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Sex Hormone-Binding Globulin as a Modulator of the Prostate "Androgenome"

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1. Introduction

Sex hormone-binding globulin (SHBG) is a sex steroid binding protein, originally described in humans as the major binding protein for estrogens and androgens in plasma (Anderson, 1974; Avvakumov, et al, 2010). By governing equilibrium conditions in plasma between bound and free sex steroids, SHBG regulates the availability of the latter to hormonally responsive tissues. Along with regulating free steroid concentrations in plasma, it is increasingly evident that SHBG also participates in other biological processes. These include, but are not limited to- activation of a rapid, membrane based steroid signaling pathway in tissues such as the prostate and breast (Rosner et al, 2010); spermatogenesis (Selva and Hammond, 2006); and a yet to be determined consequence of co-localization with oxytosin in brain cells (Caldwell et al, 2006).

Plasma based SHBG is extensively studied, especially in the context of its regulation of free steroid concentrations and epidemiologic associations. The origin of plasma SHBG is, for all intents and purposes, the liver (Khan et al, 1981; Pugeat et al, 2010) (a differentially glycosylated isoform, androgen binding protein (ABP) is synthesized in the testis (Vigersky et al, 1976)). However, we now know that SHBG is also synthesized, albeit to a much lesser degree, in certain hormonally responsive tissues (Kahn et al, 2002). Early studies demonstrated immunoreactive SHBG in the prostate and breast (Bordin & Petra 1980; Tardivel-Lacombe et al, 1984; Sinnecker et al, 1988; 1990; Meyer et al, 1994; Germain et al, 1997), though its origin (local synthesis vs. import from plasma) was unclear. Other studies demonstrated SHBG mRNA in certain nonhepatic tissues (Larrea et al, 1993; Misao et al, 1994; 1997; Moore et al, 1996; Murayama et al, 1999), and one reported both SHBG protein and mRNA together in fallopian tube tissue (Noé, 1999). In 2002, we reported that human prostate tissue expresses both SHBG mRNA and protein, as do prostate cancer cell lines (Hryb et al, 2002), suggesting that SHBG is indeed locally

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expressed by prostate cells. We therefore set out to ascertain the biological functions associated with locally expressed SHBG in the prostate. High on our list was that locally expressed SHBG could regulate the prostate cellular response to androgen signaling by modulating the expression of androgen responsive genes, referred to herein as the "androgenome".

In this chapter, we first present an overview of human SHBG gene expression, as recent studies from our group and Pinós et al, have shown it to be far more complex than previously thought. We then review our work on the effects of SHBG on the prostate androgenome, along with our most recent findings on how SHBG modulates the expression of specific and noteworthy androgen receptor (AR) responsive genes. We conclude by addressing how SHBG, through its effects on the androgenome, might affect prostate biology, and how altered SHBG expression may influence prostate cancer progression.

2. SHBG gene structure and expression

2.1 Introduction

In plasma, SHBG exists as a homodimer, whose subunits are derived from an eight-exon long transcript as a 402 amino acid precursor protein that is glycosylated (sometimes differentially) and cleaved at its amino terminus to remove a 29 amino acid signal peptide (Hammond et al, 1987; Gershagen et al, 1989; Joseph, 1994; Avvakumov et al, 2010). The same eight-exon long transcript also encodes androgen binding protein (ABP) in the testis, an alternatively glycosylated form of SHBG (not a topic of this review). The human SHBG gene is located on chromosome 17p13.1, ~30Kb from the p53 tumor suppressor gene. As a result, in most instances where hemizygous deletions of this oft-targeted chromosomal region occur in prostate tumors, it is likely that DNA sequences involving both genes are lost.

