an interplay between FKBP51, FKBP52, and peroxiredoxins in PCa cells. We therefore focus our attention on validating these protein interactions and studying their role in protection against ROS.

Peroxiredoxins constitute a family of six peroxidases (PRDX1-6) ubiquitously expressed in mammalian tissues (Rhee and Kil 2017, Rhee, Woo et al. 2018, Stancill and

Corbett 2023) that serve as antioxidant enzymes to protect cells against reactive oxygen species (ROS) (Graves, Metukuri et al. 2009). The role of peroxiredoxins in therapeutic resistance and cancer progression has been suggested by high levels of different peroxiredoxins in certain chemo- and radiotherapy resistant tumors and cancer derived cell lines. (Ishii, Warabi et al. 2012, Nystrom, Yang et al. 2012, Perkins, Poole et al. 2014).

The involvement of Peroxiredoxins in prostate cancer has been previously documented in the literature. Survival and progression of prostate cancer has been linked to overexpression of PRDX1-4 (Basu, Banerjee et al. 2011, Riddell, Bshara et al. 2011, Shiota, Yokomizo et al. 2011, Whitaker, Patel et al. 2013), and PRDX6 (Raatikainen, Aaaltomaa et al. 2015). It is important to note that PRDX1 has been corroborated to interact with AR and enhances its transactivation, which further links peroxiredoxins to AR regulation (Park, Yu et al. 2007). Upregulation of Peroxiredoxin 3 (PRDX3) protein expression, but not mRNA, was reported in PCa cells and prostatic intraepithelial neoplasia (Lin, Xu et al. 2007, Whitaker, Patel et al. 2013). Whitaker *et al.* explored the effect of PRDX3 overexpression and knockdown on oxidative stress response and found that cells resistant to hydrogen peroxide (H_2O_2) induced apoptosis had upregulated PRDX3; while knocking down PRDX3 resulted in higher susceptibility to H_2O_2 mediated apoptosis (Whitaker, Patel et al. 2013). Heatmap visualization of protein interactors across all conditions, FKBP51 with or without hormone and FKBP52 with or without hormone, revealed PRDX3 to be one of the top 50 interacting proteins (Figure 2.3).

PRDX6 is perhaps the most fitting example of the relevance of this interactome in the FKBP-SHR heterocomplex since uterine levels of PRDX6 were significantly lowered in FKBP52-KO mice when compared to wild type (Hirota 2010). Most importantly, Hirota

et al. demonstrated physical association between FKBP52 and PRDX6 in the deciduum. Further research is necessary to better understand the signaling influence by peroxiredoxins on FKBP-mediated cancer progression, but these reports posit peroxiredoxins as key regulators in cancer cell survival and treatment resistance. These studies provide insights into the mechanisms underlying therapeutic resistance and suggest that targeting peroxiredoxins could be a potential therapeutic strategy for PCa.

I hypothesize that these immunophilins interact with members of the PRDX family contributing to oxidative stress resistance, and that targeting FKBP51 and FKBP52 can sensitize PCa cells to ROS. To address the first part of this hypothesis, I performed purified recombinant protein pull-downs to assess the ability of peroxiredoxins to interact directly with each FKBP, and reciprocal co-immunoprecipitations targeting each PRDX to determine if these interactions are consistent in a larger complex of cell proteins. Furthermore, we aimed to characterize the peroxiredoxin interaction domain on FKBP52 by utilizing previously established FKBP52 single point mutants at the PPIase pocket, the TPR domain, and the Proline rich loop to verify which, if any, could abrogate these interactions. To assess FKBP52's role in oxidative stress response for PCa cells, I assessed apoptotic markers in FKBP52-KO 22RV1 cells in response to H_2O_2 treatment resulting ROS-induced apoptosis. and to verify FKBP52's role in prostate cancer cell survival in response to oxidative stress.

3. 2 Materials and Methods

3.2.1 Cell Culture

The 22RV1 FKBP52-KO cell line was obtained from Synthego and maintained in Hyclone™ RPMI 1640 media containing 25mM HEPES and L-glutamine (GE Healthcare

Life Sciences) supplemented with a final concentration of 10% FBS (Corning). Both cell lines were maintained at 37ºC in a humidified atmosphere of 5% CO_2.

3.2.2 Transient Transfections

The 22RV1 FKBP52-KO cell line was used to transfect WT FKBP52 or mutant FKBP52 along with each peroxiredoxin. Table 3.1 shows the list of expression vectors along with the protein encoded and source of the plasmid. Cells were seeded in 10mm dishes at 4x106 to reach confluency of 80% the next day. On the day of transfection, 10ug of each plasmid and lipofectamine were mixed with RPMI separately. Plasmid solution and Lipofectamine solution were mixed at a final ratio of 1:3 (DNA:Lipofectamine) and incubated for 15mins at room temperature. Cell dishes were rinsed with PBS twice and RPMI media supplemented with charcoal stripped FBS was added to each plate. Plasmids were then added in duplicates to account for DHT and EtOH treatment the next day. Media was not replaced and cells were allowed to incubate with DNA/Lipofectamine solution for 21hrs prior to lysis.

3.2.3 FLAG Purified Protein Pull-downs

Anti-FLAG M2 magnetic beads (20uL slurry; Sigma-Aldrich) were washed twice with 10 packed gel volumes of TBS for each pull-down reaction. Purified human recombinant proteins FKBP51, FKBP52, and FLAG-tagged PRDX1-6, were resuspended in TBS (50mM Tris pH7.5, 150mM NaCl) and 500ng of FKBP51 or FKBP52 were incubated alone or in combination with 500ng of each FLAG-tagged PRDX in a final concentration of 0.05% Nonidet P40. FKBP51 and FKBP52 were used as negative controls due to their lack of a FLAG tag. All samples were incubated for 2hrs at 4 ºC with

end-over-end mixing. The samples were then washed three times with TBS+0.05% Nonidet P40 and eluted by competitive elution using 3uL of 3X FLAG peptide at 5ug/uL diluted in 60uL of TBS without detergent. Elution reactions were incubated for 30mins at 4 ºC with end-over-end mixing.

3.2.4 His-tag Purified Protein Pull-downs

His-Pur Ni-NTA Magnetic Agarose Beads (4uL slurry; ThermoScientific™) were washed three times with 10 packed gel volumes of TBS for each pull-down reaction. Purified human recombinant proteins His-tagged FKBP51, His-tagged FKBP52, and FLAG-tagged PRDX1-6, were resuspended in TBS (50mM Tris pH7.5, 150mM NaCl) and 500ng of FKBP51 or FKBP52 were incubated alone or in combination with 500ng of each PRDX in a final concentration of 0.05% Nonidet-P40 and 30mM Imidazole for 1hr at 4 ºC with end-over-end mixing. In parallel, PRDXs were used as negative controls due to their lack of a FLAG tag. The samples were then washed three times with TBS+0.05% Nonidet-P40 and 50mM Imidazole and eluted by competitive elution using TBS+0.05% Nonidet-P40 and 300mM Imidazole. Elution reactions were incubated for 15mins at RT with end-over-end mixing and vortexing every 5 mins. All samples were collected and processed for gel electrophoresis and Western Blot analysis.

3.2.6 FLAG Co-Immunoprecipitations

While interaction of proteins in the absence of other proteins can provide insight into possible protein-protein interactions, it is important to further assess protein interactions in a cell-based environment for more biologically relevant results. For the Co-IP experiments, 30µL of FLAG magnetic bead slurry (Thermo Scientific) per sample were added to microcentrifuge tubes and placed into the magnet to remove storage buffer.

Resin beads were then washed twice with ice-cold TBS + 0.05% Nonidet P40 (150 mM NaCl, 50 mM Tris). Cells were washed three times with ice-cold PBS and lysed with TBS + 0.1% Nonidet P40 and protease/phosphatase inhibitor cocktail (Thermo) (150 mM NaCl, 50 mM Tris) for 30mins with gentle shaking, transferred to a microcentrifuge tube and centrifuged at maximum speed at 4°C for 20mins. The cell lysate was then pre-cleared using mouse IgG isotype agarose beads (ThermoFisher) for 1hr at 4°C with end-over-end rotation. The pre-cleared cell lysates were then incubated with anti- FLAG magnetic beads for 1hr at 4°C with end-over-end rotation. Lysate/magnetic bead complexes were washed twice with ice-cold TBS+0.05% Nonidet-P40 followed by two washes with TBS without detergent. Elution was done by competitive elution using 3uL of 3xFLAG peptide at 5ug/5uL diluted in 60uL TBS without detergent and incubated for 30mins at 4°C with end-over-end rotation. All samples were collected and processed for gel electrophoresis and Western Blot analysis.

3.2.7 Western Blotting

Samples separated by gel electrophoresis were transferred to Polyvinylidene Fluoride Membranes (PVDF, Thermo Scientific) at a constant voltage of 100V for 1.5hrs using ice-cold Transfer buffer containing 20% Methanol. Membranes were blocked using room temperature 5% skim milk in TBS-T (pH 7.4 PBS with 0.05% Tween-20) for 30mins and incubated with the following primary antibodies overnight at 4°C with gentle rocking: mouse monoclonal anti-FKBP51 Hi51B (1:5,000) and anti-FKBP52 Hi52D (1:10,000) prepared in the David F. Smith Lab, anti-FLAG M2 antibody (1:6,000; SIGMA), PRDX1-6 antibodies at varying concentrations (Proteintech), and mouse monoclonal GAPDH antibody (1:5,000; Santa Cruz Biotechnology). Membranes with primary antibodies were

washed four times for 5mins each wash in TBS-T (0.05% Tween-20) with rocking. The proteins of interest were then detected using goat anti-mouse or goat anti-rabbit AP-conjugated secondary antibodies (dilution ratio 1:10,000 Santa Cruz Biotechnology) for 1hr at room temperature. The membranes were developed with Immuno-StarTM AP substrate (BioRad) and exposed on CL-X PosureTM film (ThermoScientific).

3.2.8 Cell viability assays

FKBP52-KO 22RV1 cells were seeded in 96-well plate at $2x10^5$ cells per well to reach 70% confluence the next day. Upon reaching desired confluency, FKBP52-KO 22RV1 cells were transfected with Empty vector or WT-FKBP52 using Lipofectamine2000 at a ratio of 1:3 of DNA to Lipofectamine. The following day, DNA/Lipid complexes were removed, cells were washed twice with PBS and starved using serum-free RPMI for 24hr. After the starvation period, media was replaced with RPMI+10% FBS and cells were treated with increasing concentration of H_2O^2 (0-0.00408%) or media alone for an additional 24hrs. On the last day, cells were stained with 1ug/mL of Hoechst and PI for 30mins at 37°C to measure cell viability using ImageXpress Pico Automated Cell Imaging System (Molecular Devices).

3.2.9 Apoptotic marker array

FKBP52 KO 22RV1 cells were seeded in 6-well plates at a density of $8x10^5$ cells in 1.5mL Hyclone™ RPMI 1640 media containing 25mM HEPES and L-glutamine (GE Healthcare Life Sciences) supplemented with a final concentration of 10% FBS (Corning). The cells were transfected using Lipofectamine®2000 reagent (Invitrogen™, Thermo Scientific) with empty vector or wild type FKBP52 and incubated for 24hrs. Next, cells were washed twice with PBS and serum starved for 24hrs before treatment with 0.00272% H_2O_2 for an additional 24hrs. On the day of the assay, human apoptosis

proteome profiler antibody array kit (R&D Systems, Abingdon, UK) was used to identify apoptotic markers according to manufacturer instructions. First, nitrocellulose membranes containing capture antibodies were blocked for 1hr on a rocking platform using provided Array Buffer 1. Meanwhile, cells were lysed with provided R&D lysis solution and protein concentration was assessed by BCA assay. Of total protein lysate, 250ug were added to a final volume of 1.5ml Array Buffer 1 and incubated with blocked membranes overnight at 4°C. A fraction of cell lysate was set aside for western blot analysis of transfected FKBP52. The following day, membranes were washed three times for 10mins with 1x wash buffer. Membranes were then incubated for 1hr at RT with Detection Antibody Cocktail diluted in 1x Array buffer 2/3. Membranes were washed three times, incubated with Streptavidin-HRP for 30mins at RT, and washed three more times. HRP Chemi Reagent mix was added to all membranes which were then imaged by X-ray film exposure.

3.2.10 Apoptotic marker expression quantification

Developed films from 3.2.9 were scanned and processed using an image analysis software. Pixel densities of all duplicate spots representing each apoptosis-related protein were obtained using ImageJ. To determine differences in mean pixel intensity, two-way ANOVA followed by Bonferroni post-hoc test was applied across groups. Significant differences were accepted for $p<0.05$.

Table 3.1. Mammalian expression plasmids for Co-Immunoprecipitations.
The table summarizes the expression vectors used in this study. All plasmids contain a CMV promoter for mammalian expression. Ampicillin resistance gene is present in pCI-neo plasmids, while Kanamycin resistance gene is present in pCMV6 plasmids.

Expression vector	Gene	Description	Source
pCMV6	PRDX1	Myc-DDK-tagged human peroxiredoxin 1	Origene #RC221235
pCMV6	PRDX2	Myc-DDK-tagged human peroxiredoxin 2	Origene #RC207413
pCMV6	PRDX3	Myc-DDK-tagged human peroxiredoxin 3	Origene #RC205080
pCMV6	PRDX4	Myc-DDK-tagged human peroxiredoxin 4	Origene #RC203330
pCMV6	PRDX5	Myc-DDK-tagged human peroxiredoxin 5	Origene #RC210709
pCMV6	PRDX6	Myc-DDK-tagged human peroxiredoxin 6	Origene #RC207780

3. 3 RESULTS

3.3.1 Reciprocal Co-Immunoprecipitations validate Peroxiredoxin interactions.

In Aim 1, I identified PRDX1-6 as interacting proteins that were isolated in complex with our bait proteins, FKBP51 and FKBP52. After identifying these interactions using mass spectrometry, I verified them by employing reciprocal co-immunoprecipitations targeting PRDX1-6 as bait. It is imperative to validate the interactions identified in the previous analysis to confirm the proteins identified represent functionally relevant interactions and not the product of antibody cross-reactivity or non-specific binding to the agarose beads.

A fraction of lysate was saved to verify transfection of each FLAG-tagged peroxiredoxin and endogenous expression of FKBP51 or FKBP52. FLAG blots demonstrate successful transient transfection of all PRDXs, which can also be seen in each PRDX blot by the presence of a higher molecular weight band due to the additional tags (His-FLAG-HA) on each peroxiredoxin (Fig.3.1). Output samples from eluted Co-IP complexes demonstrate that FKBP51 and FKBP52 consistently associate with PRDX 2, 3, and 4 following incubation with 1nM DHT or Ethanol (Fig.3.1A, B). Association of FKBP52 with PRDX2 and 3 is increased in the presence of DHT when compared to

Ethanol treatment, while PRDX4 association with both FKBP51 and FKBP52 does not seem to be affected by DHT treatment Association of FKBP51 and FKBP52 with PRDX1 can also be seen, but with much less intensity than other interactions and seem to be stronger in the presence of DHT. In most experiments, PRDX5 association was not visible and was only seen when using low concentrations of NaCl and detergent. This is an indication that, if there is a PRDX5 association with the FKBP, it may be a sensitive or transient interaction, which resembles the lack of interaction seen in Aim 1.

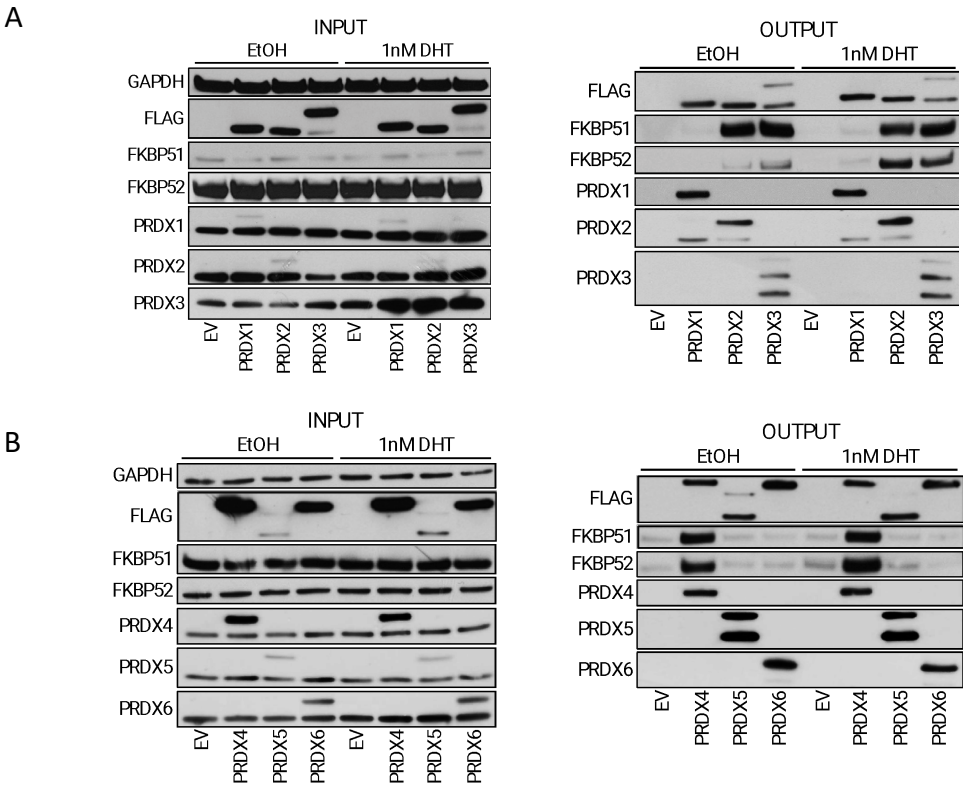

Figure 3.1. Reciprocal Co-IP targeting FLAG-tagged Peroxiredoxins 1-6.
A, B. 22RV1 cell lysates were probed for transfected FLAG-tagged PRDX1-3 **(A)** and 4-6 **(B)**, as well as endogenous FKBP51 and FKBP52 (Left). Eluted Co-IP complexes demonstrate that FKBP51 and FKBP52 associate with PRDX 2, 3, and 4 following incubations with 1nM DHT or Ethanol (Right). Association of FKBP52 with PRDX2,3 is increased with DHT, while PRDX4 association with both FKBPs is comparable following treatment with DHT or Ethanol.

3.3.2 Purified Protein Pull-downs suggest direct association for PRDX3.

Based on the interactome dataset in Aim 1 and the above co-immunoprecipitations, we then tested whether FKBP51 and FKBP52 had the ability to directly associate with any of the PRDXs in the absence of other proteins. To this end, I performed recombinant purified protein pull-down assays by two means: targeting FLAG-tagged PRDX1-6 and targeting His-tagged FKBP51 and FKBP52. All tagged recombinant purified proteins were obtained from Origene. Anti-FLAG magnetic beads (SIGMA) were used to target FLAG-tagged PRDX1-PRDX6 as bait in the presence of His-tagged FKBP52. Due to their lack of a FLAG tag, FKBPs were also incubated alone as a negative control to determine any level of nonspecific binding to the beads. Eluates processed for western blot analysis suggest a direct FKBP52 association with PRDX1-4 and PRDX6 with no visible signal from the negative control lane (Figure 3.2).

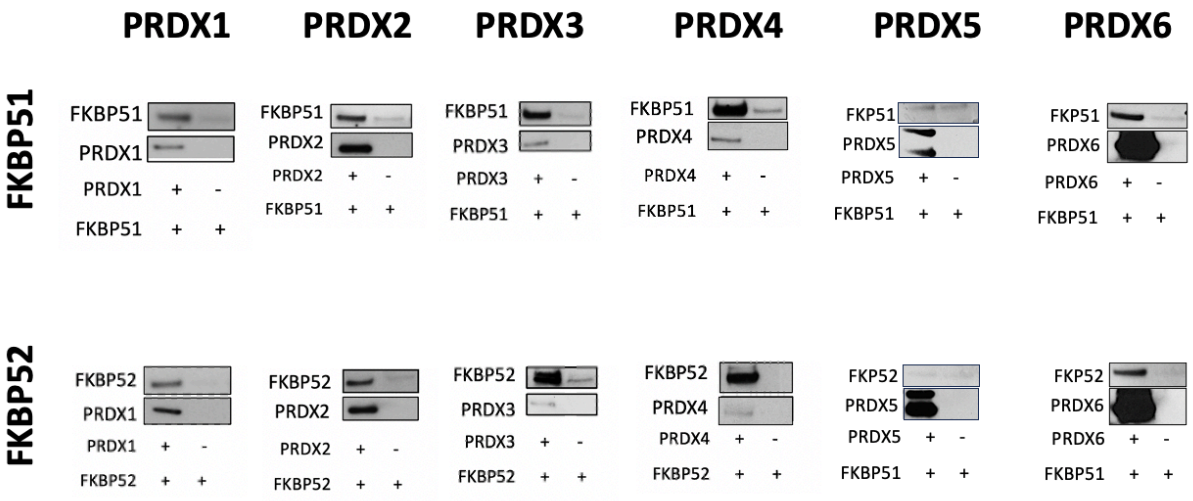

Figure 3.2. FLAG-tagged Recombinant purified protein pull-down.
Top. Input samples for all purified protein reactions demonstrate the presence of each FLAG-tagged peroxiredoxin and FKBP52, alone or in combination. **Bottom.** Anti-FLAG magnetic beads were used to pull down purified recombinant FLAG-tagged PRDX1-PRDX6 as bait. His-tagged FKBP52 was incubated with all bait proteins and alone as a negative control.

To ensure associations were consistent by other means, I performed His-tagged protein pulldowns by targeting FKBP51 and FKBP52 as bait and used FLAG-tagged PRDX1-6 as negative controls due to their lack of a His-tag. Ni-NTA magnetic beads were used to pull down His-tagged FKBP51 or FKBP52 in the presence of FLAG-tagged PRDX1-6. FKBP51 pull-downs suggest there is a direct interaction with PRDX1, PRDX3, PRDX5, and PRDX6, but not with PRDX2, and PRDX 4 (Figure 3.3A). FKBP52 pull-downs suggest there is a direct association with PRDX1, and PRDX3-6 but not with PRDX2.

