A Review of the Expression of Genes Involved in Sex Steroid Hormone Metabolism in Prostate Tissue: A Need for Epigenetic Information

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Abstract: There is strong clinical and laboratory evidence indicating that sex steroid hormones are important to the development and progression of prostate cancer, yet results from epidemiologic research conflicts. Examining gene expression in the sex steroid hormone pathway may uncover differences between cancerous and non-cancerous prostate tissues, yet our review using a pathway-oriented approach indicates that there is limited consistency across results, with the exception of *GSTP1* found in the estrogen pathway, which was under-expressed in cancerous prostate tissue. This agrees with past studies that reported *GSTP1* is methylated in prostate cancer. With new cost-effective technology, we can screen for epigenetic markers, like methylation, which can be applied in epidemiological studies. A clearer understanding of gene expression and epigenetic mechanisms in prostate cancer may contribute to improving prevention, diagnosis, and treatment.

Keywords: Prostate tissue, cancer, sex steroid hormones, methylation, *GSTP1*.

INTRODUCTION

Prostate cancer remains a major public health challenge among US men [1]. Currently, established risk factors for prostate cancer are limited to age, race, and family history [1]. Some other proposed risk factors, including circulating levels of sex steroid hormones, genetic differences, epigenetic differences, and lifestyle factors, which remain unconfirmed [2-3]. The involvement of sex steroid hormones in prostate cancer is supported by both controlled and laboratory and clinical studies [4-5]. Laboratory evidence suggests that increased testosterone (T) and estradiol (E) lead to the development of prostate cancer in rats [4-5]. Fewer cases of prostate cancer are observed among castrated men which supports the idea that lowered sex steroid hormone production may decrease prostate cancer risk, and the use of androgen ablation therapy and castration both result in the regression of metastatic prostate cancer [4, 6-8]. Given this supportive laboratory, observational, and clinical evidence, hormone involvement in prostate cancer etiology remains compelling [9-10].

Despite this evidence, the epidemiologic research is inconclusive [11-13]. The research trend has been to focus on serum levels of individual hormones and single nucleotide polymorphisms (SNPs) of genes involved in sex steroid metabolism to assess associations with prostate cancer risk and progression. In a pooled analysis of 18 prospective studies, no associations with serum T and E and prostate cancer was observed, yet in another large study, testosterone-to-sex hormone binding globulin (T:SHBG) ratios showed an association with prostate cancer risk among men over 65 years [11, 13]. Reviews of SNPs and their association with prostate cancer risk have also produced mixed results, indicating other factors are at work [14-16]. Although informative, these study methods do not provide information on tissue-specific hormone levels, or altered cellular changes in genes involved in sex steroid metabolism during cancer development and progression. Examining the expression of genes in different prostate tissue, including: normal, benign prostatic hyperplasia (BPH), and cancerous tissue, provides tissue-specific information, which will expand our current state-of-knowledge regarding the biology of prostate disease, and enhances the potential to uncover biomarkers to improve cancer prognosis. We are unaware of published articles that have extensively reviewed the expression of all genes and receptors involved in sex steroid metabolism in the prostate tissues. This is the first comprehensive review of the literature on gene expression of enzymes and receptors involved in the T

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and E metabolic pathways in normal, BPH, and cancerous prostate tissues.