2.2 The human SHBG gene transcription pattern.

Bolstered by recent reports (Nakhla et al, 2009; Pinós et al, 2009), we now know that transcription of the human SHBG gene is highly complex, as well as tissue dependent. The eight-exon long SHBG transcript is derived from a downstream promoter, designated here as P_L. In addition to the SHBG transcript, we found that at least five different mRNA species are generated through alternative splicing of exons 4-7 from the primary P_L derived transcript (Nakhla et al, 2009). Adding to the overall complexity of human SHBG gene transcription, we and others have detected at least five independent first exons in novel SHBG gene transcripts (Gershagen et al, 1989; Nakhla et al, 2009; Pinós et al, 2009). These additional first exon sequences are all located upstream of the P_L promoter, indicating that the SHBG gene utilizes at least six different promoters. We characterized transcripts derived from two of these upstream promoters, and found that they, too, undergo alternative splicing of exons 4-7. In total, from P_L and these two upstream promoters alone, we identified 19 different SHBG gene transcripts (Nakhla et al, 2009); Pinós et al. describe additional transcripts arising from other SHBG gene promoters (Pinós et al, 2009). However, apart from the singular transcript encoding SHBG itself, it is unclear whether any other SHBG gene transcript encodes a functional protein in humans, or whether they might act to regulate expression of the SHBG transcript.

2.3 SHBG expression in normal prostate tissue and the LNCaP prostate cancer cell line

Our analyses also included a detailed look at the SHBG expression patterns in normal human prostate tissue and the LNCaP prostate cancer cell line (Nakhla et al, 2009). Focusing on P_L, we found that only the eight exon long SHBG transcript is generated in normal prostate tissue. This suggests that alternatively spliced P_L–derived species are either not present, that they exist at levels undetectable by our RT-PCR assay, or that they are synthesized in minor cellular populations within normal prostate tissue. Compared to normal liver tissue, quantitative PCR analysis revealed that normal prostate expresses only $1/1000^{th}$ the abundance of total P_L-derived transcripts. Even taking into account the relative complexity of the P_L-transcript expression pattern in normal liver, with the SHBG transcript being most abundant, these findings are in concordance with hepatic SHBG being synthesized for global use (plasma), and prostate SHBG being synthesized for local, or intracellular use. Normal prostate revealed a low abundance of transcripts derived from the two upstream promoters we examined. In striking comparison, the LNCaP prostate cancer cell line exhibited a dramatic relative increase in both the number of alternatively spliced transcripts and transcripts from upstream promoters. The reasons behind these differences in SHBG gene transcription profiles are unclear, they could reflect the clonality of LNCaP cells vs. whole prostate tissue, dysregulation of global RNA processing in LNCaP, and/or changes in specific SHBG mRNA processing elements, among other possibilities. Taken together, the SHBG gene may be a valuable provider of diagnostic, prognostic, and predictive biomarkers for individuals with prostate cancer.

3. SHBG and its effects on the prostate "androgenome"

3.1 Introduction

Because SHBG binds androgens, we hypothesized that a major function of locally expressed SHBG in prostate cells might be to regulate the androgenome. We set out to investigate two different scenarios by which SHBG could influence androgen signaling. First was that locally synthesized SHBG could modulate the binding of androgen to the androgen receptor (AR) by acting as a steroid sequestering agent. For example, in the same way that plasma SHBG regulates the concentrations of plasma free steroids, intracellular SHBG could regulate intraprostatic free testosterone and dihydrotestosterone (DHT). Perhaps relevant to prostate cancer progression, this model predicts that diminished intracellular SHBG would allow for increased free intracellular DHT and hence increase the effect of intracellular androgens.

The second scenario envisions that locally expressed SHBG can participate, in an autocrine/paracrine manner, in a rapid, membrane based signaling pathway in prostate cells (Kahn et al, 2002; Kahn et al, 2003; Rosner et al, 2010). The initial steps of this pathway are well established biochemically, however little is understood about its biologic functions. Briefly, SHBG, in its steroid-free configuration, binds to a high affinity, but yet to be cloned membrane receptor (R_{SHBG}), forming a bipartite complex (SHBG-R_{SHBG}). Subsequently, DHT binds to and activates the SHBG-R_{SHBG} complex causing a rapid induction of cAMP and the activation of protein kinase A. This occurs independently of the AR.

3.2 Functional microarray analysis

We developed a functional microarray approach to ascertain the effects of SHBG on the androgenome of LNCaP cells (Kahn et al, 2008). Using an inducible system that enabled

SHBG overexpression in an engineered human LNCaP prostate cancer cell line, we specifically addressed the two scenarios described above. Using appropriate controls, SHBG effects on AR-mediated signaling would be evident by the altered expression of genes that are responsive to DHT treatment. And, those genes whose expression was sensitive to R_{SHBG} signaling would show changes only under conditions that activate R_{SHBG} (SHBG followed by DHT binding), but not in the presence of either SHBG or DHT alone.