Interestingly, the negative control His-tag pull-down targeting FLAG-tagged PRDX1-6, confirmed the presence of nonspecific interactions to the Ni-NTA beads, in this case PRDX2, PRDX3, PRDX5, and PRDX6. Continuous troubleshooting led to the modification of the binding buffer for the His-tag pull-downs and ultimately resulted in the addition of glycerol. Although not entirely clear, glycerol has been used as a stabilizer for proteins outside of their native environment. Addition of glycerol to the binding buffer reduced or completely abrogated non-specific binding of PRDX2, PRDX3, and PRDX5 (Figure 3.4C) when compared to previous pulldowns (Figure 3.3). Despite this improvement, PRDX1 was now able to bind non-specifically to the Ni-NTA magnetic beads, which had not been seen throughout these experiments (Figure 3.4C). Furthermore, the association of FKBP51 with PRDX5 and FKBP52's association with PRDX4 seem to be lost with the addition of glycerol. Although PRDX1 and PRDX6 seem to associate with FKBP51 and FKBP52 across different conditions for pull-downs, they are also present throughout the negative control reactions. Overall, PRDX3 association with FKBP51 and FKBP52 was observed consistently in all pull-down experiments

regardless of the buffer composition and without any visible non-specific interaction with the Ni-NTA beads.

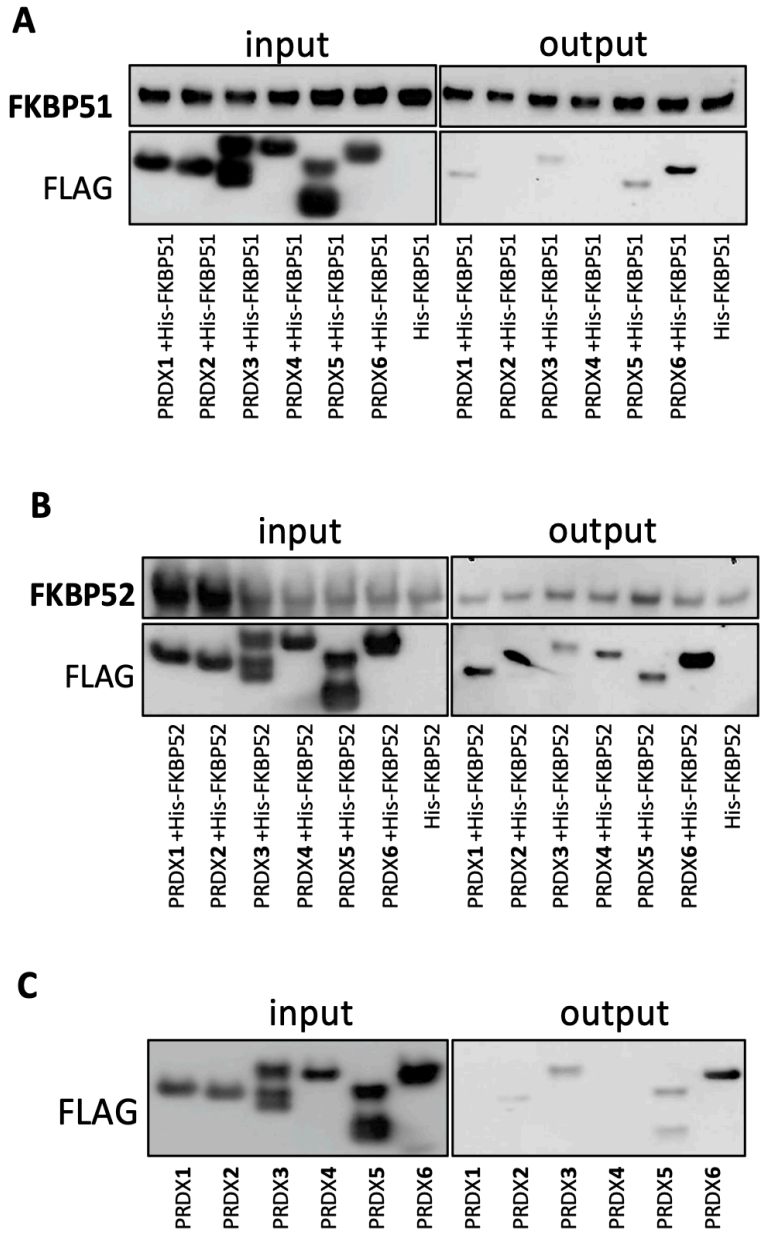

Figure 3.3. His-tagged Recombinant purified protein pull-down.
Ni-NTA magnetic beads were used to pull down His-tagged FKBP51 or FKBP52 in the presence of FLAG-tagged PRDX1-6. **(A, B).** FKBP51-His tag and is seen interacting with PRDX1, PRDX3, PRDX5, and PRDX6 (**A**). FKBP52-His tag pull-down suggests direct interaction with PRDX1, PRDX3, and PRDX6 (**B**). FLAG-tagged PRDX1-6 lacking His-tag were incubated alone with Ni-NTA magnetic beads as negative control to check for non-specific binding of non-target proteins to beads (**C**).

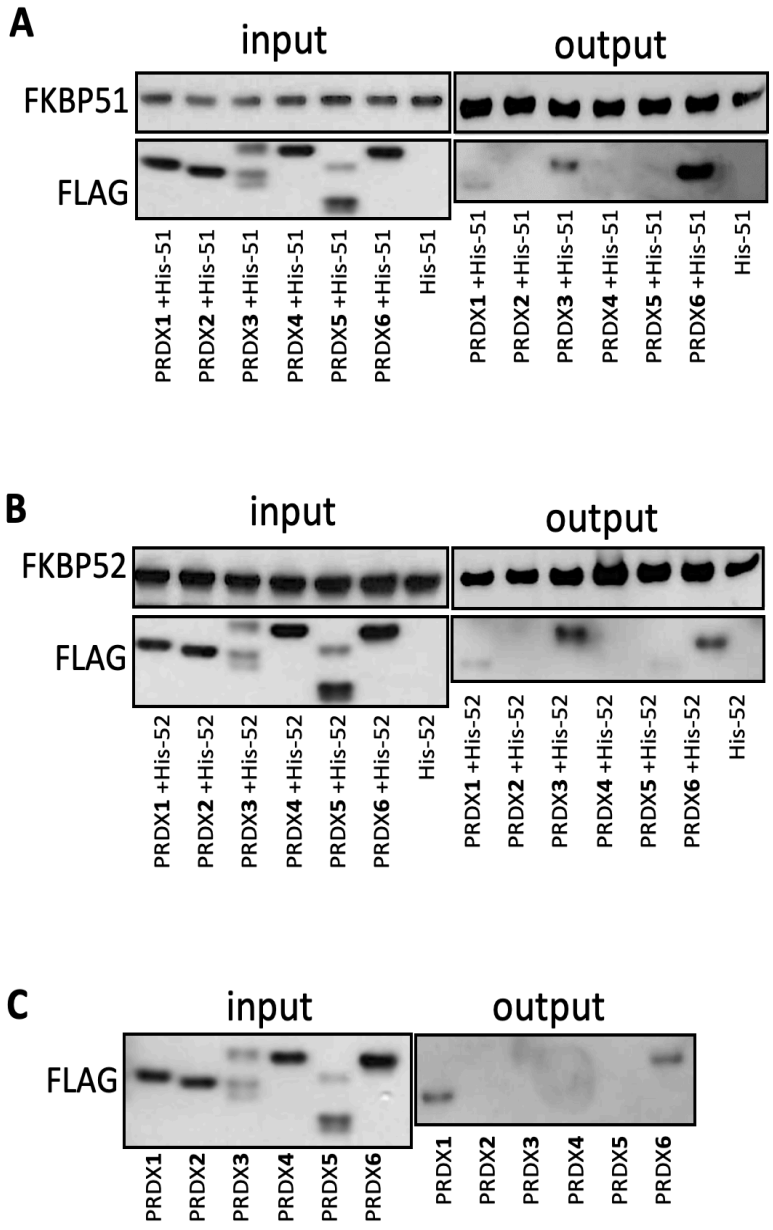

Figure 3.4. His-tagged Recombinant purified protein pull-down.
Ni-NTA magnetic beads were used to pull down His-tagged FKBP51 or FKBP52 in the presence of FLAG-tagged PRDX1-6. Addition of glycerol to the binding buffer reduced non-specific binding when compared to previous pulldowns (Figure 3.3). **A, B.** FKBP51-His tag and FKBP52-His tag pull-down suggest direct interaction with PRDX1, PRDX3, and PRDX6. **C.** FLAG-tagged PRDX1-6 lacking His-tag were incubated alone with Ni-NTA magnetic beads as negative control to check for non-specific binding of non-target proteins to beads.

3.3.3 FKBP52 differentially regulates apoptosis-related proteins under H_2O_2-induced oxidative stress

To determine the effects H_2O_2 on cell viability and apoptosis-related protein expression in the presence or absence of FKBP52, FKBP52KO-22RV1 cells were transfected with EV or WT-FKBP52 and subjected to H_2O_2 treatment for 24hrs. For viability assays, transfected cells were serum-starved for 24hrs followed by increasing concentrations of H_2O_2 (0-0.00408%) for an additional 24hrs. As determined by counterstain with PI and Hoechst, 0.00272% H_2O_2 treatment resulted in approximately 50% cell death in WT-FKBP52 and EV 22RV1 cells (Figure 3.5A). This concentration of H_2O_2 was used for subsequent assays to determine its effect on apoptotic pathways.

To assess H_2O_2-induced oxidative stress on apoptosis-related protein expression in the presence or absence of FKBP52, FKBP52-KO 22RV1 cells were transfected with empty vector or WT-FKBP52 and treated with 0.00272% H_2O_2 for 24hrs. Lysates were analyzed using an apoptotic marker array detection kit. Proteome Profiler Human Apoptosis Array Kit (R&D ARY009) was used to detect expression of 35 apoptosis-related proteins simultaneously. In these arrays, FKBP52-KO 22RV1 cells transfected with FKBP52 showed downregulation of several pro-apoptotic markers under H_2O_2 stress conditions. Cleaved Caspase-3, Phospho-P53(S15), Phospho-P53(S392), Phospho-P53(S46), and Phosphor-P53-Rad17(S635) were downregulated to near or below untreated levels after treatment to induce oxidative stress in cells transfected with WT FKBP52 (Figure 3.6). Pro-apoptotic markers p21 and p27 were significantly upregulated under oxidative stress conditions in the absence of FKBP52 and, although re-introducing FKBP52 did not decrease expression of these proteins to mimic levels in untreated cells, FKBP52 expression did significantly decrease expression of p21 and p27 when compared to EV (Figure 3.7; Table 3.2). Interestingly, catalase, which protects cells against apoptosis, was upregulated in FKBP52-KO 22RV1 cells expressing FKBP52 under stress

conditions (Figure 3.6). Taken together, the expression levels of these apoptosis-related proteins suggest a protective roll for FKBP52 in oxidative stress management.

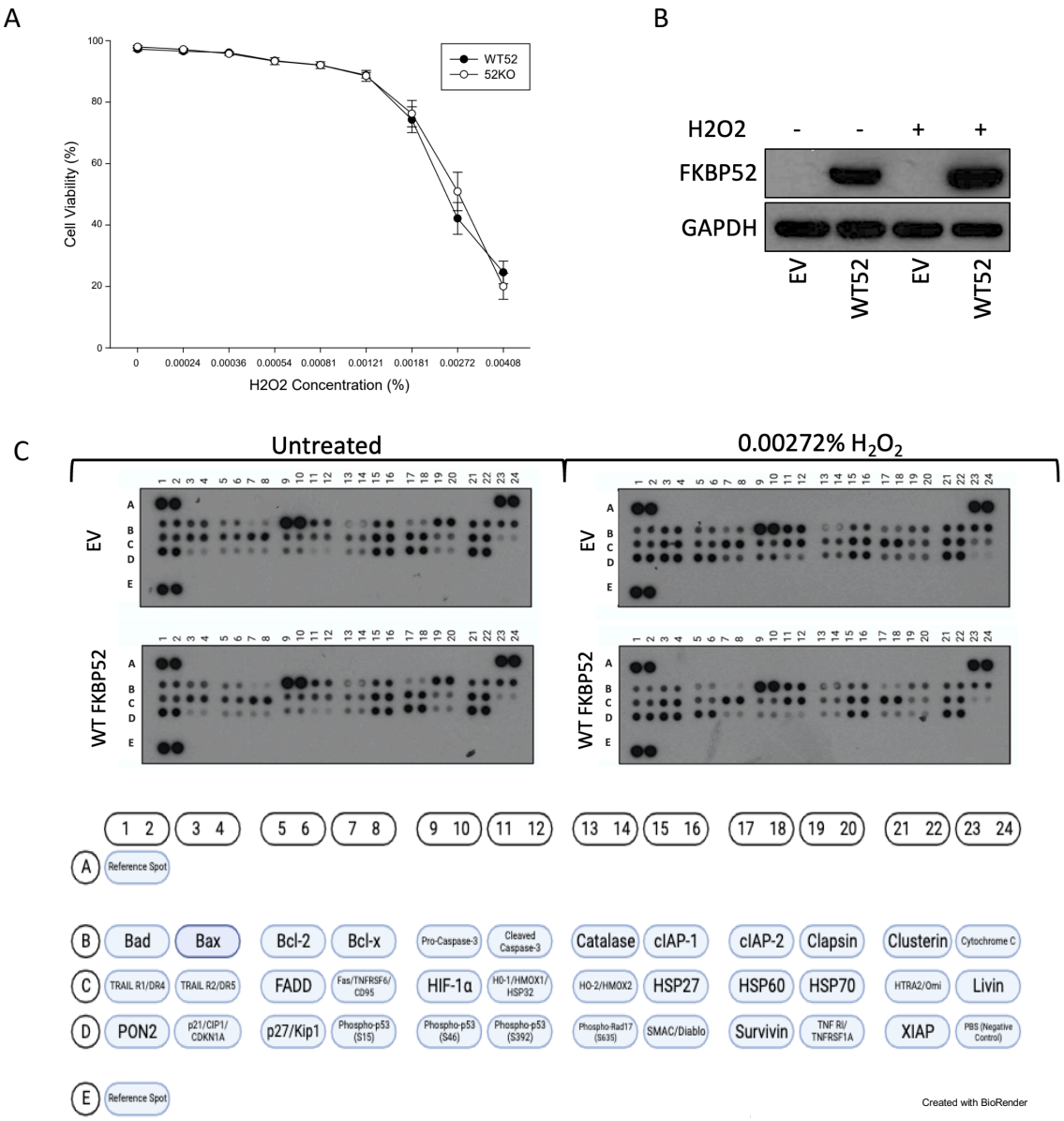

Figure 3.5. Effects of FKBP52 expression in H_2O_2 -induced oxidative stress.
52KO-22RV1 cells transfected with empty vector or WT-FKBP52 were used to study the effects of H_2O_2 on cell viability and apoptotic marker expression **(A).** 52KO-22RV1 cells with or without FKBP52 were incubated with increasing concentrations of H_2O_2 for 24 hours before measuring cell viability. **B, C.** 52-KO 22RV1 duplicate cells transfected with empty vector or WT-FKBP52 were treated with 0.00272% H_2O_2 for 24hrs and lysed for WB verification of FKBP52 expression (**B**) or for apoptosis marker expression using Human Proteome Profiler Apoptosis Array kit (**C**).

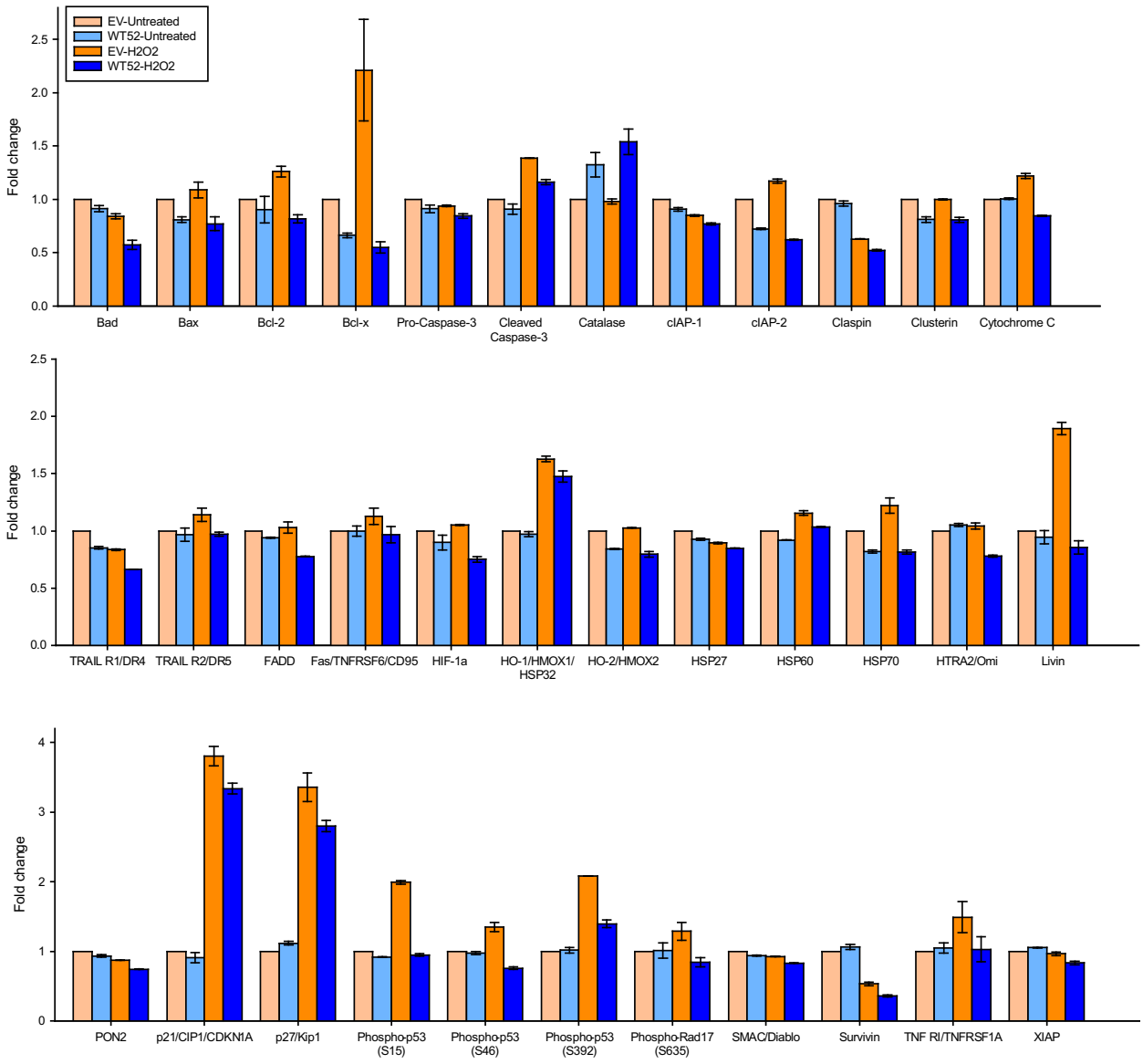

Figure 3.6. Quantification of relative levels of 35 Human Apoptosis related proteins. Proteome Profiler Human Apoptosis Array kit was used to process 22RV1 lysates following H_2O_2 treatment in the presence or absence of WT-FKBP52. Pixel intensity for each of the 35 protein dots across four conditions was quantified using ImageJ and statistical analysis consisted of two-way ANOVAs followed by Bonferroni post-hoc test to reveal statistical significance across groups. For fold-change quantification and visualization, pixel intensity for each protein was normalized to EV-untreated samples.

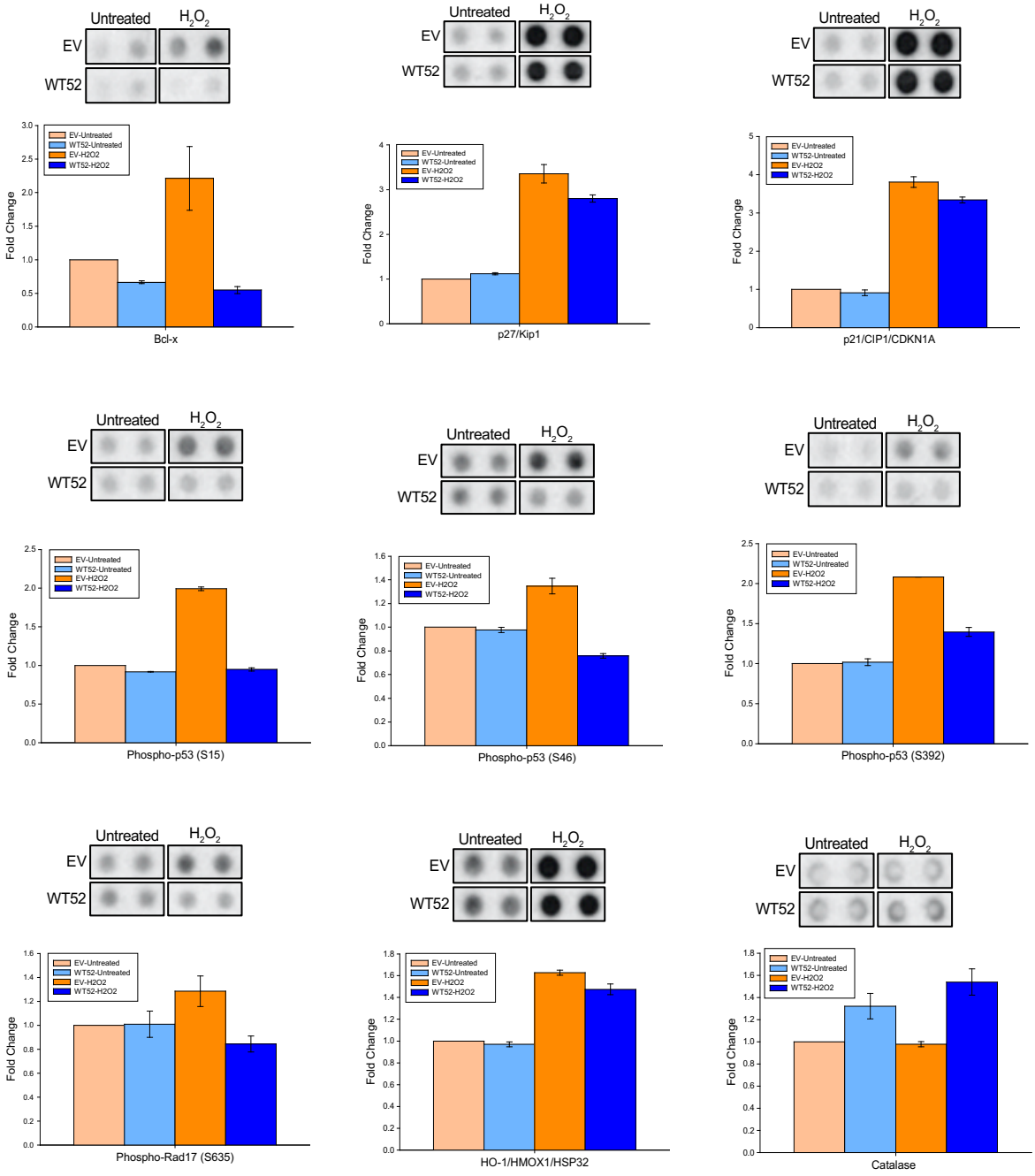

Figure 3.7. FKBP52 differentially regulates several apoptosis-related proteins in H_2O_2-induced oxidative stress.