METHODS

The literature was reviewed for expression (mRNA and protein levels) of the following genes: Androgen receptor (*AR*); hydroxysteroid (17-β) dehydrogenase types 1, 2, 3, 4,7, 8, and 12 (*HSD17B1, HSD17B2, HSD17B3, HSD17B4, HSD17B7, HSD17B8, HSD17B12)*; steroid 5 α-reductase (*SRD5A1, SRD5A2*); the cytochrome P450, CYP3A family (*CYP3A4, CYP3A5, CYP3A7*, and *CYP3A43*); 3-α hydroxysteroid dehydrogenase type 2 and 3 (*AKR1C3* and *AKR1C2*); hydroxy-Δ-5-steroid dehydrogenase, 3 β- and steroid Δ-isomerase 1 and (*HSD3B1*); hydroxy-Δ-5-steroid dehydrogenase, 3-β and steroid Δ-isomerase 2 (*HSD3B2);* UDP Glucuronosyl Transferase 15 and 17 (*UGT2B15* and *UGT2B17)*; Estrogen Receptors α, β, and g-protein coupled (*ERS1, ERS2, GPER*); Aromatase (*CYP19A1*); cytochrome P450, family 1, subfamily A and B, polypeptide 1 (*CYP1B1, CYP1A1*, and *CYP1A2)*; NAD(P)H dehydrogenase quinone 1 (*NQO1*); Catechol-O-methyltransferase (*COMT*); Hydroxysteroid sulfotransferase 1A1, 1A3, 2B1a, and 2B1b (*SULT1A1, SULT1A3, SULT2B1a, SULT2B1b*); Glutathione S-transferase theta 1 (*GSTT1*); Glutathione S-transferase mu 1 (*GSTM1*); and, Glutathione S-transferase pi 1 (*GSTP1*). We did not review *HSD17B5, HSD17B9*, and *HSD17B10,* since this pathway is preliminary. Literature searches were conducted in PubMed and were limited to English language and human prostate tissues only. No cell studies were included since cell lines behave differently than tissues. A total of 85 studies were retrieved from primary searches and selected references. Comparisons of expression levels between different tissue types (cancer, BPH, or normal) and between age, race/ethnicity, sex steroid hormone levels, and cancer treatment were also reviewed.

RESULTS

The pathways in Figure **1** pictures the reviewed literature of the genes coding for enzymes and receptors involved in E and T metabolism in the prostate tissue [10, 17-19]. Based on the collective information obtained, we constructed a plausible gene network for E and T expression. Past studies have only included portions of pathways, or the T or E pathways alone, and often do not include preliminary pathways. The dashed line from androstenedione to DHT indicates that this reaction is preliminary.

A supplemental materials that includes a table of the 81 studies and cited references retrieved from the literature searches and includes, the gene chromosomal location, the type of tissue analyzed, a summary of expression study findings, and the laboratory method used and additional referenced citations is located at: <**Note for Author: The address of the web page would be added after the finalization of the manuscript**>. Overall, gene expression results were conflicting or scant, with the exception of down-regulation or the absence of *GSTP1* in prostate tissues. In our search, no expression studies were uncovered for *HSD17B8* and *NQO1,* and, no single study simultaneously assessed and reported the expression of all genes collectively. Laboratory methods used to examine protein levels included immunostaining (IS), immunohistochemistry (IHC) and *in situ* hybridization (ISH). Laboratory methods that measure mRNA included microarrays (cDNA, oligonuclotide, and tissue), northern blots, and real-time polymerase chain reaction (real-time PCR). Few studies examined the association of expression results with age, race, sex hormones, cancer stage, or cancer treatment administration and treatment outcome.

Glutathione S-transferase enzymes coded by *GSTT1, GSTM1, GSTM2-5,* and *GSTP1* form glutathione conjugates from E1/E2-2,3 and 3,4 quinones, which deactivate quinones to prevent DNA adducts [10, 20-21]. Studies examining *GSTT1, GSTM1, GSTM2-5* were scant or inconsistent. Six studies found that *GSTP1* was consistently not expressed or was down-regulated in cancerous tissues [22-26]. Another study reported lower *GSTP1* expression using one microarray method and higher expression in cancerous tissues using another [27]. *GSTP1* was up-regulated in prostate cancer treated with finasteride [28-29].

DISCUSSION

In the present study, the literature was reviewed for studies that provided information on the expression of genes involved in sex steroid metabolism activity in normal and diseased prostate tissues. Overall, we found that studies were scant or results were conflicting, with the exception of down-regulation or minimal expression of *GSTP1* in cancerous tissues [22-28, 30]. No studies collectively reported on the expression of all genes coding for enzymes found in sex steroid metabolism in prostate tissues (Figure **1**). Conflicting results and the absence of a complete reported transcriptome analysis across gene