3.3 Generation of the inducible L5S2 and vector control L5V4 clonal cell lines

The two clonal cell lines, L5S2 and L5V4, formed the core of our studies. The inducible L5S2 clonal cell line, which reproducibly overexpresses SHBG in response to Ponasterone A (PonA), was indirectly derived from LNCaP cells through an intermediate cell line, L5. L5 was generated by stably transfecting LNCaP cells with the plasmid, pVgRXR (Invitrogen, Carlsbad, CA). pVgRXR encodes a hybrid transactivator that is activated by PonA. This transactivator recognizes and directs transcription from a promoter within a second plasmid, pINDhygro (Invitrogen). L5S2 was generated by stably transfecting L5 cells with a pINDhygro construct that contains the full length human SHBG cDNA coding sequence cloned directly downstream of the inducible promoter. The L5V4 vector control cell line was generated by stably transfecting L5 cells with the empty vector, pINDhygro. As such, L5S2 and L5V4, both being derived directly from the L5 subclone, were considered nearly isogenic. Titration experiments revealed maximal SHBG induction was approached in L5S2 cells upon treatment with 10 μM PonA for 24 hrs, similar treatment of L5V4 cells had no effect on SHBG expression (data not shown).

Table 1 shows the effect of treatment conditions on SHBG expression in the L5S2 inducible, and L5V4 vector control cell lines. L5S2 cells treated for 24 hrs with 10 μM PonA reproducibly exhibit an 80+-fold induction over basal L5S2 levels in either the absence or presence of 10 nM DHT. The inducing agent, PonA by itself has only a very slight effect on SHBG expression.

3.4 Effects of SHBG overexpression on the LNCaP androgenome

The global effect of SHBG overexpression on gene expression in LNCaP cells following 10 nM DHT treatment is summarized in Table 2. L5S2 cells were induced with PonA for 24 hrs, then treated with 10 nM DHT for another 24 hrs (this being the same DHT treatment condition that induces R_{SHBG} signaling). Approximately 3000 genes displayed at least a 20% difference in expression when compared to similarly treated L5V4 vector control cells, with slightly over 1700 genes showing at least a 50% increase in expression, or a 33% decrease in expression. Thus, SHBG, when expressed at high levels in LNCaP cells, does affect the androgenome.

3.5 SHBG effects on c-myc, TIMP2, GPR30, and STAMP4 expression

Having demonstrated a global effect of SHBG on the androgen response of LNCaP cells, we performed a series of qPCR experiments to confirm our microarray results. We investigated a select group of four genes of potential importance in prostate cancer and hormonal signaling- c-myc, TIMP2 (tissue inhibitor of metalloproteinase 2), GPR30 (G protein-coupled receptor 30), and STEAP4 (six-transmembrane epithelial antigen of prostate 4, also known as STAMP2 (six transmembrane protein of prostate 2)), each of which displayed a sensitivity to

CELL LINE AND TREATMENT CONDITIONS	EFFECT TESTED	SHBG Fold expression change	P.Value	B value
L5S2: 10uM PonA vs. L5V4: 10uM PonA	Pon A induction	83.7	1.34E-19	38.3
L5S2: 10nM DHT, 10uM PonA vs. L5V4: 10nM DHT, 10uM PonA	Pon A induction and DHT	89.3	1.03E-19	39.8
L5V4: 10uM PonA vs. L5V4: Mock treated	Pon A effects alone	1.57	4.73E-05	7.02
L5S2: Mock treated vs. L5V4: Mock treated	Leakiness of L5S2 cells	4.82	5.53E-13	26.8
L5V4: 10nM DHT, 10uM PonA vs. L5V4: 10uM PonA	DHT on L5V4 cells	N/D	N/A	N/A
L5S2: 10nM DHT, 10uM PonA vs. L5S2: 10uM PonA	DHT on L5S2 cells	N/D	N/A	N/A