To determine the role of FKBP52 expression on apoptotic markers under oxidative stress conditions, FKBP52KO cells were transfected with EV or WT52 and treated with a low concentration of H_2O_2 for 24hrs. Visually notable differences in marker expression supported by two-way ANOVAs and Bonferroni post-hoc test are visualized here with their respective fold-change bar graphs. All pro-apoptotic markers were significantly upregulated after H_2O_2 treatment in the absence of FKBP52. Exogenous expression of FKBP52 significantly decreased expression of these pro-apoptotic markers.

Table 3.2. P-values of the Proteome Profiler Human Apoptosis Array kit.
Statistical analysis was performed across four conditions to identify significant differences in pixel density directly correlated with expression of various apoptosis-related proteins. Values in red denote any p values below 0.05.

	Statistical comparison between groups			
	EV untreated vs EV H2O2	**WT52 untreated vs WT52 H2O2**	**EV vs WT52 untreated**	**EV vs WT52 H2O2**
Bad	0.022	0.001	0.113	0.003
Bax	0.019	0.239	0.209	0.003
Bcl-2	0.70	0.464	0.019	0.003
Bcl-x	0.083	0.787	0.482	0.028
Pro-Caspase-3	0.042	0.037	0.014	0.012
Cleaved Caspase-3	0.002	0.010	0.0174	0.015
Catalase	0.780	0.042	0.013	0.002
cIAP-1	0.007	0.010	0.034	0.055
cIAP-2	0.969	No dif	No dif	No dif
Claspin	<0.001	<0.001	0.135	0.008
Clusterin	0.922	0.922	<0.001	<0.001
Cytochrome C	0.003	0.010	0.872	<0.001
TRAIL R1/DR4	0.017	0.866	0.349	0.008
TRAIL R2/DR5	<0.001	<0.001	<0.001	<0.001
FADD	0.460	0.009	0.14	0.002
Fas/TNFRSF6/CD95	0.065	No dif	No dif	No dif
HIF-1α	0.446	0.073	0.151	0.007
HO-1/HMOX1/HSP32	<0.001	<0.001	0.429	0.011
HO-2/HMOX2	0.199	0.056	<0.001	<0.001
HSP27	0.002	0.007	0.007	0.038
HSP60	0.002	0.005	0.018	0.004
HSP70	0.010	0.980	0.019	0.001
HTRA2/Omi	0.041	<0.001	0.022	<0.001
Livin	0.001	0.456	0.609	<0.001
PON2	<0.001	<0.001	0.004	<0.001
p21/CIP1/CDKN1A	<0.001	<0.001	0.298	0.004
p27/Kip1	<0.001	<0.001	0.152	0.001
Phospho-p53 (S15)	<0.001	0.482	0.108	<0.001
Phospho-p53 (S46)	0.002	0.012	0.654	<0.001
Phospho-p53 (S392)	<0.001	0.005	0.821	<0.001
Phospho-Rad17 (S635)	0.012	0.064	0.970	0.002
SMAC/Diablo	0.012	0.003	0.019	0.005
Survivin	<0.001	<0.001	0.044	0.001
TNFRI/TNFRSF1A	0.014	0.807	0.728	0.015
XIAP	0.276	0.002	0.145	0.010
Reference Spots	0.073			

CHAPTER 4: QUANTITATIVE COMPARISON OF FKBP51 AND FKBP52 INTERACTOMES

4.1 RATIONALE

An initial interest of the current project was to elucidate proteins present in the FKBP51 and FKBP52 protein complexes in the presence or absence of hormone. MS/MS analysis revealed novel proteins associated with one or both of our target proteins and in vitro pull-down assays further validated some of these associations. These proteomic analyses resulted in the identification of common and unique protein interactors for FKBP51 and FKBP52. Although the subsequent validation and functional assays conducted provided an additional level of confidence that these novel interactors play a role in the regulatory AR heterocomplex in PCa, it is still unclear how the pieces fit together in the larger model. Additionally, the current literature demonstrates unclear but vital functions for FKBP51 and FKBP52, as evident by the lethality of the double KO in the embryonic stages in mice (Storer, Dickey et al. 2011). This demonstrates a need to implement more efficient methods to elucidate the intricate signaling network in which these proteins play a role.

To determine how the associated protein networks may influence specific signaling pathways, Ingenuity Pathway Analysis (IPA) was applied to identify signaling differences downstream to the PPIs experimentally obtained. Therefore, we performed a quantitative comparison of the two interactomes to better understand common and distinct functions between the two. Although the analysis match for this software currently comprises only gene expression datasets, these datasets can serve as a source of biological insights from our proteomic analysis given that the data matches are established at the biological entity level.

4.2 METHODS

A total of 755 proteins were originally observed in the raw spectral count matrix of the FKBP51 and FKBP52 proteomic analysis. Removal of duplicate proteins, keratin family of proteins, or proteins without accession numbers resulted in 395 unique proteins that were used for downstream analysis. The top 100 proteins from the FKBP51 and FKBP52 interactomes were selected and the log2 fold-change values for each protein comparing EtOH and DHT treated cells were further analyzed by IPA (Qiagen, USA). With the use of this IPA software, we were able to match our dataset to a collection of over 700 pre-defined canonical pathways to find overlap between our interactome and their knowledge base.

4.3 RESULTS

4.3.1 FKBP51 and FKBP52 share 45 common interactors

A visual representation of the common and unique protein interactions from FKBP51 and FKBP52 proteomic analysis shows there are 45 proteins that associate with both proteins and 55 unique protein interactions in the complexes isolated (Figure 4.1). Whether these associations are through direct or indirect interactions is yet to be determined. Venn diagram was generated by free source site ugent.be by uploading the gene list of our top 100 interactions.

Three separate analyses were also conducted to match canonical pathways to the dataset of associated network of proteins for FKBP51, FKBP52, and common interactions. Pathways from IPA were narrowed down based on a pre-set -log(p-value) of 1.3 or above. The canonical pathways are determined by considering the activation status of at least one key molecule when the pathway is activated, as well as the relationships

between these molecules (i.e., activation and inhibition as suggested by literature findings). This process generates an activity pattern for the molecules and the end-point functions in the pathway. The following dataset provides multiple directions to pursue studies to identify downstream events regulated by FKBP51 and FKBP52's unique and common interactors. However, for the purpose of my study, I will focus on pathways predicted to have a role in oxidative stress and/or cancer progression as suggested by the current literature.

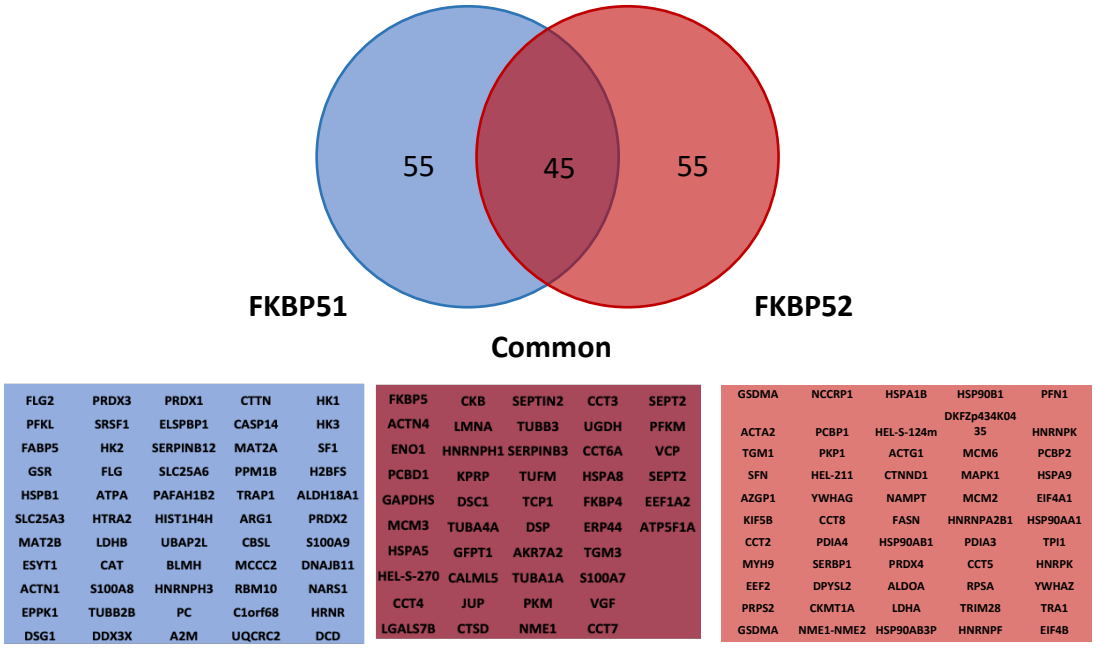

Figure 4.1. Venn diagram of common vs unique FKBP51 and FKBP52 interactors.
Top 100 protein interactors from proteomic analysis are visually represented to show the number of proteins different and shared by their networks. The diagram was generated by free source site ugent.be.

4.3.2 NRF2-mediated oxidative stress response is differentially regulated by FKBP51 and FKBP52 protein networks

IPA for the three datasets identified several molecules that link the three protein networks to NRF2-oxidative stress response pathways. NRF2-mediated oxidative stress response pathway plays a central role in regulating multiple antioxidant enzymes involved in elimination of oxidative stress and protective mechanisms against other stresses including inflammatory, metabolic, and xenobiotic stresses. Based on the top 100 FKBP51 associated proteins, our current analysis identified four molecules that are involved in the NRF2-mediated oxidative stress response pathway including CAT, DNAJB11, GSR, PRDX1. This analysis suggests NRF2 oxidative stress response is inhibited in the FKBP51 associated network of proteins (Figure 4.5). Additionally, out of the four molecules initially found to participate in this pathway, three (PRDX1, GSR, and Hsp22/Hsp40/Hsp90) seem to decrease in activity, as seen by the light green color. On the other hand, pink on CAT denotes a slight increase in measurement or activity. White bar representing the z-score indicates the pathway has a z-scores at or very close to 0 or has with fewer than four analysis-ready molecules in our dataset, rendering it ineligible for analysis (Figure 4.2)

The FKBP52 IPA identified ACTA2, ACTG1, HSP90AA1, HSP90AB1, HSP90B1, and MAPK1 from the top 100 interactors to play a role in NRF2 oxidative mediated stress response. As suggested by the pathway analysis, the NRF2 oxidative stress pathway is activated in response to the FKBP52 network of proteins (Figure 4.6). In contrast to the downstream effect of FKBP51 associated network, in FKBP52 protein network, there is a decrease in cytoplasmic ERK1/2 expression which, in turn, is predicted to inhibit

downstream phosphorylation of NRF2 (NFE2L2). Upon phosphorylation, NRF2 dissociates from KEAP1 and translocates to the nucleus to initiate antioxidative pathways, as well as promote cell survival and tumorigenesis. Inside the nucleus, ERK1/2 expression is also downregulated, although data is inconclusive on how this may affect downstream activation of the transcription coactivator CBP and p300. The canonical pathway bar is also represented in white due to a z-score that is at or near 0 (Figure 4.3)

The third analysis for canonical pathway prediction based on FKBP51 and FKBP52 common interactors identified four molecules: AKR7A2, CCT7, FKBP5, and VCP that are also involved in NRF2 mediated oxidative stress response (Figure 4.4). In this third comparison, cytoplasmic signaling appears similar to the FKBP51 downstream effect, while the nuclear expression patterns and activity differ slightly (Figure 4.7). Most notably, FKBP5 shows a decrease in activity and AFAR shows a slight increase.

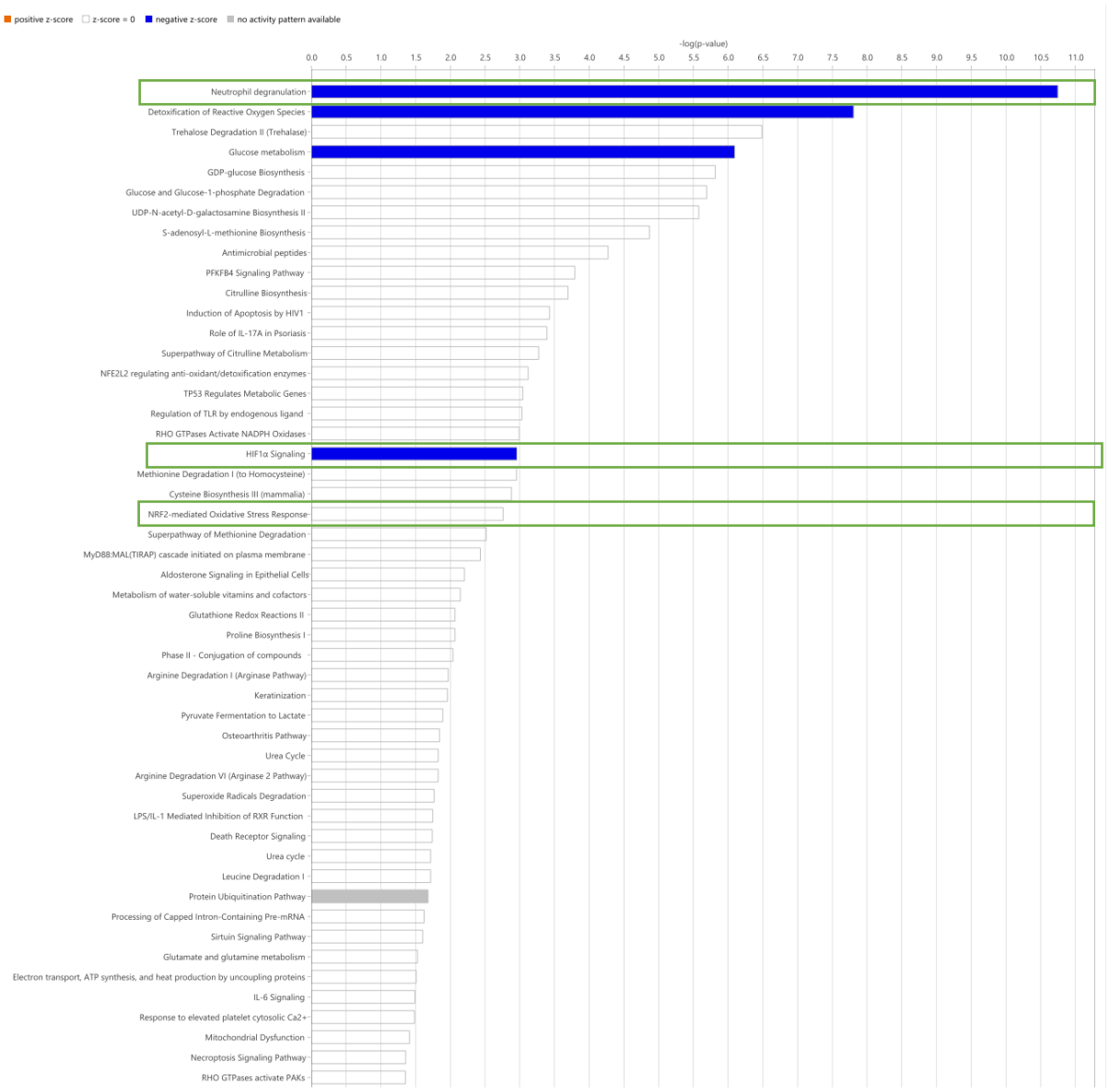

Figure 4.2. Top 50 pathways from unique FKBP51 interactors.
Pathways found associated across FKBP51 PPI dataset were organized based on the default -log(p-value) of 1.3 or above, with the most significant pathways are at the top. Only pathways that met the threshold were included in the bar chart. Two pathways of interest are highlighted in green box.

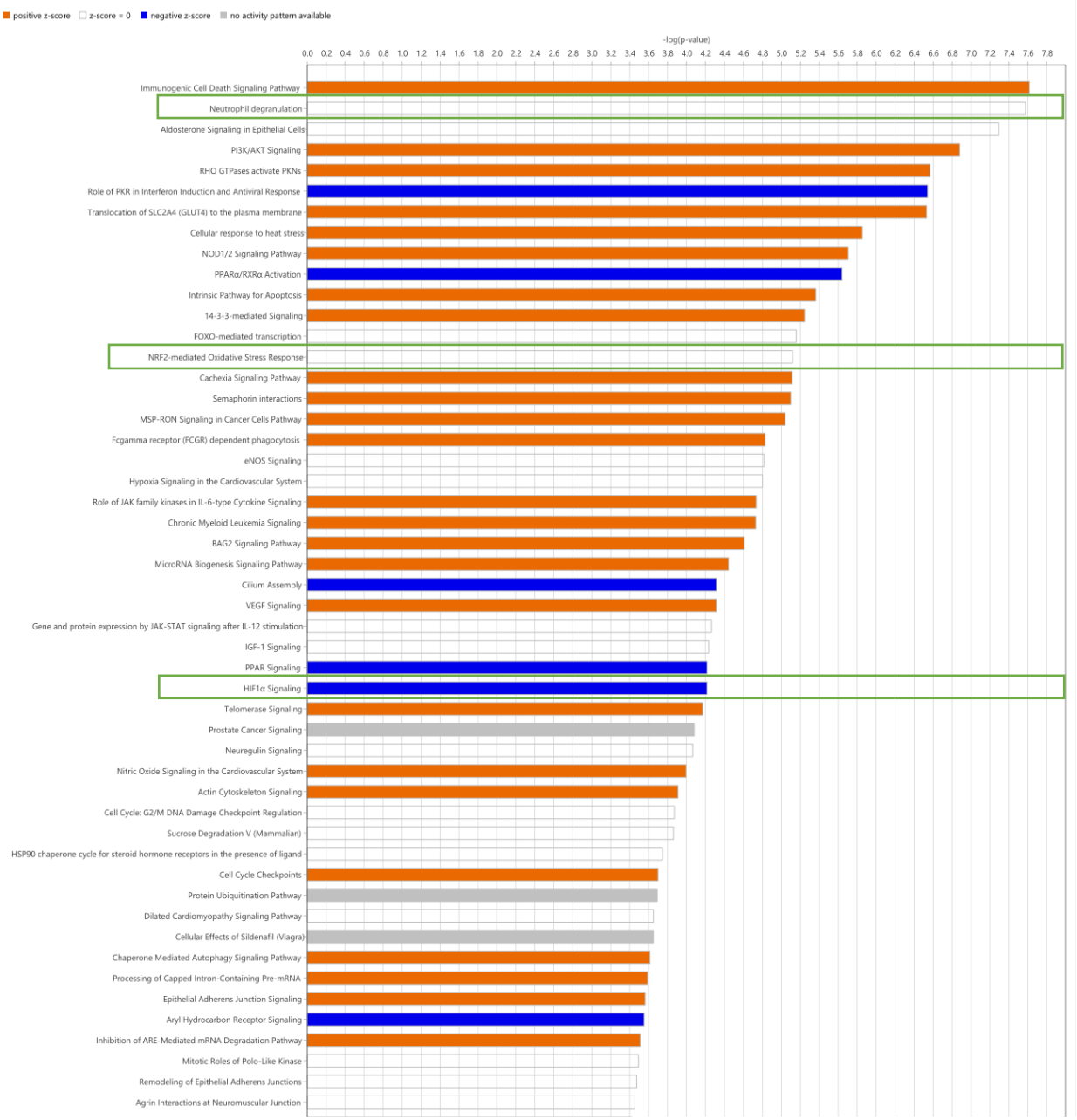

Figure 4.3. Top 50 pathways from unique FKBP52 interactors.
Pathways found associated across FKBP51 PPI dataset were organized based on the default -log(p-value) of 1.3 or above, with the most significant pathways are at the top. Only pathways that met the threshold were included in the bar chart. Two pathways of interest are highlighted in green box.

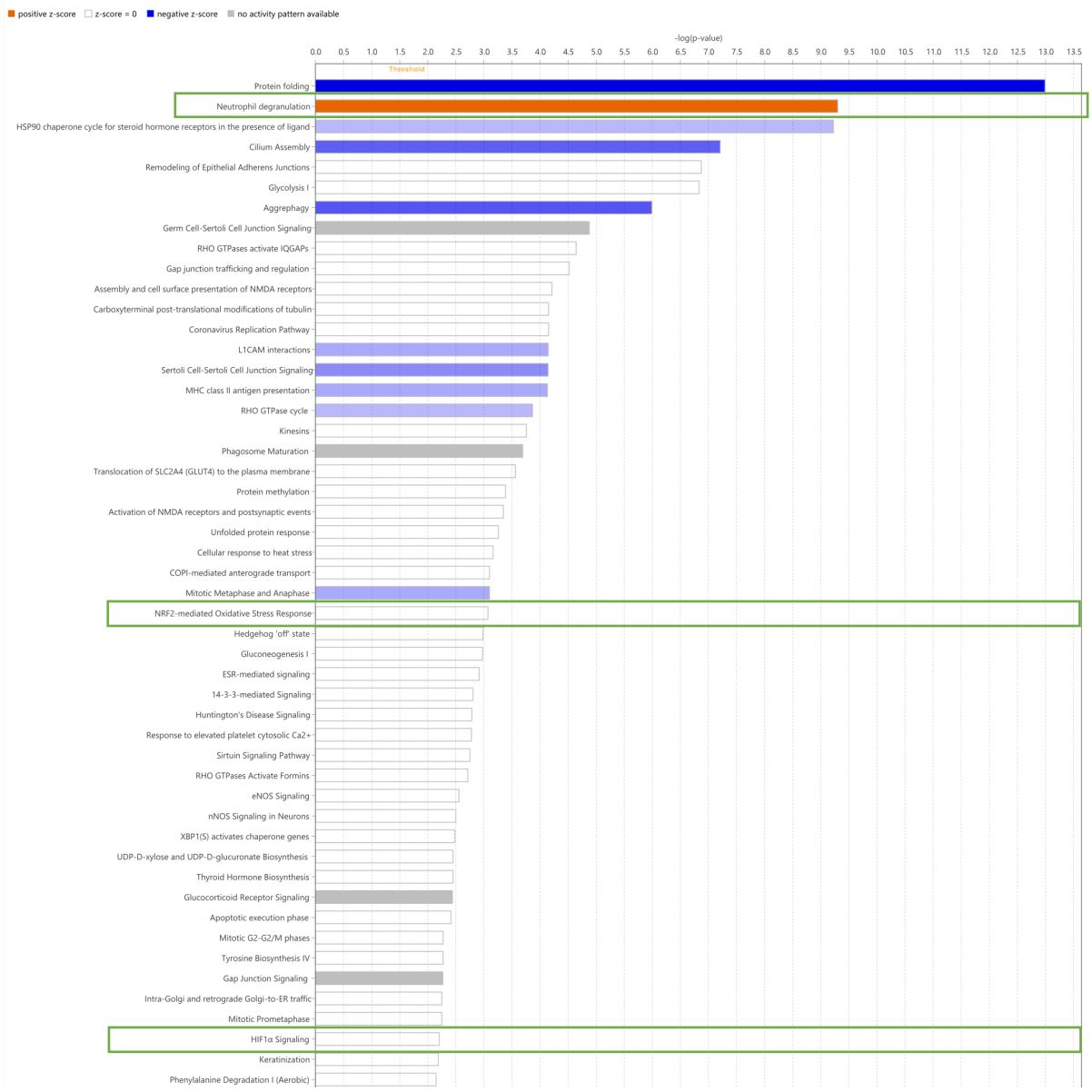

Figure 4.4 Top 50 pathways from common FKBP51 and FKBP52 interactors.
Pathways found associated across FKBP51 PPI dataset were organized based on the default -log(p-value) of 1.3 or above, with the most significant pathways are at the top. Only pathways that met the threshold were included in the bar chart. Two pathways of interest are highlighted in green box.

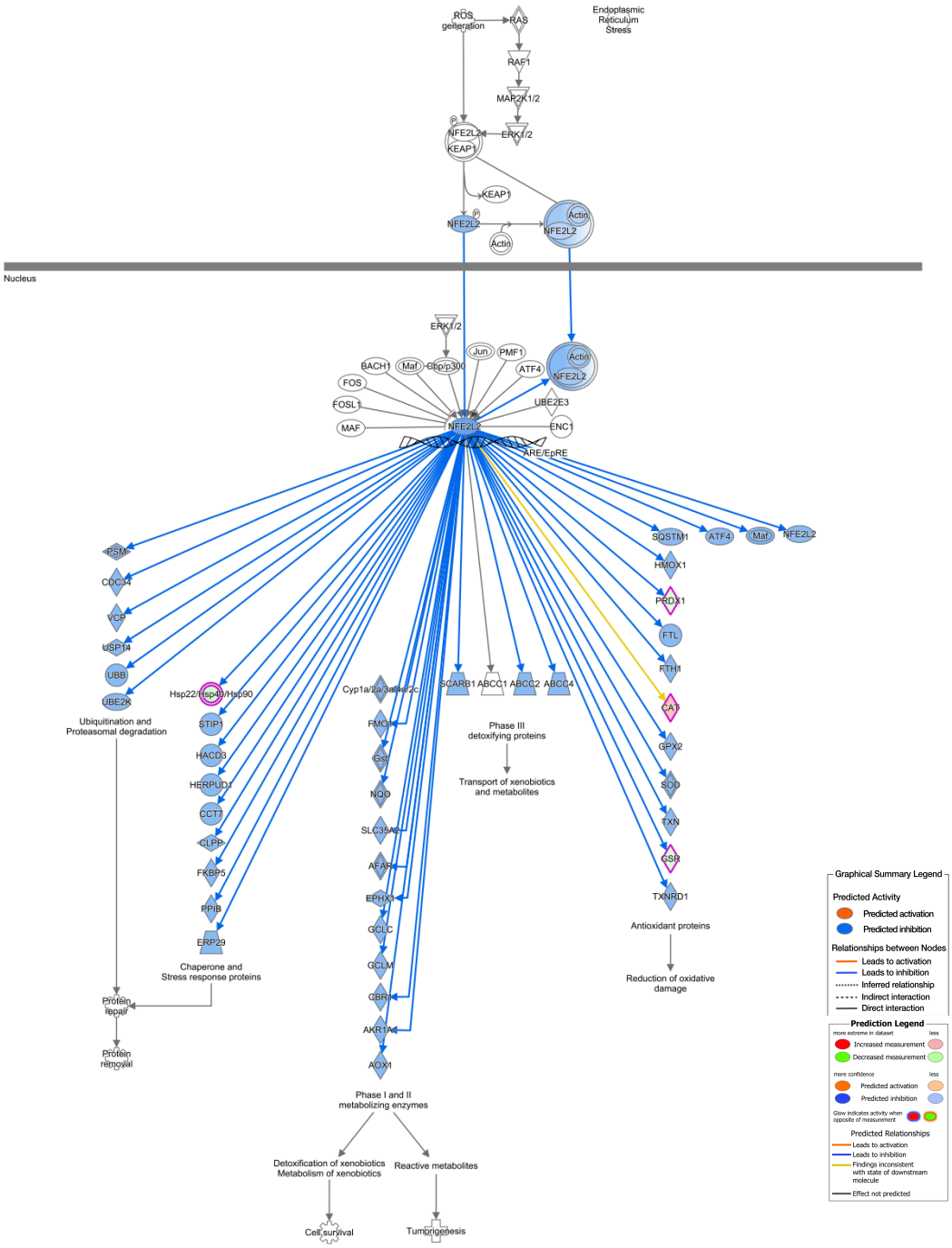

Figure 4.5. NRF2-mediated oxidative stress response regulated by FKBP51 network. Four molecules (highlighted in pink) from the FKBP51 associated network of proteins were found to play a role in oxidative stress response through the KEAP1/NRF2 pathway. The shapes represent the type of proteins, while the line pattern differentiates between direct and indirect interactions. The different colors represent inhibition or activation, and color intensity represents the relative magnitude of change in protein expression.

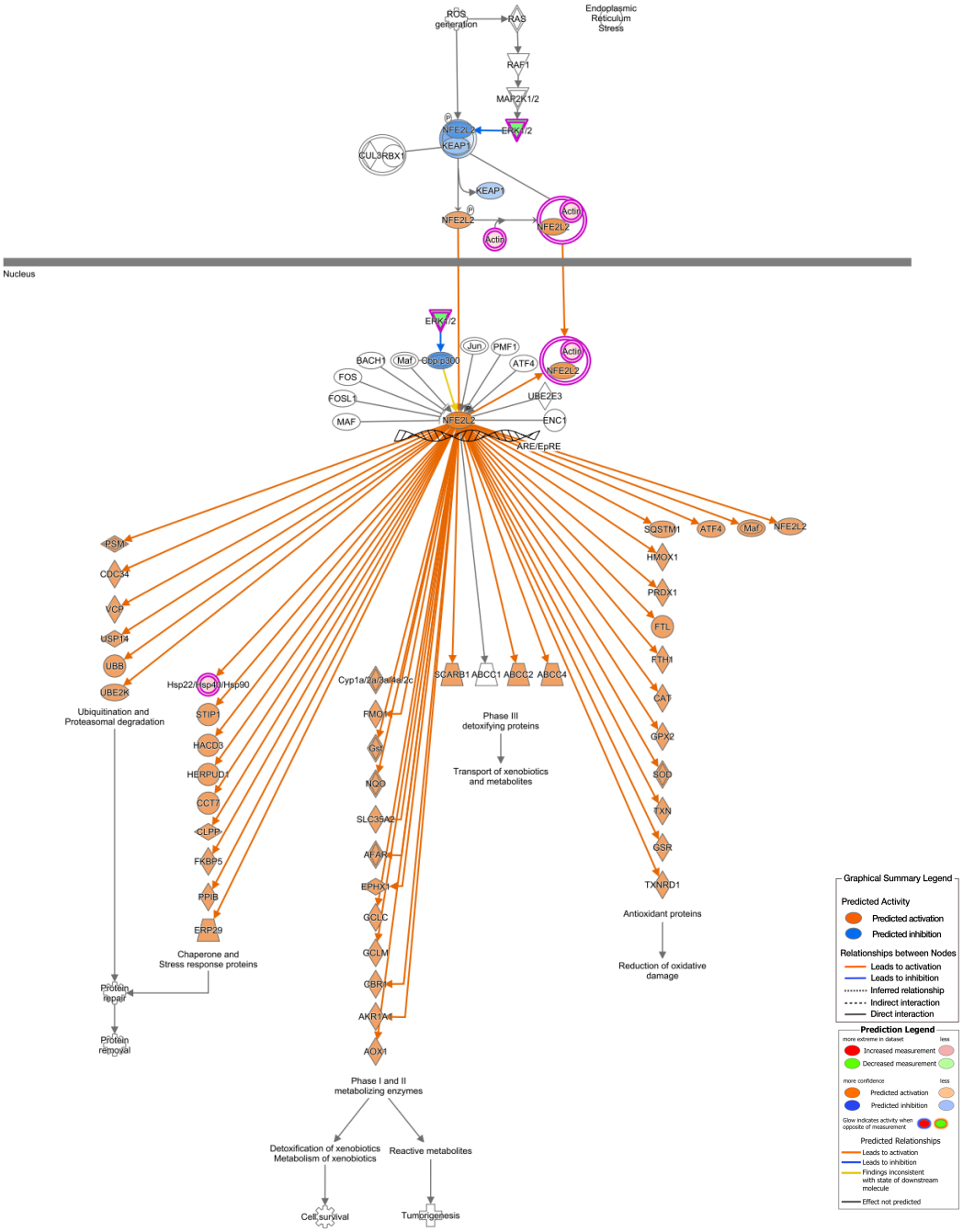

Figure 4.6. NRF2-mediated oxidative stress response regulated by FKBP52 network. Six molecules (highlighted in pink) from the FKBP52 associated network of proteins were found to play a role in oxidative stress response through the KEAP1/NRF2 pathway. The shapes represent the type of proteins, while the line pattern differentiates between direct and indirect interactions. The different colors represent inhibition or activation, and color intensity represents the relative magnitude of change in protein expression. The pathway is predicted to be activated in response to the FKBP52 protein network.

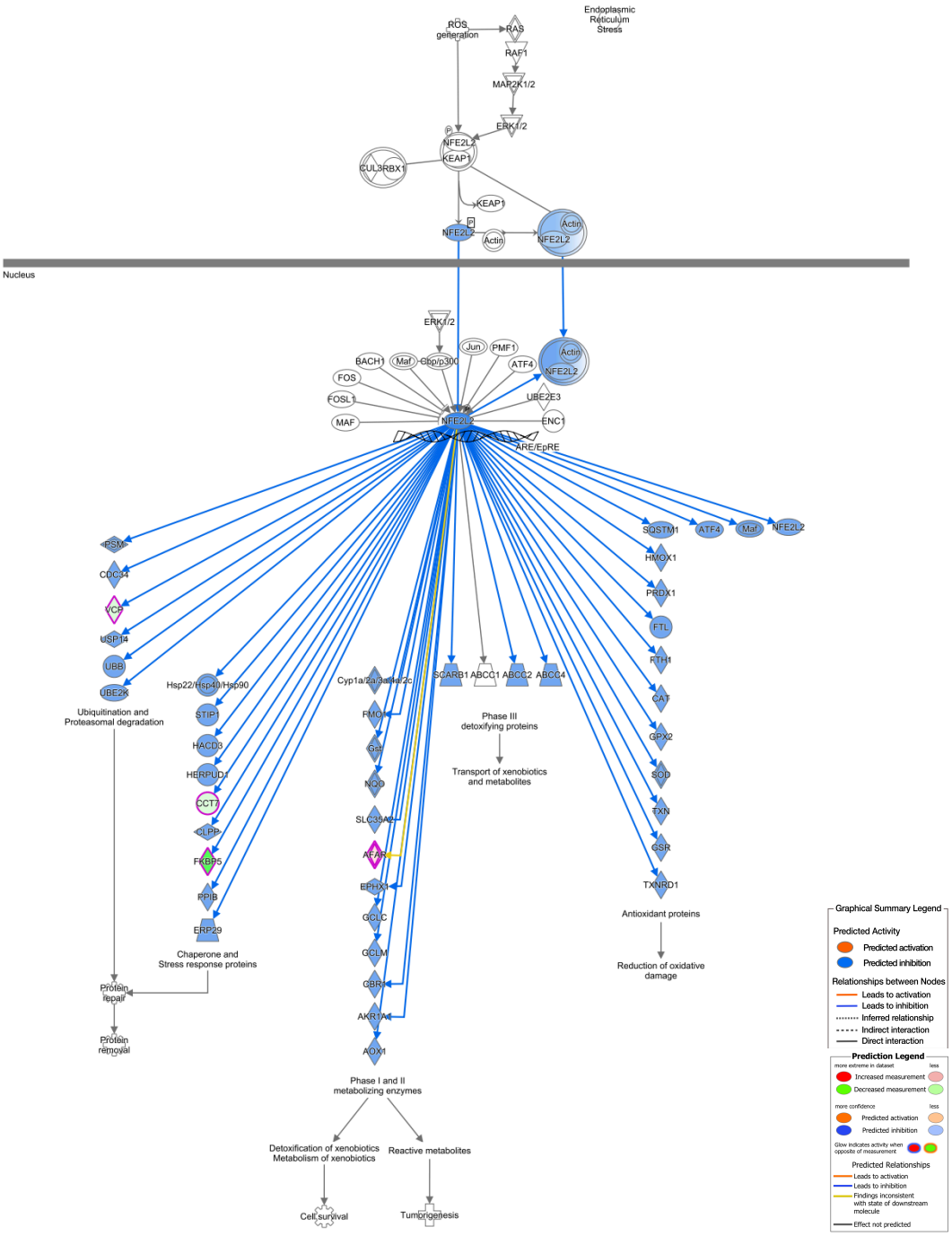

Figure 4.7. NRF2-mediated oxidative stress response regulated by common FKBP51 and FKBP52 network.
Four molecules (highlighted in pink) from the FKBP51 associated network of proteins were found to play a role in oxidative stress response through the KEAP1/NRF2 pathway. The shapes represent the type of proteins, while the line pattern differentiates between direct and indirect interactions. The different colors represent inhibition or activation, and color intensity represents the relative magnitude of change in protein expression.

4.3.3 Neutrophil degranulation is differentially regulated by FKBP51 and FKBP52 protein networks

A second pathway was identified amongst the top most significant with a -log(p-value above 1.3 (Figure 4.4), and which was present across the three datasets analyzed. Neutrophil degranulation was found at the first or second place from top to bottom for FKBP51 and FKBP52 unique interactors (Figure 4.2, Figure 4.3), as well as for the common interactors pathways (Figure 4.4). Neutrophils have diverse functions in disease and play a role in host defense by exhibiting antibacterial, antifungal, and antiviral functions, but most recently have been implicated in survival of tumor cells by facilitating the aggregation of these cells (Uribe-Querol and Rosales 2015). Our results indicate differential regulation across all granule specificities as a consequence of the FKBP network that is analyzed (Figure 4.8).

The IPA for FKBP51 matched 13 molecules from the top 100 interactors to play a role in neutrophil degranulation: ARG1, CAT, DDX3X, DSG1, FABP5, FLG2, HK3, HRNR, PAFAH1B2, PFKL, S100A8, S100A9, and SERPINB12. These molecules play roles in the varying classes of granules and throughout the degranulation pathway. Azurophilic granule lumen proteins, which contain proteins and enzymes toxic to microorganisms, show an overall decreased measurement in our dataset when compared to the software knowledge base. However, the membrane azurophil granule proteins remain unaltered. Specific granule proteins contain proteins and enzymes involved in chemotaxis, phagocytosis, and antimicrobial activity, while tertiary granule proteins can degrade components of the extracellular matrix. For both of these subsets we get an overall, but slight, increased measurement of proteins, as suggested by the pink coloration. Secretory granules are rich in transmembrane receptors and do not contain many antibacterial proteins. It is predicted in this analysis that there is a marked significant decrease in secretory granule proteins, as well as the ficolin-rich granule proteins (Figure 4.8A).

Pathway analysis for downstream regulation of FKBP52 network of proteins shows a more drastic decrease in the measurement for Azurophil granule lumen proteins, but not for their membrane counterpart, which is similar to the FKBP51 effects on this subset of granule proteins (Figure 4.8B). The specific granule proteins, whether located in the lumen or membrane, registered no difference in our dataset when compared to the software knowledge base. Tertiary granule lumen proteins demonstrate a slight decrease while their membrane counterparts remain unchanged. At the secretory granule level, for ficolin-rich and others, there are contradicting effects. Ficolin-rich granule and secretory granule lumen proteins are predicted to have a decrease and increase within the same subset depending on the specific proteins or enzymes, while ficolin-rich granule proteins from the membrane show only a decrease in measurement.

Common interactor IPA for FKBP51 and FKBP52 suggest a similar pattern of regulation for Azurophil granule lumen proteins as the individual analysis showing decrease in measurement, but no change for the membrane Azurophil granule proteins. Specific granule lumen proteins suggest there is differential regulation in the proteins as seen by the gradient in color representing an increase and a decrease. Tertiary granule lumen proteins show the highest measurement for this combined analysis than for the individual ones. However, similar to the individual IPAs for unique interactors, the ficolin-rich granule lumen proteins and the secretory lumen proteins are shown to have a mixture increase and decrease regulation of specific proteins and enzymes present in this subset. The specific proteins differentially regulated in this class of granule was not determined in this analysis.

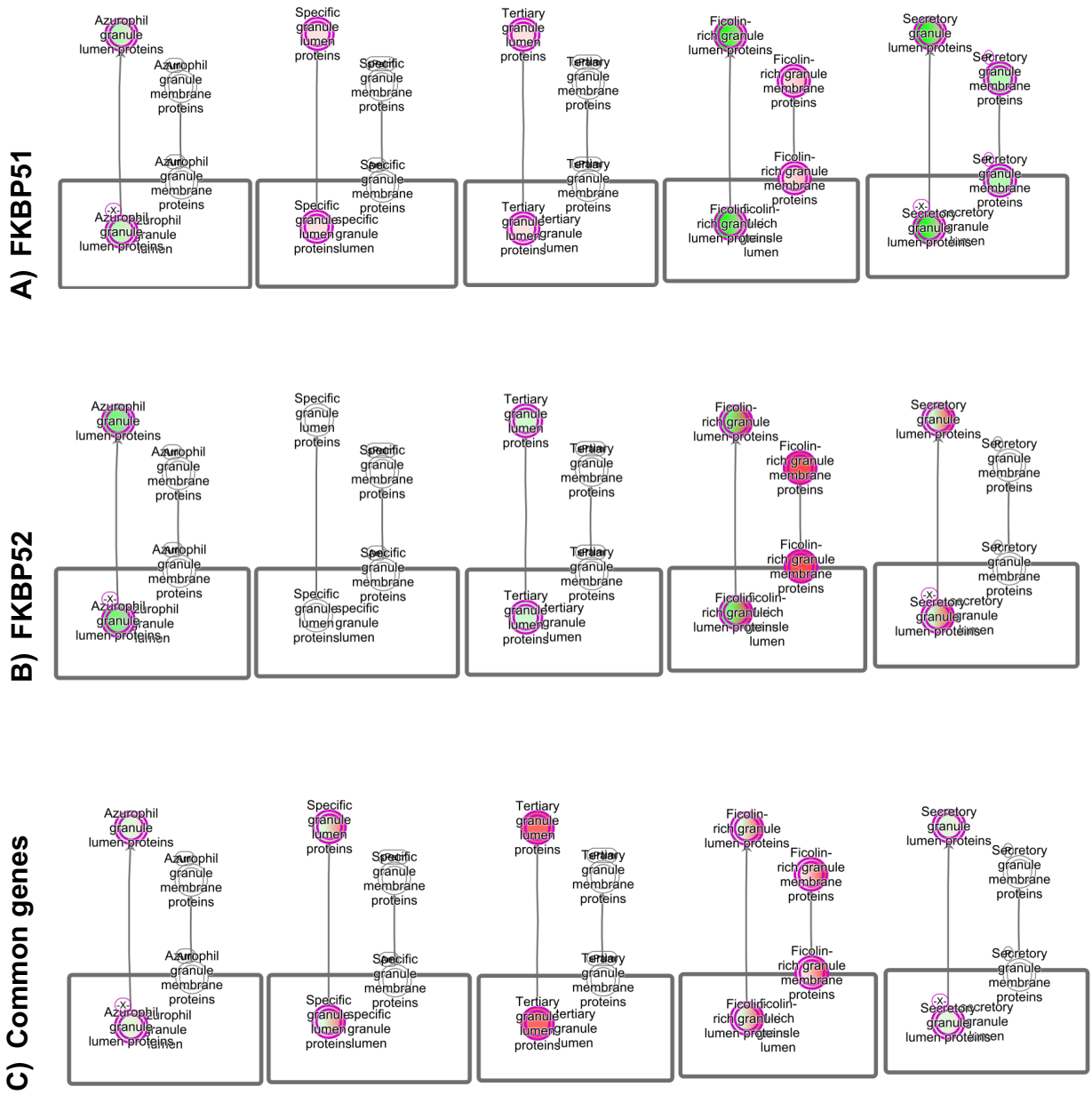

Figure 4.8. Neutrophil granules differentially regulated by FKBP51 and FKBP52 protein networks

FKBP51 and FKBP52 protein networks demonstrate differential regulation across all granule specificities.

4.3.4 HIF-1α pathway is differentially regulated by FKBP51 and FKBP52 protein networks

A third pathway was identified amongst the 50 most significant pathways in which molecules from our interactome play a role (Figure 4.2, Figure 4.3). Seven molecules suggested to interact with FKBP51 that were found to be involved in this pathway are Hexokinase 1-3, Heat-shock protein 70 (Hsp70) member 5 and 8, lactate dehydrogenase B (LDHB), and pyruvate kinase M1/2 (PKM) (Figure 4.9). There was a decreased abundance of these molecules when compared to the expected increase necessary to induce activation of the pathway. As a result of this decrease in abundance, the hypoxia-inducible factor 1 alpha (HIF-1α) signaling was predicted to be inhibited downstream of FKBP51 network of proteins. In the case of FKBP52, five molecules suggested to interact with FKBP52 were found to play a role in HIF-1α. HSP90, HSP70 member 9 and 1A, lactate dehydrogenase A (LDHA), and mitogen-activated protein kinase 1 (MAPK1) were found to be abundant, except for MAPK1 (Figure 4.10). Despite the requirement of MAPK1 expression for the transactivation activity of HIF-1α, it was predicted that FKBP52 network of proteins can activate HIF-1α pathway. Under hypoxic conditions, HIF-1α signaling induces the expression of genes associated with metabolism, angiogenesis, invasion, and cell survival. Therefore, increased HIF-1α signaling may be associated with poor PCa prognosis.