L5V4 vector control cells and inducible L5S2 cells were each seeded into two groups of multiple six well plates in RPMI-1640 medium (Mediatech, Herndon, VA) supplemented with 1mM sodium pyruvate (Mediatech), 100 units/ml of Penicillin-Streptomycin (Invitrogen), and 10% charcoal stripped fetal calf serum (Gemini Bio-Products, Woodland, CA) for 24 hours. One group was then treated with the inducing agent, PonA (10μM)(Invitrogen), and the other treated with an equal volume of carrier ethanol, for 24 hr. Triplicate wells from the PonA-treated cells were then treated for an additional 24 hr with either carrier or 10 nM DHT, giving six treatment conditions-

A. L5V4 vector control cells treated with carrier alone (mock treated)
B. L5V4 vector control cells treated with 10uM PonA 24 hrs
C. L5V4 vector control cells treated with 10uM PonA + 10nM DHT 24 hrs
D. L5S2 inducible SHBG cells treated with carrier alone (mock treated)
E. L5S2 inducible SHBG cells treated with 10uM Pon A 24 hrs
F. L5S2 inducible SHBG cells treated with 10uM PonA + 10nM DHT 24 hrs

Total RNA was isolated with Trizol (Invitrogen) followed by a Qiagen clean up procedure (Qiagen, Valencia, CA). RNA integrity was assessed using an Agilent 2100 Bioanalyzer and RNA 6000 Nano Lab Chip LabChips (Agilent, Palo Alto, CA). RNA samples showed a 260/280 ratio between 1.8 and 2.0 and 28S:18S ratio of 1.5 and higher. Each triplicate RNA preparation was used in a single microarray analysis. First-strand cDNAs were synthesized from 5 μg of each RNA sample using a T7-Oligo(dT) promoter primer and SuperScript II. After RNase H-mediated second-stranded cDNA synthesis, double-stranded cDNAs were purified using a GeneChip sample clean-up module. Biotinylated complementary RNAs (cRNAs) were generated by in vitro transcription using T7 RNA Polymerase and a biotinylated nucleotide analog/ribonucleotide mix. Biotinylated cRNAs were cleaned up, fragmented, and hybridized to Affymetrix Human Genome U133 Plus 2.0 Array chips, representing 54675 transcripts (Affymetrix, Santa Clara, CA), at 45°C for 16 h with constant rotation at 60 rpm. Chips were processed using an Affymetrix fluidics station and scanned on an Affymetrix scanner 3000 with workstation. Images were processed with GeneChip Operating Software (GCOS) and raw data were analyzed with GeneSpring 7.2 software (Silicon Genetics, Redwood City, CA) to identify differentially expressed genes between conditions. Data were normalized to the 50th percentile of measurements taken from the chip to reduce chip-wide variations in intensity. Each gene was normalized to the average measurement of the gene throughout the experiment to enable comparison of relative changes in gene expression levels between different conditions. Data filtration was performed based on flags, present or marginal. Shown are the changes in SHBG gene expression between given cell lines and treatment conditions as determined by microarray analysis. B value: Bayesian log odds score. ND= none detected; NA= not applicable

Table 1. Effect of treatment conditions on SHBG expression in L5V4 and L5S2 cells

Total Number of Induced Genes	Total Number of Repressed Genes	Genes Induced > 1.5-fold	Genes expressed <0.66-fold (repressed)
1250	1770	665	1068

L5S2 inducible and L5V4 vector control cells were treated with 10μM PonA for 24 hours, and then stimulated with 10nM DHT for an additional 24 hours. Total number of induced and repressed genes include those displaying at least a 20% difference in transcript abundance between similarly treated L5S2 and L5V4 cells, as determined by microarray analysis.

Table 2. Global effects of SHBG overexpression on DHT-treated (24 hr.) LNCaP cells