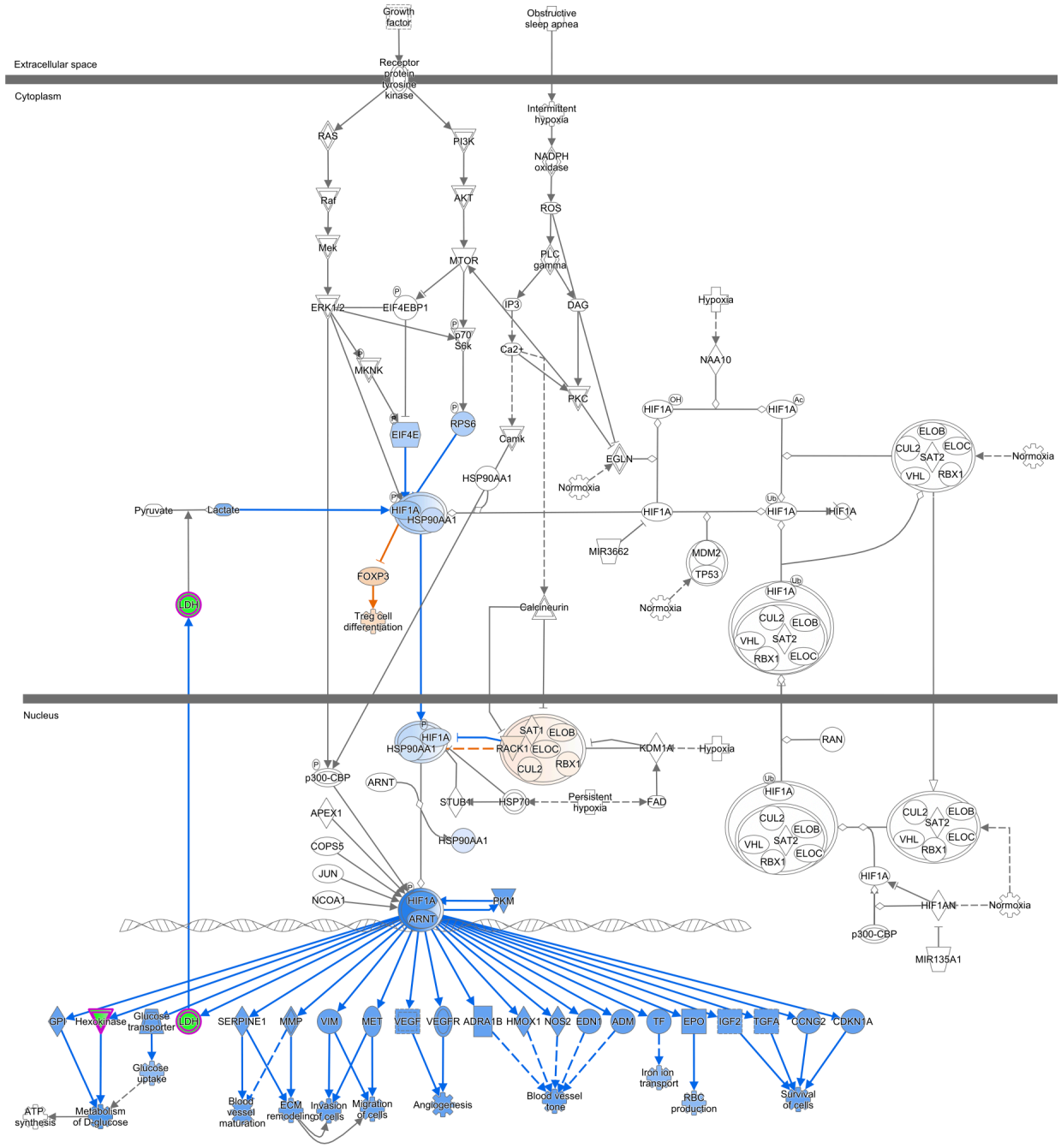

Figure 4.9. HIF-1α signaling pathway downstream regulation by FKBP51
Molecules present in the FKBP51 interactome found to play a role in HIF-1α pathway are highlighted in pink. IPA analysis predicts an inhibitory effect on this pathway in response to the molecules present and their abundance.

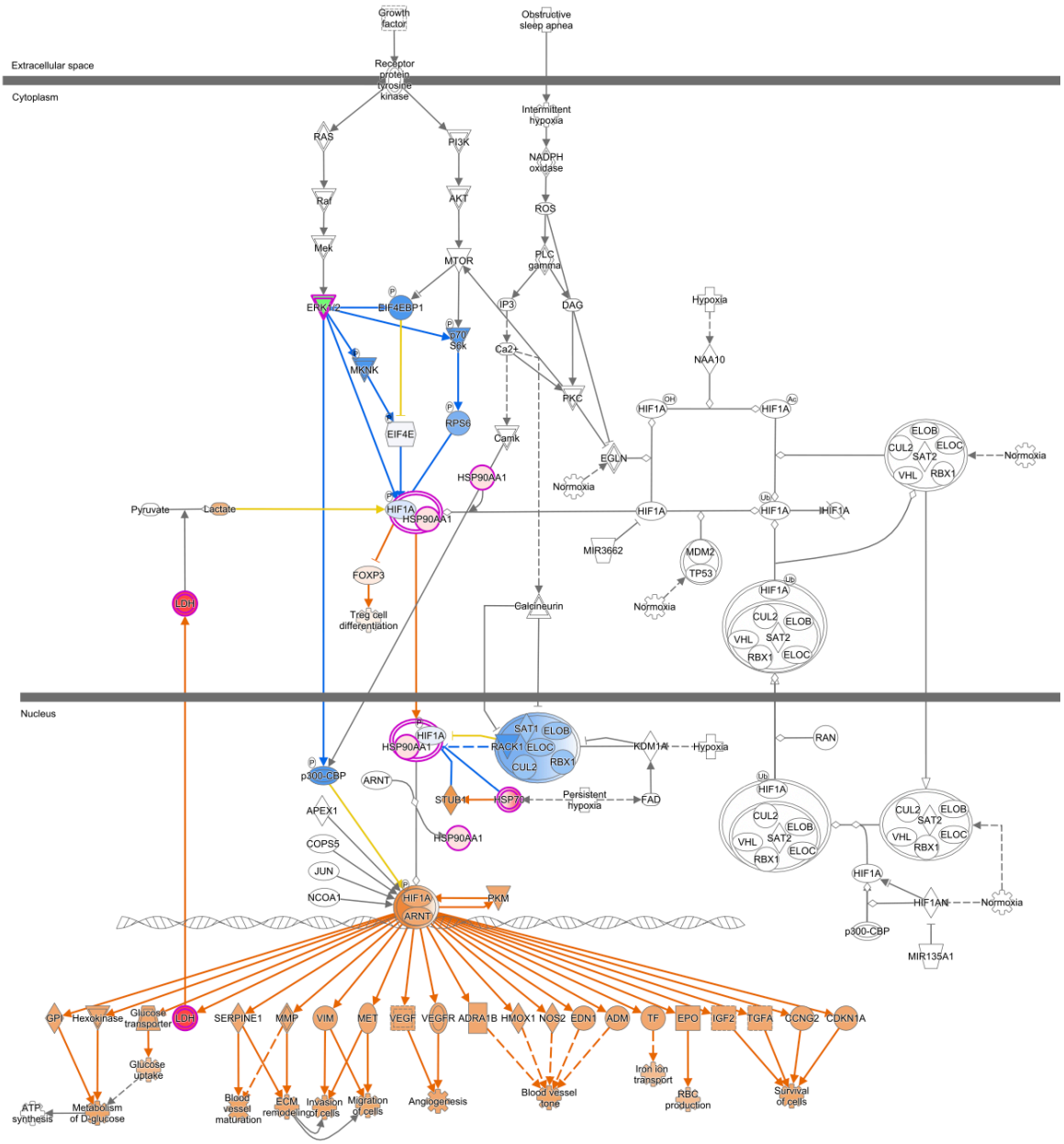

Figure 4.10. HIF-1α signaling pathway downstream regulation by FKBP52
Molecules present in the FKBP52 interactome found to play a role in HIF-1α pathway are highlighted in pink. IPA analysis predicts an activation of this pathway in response to the molecules present and their abundance.

CHAPTER 5: DISCUSSION

AR signaling, amongst many other cellular processes, is not dependent on individual proteins, but rather orchestrated by a coordinated protein network. Understanding how these proteins interact within larger complexes and influence one another is pivotal in unraveling complex cellular mechanisms. Chaperone proteins, such as FKBP51 and FKBP52, have been long documented as regulators of AR and other SHR functions in normal physiology and disease (Kang, Hong et al. 2008, Baker, Ozsan et al. 2018, Zgajnar, De Leo et al. 2019). FKBP51 and FKBP52 are closely related homologs that share 70% amino acid sequence similarity (Figure 1.1), and similar domain conformations have been suggested by three-dimensional crystallographic structures (Sinars, Cheung-Flynn et al. 2003, Wu, Li et al. 2004). Despite these similarities, both proteins exert opposite regulatory effects on their SHR client proteins. It is likely, then, that these differences are cell and organ-specific regulatory mechanisms that require the cooperation of the protein networks unique to each cell line or model. Hence, the overarching goal of this project was to dissect FKBP51 and FKBP52 protein complexes and their networks in PCa that may play a role in FKBP51- or FKP52-mediated AR signaling.

Here, we implemented a MS-based experimental approach to explore PPI networks influencing AR signaling *in vitro* using a PCa cell line. Co-IP experiments targeting FKBP51 or FKBP52 in the presence or absence of the hormone ligand, revealed 395 protein interactions. Protein interactions well-documented in the literature were concluded to be positive indicators of the physiological relevance of the complexes isolated. Additional interactors in the dataset included peroxiredoxins, which play a crucial

role in regulating reactive oxygen species (ROS) and protecting cells from oxidative stress. This observation is consistent with previous studies from our lab in which a pathway analysis identified dysregulation of oxidative phosphorylation as the major pathway affected in the absence of FKBP52 (Ortiz 2021).

Reciprocal co-immunoprecipitations were implemented to verify FKBP51 and FKBP52 association with the peroxiredoxin family of proteins and confirmed the association with PRDX1-4 (Figure 3.1A, B right), while associations with PRDX5 and PRDX6 was dependent on the conditions used to carry out the co-Ips (Figure 3.1A, B right). PRDX6 has previously been associated with FKBP52 and a physical association between the two proteins has also been reported in the deciduum (Hirota 2010). Although our reports are not consistent with Hirota et al, it is important to highlight the conditions for co-IPs are typically not physiologically similar to those inside of a cell, but rather simplified artificial methods to gain insight on the potential associations of specific proteins. With that said, our lack of PRDX5 and PRDX6 association with FKBP51 or FKBP52 does not negate the possibility of an *in vivo* association and collaboration in a PCa setting.

Similar contradictions were observed in the purified protein pull downs. Unlike the cell-based co-IPs, purified protein pull-downs allow us to study the potential and direct physical interaction between two proteins. In the FLAG pull downs targeting FLAG-tagged peroxiredoxins, PRDX1-4 and PRDX6 were confirmed as having a direct interaction with FKBP51 and FKBP52 (Figure 3.2). Upon further experimentation, a question arose as to whether the consistent binding of all PRDXs could be attributed to non-specific binding to the FLAG-bead system, or if it was due to the ability of the proteins to interact directly.

We therefore sought a different method to purify the complexes *in vitro* and in the absence of other proteins by using Ni-NTA beads to target FKBP51 and FKBP52. Non-specific binding of PRDX1-6 to the Ni-NTA resin in the absence of an appropriate antibody against PRDX1-6 confirmed an issue of non-specific binding and rendered the previous FLAG pull-down results questionable.

Continuous troubleshooting led to the addition of glycerol to the binding buffer, which, along with a slight increase in detergent concentration (0.05% to 0.075%), allowed us to remove three of the five non-specifically bound PRDXs (Figure 3.4). Despite ongoing issues with non-specific binding, PRDX3 has consistently been observed as an interactor with FKBP51 and FKBP52 in the presence or absence of other proteins. This comes as no surprise given the suggested critical role PRDX3 has been reported to have in PCa protection and cell survival (Whitaker, Patel et al. 2013).

Considering the crucial role peroxiredoxins play in oxidative pathways and our recent interactome analysis, we wanted to determine if the protective effect of PRDXs under oxidative stress conditions, especially PRDX3, were in part due to their association with FKBP51 and FKBP52. To study the potential role for FKBP52 in oxidative stress protection in PCa cells, we measured the expression of 35 apoptosis-related proteins under stress conditions and in the presence or absence of FKBP52. Among the most noticeable proteins differentially expressed under stress conditions and affected by FKBP52 expression were the pro-apoptotic p21, p27, and P53 phosphorylated at four independent sites (S15, S46, S392, S635). Interestingly, anti-apoptotic catalase and Livin expression was also altered under the same conditions.

Tumor suppressor P53 is known to regulate cell cycle, survival, DNA repair, autophagy, and apoptosis upon sever DNA damage (Yogosawa and Yoshida 2018). The stress-induced phosphorylation of p53 at multiple residues has been demonstrated to be a key regulation step for p53-mediated cellular response to stress and apoptosis induction (Wang and Chen 2003). Phosphorylation at Ser15, 20, 37, and 46 is reported to induce apoptosis in osteosarcoma and melanoma models (Zajkowicz, Gdowicz-Klosok et al. 2015, Wang, Meng et al. 2018). Furthermore, phosphorylation at S392 can promote the ability of p53 to suppress cell growth (Sakaguchi, Sakamoto et al. 1997, Cox and Meek 2010). Additionally, p53 regulates expression of p21 and p27, which can act as tumor suppressors or oncogenes depending on the cellular context, subcellular localization, and posttranslational modifications.

Expression of these apoptosis related proteins was measured after inducing oxidative stress by H_2O_2 treatment and in the presence or absence of FKBP52 (Figure 3.7). Induction of oxidative stress was expected with a significant increase in tumor suppressor and pro-apoptotic markers (i.e., phosphorylated p53, p21, p27) after 24hr treatment in FKBP52 KO 22RV1 cells. Interestingly, expression of FKBP52 under the same conditions showed a decrease in all of these pro-apoptotic markers, suggesting the role for FKBP52 in protecting PCa cells under stress conditions and preventing apoptosis.

The resistance to apoptosis is suggested to involve mechanisms mediated by mitochondria and that are dependent on caspases. Overexpression of Bcl-2, Bcl-xL, and catalase proteins has previously been shown to reduce cell death induced by NiCl2, while knockdown of their expression sensitizes cells to nickel-induced apoptosis (Yang, Li et al. 2013). This suggests that the expression of Bcl-2, Bcl-xL, and catalase proteins plays a

crucial role in apoptosis resistance. Our data shows that expression of catalase is increased before and after induction of oxidative stress, but only in the presence of FKBP52. Bcl-x on the other hand, demonstrates a similar expression pattern to the pro-apoptotic markers discussed above. Bcl-x gene has two isoforms: Bcl-XL and a Bcl-XS which demonstrate pro-survival and pro-apoptotic roles, respectively. Due to their documented role in regulating apoptosis differentially, we can assume that the isoform that is measured, is representative of the pro-apoptotic isoform, Bcl-XS. Taking these observations into consideration, we suggest that downregulation of apoptotic markers and upregulation of anti-apoptotic proteins in prostate cancer cells undergoing oxidative stress may be partly mediated by FKBP52 via its association with PRDX3.

While the initial goal of this project was to identify global PPIs for FKBP51 and FKBP52, our overarching goal is to pursue a comparative approach to understand the common and unique roles these immunophilins play in normal physiology and disease. In line with this goal we sought to discern the downstream effect of these protein interaction on specific pathways important for normal physiology and disease progression. In addition to the identified protein interactions in this interactome, we conducted a interaction analysis based on data from published PPI databases (Figure 1.2). BioGRID protein interaction database was accessed on September 25, 2022, at 2:32 a.m. UTC to generate a Venn diagram of protein interactions that are shared or unique to FKBP51 and FKBP52 (Figure 2.6). We filtered the database to show only interactors with physical evidence (LTP or HTP), although distinction between direct interaction or interaction through complexes could not be concluded. A similar analysis was conducted using only the interactors reported in Aim1 to generate a Venn diagram of common and

unique interactions (Figure 4.1). It is important to note that the reported associations in the published dataset do not include some of the interacting proteins we report in our studies, as is the case for peroxiredoxin 3. Protein interactions that match the literature search include, but are not limited to, Mini chromosome maintenance protein MCM3, Heterogenous nuclear ribonucleoprotein H1 (HNRNPH1), and Hsp70 family A member 8 (HSPA8). The presence of these common interactors in the literature as well as our dataset, provide us with a level of confidence that the complexes herein reported are physiologically relevant, while the novel interactors provide us with potential and alternative targets against PCa that have not been previously reported.

To perform the comparative pathway analysis for FKBP51 and FKBP52 interactomes, we conducted an Ingenuity Pathway Analysis (IPA) which can detect the pathway overlap of our significant dataset molecules and predict the activation or inhibition of those pathways in our dataset. This algorithm considers that up-regulated proteins do not always activate a specific pathway, given the increasing reports of protein overexpression resulting in inhibition of a particular pathway. Three pathways that are involved in oxidative stress and PCa progression were found to be amongst the top 50 most significant pathways for the three PPI datasets analyzed and became the focus of our third aim.

NRF2-mediated oxidative stress response protects tissue from oxidative and cytotoxic injury and is a key pathway for protecting tissue against endogenous and exogenous stress. The NRF2 pathway has been reported to protect against a range of chronic diseases, such as cardiovascular, neurodegenerative, and respiratory diseases (Ooi, Goh et al. 2017, Cuadrado, Pajares et al. 2020, Cuadrado 2022). Upon oxidative

stress, NRF2 translocates to the nucleus and induces the expression of antioxidant responsive elements, such as the PRDXs, which were identified in our interactome. In the context of cancer and the increase of oxidative stress, this pathway is hijacked to promote PCa survival and aggressiveness. Our IPA analysis predicted that 51 would inhibit the NRF2 signaling pathway, shown by the decrease of downstream interactors involved in the stress response: PRDX1 and GSR (Figure 4.5). Disruption of NRF2 activity, elevated ROS and increased DNA damage have been reported as responsible factors for the oncogenic transformation at the initial stage of human PCa (Frohlich et al., 2008). In the case of 52, the IPA analysis predicted activation of the NRF2 pathway, thus leading to the promotion of the cell survival (Figure 4.6). The activation of NRF2 pathway downstream of FKBP52 protein interactions may be correlated to the protective effect FKBP52 was reported to have in the presence of oxidative stress conditions in this study. A comparison of apoptosis-related proteins is necessary for FKBP51 to corroborate the decreased activity reported in the FKBP51 IPA for NRF2-mediated oxidative stress response (Figure 4.5).

In addition to the NRF2 pathway, we found that neutrophil degranulation is a common pathway regulated by molecules present in the FKBP51, FKBP52, and common analysis. Neutrophils contribute to the clearance of apoptotic cell debris, which supports tissue regeneration and angiogenesis following tissue damage (Castanheira and Kubes 2019). In individuals with breast and prostate cancers, the presence of tumor cell clusters in the blood has been linked to unfavorable survival outcomes (Aceto, Bardia et al. 2014). Of relevance to our research, neutrophils have been reported to contribute to PCa progression by releasing growth factors such as epidermal growth factor and hepatocyte

growth factor (HGF), and by eliminating senescence through IL-1 receptor antagonist (IL-1RA) (Fan, Lee et al. 2020).

The IPA analysis also revealed HIF-1α signaling pathway to be enriched with several molecules from our initial interactome. It is well established that this pathway contributes to metastatic cancer progression by stabilization of transcription factor HIF-1α in response to low oxygen levels. However, there is limited knowledge regarding the mechanism through which heterogeneous hypoxia contributes to cancer progression, primarily due to the inadequacy of existing experimental models in replicating the complex nature of hypoxia. The HIF-1α signaling was predicted to be inhibited by FKBP51 and activated by FKBP52 (Figure 4.9, Figure 4.10). Under hypoxic conditions, HIF-1α signaling induces the expression of genes associated with metabolism, angiogenesis, invasion and cell survival (Chen and Sang 2016). Therefore, increased HIF-1α signaling is associated with poor PCa prognosis. Horii et al. demonstrated that HIF-1α interacts with AR to activate prostate-specific antigen (PSA) expression in LNCaP PCa cells under hypoxic conditions (Horii, Suzuki et al. 2007). On the other hand, Knockdown of HIF-1α enhanced the effect of DDP on PCa cells by inducing cell death through ROS overproduction (Gu, Li et al. 2017). In line with these findings, our IPA analysis suggests that FKBP52, a known positive regulator of AR, plays a key role in regulating these pathways to promote oxidative stress protection in PCa. Therefore, the protective role of FKBP52 against oxidative stress-induced apoptosis that we report here, may be due to its predicted activation of the HIF-1α pathway. These findings further support our idea that FKBP52 is a promising target to treat PCa given the growing list of interacting proteins and the multitude of pathways these may regulate.

Several potential limitations also need to be acknowledged for in these experimental procedures. As with all PPI assays, we cannot exclude the possibility that some of the associations identified in Aim1 and verified in Aim 2 may be false positives. Additionally, the use of an adherent cell line with monolayer characteristics, such as 22RV1, to study apoptosis-related responses to oxidative damage does not offer the most physiologically relevant model to study hypoxia. The use of organoids from the same cell line may be more suitable to mimic the heterogenous hypoxia environment reported in tumors.

Going forward, these interactions will serve as the basis for future projects involving the cooperation of chaperones, FKBP51 and FKBP52, in AR-mediated disease progression, as well as other steroid hormone receptor pathways. To expand on the findings described in aim 2, a comparison of FKP51 in oxidative stress response after H_2O_2 treatment should be assessed. Additionally, the role of both FKBPs in oxidative stress-induced apoptosis should be further assessed *in vitro* with the use of organoids to compare their response in a more physiologically relevant model. Given the supportive role FKBP52 has been reported to play in PRDX6 protein stability, and the protective role PRDX3 and FKBP52 have shown against oxidative stress, knock-down cell lines of FKBP52 can be used to study if there is also a correlation between FKBP52 expression and PRDX1-6 expression and protein stability. Given that the role of FKBP51 in oxidative stress-induced apoptosis has yet to be determined, we are not certain the same experimental approach may be needed for FKBP51, though these results may lead us in a different direction.

The protein interactions presented in this thesis emphasize the intricate network of proteins that is not fully understood in PCa, and that also contributes to a deeper understanding of cellular signaling networks in various types of cancer or other hormone diseases.

BIBLIOGRAPHY

Acar, N. and I. Ustunel (2015). "Expression of 52-kDa FK506-binding protein (FKBP52) in human placenta complicated by preeclampsia and intrauterine growth restriction." Anal Quant Cytopathol Histpathol **37**(2): 87-95.

Aceto, N., A. Bardia, D. T. Miyamoto, M. C. Donaldson, B. S. Wittner, J. A. Spencer, M. Yu, A. Pely, A. Engstrom, H. Zhu, B. W. Brannigan, R. Kapur, S. L. Stott, T. Shioda, S. Ramaswamy, D. T. Ting, C. P. Lin, M. Toner, D. A. Haber and S. Maheswaran (2014). "Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis." Cell **158**(5): 1110-1122.

Agoulnik, I. U. and N. L. Weigel (2008). "Androgen receptor coactivators and prostate cancer." Adv Exp Med Biol **617**: 245-255.

Aranda, A. and A. Pascual (2001). "Nuclear hormone receptors and gene expression." Physiol Rev **81**(3): 1269-1304.

Bailus, B. J., S. M. Scheeler, J. Simons, M. A. Sanchez, K. T. Tshilenge, J. Creus-Muncunill, S. Naphade, A. Lopez-Ramirez, N. Zhang, K. Lakshika Madushani, S. Moroz, A. Loureiro, K. H. Schreiber, F. Hausch, B. K. Kennedy, M. E. Ehrlich and L. M. Ellerby (2021). "Modulating FKBP5/FKBP51 and autophagy lowers HTT (huntingtin) levels." Autophagy **17**(12): 4119-4140.

Bajor, M., A. O. Zych, A. Graczyk-Jarzynka, A. Muchowicz, M. Firczuk, L. Trzeciak, P. Gaj, A. Domagala, M. Siernicka, A. Zagozdzon, P. Siedlecki, M. Kniotek, P. C. O'Leary, J. Golab and R. Zagozdzon (2018). "Targeting peroxiredoxin 1 impairs growth of breast cancer cells and potently sensitises these cells to prooxidant agents." Br J Cancer **119**(7): 873-884.

Baker, J. D., I. Ozsan, S. Rodriguez Ospina, D. Gulick and L. J. Blair (2018). "Hsp90 Heterocomplexes Regulate Steroid Hormone Receptors: From Stress Response to Psychiatric Disease." Int J Mol Sci **20**(1).

Basu, A., H. Banerjee, H. Rojas, S. R. Martinez, S. Roy, Z. Jia, M. B. Lilly, M. De Leon and C. A. Casiano (2011). "Differential expression of peroxiredoxins in prostate cancer: consistent upregulation of PRDX3 and PRDX4." Prostate **71**(7): 755-765.

Bauder, M., C. Meyners, P. L. Purder, S. Merz, W. O. Sugiarto, A. M. Voll, T. Heymann and F. Hausch (2021). "Structure-Based Design of High-Affinity Macrocyclic FKBP51 Inhibitors." J Med Chem **64**(6): 3320-3349.

Bevan, C. L., S. Hoare, F. Claessens, D. M. Heery and M. G. Parker (1999). "The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1." Mol Cell Biol **19**(12): 8383-8392.

Binder, E. B., R. G. Bradley, W. Liu, M. P. Epstein, T. C. Deveau, K. B. Mercer, Y. Tang, C. F. Gillespie, C. M. Heim, C. B. Nemeroff, A. C. Schwartz, J. F. Cubells and K. J. Ressler (2008). "Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults." JAMA **299**(11): 1291-1305.

Blair, L. J., J. D. Baker, J. J. Sabbagh and C. A. Dickey (2015). "The emerging role of peptidyl-prolyl isomerase chaperones in tau oligomerization, amyloid processing, and Alzheimer's disease." J Neurochem **133**(1): 1-13.

Blair, L. J., B. A. Nordhues, S. E. Hill, K. M. Scaglione, J. C. O'Leary, 3rd, S. N. Fontaine, L. Breydo, B. Zhang, P. Li, L. Wang, C. Cotman, H. L. Paulson, M. Muschol, V. N. Uversky, T. Klengel, E. B. Binder, R. Kayed, T. E. Golde, N. Berchtold and C. A. Dickey (2013). "Accelerated neurodegeneration through chaperone-mediated oligomerization of tau." J Clin Invest **123**(10): 4158-4169.

Bohl, C. E., W. Gao, D. D. Miller, C. E. Bell and J. T. Dalton (2005). "Structural basis for antagonism and resistance of bicalutamide in prostate cancer." Proc Natl Acad Sci U S A **102**(17): 6201-6206.

Boonying, W., A. Joselin, E. Huang, D. Qu, F. Safarpour, G. O. Iyirhiaro, Y. R. Gonzalez, S. M. Callaghan, R. S. Slack, D. Figeys, Y. H. Chung and D. S. Park (2019). "Pink1 regulates FKBP5 interaction with AKT/PHLPP and protects neurons from neurotoxin stress induced by MPP(.)." J Neurochem **150**(3): 312-329.

Bracher, A., C. Kozany, A. Hahle, P. Wild, M. Zacharias and F. Hausch (2013). "Crystal structures of the free and ligand-bound FK1-FK2 domain segment of FKBP52 reveal a flexible inter-domain hinge." J Mol Biol **425**(22): 4134-4144.

Buchner, J., T. Weikl, H. Bugl, F. Pirkl and S. Bose (1998). "Purification of Hsp90 partner proteins Hop/p60, p23, and FKBP52." Methods Enzymol **290**: 418-429.

Bulun, S. E., Y. H. Cheng, P. Yin, G. Imir, H. Utsunomiya, E. Attar, J. Innes and J. Julie Kim (2006). "Progesterone resistance in endometriosis: link to failure to metabolize estradiol." Mol Cell Endocrinol **248**(1-2): 94-103.

Callebaut, I., J. M. Renoir, M. C. Lebeau, N. Massol, A. Burny, E. E. Baulieu and J. P. Mornon (1992). "An immunophilin that binds M(r) 90,000 heat shock protein: main structural features of a mammalian p59 protein." Proc Natl Acad Sci U S A **89**(14): 6270-6274.

Castanheira, F. V. S. and P. Kubes (2019). "Neutrophils and NETs in modulating acute and chronic inflammation." Blood **133**(20): 2178-2185.

Ceder, Y., A. Bjartell, Z. Culig, M. A. Rubin, S. Tomlins and T. Visakorpi (2016). "The Molecular Evolution of Castration-resistant Prostate Cancer." Eur Urol Focus **2**(5): 506-513.

Chambraud, B., C. Byrne, G. Meduri, E. E. Baulieu and J. Giustiniani (2022). "FKBP52 in Neuronal Signaling and Neurodegenerative Diseases: A Microtubule Story." Int J Mol Sci **23**(3).

Chambraud, B., N. Rouviere-Fourmy, C. Radanyi, K. Hsiao, D. A. Peattie, D. J. Livingston and E. E. Baulieu (1993). "Overexpression of p59-HBI (FKBP59), full length and domains, and characterization of PPIase activity." Biochem Biophys Res Commun **196**(1): 160-166.

Chang, C., S. O. Lee, R. S. Wang, S. Yeh and T. M. Chang (2013). "Androgen receptor (AR) physiological roles in male and female reproductive systems: lessons learned from AR-knockout mice lacking AR in selective cells." Biol Reprod **89**(1): 21.

Check, J. H., E. Dix, C. Wilson and D. Check (2010). "Progesterone receptor antagonist therapy has therapeutic potential even in cancer restricted to males as evidenced from murine testicular and prostate cancer studies." Anticancer Res **30**(12): 4921-4923.

Chen, S. and N. Sang (2016). "Hypoxia-Inducible Factor-1: A Critical Player in the Survival Strategy of Stressed Cells." J Cell Biochem **117**(2): 267-278.

Chen, S., W. P. Sullivan, D. O. Toft and D. F. Smith (1998). "Differential interactions of p23 and the TPR-containing proteins Hop, Cyp40, FKBP52 and FKBP51 with Hsp90 mutants." Cell Stress Chaperones **3**(2): 118-129.

Chen, Y., Z. Liu, Y. Wang, J. Zhuang, Y. Peng, X. Mo, J. Chen, Y. Shi, M. Yu, W. Cai, Y. Li, X. Zhu, W. Yuan, Y. Li, F. Li, Z. Zhou, G. Dai, X. Ye, Y. Wan, Z. Jiang, P. Zhu, X. Fan and X. Wu (2020). "FKBP51 induces p53-dependent apoptosis and enhances drug sensitivity of human non-small-cell lung cancer cells." Exp Ther Med **19**(3): 2236-2242.

Cheung-Flynn, J., V. Prapapanich, M. B. Cox, D. L. Riggs, C. Suarez-Quian and D. F. Smith (2005). "Physiological role for the cochaperone FKBP52 in androgen receptor signaling." Mol Endocrinol **19**(6): 1654-1666.

Cheung-Flynn, J., V. Prapapanich, M. B. Cox, D. L. Riggs, C. Suarez-Quian and D. F. Smith (2005). "Physiological Role for the Cochaperone FKBP52 in Androgen Receptor Signaling." Molecular Endocrinology **19**(6): 1654-1666.

Cheung-Flynn, J., P. J. Roberts, D. L. Riggs and D. F. Smith (2003). "C-terminal Sequences outside the Tetratricopeptide Repeat Domain of FKBP51 and FKBP52 Cause Differential Binding to Hsp90." J Biol Chem **278**(19): 17388-17394.

Cluning, C., B. K. Ward, S. L. Rea, A. Arulpragasam, P. J. Fuller and T. Ratajczak (2013). "The helix 1-3 loop in the glucocorticoid receptor LBD is a regulatory element for FKBP cochaperones." Mol Endocrinol **27**(7): 1020-1035.

Cordani, M., G. Butera, R. Pacchiana and M. Donadelli (2017). "Molecular interplay between mutant p53 proteins and autophagy in cancer cells." Biochim Biophys Acta Rev Cancer **1867**(1): 19-28.

Cox, M. B., D. L. Riggs, M. Hessling, F. Schumacher, J. Buchner and D. F. Smith (2007). "FK506-binding protein 52 phosphorylation: a potential mechanism for regulating steroid hormone receptor activity." Mol Endocrinol. **21**(12): 2956-2967. Epub 2007 Aug 2923.

Cox, M. L. and D. W. Meek (2010). "Phosphorylation of serine 392 in p53 is a common and integral event during p53 induction by diverse stimuli." Cell Signal **22**(3): 564-571.

Criado-Marrero, M., N. T. Gebru, L. A. Gould, D. M. Blazier, Y. Vidal-Aguiar, T. M. Smith, S. S. Abdelmaboud, L. B. Shelton, X. Wang, J. Dahrendorff, D. Beaulieu-Abdelahad, C. A. Dickey and L. J. Blair (2021). "FKBP52 overexpression accelerates hippocampal-dependent memory impairments in a tau transgenic mouse model." NPJ Aging Mech Dis **7**(1): 9.

Criado-Marrero, M., T. Rein, E. B. Binder, J. T. Porter, J. Koren, 3rd and L. J. Blair (2018). "Hsp90 and FKBP51: complex regulators of psychiatric diseases." Philos Trans R Soc Lond B Biol Sci **373**(1738).

Cuadrado, A. (2022). "Brain-Protective Mechanisms of Transcription Factor NRF2: Toward a Common Strategy for Neurodegenerative Diseases." Annu Rev Pharmacol Toxicol **62**: 255-277.

Cuadrado, A., M. Pajares, C. Benito, J. Jimenez-Villegas, M. Escoll, R. Fernandez-Gines, A. J. Garcia Yague, D. Lastra, G. Manda, A. I. Rojo and A. T. Dinkova-Kostova (2020). "Can Activation of NRF2 Be a Strategy against COVID-19?" Trends Pharmacol Sci **41**(9): 598-610.

Cyr, D. M. (1995). "Cooperation of the molecular chaperone Ydj1 with specific Hsp70 homologs to suppress protein aggregation." FEBS Lett **359**(2-3): 129-132.

D'Amato, N. C., M. A. Gordon, B. Babbs, N. S. Spoelstra, K. T. Carson Butterfield, K. C. Torkko, V. T. Phan, V. N. Barton, T. J. Rogers, C. A. Sartorius, A. Elias, J. Gertz, B. M. Jacobsen and J. K. Richer (2016). "Cooperative Dynamics of AR and ER Activity in Breast Cancer." Mol Cancer Res **14**(11): 1054-1067.

Dannlowski, U., H. J. Grabe, K. Wittfeld, J. Klaus, C. Konrad, D. Grotegerd, R. Redlich, T. Suslow, N. Opel, P. Ohrmann, J. Bauer, P. Zwanzger, I. Laeger, C. Hohoff, V. Arolt, W. Heindel, M. Deppe, K. Domschke, K. Hegenscheid, H. Volzke, D. Stacey, H. Meyer Zu Schwabedissen, H. Kugel and B. T. Baune (2015). "Multimodal imaging of a tescalcin (TESC)-regulating polymorphism (rs7294919)-specific effects on hippocampal gray matter structure." Mol Psychiatry **20**(3): 398-404.

Davies, T. H., Y. M. Ning and E. R. Sanchez (2002). "A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins." J Biol Chem **277**(7): 4597-4600.

De Leon, J. T., A. Iwai, C. Feau, Y. Garcia, H. A. Balsiger, C. L. Storer, R. M. Suro, K. M. Garza, S. Lee, Y. S. Kim, Y. Chen, Y. M. Ning, D. L. Riggs, R. J. Fletterick, R. K. Guy, J. B. Trepel, L. M. Neckers and M. B. Cox (2011). "Targeting the regulation of androgen

receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells." Proc Natl Acad Sci U S A **108**(29): 11878-11883.

De Leon, J. T., A. Iwai, C. Feau, Y. Garcia, H. A. Balsiger, C. L. Storer, R. M. Suro, K. M. Garza, S. Lee, Y. Sang Kim, Y. Chen, Y. M. Ning, D. L. Riggs, R. J. Fletterick, R. K. Guy, J. B. Trepel, L. M. Neckers and M. B. Cox (2011). "Targeting the regulation of androgen receptor signaling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells." Proc Natl Acad Sci U S A **108**(29): 11878-11883.

Denny, W. B., D. L. Valentine, P. D. Reynolds, D. F. Smith and J. G. Scammell (2000). "Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding." Endocrinology **141**(11): 4107-4113.

Dinsdale, N. L. and B. J. Crespi (2021). "Endometriosis and polycystic ovary syndrome are diametric disorders." Evol Appl **14**(7): 1693-1715.

Dong, J., Y. Jiao, W. Mu, B. Lu, M. Wei, L. Sun, S. Hu, B. Cui, X. Liu, Z. Chen and Y. Zhao (2017). "FKBP51 decreases cell proliferation and increases progestin sensitivity of human endometrial adenocarcinomas by inhibiting Akt." Oncotarget **8**(46): 80405-80415.

Dunyak, B. M. and J. E. Gestwicki (2016). "Peptidyl-Proline Isomerases (PPIases): Targets for Natural Products and Natural Product-Inspired Compounds." J Med Chem **59**(21): 9622-9644.

Ebong, I. O., V. Beilsten-Edmands, N. A. Patel, N. Morgner and C. V. Robinson (2016). "The interchange of immunophilins leads to parallel pathways and different intermediates in the assembly of Hsp90 glucocorticoid receptor complexes." Cell Discov **2**: 16002.

Echeverria, P. C., G. Mazaira, A. Erlejman, C. Gomez-Sanchez, G. Piwien Pilipuk and M. D. Galigniana (2009). "Nuclear import of the glucocorticoid receptor-hsp90 complex through the nuclear pore complex is mediated by its interaction with Nup62 and importin beta." Mol Cell Biol **29**(17): 4788-4797.

Ekpenyong, O., C. Cooper, J. Ma, N. C. Guy, A. N. Payan, F. Ban, A. Cherkasov, M. B. Cox, D. Liang and H. Xie (2020). "Bioanalytical Assay Development and Validation for the Pharmacokinetic Study of GMC1, a Novel FKBP52 Co-chaperone Inhibitor for Castration Resistant Prostate Cancer." Pharmaceuticals (Basel) **13**(11).

Engelhardt, C., B. Boulat, M. Czisch and M. V. Schmidt (2021). "Lack of FKBP51 Shapes Brain Structure and Connectivity in Male Mice." J Magn Reson Imaging **53**(5): 1358-1365.

Estebanez-Perpina, E., J. M. Moore, E. Mar, E. Delgado-Rodrigues, P. Nguyen, J. D. Baxter, B. M. Buehrer, P. Webb, R. J. Fletterick and R. K. Guy (2005). "The molecular mechanisms of coactivator utilization in ligand-dependent transactivation by the androgen receptor." J Biol Chem **280**(9): 8060-8068.

Fan, C. Y., H. Y. Ren, P. Lee, A. J. Caplan and D. M. Cyr (2005). "The type I Hsp40 zinc finger-like region is required for Hsp70 to capture non-native polypeptides from Ydj1." J Biol Chem **280**(1): 695-702.

Fan, Y. C., K. D. Lee and Y. C. Tsai (2020). "Roles of Interleukin-1 Receptor Antagonist in Prostate Cancer Progression." Biomedicines **8**(12).

Fani, N., D. Gutman, E. B. Tone, L. Almli, K. B. Mercer, J. Davis, E. Glover, T. Jovanovic, B. Bradley, I. D. Dinov, A. Zamanyan, A. W. Toga, E. B. Binder and K. J. Ressler (2013). "FKBP5 and attention bias for threat: associations with hippocampal function and shape." JAMA Psychiatry **70**(4): 392-400.

Federer-Gsponer, J. R., C. Quintavalle, D. C. Muller, T. Dietsche, V. Perrina, T. Lorber, D. Juskevicius, E. Lenkiewicz, T. Zellweger, T. Gasser, M. T. Barrett, C. A. Rentsch, L. Bubendorf and C. Ruiz (2018). "Delineation of human prostate cancer evolution identifies chromothripsis as a polyclonal event and FKBP4 as a potential driver of castration resistance." J Pathol **245**(1): 74-84.

Fischer, G. (1994). "Peptidyl-Prolyl &/trans Isomerases and Their Effectors." 1415-1436 .

Fischer, G., H. Bang and C. Mech (1984). "[Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides]." Biomed Biochim Acta. **43**(10): 1101-1111.

Fliss, A. E., J. Rao, M. W. Melville, M. E. Cheetham and A. J. Caplan (1999). "Domain requirements of DnaJ-like (Hsp40) molecular chaperones in the activation of a steroid hormone receptor." J Biol Chem **274**(48): 34045-34052.

Fries, G. R., N. C. Gassen and T. Rein (2017). "The FKBP51 Glucocorticoid Receptor Co-Chaperone: Regulation, Function, and Implications in Health and Disease." Int J Mol Sci **18**(12).

Gaali, S., A. Kirschner, S. Cuboni, J. Hartmann, C. Kozany, G. Balsevich, C. Namendorf, P. Fernandez-Vizarra, C. Sippel, A. S. Zannas, R. Draenert, E. B. Binder, O. F. Almeida, G. Ruhter, M. Uhr, M. V. Schmidt, C. Touma, A. Bracher and F. Hausch (2015). "Selective inhibitors of the FK506-binding protein 51 by induced fit." Nat Chem Biol **11**(1): 33-37.

Galat, A. (1993). "Peptidylproline cis-trans-isomerases: immunophilins." Eur J Biochem **216**(3): 689-707.

Galigniana, M. D., A. G. Erlejman, M. Monte, C. Gomez-Sanchez and G. Piwien-Pilipuk (2010). "The hsp90-FKBP52 complex links the mineralocorticoid receptor to motor proteins and persists bound to the receptor in early nuclear events." Mol Cell Biol **30**(5): 1285-1298.

Galigniana, M. D., J. M. Harrell, P. J. Murphy, M. Chinkers, C. Radanyi, J. M. Renoir, M. Zhang and W. B. Pratt (2002). "Binding of hsp90-associated immunophilins to

cytoplasmic dynein: direct binding and in vivo evidence that the peptidylprolyl isomerase domain is a dynein interaction domain." Biochemistry **41**(46): 13602-13610.

Galigniana, M. D., C. Radanyi, J. M. Renoir, P. R. Housley and W. B. Pratt (2001). "Evidence that the peptidylprolyl isomerase domain of the hsp90-binding immunophilin FKBP52 is involved in both dynein interaction and glucocorticoid receptor movement to the nucleus." J Biol Chem **276**(18): 14884-14889.

Gan, Y. L., C. Y. Wang, R. H. He, P. C. Hsu, H. H. Yeh, T. H. Hsieh, H. C. Lin, M. Y. Cheng, C. J. Jeng, M. C. Huang and Y. H. Lee (2022). "FKBP51 mediates resilience to inflammation-induced anxiety through regulation of glutamic acid decarboxylase 65 expression in mouse hippocampus." J Neuroinflammation **19**(1): 152.

Gellersen, B., I. A. Brosens and J. J. Brosens (2007). "Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives." Semin Reprod Med **25**(6): 445-453.

Gellersen, B. and J. J. Brosens (2014). "Cyclic decidualization of the human endometrium in reproductive health and failure." Endocr Rev **35**(6): 851-905.

Gelmann, E. P. (2002). "Molecular biology of the androgen receptor." J Clin Oncol **20**(13): 3001-3015.

Ghartey-Kwansah, G., Z. Li, R. Feng, L. Wang, X. Zhou, F. Z. Chen, M. M. Xu, O. Jones, Y. Mu, S. Chen, J. Bryant, W. B. Isaacs, J. Ma and X. Xu (2018). "Comparative analysis of FKBP family protein: evaluation, structure, and function in mammals and Drosophila melanogaster." BMC Dev Biol **18**(1): 7.

Giovannelli, P., M. Di Donato, G. Galasso, E. Di Zazzo, A. Bilancio and A. Migliaccio (2018). "The Androgen Receptor in Breast Cancer." Front Endocrinol (Lausanne) **9**: 492.