SHBG overexpression in response to DHT, as detected by microarray analysis (data not shown). The qPCR results corroborated our microarray results for these four genes. DHT treatment of induced L5S2 cells resulted in nearly a one-third decrease in c-myc gene transcript abundance compared to mock-treated L5S2 cells, whereas similar DHT treatment caused a 20% increase in L5V4 vector control cells compared to mock-treated L5V4 cells. Given that elevated c-myc gene expression is a hallmark of many prostate tumors (Gurel et al, 2008), the effect of increased SHBG serving to decrease c-myc gene expression is intriguing and warrants further investigation. We note that slightly higher levels of c-myc are seen in unstimulated L5S4 vs. L5V2 cells, this observation requires clarification. DHT treatment also caused a decrease in the abundance of TIMP2 gene transcripts in L5S2 cells, whereas there was little change in similarly treated L5V4 cells. This result is of interest, as TIMP2 expression has been correlated with advanced prostate cancer stage and recurrence (Ross et al, 2003). SHBG overexpression markedly amplified the DHT-mediated decrease in cellular levels of GPR30 gene transcripts; GPR30 is a membrane receptor for estrogen that releases epidermal growth factor-related ligands, thereby inducing signaling via the epidermal growth factor receptor (Wang et al, 2010). And, SHBG overexpression displayed a dramatic effect on STEAP4 transcript levels in LNCaP cells- DHT treatment resulted in 1000-fold higher levels in L5S2 cells than in similarly treated L5V4 cells. STEAP4 is an emerging player in metabolic syndrome and glucose transport (Wellen et al, 2007), and provocatively, SHBG has been linked to metabolic syndrome (Pugeat et al, 2007). While its expression is often elevated in prostate cancer cells (Korkmaz, et al, 2005) it is still unclear how STEAP4 expression may contribute to prostate cancer progression. This, and the exquisite responsiveness of STEAP4 expression levels to SHBG are areas that beg further investigation.

3.6 SHBG effects on the expression of AR co-regulators, including FKBP5

We next turned our attention to whether SHBG might indirectly affect AR activation by modulating the expression of AR co-regulators in response to DHT. Working with a detailed list of 186 AR co-regulators kindly provided by Dr. Donald Tindall and Dr. Hannelore Heemers (for review, see Heemers and Tindall, 2007), we examined our microarray data for those whose expression was affected by SHBG overexpression following 24 hr. DHT exposure. AR co-regulators displaying at least a 20% difference are listed in Table 3.

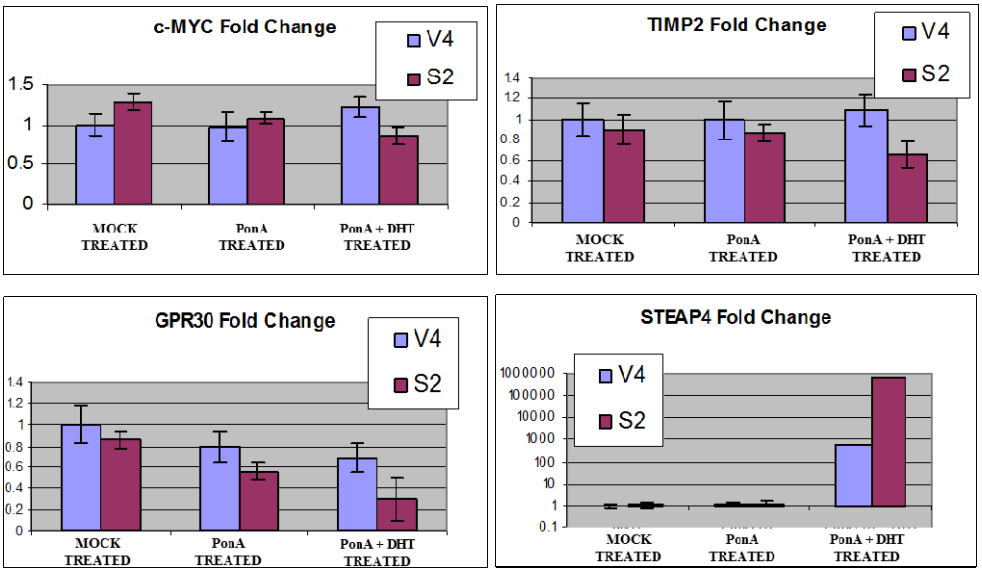
Of the 20 AR co-regulators whose expression was markedly changed by the presence of SHBG, eight were upregulated and 12 were downregulated. The greatest difference was in expression of the FKBP5 (FK506 binding protein 5) gene, which was elevated 3.77-fold. Because FKBP5 is a known early androgen responsive gene whose expression is rapidly induced by DHT treatment (Jääskeläinen et al, 2011), we examined its expression level in

PonA-treated L5S2 and L5V4 cells after only 4 hrs of incubation with DHT. Indeed, in L5V4 cells, FKBP5 was induced to high levels at this earlier time point, whereas its induction was dampened by the presence of SHBG in L5S2 cells (data not shown). This suggests that SHBG overexpression not only diminishes the amplitude of DHT-mediated FKBP5 induction, it also shifts the response curve to the right. This is an intriguing finding, as FKBP5 has been shown to be a limiting component of the HSP90 chaperone supercomplex that maintains the AR in its ligand binding state (Ni et al, 2010).