Giustiniani, J., B. Chambraud, E. Sardin, O. Dounane, K. Guillemeau, H. Nakatani, D. Paquet, A. Kamah, I. Landrieu, G. Lippens, E. E. Baulieu and M. Tawk (2014). "Immunophilin FKBP52 induces Tau-P301L filamentous assembly in vitro and modulates its activity in a model of tauopathy." Proc Natl Acad Sci U S A **111**(12): 4584-4589.

Giustiniani, J., K. Guillemeau, O. Dounane, E. Sardin, I. Huvent, A. Schmitt, M. Hamdane, L. Buee, I. Landrieu, G. Lippens, E. E. Baulieu and B. Chambraud (2015). "The FK506-binding protein FKBP52 in vitro induces aggregation of truncated Tau forms with prion-like behavior." FASEB J **29**(8): 3171-3181.

Giustiniani, J., M. Sineus, E. Sardin, O. Dounane, M. Panchal, V. Sazdovitch, C. Duyckaerts, B. Chambraud and E. E. Baulieu (2012). "Decrease of the immunophilin FKBP52 accumulation in human brains of Alzheimer's disease and FTDP-17." J Alzheimers Dis **29**(2): 471-483.

Graves, J. A., M. Metukuri, D. Scott, K. Rothermund and E. V. Prochownik (2009). "Regulation of reactive oxygen species homeostasis by peroxiredoxins and c-Myc." J Biol Chem **284**(10): 6520-6529.

Grino, P. B., J. E. Griffin and J. D. Wilson (1990). "Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone." Endocrinology **126**(2): 1165-1172.

Gu, J., Y. Li, J. Zeng, B. Wang, K. Ji, Y. Tang and Q. Sun (2017). "Knockdown of HIF-1alpha by siRNA-expressing plasmid delivered by attenuated Salmonella enhances the antitumor effects of cisplatin on prostate cancer." Sci Rep **7**(1): 7546.

Guy, N. C., Y. A. Garcia and M. B. Cox (2015). "Therapeutic Targeting of the FKBP52 Co-Chaperone in Steroid Hormone Receptor-Regulated Physiology and Disease." Curr Mol Pharmacol **9**(2): 109-125.

Guzeloglu-Kayisli, O., N. Semerci, X. Guo, K. Larsen, A. Ozmen, S. Arlier, D. Mutluay, C. Nwabuobi, B. Sipe, I. Buhimschi, C. Buhimschi, F. Schatz, U. A. Kayisli and C. J. Lockwood (2021). "Decidual cell FKBP51-progesterone receptor binding mediates maternal stress-induced preterm birth." Proc Natl Acad Sci U S A **118**(11).

Habara, M., Y. Sato, T. Goshima, M. Sakurai, H. Imai, H. Shimizu, Y. Katayama, S. Hanaki, T. Masaki, M. Morimoto, S. Nishikawa, T. Toyama and M. Shimada (2022). "FKBP52 and FKBP51 differentially regulate the stability of estrogen receptor in breast cancer." Proc Natl Acad Sci U S A **119**(15): e2110256119.

Harding, M. W., A. Galat, D. E. Uehling and S. L. Schreiber (1989). "A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase." Nature **341**(6244): 758-760.

Harris, D. C., Y. A. Garcia, C. S. Samaniego, V. W. Rowlett, N. R. Ortiz, A. N. Payan, T. Maehigashi and M. B. Cox (2019). "Functional Comparison of Human and Zebra Fish FKBP52 Confirms the Importance of the Proline-Rich Loop for Regulation of Steroid Hormone Receptor Activity." Int J Mol Sci **20**(21).

Hartl, F. U. and M. Hayer-Hartl (2002). "Molecular chaperones in the cytosol: from nascent chain to folded protein." Science **295**(5561): 1852-1858.

Hartmann, J., K. V. Wagner, S. Gaali, A. Kirschner, C. Kozany, G. Ruhter, N. Dedic, A. S. Hausl, L. Hoeijmakers, S. Westerholz, C. Namendorf, T. Gerlach, M. Uhr, A. Chen, J. M. Deussing, F. Holsboer, F. Hausch and M. V. Schmidt (2015). "Pharmacological Inhibition of the Psychiatric Risk Factor FKBP51 Has Anxiolytic Properties." J Neurosci **35**(24): 9007-9016.

Hartmann, J., K. V. Wagner, C. Liebl, S. H. Scharf, X. D. Wang, M. Wolf, F. Hausch, T. Rein, U. Schmidt, C. Touma, J. Cheung-Flynn, M. B. Cox, D. F. Smith, F. Holsboer, M. B. Muller and M. V. Schmidt (2012). "The involvement of FK506-binding protein 51

(FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress." Neuropharmacology **62**(1): 332-339.

Hausl, A. S., L. M. Brix, J. Hartmann, M. L. Pohlmann, J. P. Lopez, D. Menegaz, E. Brivio, C. Engelhardt, S. Roeh, T. Bajaj, L. Rudolph, R. Stoffel, K. Hafner, H. M. Goss, J. Reul, J. M. Deussing, M. Eder, K. J. Ressler, N. C. Gassen, A. Chen and M. V. Schmidt (2021). "The co-chaperone Fkbp5 shapes the acute stress response in the paraventricular nucleus of the hypothalamus of male mice." Mol Psychiatry **26**(7): 3060-3076.

He, B., J. A. Kemppainen, J. J. Voegel, H. Gronemeyer and E. M. Wilson (1999). "Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain." J Biol Chem **274**(52): 37219-37225.

He, Z., L. Li and S. Luan (2004). "Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in Arabidopsis." Plant Physiol **134**(4): 1248-1267.

Heemers, H. V. and D. J. Tindall (2007). "Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex." Endocr Rev **28**(7): 778-808.

Heinlein, C. A. and C. Chang (2002). "Androgen receptor (AR) coregulators: an overview." Endocr Rev **23**(2): 175-200.

Hendrick, J. P., T. Langer, T. A. Davis, F. U. Hartl and M. Wiedmann (1993). "Control of folding and membrane translocation by binding of the chaperone DnaJ to nascent polypeptides." Proc Natl Acad Sci U S A **90**(21): 10216-10220.

Hildenbrand, Z. L., S. K. Molugu, N. Herrera, C. Ramirez, C. Xiao and R. A. Bernal (2011). "Hsp90 can accommodate the simultaneous binding of the FKBP52 and HOP proteins." Oncotarget **2**(1-2): 43-58.

Hirota, Y., N. Acar, S. Tranguch, K. E. Burnum, H. Xie, A. Kodama, Y. Osuga, I. Ustunel, D. B. Friedman, R. M. Caprioli, T. Daikoku and S. K. Dey (2010). "Uterine FK506-binding protein 52 (FKBP52)-peroxiredoxin-6 (PRDX6) signaling protects pregnancy from overt oxidative stress." Proc Natl Acad Sci U S A **107**(35): 15577-15582.

Hirota, Y., S. Tranguch, T. Daikoku, A. Hasegawa, Y. Osuga, Y. Taketani and S. K. Dey (2008). "Deficiency of immunophilin FKBP52 promotes endometriosis." Am J Pathol **173**(6): 1747-1757.

Hohfeld, J., Y. Minami and F. U. Hartl (1995). "Hip, a novel cochaperone involved in the eukaryotic Hsc70/Hsp40 reaction cycle." Cell **83**(4): 589-598.

Hong, C., T. Li, F. Zhang, X. Wu, X. Chen, X. Cui, G. Zhang and Y. Cui (2017). "Elevated FKBP52 expression indicates a poor outcome in patients with breast cancer." Oncol Lett **14**(5): 5379-5385.

Hong, J., S. T. Kim, S. Tranguch, D. F. Smith and S. K. Dey (2007). "Deficiency of co-chaperone immunophilin FKBP52 compromises sperm fertilizing capacity." Reproduction. **133**(2): 395-403.

Horii, K., Y. Suzuki, Y. Kondo, M. Akimoto, T. Nishimura, Y. Yamabe, M. Sakaue, T. Sano, T. Kitagawa, S. Himeno, N. Imura and S. Hara (2007). "Androgen-dependent gene expression of prostate-specific antigen is enhanced synergistically by hypoxia in human prostate cancer cells." Mol Cancer Res **5**(4): 383-391.

Hou, J. and L. Wang (2012). "FKBP5 as a selection biomarker for gemcitabine and Akt inhibitors in treatment of pancreatic cancer." PLoS One **7**(5): e36252.

Huang, X., C. H. Chau and W. D. Figg (2012). "Challenges to improved therapeutics for metastatic castrate resistant prostate cancer: from recent successes and failures." J Hematol Oncol **5**: 35.

Hubler, T. R., W. B. Denny, D. L. Valentine, J. Cheung-Flynn, D. F. Smith and J. G. Scammell (2003). "The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progestin and attenuates progestin responsiveness." Endocrinology **144**(6): 2380-2387.

Ishii, T., E. Warabi and T. Yanagawa (2012). "Novel roles of peroxiredoxins in inflammation, cancer and innate immunity." J Clin Biochem Nutr **50**(2): 91-105.

Jaaskelainen, T., H. Makkonen and J. J. Palvimo (2011). "Steroid up-regulation of FKBP51 and its role in hormone signaling." Curr Opin Pharmacol **11**(4): 326-331.

Jagtap, P. K. A., S. Asami, C. Sippel, V. R. I. Kaila, F. Hausch and M. Sattler (2019). "Corrigendum: Selective Inhibitors of FKBP51 Employ Conformational Selection of Dynamic Invisible States." Angew Chem Int Ed Engl **58**(39): 13619.

Jiang, W., S. Cazacu, C. Xiang, J. C. Zenklusen, H. A. Fine, M. Berens, B. Armstrong, C. Brodie and T. Mikkelsen (2008). "FK506 binding protein mediates glioma cell growth and sensitivity to rapamycin treatment by regulating NF-kappaB signaling pathway." Neoplasia **10**(3): 235-243.

Jinwal, U. K., J. Koren, III, S. I. Borysov, A. B. Schmid, J. F. Abisambra, L. J. Blair, A. G. Johnson, J. R. Jones, C. L. Shults, J. C. O'Leary, III, Y. Jin, J. Buchner, M. B. Cox and C. A. Dickey (2010). "The Hsp90 cochaperone, FKBP51, increases Tau stability and polymerizes microtubules." J.Neurosci. **30**(2): 591-599.

Joshi, N. R., E. H. Miyadahira, Y. Afshar, J. W. Jeong, S. L. Young, B. A. Lessey, P. C. Serafini and A. T. Fazleabas (2017). "Progesterone Resistance in Endometriosis Is Modulated by the Altered Expression of MicroRNA-29c and FKBP4." J Clin Endocrinol Metab **102**(1): 141-149.

Kang, C. B., Y. Hong, S. Dhe-Paganon and H. S. Yoon (2008). "FKBP family proteins: immunophilins with versatile biological functions." Neurosignals **16**(4): 318-325.

Ketefian, A., M. R. Jones, R. M. Krauss, Y. D. Chen, R. S. Legro, R. Azziz and M. O. Goodarzi (2016). "Association study of androgen signaling pathway genes in polycystic ovary syndrome." Fertil Steril **105**(2): 467-473 e464.

Kinter, K. J. and A. A. Anekar (2020). Biochemistry, Dihydrotestosterone. StatPearls. Treasure Island (FL).

Kolos, J. M., A. M. Voll, M. Bauder and F. Hausch (2018). "FKBP Ligands-Where We Are and Where to Go?" Front Pharmacol **9**: 1425.

Koochekpour, S. (2010). "Androgen receptor signaling and mutations in prostate cancer." Asian J Androl **12**(5): 639-657.

Kumar, R. and I. J. McEwan (2012). "Allosteric modulators of steroid hormone receptors: structural dynamics and gene regulation." Endocr Rev **33**(2): 271-299.

Kumari, S., D. Senapati and H. V. Heemers (2017). "Rationale for the development of alternative forms of androgen deprivation therapy." Endocr Relat Cancer **24**(8): R275-R295.

Kundrat, L. and L. Regan (2010). "Identification of residues on Hsp70 and Hsp90 ubiquitinated by the cochaperone CHIP." J Mol Biol **395**(3): 587-594.

Langer, T., C. Lu, H. Echols, J. Flanagan, M. K. Hayer and F. U. Hartl (1992). "Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding." Nature **356**(6371): 683-689.

Langley, E., J. A. Kemppainen and E. M. Wilson (1998). "Intermolecular NH2-/Carboxyl-terminal Interactions in Androgen Receptor Dimerization Revealed by Mutations That Cause Androgen Insensitivity." J. Biol. Chem. **273**(1): 92-101.

Laplante, M. and D. M. Sabatini (2012). "mTOR signaling in growth control and disease." Cell **149**(2): 274-293.

Leitao, B., M. C. Jones, L. Fusi, J. Higham, Y. Lee, M. Takano, T. Goto, M. Christian, E. W. Lam and J. J. Brosens (2010). "Silencing of the JNK pathway maintains progesterone receptor activity in decidualizing human endometrial stromal cells exposed to oxidative stress signals." FASEB J **24**(5): 1541-1551.

Li, H., Y. L. Jiao, R. F. Zhou, S. Liu, B. Cui, L. C. Wang, X. W. Liu and Y. R. Zhao (2020). "FKBP51 acts as a biomarker of early metastasis and is related to carmustine sensitivity in human glioma cells." Eur Rev Med Pharmacol Sci **24**(17): 8918-8930.

Li, J., J. Soroka and J. Buchner (2012). "The Hsp90 chaperone machinery: conformational dynamics and regulation by co-chaperones." Biochim Biophys Acta **1823**(3): 624-635.

Li, L., Z. Lou and L. Wang (2011). "The role of FKBP5 in cancer aetiology and chemoresistance." Br J Cancer **104**(1): 19-23.

Liang, S., X. Bian, D. Liang, J. C. Sivils, L. M. Neckers, M. B. Cox and H. Xie (2016). "Solution formulation development and efficacy of MJC13 in a preclinical model of castration-resistant prostate cancer." Pharm Dev Technol **21**(1): 121-126.

Lin, J. F., J. Xu, H. Y. Tian, X. Gao, Q. X. Chen, Q. Gu, G. J. Xu, J. D. Song and F. K. Zhao (2007). "Identification of candidate prostate cancer biomarkers in prostate needle biopsy specimens using proteomic analysis." Int J Cancer **121**(12): 2596-2605.

Liu, L., J. Cheng, F. Wei, L. Pang, Z. Zhi, W. Yang and W. Tan (2021). "The Influence Mechanism of Abnormal Immunophilin FKBP52 on the Expression Levels of PR-A and PR-B in Endometriosis Based on Endometrial Stromal Cell Model in Vitro." Organogenesis **17**(1-2): 1-13.

Lonergan, P. E. and D. J. Tindall (2011). "Androgen receptor signaling in prostate cancer development and progression." J Carcinog **10**: 20.

Lousse, J. C., A. Van Langendonckt, S. Defrere, R. G. Ramos, S. Colette and J. Donnez (2012). "Peritoneal endometriosis is an inflammatory disease." Front Biosci (Elite Ed) **4**(1): 23-40.

Lu, Y., F. Feng, Y. Yang, X. Gao, J. Cui, C. Zhang, F. Zhang, Z. Xu, J. Qv, C. Wang, Z. Zeng, Y. Zhu and Y. Yang (2013). "LINE-1 ORF-1p functions as a novel androgen receptor co-activator and promotes the growth of human prostatic carcinoma cells." Cell Signal **25**(2): 479-489.

Madureira, P. A. and D. M. Waisman (2013). "Annexin A2: the importance of being redox sensitive." Int J Mol Sci **14**(2): 3568-3594.

Maeda, K., M. Habara, M. Kawaguchi, H. Matsumoto, S. Hanaki, T. Masaki, Y. Sato, H. Matsuyama, K. Kunieda, H. Nakagawa and M. Shimada (2022). "FKBP51 and FKBP52 regulate androgen receptor dimerization and proliferation in prostate cancer cells." Mol Oncol **16**(4): 940-956.

Mange, A., E. Coyaud, C. Desmetz, E. Laurent, B. Beganton, P. Coopman, B. Raught and J. Solassol (2019). "FKBP4 connects mTORC2 and PI3K to activate the PDK1/Akt-dependent cell proliferation signaling in breast cancer." Theranostics **9**(23): 7003-7015.

Marivoet, S., P. Van Dijck, G. Verhoeven and W. Heyns (1992). "Interaction of the 90-kDa heat shock protein with native and in vitro translated androgen receptor and receptor fragments." Mol Cell Endocrinol **88**(1-3): 165-174.

Matias, P. M., P. Donner, R. Coelho, M. Thomaz, C. Peixoto, S. Macedo, N. Otto, S. Joschko, P. Scholz, A. Wegg, S. Basler, M. Schafer, U. Egner and M. A. Carrondo (2000). "Structural evidence for ligand specificity in the binding domain of the human androgen

receptor. Implications for pathogenic gene mutations." J Biol Chem **275**(34): 26164-26171.

Meduri, G., K. Guillemeau, O. Dounane, V. Sazdovitch, C. Duyckaerts, B. Chambraud, E. E. Baulieu and J. Giustiniani (2016). "Caspase-cleaved Tau-D(421) is colocalized with the immunophilin FKBP52 in the autophagy-endolysosomal system of Alzheimer's disease neurons." Neurobiol Aging **46**: 124-137.

Meng, W., J. Meng, H. Jiang, X. Feng, D. Wei and Q. Ding (2020). "FKBP4 Accelerates Malignant Progression of Non-Small-Cell Lung Cancer by Activating the Akt/mTOR Signaling Pathway." Anal Cell Pathol (Amst) **2020**: 6021602.

Miyata, Y., B. Chambraud, C. Radanyi, J. Leclerc, M. C. Lebeau, J. M. Renoir, R. Shirai, M. G. Catelli, I. Yahara and E. E. Baulieu (1997). "Phosphorylation of the immunosuppressant FK506-binding protein FKBP52 by casein kinase II: regulation of HSP90-binding activity of FKBP52." Proc Natl Acad Sci U S A **94**(26): 14500-14505.

Morishima, Y., K. C. Kanelakis, P. J. Murphy, E. R. Lowe, G. J. Jenkins, Y. Osawa, R. K. Sunahara and W. B. Pratt (2003). "The hsp90 cochaperone p23 is the limiting component of the multiprotein hsp90/hsp70-based chaperone system in vivo where it acts to stabilize the client protein: hsp90 complex." J Biol Chem **278**(49): 48754-48763.

Nadal, M., S. Prekovic, N. Gallastegui, C. Helsen, M. Abella, K. Zielinska, M. Gay, M. Vilaseca, M. Taules, A. B. Houtsmuller, M. E. van Royen, F. Claessens, P. Fuentes-Prior and E. Estebanez-Perpina (2017). "Structure of the homodimeric androgen receptor ligand-binding domain." Nat Commun **8**: 14388.

Nair, S. C., R. A. Rimerman, E. J. Toran, S. Chen, V. Prapapanich, R. N. Butts and D. F. Smith (1997). "Molecular cloning of human FKBP51 and comparisons of immunophilin interactions with Hsp90 and progesterone receptor." Mol Cell Biol **17**(2): 594-603.

Neckers, L. (2007). "Heat shock protein 90: the cancer chaperone." J Biosci **32**(3): 517-530.

Ni, L., C. S. Yang, D. Gioeli, H. Frierson, D. O. Toft and B. M. Paschal (2010). "FKBP51 promotes assembly of the Hsp90 chaperone complex and regulates androgen receptor signaling in prostate cancer cells." Mol Cell Biol **30**(5): 1243-1253.

Nystrom, T., J. Yang and M. Molin (2012). "Peroxiredoxins, gerontogenes linking aging to genome instability and cancer." Genes Dev **26**(18): 2001-2008.

Ooi, B. K., B. H. Goh and W. H. Yap (2017). "Oxidative Stress in Cardiovascular Diseases: Involvement of Nrf2 Antioxidant Redox Signaling in Macrophage Foam Cells Formation." Int J Mol Sci **18**(11).

Oroz, J., B. J. Chang, P. Wysoczanski, C. T. Lee, A. Perez-Lara, P. Chakraborty, R. V. Hofele, J. D. Baker, L. J. Blair, J. Biernat, H. Urlaub, E. Mandelkow, C. A. Dickey and M.

Zweckstetter (2018). "Structure and pro-toxic mechanism of the human Hsp90/PPIase/Tau complex." Nat Commun **9**(1): 4532.

Ortiz, N. R. (2021). A genomic approach to assessing the FKBP52-regulated Androgen receptor cistrome and transcriptome, University of Texas at El Paso.

Park, S. Y., X. Yu, C. Ip, J. L. Mohler, P. N. Bogner and Y. M. Park (2007). "Peroxiredoxin 1 interacts with androgen receptor and enhances its transactivation." Cancer Res **67**(19): 9294-9303.

Peattie, D. A., M. W. Harding, M. A. Fleming, M. T. DeCenzo, J. A. Lippke, D. J. Livingston and M. Benasutti (1992). "Expression and characterization of human FKBP52, an immunophilin that associates with the 90-kDa heat shock protein and is a component of steroid receptor complexes." Proc Natl Acad Sci U S A **89**(22): 10974-10978.

Pei, H., L. Li, B. L. Fridley, G. D. Jenkins, K. R. Kalari, W. Lingle, G. Petersen, Z. Lou and L. Wang (2009). "FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt." Cancer Cell **16**(3): 259-266.

Pei, H., L. Li, B. L. Fridley, G. D. Jenkins, K. R. Kalari, W. Lingle, G. Petersen, Z. Lou and L. Wang (2009). "FKBP51 affects cancer cell response to chemotherapy by negatively regulating Akt." Cancer Cell **16**(3): 259-266.

Periyasamy, S., T. Hinds, Jr., L. Shemshedini, W. Shou and E. R. Sanchez (2010). "FKBP51 and Cyp40 are positive regulators of androgen-dependent prostate cancer cell growth and the targets of FK506 and cyclosporin A." Oncogene **29**(11): 1691-1701.

Periyasamy, S., M. Warrier, M. P. Tillekeratne, W. Shou and E. R. Sanchez (2007). "The immunophilin ligands cyclosporin A and FK506 suppress prostate cancer cell growth by androgen receptor-dependent and -independent mechanisms." Endocrinology **148**(10): 4716-4726.

Perkins, A., L. B. Poole and P. A. Karplus (2014). "Tuning of peroxiredoxin catalysis for various physiological roles." Biochemistry **53**(49): 7693-7705.

Pirkl, F. and J. Buchner (2001). "Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40." J Mol Biol **308**(4): 795-806.

Pirkl, F. and J. Buchner (2001). "Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40." J Mol Biol **308**(4): 795-806.

Pratt, W. B., A. M. Silverstein and M. D. Galigniana (1999). "A model for the cytoplasmic trafficking of signalling proteins involving the hsp90-binding immunophilins and p50cdc37." Cell Signal **11**(12): 839-851.

Prescott, J. and G. A. Coetzee (2006). "Molecular chaperones throughout the life cycle of the androgen receptor." Cancer Lett **231**(1): 12-19.

Qiu, B., Z. Zhong, S. Righter, Y. Xu, J. Wang, R. Deng, C. Wang, K. E. Williams, Y. Y. Ma, G. Tsechpenakis, T. Liang and W. Yong (2022). "FKBP51 modulates hippocampal size and function in post-translational regulation of Parkin." Cell Mol Life Sci **79**(3): 175.

Quintá, H. R., D. Maschi, C. Gomez-Sanchez, G. Piwien Pilipuk and M. D. Galigniana (2010). "Subcellular rearrangement of hsp90-binding immunophilins accompanies neuronal differentiation and neurite outgrowth." J Neurochem **115**: 716-734.

Raatikainen, S., S. Aaaltomaa, V. Karja and Y. Soini (2015). "Increased Peroxiredoxin 6 Expression Predicts Biochemical Recurrence in Prostate Cancer Patients After Radical Prostatectomy." Anticancer Res **35**(12): 6465-6470.

Rafiei, S., K. Tiedemann, S. Tabaries, P. M. Siegel and S. V. Komarova (2015). "Peroxiredoxin 4: a novel secreted mediator of cancer induced osteoclastogenesis." Cancer Lett **361**(2): 262-270.

Rhee, S. G. and I. S. Kil (2017). "Multiple Functions and Regulation of Mammalian Peroxiredoxins." Annu Rev Biochem **86**: 749-775.

Rhee, S. G., H. A. Woo and D. Kang (2018). "The Role of Peroxiredoxins in the Transduction of H(2)O(2) Signals." Antioxid Redox Signal **28**(7): 537-557.

Riddell, J. R., W. Bshara, M. T. Moser, J. A. Spernyak, B. A. Foster and S. O. Gollnick (2011). "Peroxiredoxin 1 controls prostate cancer growth through Toll-like receptor 4-dependent regulation of tumor vasculature." Cancer Res **71**(5): 1637-1646.

Riggs, D. L., M. B. Cox, H. L. Tardif, M. Hessling, J. Buchner and D. F. Smith (2007). "Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling." Mol Cell Biol **27**(24): 8658-8669.

Riggs, D. L., M. B. Cox, H. L. Tardif, M. Hessling, J. Buchner and D. F. Smith (2007). "Noncatalytic role of the FKBP52 peptidyl-prolyl isomerase domain in the regulation of steroid hormone signaling." Mol Cell Biol. **27**(24): 8658-8669. Epub 2007 Oct 8615.

Riggs, D. L., P. J. Roberts, S. C. Chirillo, J. Cheung-Flynn, V. Prapapanich, T. Ratajczak, R. Gaber, D. Picard and D. F. Smith (2003). "The Hsp90-binding peptidylprolyl isomerase FKBP52 potentiates glucocorticoid signaling in vivo." EMBO J **22**(5): 1158-1167.

Romano, S., Y. Xiao, M. Nakaya, A. D'Angelillo, M. Chang, J. Jin, F. Hausch, M. Masullo, X. Feng, M. F. Romano and S. C. Sun (2015). "FKBP51 employs both scaffold and isomerase functions to promote NF-kappaB activation in melanoma." Nucleic Acids Res **43**(14): 6983-6993.

Rostam, M. A., T. J. Piva, H. B. Rezaei, D. Kamato, P. J. Little, W. Zheng and N. Osman (2015). "Peptidyl-prolyl isomerases: functionality and potential therapeutic targets in cardiovascular disease." Clin Exp Pharmacol Physiol **42**(2): 117-124.

Rotoli, D., L. Diaz-Flores, R. Gutierrez, M. Morales, J. Avila and P. Martin-Vasallo (2022). "AmotL2, IQGAP1, and FKBP51 Scaffold Proteins in Glioblastoma Stem Cell Niches." J Histochem Cytochem **70**(1): 9-16.

Rouviere, N., M. Vincent, C. T. Craescu and J. Gallay (1997). "Immunosuppressor binding to the immunophilin FKBP59 affects the local structural dynamics of a surface beta-strand: time-resolved fluorescence study." Biochemistry **36**(24): 7339-7352.

Ruiz-Estevez, M., J. Staats, E. Paatela, D. Munson, N. Katoku-Kikyo, C. Yuan, Y. Asakura, R. Hostager, H. Kobayashi, A. Asakura and N. Kikyo (2018). "Promotion of Myoblast Differentiation by Fkbp5 via Cdk4 Isomerization." Cell Rep **25**(9): 2537-2551 e2538.

Russo, D., F. Merolla, M. Mascolo, G. Ilardi, S. Romano, S. Varricchio, V. Napolitano, A. Celetti, L. Postiglione, P. P. Di Lorenzo, L. Califano, G. O. Dell'Aversana, F. Astarita, M. F. Romano and S. Staibano (2017). "FKBP51 Immunohistochemical Expression: A New Prognostic Biomarker for OSCC?" Int J Mol Sci **18**(2).

Sabbagh, J. J., R. A. Cordova, D. Zheng, M. Criado-Marrero, A. Lemus, P. Li, J. D. Baker, B. A. Nordhues, A. L. Darling, C. Martinez-Licha, D. A. Rutz, S. Patel, J. Buchner, J. W. Leahy, J. Koren, 3rd, C. A. Dickey and L. J. Blair (2018). "Targeting the FKBP51/GR/Hsp90 Complex to Identify Functionally Relevant Treatments for Depression and PTSD." ACS Chem Biol **13**(8): 2288-2299.

Sack, J. S., K. F. Kish, C. Wang, R. M. Attar, S. E. Kiefer, Y. An, G. Y. Wu, J. E. Scheffler, M. E. Salvati, S. R. Krystek, Jr., R. Weinmann and H. M. Einspahr (2001). "Crystallographic structures of the ligand-binding domains of the androgen receptor and its T877A mutant complexed with the natural agonist dihydrotestosterone." Proc Natl Acad Sci U S A **98**(9): 4904-4909.

Sakaguchi, K., H. Sakamoto, M. S. Lewis, C. W. Anderson, J. W. Erickson, E. Appella and D. Xie (1997). "Phosphorylation of serine 392 stabilizes the tetramer formation of tumor suppressor protein p53." Biochemistry **36**(33): 10117-10124.

Scheufler, C., A. Brinker, G. Bourenkov, S. Pegoraro, L. Moroder, H. Bartunik, F. U. Hartl and I. Moarefi (2000). "Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine." Cell **101**(2): 199-210.

Schmidt, L. J., K. M. Regan, S. K. Anderson, Z. Sun, K. V. Ballman and D. J. Tindall (2009). "Effects of the 5 alpha-reductase inhibitor dutasteride on gene expression in prostate cancer xenografts." Prostate **69**(16): 1730-1743.

Schroer, T. A. (2004). "Dynactin." Annu Rev Cell Dev Biol **20**: 759-779.

Shiota, M., A. Yokomizo, E. Kashiwagi, A. Takeuchi, N. Fujimoto, T. Uchiumi and S. Naito (2011). "Peroxiredoxin 2 in the nucleus and cytoplasm distinctly regulates androgen receptor activity in prostate cancer cells." Free Radic Biol Med **51**(1): 78-87.

Shirane-Kitsuji, M. and K. I. Nakayama (2014). "Mitochondria: FKBP38 and mitochondrial degradation." Int J Biochem Cell Biol **51**: 19-22.

Sikorski, R. S., M. S. Boguski, M. Goebl and P. Hieter (1990). "A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis." Cell **60**(2): 307-317.

Simental, J. A., M. Sar, M. V. Lane, F. S. French and E. M. Wilson (1991). "Transcriptional activation and nuclear targeting signals of the human androgen receptor." J Biol Chem **266**(1): 510-518.

Sinars, C. R., J. Cheung-Flynn, R. A. Rimerman, J. G. Scammell, D. F. Smith and J. Clardy (2003). "Structure of the large FK506-binding protein FKBP51, an Hsp90-binding protein and a component of steroid receptor complexes." Proc Natl Acad Sci U S A **100**(3): 868-873.

Sivils, J. C., C. L. Storer, M. D. Galigniana and M. B. Cox (2011). "Regulation of steroid hormone receptor function by the 52-kDa FK506-binding protein (FKBP52)." Curr Opin Pharmacol **19**: 19.

Smedlund, K. B., E. R. Sanchez and T. D. Hinds, Jr. (2021). "FKBP51 and the molecular chaperoning of metabolism." Trends Endocrinol Metab **32**(11): 862-874.

Smith, D. F. (2004). "Tetratricopeptide repeat cochaperones in steroid receptor complexes." Cell Stress Chap **9**(2): 109-121.

Solassol, J., A. Mange and T. Maudelonde (2011). "FKBP family proteins as promising new biomarkers for cancer." Curr Opin Pharmacol **11**(4): 320-325.

Solit, D. B., H. I. Scher and N. Rosen (2003). "Hsp90 as a therapeutic target in prostate cancer." Semin Oncol **30**(5): 709-716.

Somarelli, J. A., S. Y. Lee, J. Skolnick and R. J. Herrera (2008). "Structure-based classification of 45 FK506-binding proteins." Proteins **72**(1): 197-208.

Song, S. and Y. Tan (2019). "Expression of FKBP52 in the ovaries of PCOS rats." Int J Mol Med **43**(2): 868-878.

Sramkoski, R. M., T. G. Pretlow, 2nd, J. M. Giaconia, T. P. Pretlow, S. Schwartz, M. S. Sy, S. R. Marengo, J. S. Rhim, D. Zhang and J. W. Jacobberger (1999). "A new human prostate carcinoma cell line, 22Rv1." In Vitro Cell Dev Biol Anim **35**(7): 403-409.

Staibano, S., M. Mascolo, G. Ilardi, M. Siano and G. De Rosa (2011). "Immunohistochemical analysis of FKBP51 in human cancers." Curr Opin Pharmacol **11**(4): 338-347.

Stancill, J. S. and J. A. Corbett (2023). "Hydrogen peroxide detoxification through the peroxiredoxin/thioredoxin antioxidant system: A look at the pancreatic beta-cell oxidant defense." Vitam Horm **121**: 45-66.

Stewart, M. (2007). "Molecular mechanism of the nuclear protein import cycle." Nat Rev Mol Cell Biol **8**(3): 195-208.

Storer, C. L. (2010). A Novel Synergistic Relationship between FKBP52 and Beta Catenin. El Paso, TX, The University of Texas at El Paso.

Storer, C. L., C. A. Dickey, M. D. Galigniana, T. Rein and M. B. Cox (2011). "FKBP51 and FKBP52 in signaling and disease." Trends Endocrinol Metab **22**(12): 481-490.

Suh, J. H., A. Chattopadhyay, D. H. Sieglaff, C. Storer Samaniego, M. B. Cox and P. Webb (2015). "Similarities and Distinctions in Actions of Surface-Directed and Classic Androgen Receptor Antagonists." PLoS One **10**(9): e0137103.

Tan, M. H. E., J. Li, H. E. Xu, K. Melcher and E.-l. Yong (2014). "Androgen receptor: structure, role in prostate cancer and drug discovery." Acta Pharmacologica Sinica **36**: 3.

Tao, Y. J. and W. Zheng (2011). "Chaperones and the maturation of steroid hormone receptor complexes." Oncotarget **2**(3): 104-106.

Thapa, P., H. Jiang, N. Ding, Y. Hao, A. Alshahrani and Q. Wei (2023). "The Role of Peroxiredoxins in Cancer Development." Biology (Basel) **12**(5).

Tranguch, S., J. Cheung-Flynn, T. Daikoku, V. Prapapanich, M. B. Cox, H. Xie, H. Wang, S. K. Das, D. F. Smith and S. K. Dey (2005). "Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation." Proc Natl Acad Sci U S A. **102**(40): 14326-14331. Epub 12005 Sep 14321.

Tranguch, S., J. Cheung-Flynn, T. Daikoku, V. Prapapanich, M. B. Cox, H. Xie, H. Wang, S. K. Das, D. F. Smith and S. K. Dey (2005). "Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation." Proc Natl Acad Sci U S A **102**(40): 14326-14331.

Tranguch, S., H. Wang, T. Daikoku, H. Xie, D. F. Smith and S. K. Dey (2007). "FKBP52 deficiency-conferred uterine progesterone resistance is genetic background and pregnancy stage specific." J Clin Invest. **117**(7): 1824-1834.

Tufano, M., E. Cesaro, R. Martinelli, R. Pacelli, S. Romano and M. F. Romano (2021). "FKBP51 Affects TNF-Related Apoptosis Inducing Ligand Response in Melanoma." Front Cell Dev Biol **9**: 718947.

Uribe-Querol, E. and C. Rosales (2015). "Neutrophils in Cancer: Two Sides of the Same Coin." J Immunol Res **2015**: 983698.

Van Duyne, G. D., R. F. Standaert, P. A. Karplus, S. L. Schreiber and J. Clardy (1991). "Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex." Science **252**(5007): 839-842.

Veldscholte, J., C. A. Berrevoets, N. D. Zegers, T. H. van der Kwast, J. A. Grootegoed and E. Mulder (1992). "Hormone-induced dissociation of the androgen receptor-heat-shock protein complex: use of a new monoclonal antibody to distinguish transformed from nontransformed receptors." Biochemistry **31**(32): 7422-7430.

Voll, A. M., C. Meyners, M. C. Taubert, T. Bajaj, T. Heymann, S. Merz, A. Charalampidou, J. Kolos, P. L. Purder, T. M. Geiger, P. Wessig, N. C. Gassen, A. Bracher and F. Hausch (2021). "Macrocyclic FKBP51 Ligands Define a Transient Binding Mode with Enhanced Selectivity." Angew Chem Int Ed Engl **60**(24): 13257-13263.

Wang, C. and J. Chen (2003). "Phosphorylation and hsp90 binding mediate heat shock stabilization of p53." J Biol Chem **278**(3): 2066-2071.

Wang, C. C., S. C. Chai and M. R. Holahan (2010). "Effect of stimulus pre-exposure on inhibitory avoidance retrieval-associated changes in the phosphorylated form of the extracellular signal-regulated kinase-1 and -2 (pERK1/2)." Neurobiol Learn Mem **93**(1): 66-76.

Wang, W., F. B. Meng, Z. X. Wang, X. Li and D. S. Zhou (2018). "Selenocysteine inhibits human osteosarcoma cells growth through triggering mitochondrial dysfunction and ROS-mediated p53 phosphorylation." Cell Biol Int **42**(5): 580-588.

Wei, M., Y. Gao, B. Lu, Y. Jiao, X. Liu, B. Cui, S. Hu, L. Sun, S. Mao, J. Dong, L. Yan, Z. Chen and Y. Zhao (2018). "FKBP51 regulates decidualization through Ser473 dephosphorylation of AKT." Reproduction **155**(3): 283-295.

Whitaker, H. C., D. Patel, W. J. Howat, A. Y. Warren, J. D. Kay, T. Sangan, J. C. Marioni, J. Mitchell, S. Aldridge, H. J. Luxton, C. Massie, A. G. Lynch and D. E. Neal (2013). "Peroxiredoxin-3 is overexpressed in prostate cancer and promotes cancer cell survival by protecting cells from oxidative stress." Br J Cancer **109**(4): 983-993.

Wilson, E. M. and F. S. French (1976). "Binding properties of androgen receptors. Evidence for identical receptors in rat testis, epididymis, and prostate." J Biol Chem **251**(18): 5620-5629.

Wochnik, G. M., J. Ruegg, G. A. Abel, U. Schmidt, F. Holsboer and T. Rein (2005). "FK506-binding proteins 51 and 52 differentially regulate dynein interaction and nuclear translocation of the glucocorticoid receptor in mammalian cells." J Biol Chem **280**(6): 4609-4616.

Wolf, I. M., S. Periyasamy, T. Hinds, Jr., W. Yong, W. Shou and E. R. Sanchez (2009). "Targeted ablation reveals a novel role of FKBP52 in gene-specific regulation of glucocorticoid receptor transcriptional activity." J Steroid Biochem Mol Biol **113**(1-2): 36-45.

Wu, B., P. Li, Y. Liu, Z. Lou, Y. Ding, C. Shu, S. Ye, M. Bartlam, B. Shen and Z. Rao (2004). "3D structure of human FK506-binding protein 52: Implications for the assembly of the glucocorticoid receptor/Hsp90/immunophilin heterocomplex." Proc Natl Acad Sci U S A **101**(22): 8348-8353.

Xia, Z., G. Zhang, C. Wang and Y. Feng (2021). "The role of FKBP51 in the prognosis of ulcerative colitis-associated colorectal cancer." Adv Med Sci **66**(1): 89-97.

Xie, P., H. R. Kranzler, J. Poling, M. B. Stein, R. F. Anton, L. A. Farrer and J. Gelernter (2010). "Interaction of FKBP5 with childhood adversity on risk for post-traumatic stress disorder." Neuropsychopharmacology **35**(8): 1684-1692.

Yang, H., Y. Zhou, B. Edelshain, F. Schatz, C. J. Lockwood and H. S. Taylor (2012). "FKBP4 is regulated by HOXA10 during decidualization and in endometriosis." Reproduction **143**(4): 531-538.

Yang, Y. X., X. L. Li, L. Wang, S. Y. Han, Y. R. Zhang, P. Pratheeshkumar, X. Wang, J. Lu, Y. Q. Yin, L. J. Sun, A. Budhraja, A. J. Hitron and S. Z. Ding (2013). "Anti-apoptotic proteins and catalase-dependent apoptosis resistance in nickel chloride-transformed human lung epithelial cells." Int J Oncol **43**(3): 936-946.

Yang, Z., I. M. Wolf, H. Chen, S. Periyasamy, Z. Chen, W. Yong, S. Shi, W. Zhao, J. Xu, A. Srivastava, E. R. Sanchez and W. Shou (2006). "FK506-binding protein 52 is essential to uterine reproductive physiology controlled by the progesterone receptor A isoform." Mol Endocrinol. **20**(11): 2682-2694. Epub 2006 Jul 2627.

Yao, Y. L., Y. C. Liang, H. H. Huang and W. M. Yang (2011). "FKBPs in chromatin modification and cancer." Curr Opin Pharmacol **11**(4): 301-307.

Yogosawa, S. and K. Yoshida (2018). "Tumor suppressive role for kinases phosphorylating p53 in DNA damage-induced apoptosis." Cancer Sci **109**(11): 3376-3382.

Yong, W., Z. Yang, S. Periyasamy, H. Chen, S. Yucel, W. Li, L. Y. Lin, I. M. Wolf, M. J. Cohn, L. S. Baskin, E. R. Sanchez and W. Shou (2007). "Essential role for Co-chaperone Fkbp52 but not Fkbp51 in androgen receptor-mediated signaling and physiology." J Biol Chem. **282**(7): 5026-5036. Epub 2006 Dec 5021.

Yu, J., L. Sun, T. Hao, B. Zhang, X. Chen, H. Li, Z. Zhang, S. Zhu, C. Quan, Y. Niu and Z. Shang (2019). "Restoration of FKBP51 protein promotes the progression of castration resistant prostate cancer." Ann Transl Med **7**(23): 729.

Yun, J. Y., M. J. Jin, S. Kim and S. H. Lee (2022). "Stress-related cognitive style is related to volumetric change of the hippocampus and FK506 binding protein 5 polymorphism in post-traumatic stress disorder." Psychol Med **52**(7): 1243-1254.

Zajkowicz, A., A. Gdowicz-Klosok, M. Krzesniak, D. Scieglinska and M. Rusin (2015). "Actinomycin D and nutlin-3a synergistically promote phosphorylation of p53 on serine 46 in cancer cell lines of different origin." Cell Signal **27**(9): 1677-1687.

Zannas, A. S., T. Wiechmann, N. C. Gassen and E. B. Binder (2016). "Gene-Stress-Epigenetic Regulation of FKBP5: Clinical and Translational Implications." Neuropsychopharmacology **41**(1): 261-274.

Zgajnar, N. R., S. A. De Leo, C. M. Lotufo, A. G. Erlejman, G. Piwien-Pilipuk and M. D. Galigniana (2019). "Biological Actions of the Hsp90-binding Immunophilins FKBP51 and FKBP52." Biomolecules **9**(2).

Zhou, Z. X., M. Sar, J. A. Simental, M. V. Lane and E. M. Wilson (1994). "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 **269**(18): 13115-13123.

Zong, S., Y. Jiao, X. Liu, W. Mu, X. Yuan, Y. Qu, Y. Xia, S. Liu, H. Sun, L. Wang, B. Cui, X. Liu, P. Li and Y. Zhao (2021). "FKBP4 integrates FKBP4/Hsp90/IKK with FKBP4/Hsp70/RelA complex to promote lung adenocarcinoma progression via IKK/NF-kappaB signaling." Cell Death Dis **12**(6): 602.

VITA

Olga Soto was born in Odessa, TX and raised in El Paso TX. She attended The University of Texas at El Paso (UTEP) where she earned a Bachelor of Science in Cellular and Molecular Biochemistry in December 2014. She continued to pursue a Doctor of Philosophy in Biological Sciences in the same university.

While attending UTEP for her doctoral studies, Olga earned a fellowship from the Hispanic Alliance for Graduate Education and the Professoriate (HAGEP) from January 2021 to December 2022. This fellowship was awarded in collaboration with The City of College of New York to promote the advancement of historically underrepresented graduate students towards faculty positions in STEM. During this time, she underwent teaching training sessions and presented at multiple conferences, including Society for Organizational Learning (SOL) and Society for the Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS). This opportunity provided Olga the experience of developing and teaching courses at El Paso Community College (EPCC) under the guidance of a tenured professor.

During her doctoral studies, Olga published a first-author review article titled “Structure and Function of the TPR-Domain Immunophilins FKBP51 and FKBP52 in Normal Physiology and Disease” in Journal of Cellular Biochemistry in April 2023. Additionally, Olga attended conferences such as Society for Basic Urology Research (SBUR), American Association for Cancer Research (AACR), and Midwest Stress Response and Molecular Chaperone Meeting (MWSM).

At the present time, Olga is exploring career options, which include, but are not restricted to, scientific writing or medical science liaison positions with the goal to contribute to promoting and improving scientific communication.

Contact Information: soto.ob@gmail.com