Gene	**Gene name**	**Fold change**
		(L5S2 vs. L5V4)
FKBP5	FK506 binding protein 5	3.77
HIPK3	homeodomain interacting protein kinase 3	1.55
APPBP2	amyloid beta precursor protein (cytoplasmic tail) binding protein 2	1.49
NCOR1	nuclear receptor co-repressor 1	1.46
RNF14	ring finger protein 14	1.45
HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	1.41
NCOR1	nuclear receptor co-repressor 1	1.39
CDC37	CDC37 cell division cycle 37 homolog (S. cerevisiae)	1.36
SRA1	steroid receptor RNA activator 1	1.28
TADA3L	transcriptional adaptor 3 (NGG1 homolog, yeast)-like	0.78
CDK7	cyclin-dependent kinase 7 (MO15 homolog, Xenopus laevis, cdk-activating kinase)	0.76
BAG1	BCL2-associated athanogene	0.73
RAN	RAN, member RAS oncogene family	0.72
NONO	non-POU domain containing, octamer-binding	0.72
MMS19L	MMS19-like (MET18 homolog, S. cerevisiae)	0.69
SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	0.68
UBE1C	ubiquitin-activating enzyme E1C (UBA3 homolog, yeast)	0.65
RBM9	RNA binding motif protein 9	0.63
JDP2	Jun dimerization protein 2	0.62
CRSP2	cofactor required for Sp1 transcriptional activation, subunit 2, 150kDa	0.59

Table 3. Effect of SHBG on AR Co-regulator gene expression following 24 hr. DHT treatment of LNCaP cells.

These results support the view that endogenously expressed SHBG plays a significant role in orchestrating the LNCaP androgenome. It is noted that our experimental strategy utilized unusually high SHBG concentrations, thus we need to investigate further how normal levels of SHBG expression influence the LNCaP androgenome. More detailed time course analyses are also necessary to ascertain how SHBG affects the timing of AR activation, and how perturbations in SHBG expression might affect androgen induced events. This is especially critical considering that SHBG influences the expression of specific AR co-regulators, which could impact the timing of events required for AR activation.



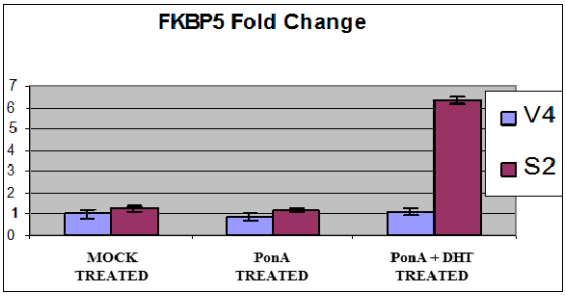
Taqman assay qPCR amplifications were performed in triplicate, using primers specific for the indicated genes. cDNA templates were generated from the same RNAs as were used for microarray analysis. Assays were run in an ABI PRISMR 7700 Sequence Detection System machine. Data were extracted and amplification plots generated with ABI SDS software. Threshold cycle (Ct) scores were averaged for subsequent calculations of relative expression values. Specific gene Ct values were normalized to GAPDH Ct values for each cell line and treatment condition, and standard deviations were calculated. Specific gene expression comparisons (fold change) are presented as the ratios of normalized Ct values for L5V4 or L5S2 cells under given treatment conditions to the normalized Ct value of the specific gene in mock-treated L5V4 vector control cells.

Fig. 1. Effect of SHBG on the expression of selected genes involved in prostate cancer progression and hormone signaling following 24 hr. DHT treatment of LNCaP cells - qPCR analysis of L5S2 and L5V4 cells.

3.7 SHBG overexpression and the R_{SHBG} pathway

Finally, our functional microarray strategy provided a means (overexpression of SHBG followed by DHT treatment) to detect genes whose altered expression is consistent with having been activated via the R_{SHBG} pathway. Just over 1000 genes displayed a pattern of not showing a significant response to either elevated SHBG expression or 24 hr. DHT treatment alone, while changing expression (a >50% induction or > 33% reduction) when SHBG was

induced in L5S2 cells, followed by the addition of DHT in a manner consistent with the activation of R_{SHBG} signaling (data not shown). However, before we can assign any biologic function to R_{SHBG} signaling in prostate cells, we need to confirm these results in order to differentiate between the activation of R_{SHBG} signaling and a delay in AR mediated expression changes for specific genes in response to SHBG overexpression, as was the case for FKBP5.



See legend to Figure 1

Fig. 2. Effect of SHBG on FKBP5 gene transcript levels following 24 hr. DHT treatment of LNCaP cells

4. Conclusions

The human SHBG gene is expressed at the mRNA and protein levels in prostate cells. Considering its sex hormone binding properties, we examined its ability to affect the androgenome of prostate cells. Using a functional microarray approach, we have obtained evidence that indeed, SHBG does affect the expression of DHT-responsive genes. In LNCaP cells, SHBG overexpression exerts global effects that include genes involved in prostate cancer (eg. c-myc and TIMP2), hormonal signaling (eg. GPR30), and the expression of AR co-regulators (eg. FKBP5), among others.

The scope of SHBG's influence on the androgenome appears to be broad and complex, involving many aspects of AR activation. Two possible mechanisms include the binding and sequestering of intracellular androgen, and the indirect modulation of AR co-regulator expression. It remains to be determined whether signaling through R_{SHBG} is also involved.

Given the ability of prostate cells to greatly ramp up their expression of endogenously synthesized SHBG (we have overexpressed SHBG in LNCaP, PC3, and DU145 cells), this raises the question of whether they regulate their androgenome by modulating intracellular SHBG levels. It is likely that a decrease in intracellular SHBG levels results in an equilibrium shift towards increased free intracellular testosterone and free intracellular DHT. We speculate that in those prostate cancer cells which undergo deletions of the SHBG/p53 locus, SHBG expression will be reduced. Deletions of the SHBG/p53 locus could thus provide a genetic means by which prostate cancer patients placed on current androgen ablation therapies can progress- enabling cells to survive under conditions of diminished androgen due to the relative increase in free intracellular androgen available to activate AR-mediated signaling. If this speculation is confirmed, it will be of interest to see how such patients respond to newer therapies that target testosterone and DHT biosynthesis, such as abiraterone.

It is intriguing to also speculate that, in addition to the prostate, locally expressed SHBG plays a functional role in the hormone response of other tissues. We have preliminary evidence that endogenously expressed SHBG can modulate the estrogen response of human breast cells (Kahn et al, 2008). And, if plasma SHBG levels provide a clue into how altered SHBG expression may contribute to the disease state at a cellular level, it will be of great interest to investigate whether there is a connection between tissue specific SHBG expression and Type 2 diabetes. This does not detract in any way from the importance of plasma SHBG levels on androgen and estrogen responsiveness in humans. Instead, it serves to broaden the scope of SHBG influence on the response to sex steroids to the individual tissue and cellular level.

5. Acknowledgements

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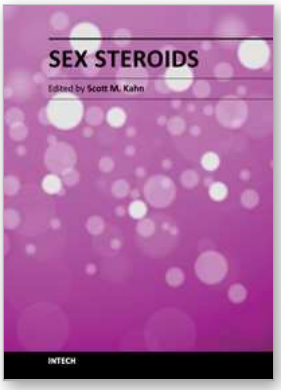

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This book, entitled "Sex Steroids", features a valuable collection of reviews and research articles written by experts in signal transduction, cellular biology, diseases and disorders. "Sex Steroids" is comprised of four sections, "The Biology of Sex Steroids", "Sex Steroids, Memory, and the Brain", "Sex Steroids and the Immune Response", and "Therapy"; individual chapters address a broad range of recognized and predicted functions and applications of sex steroids. "Sex Steroids" is intended to provide seasoned veterans as well as newcomers to this area of research with informative, resourceful, and provocative insights. Readers of "Sex Steroids" should emerge with an appreciation and understanding of the multitude and complexity of biologic processes attributed to these important hormones, and possible future directions of research in this fascinating and ever evolving field.

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