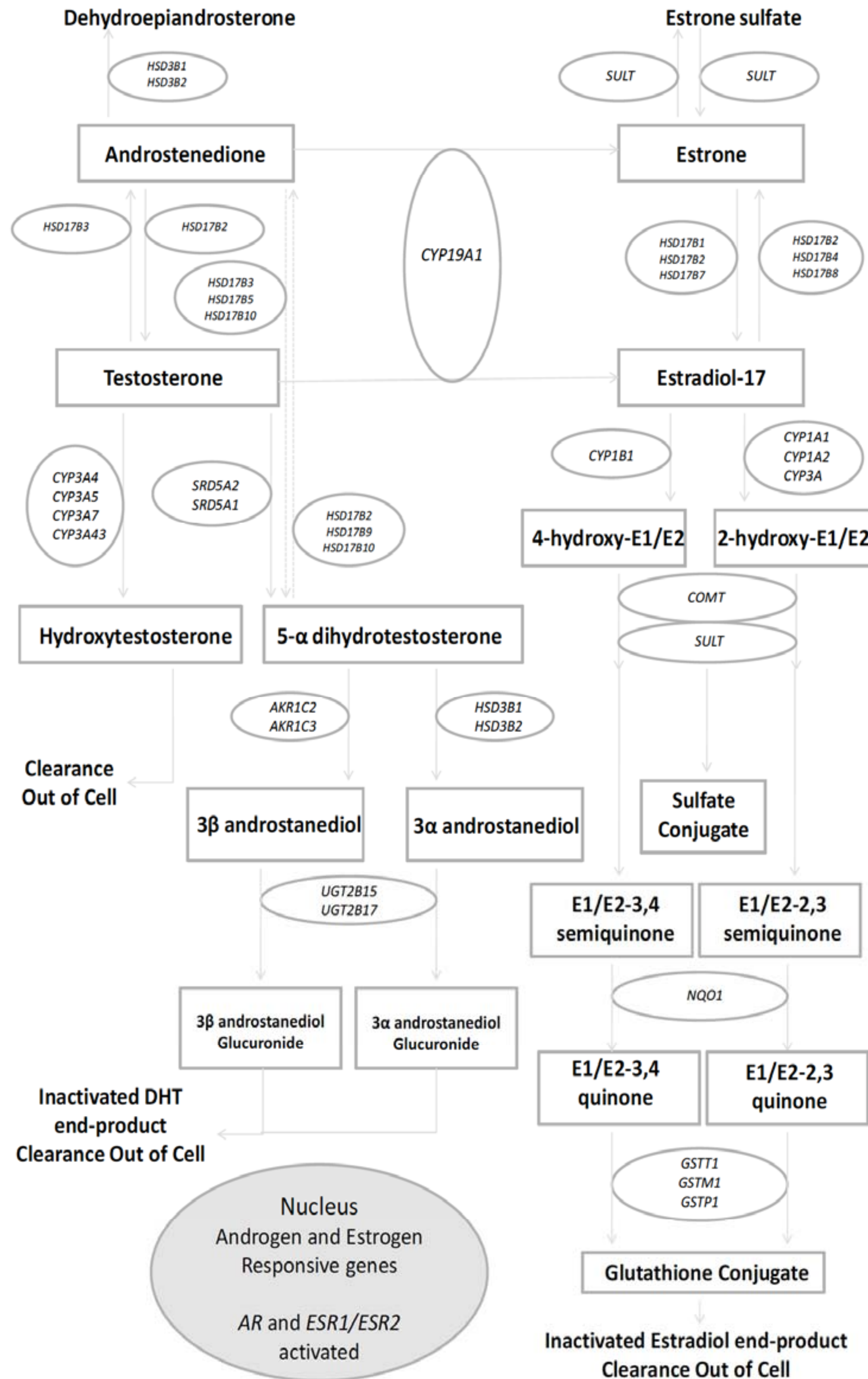

Figure 1: Androgen and Estrogen Metabolism in Prostate Cells.
Grey circle: Cell Nucleus; White circles: Genes coding for enzymes; Boxes: Hormone Metabolites.

expression studies constitute a gap in the current research. These results suggest a strong need to more thoroughly investigate genomic signatures of expression in addition to other mechanisms of action. There is a growing interest in examining epigenetic mechanisms, particularly methylation, histone modification, and the presence of miRNAs, rather than gene expression alone, to uncover how sex steroid hormones contribute to prostate cancer etiology.

As mentioned above, the most striking result of our literature review revealed a decrease in expression of

GSTP1. This gene has been widely investigated in prostate tissues, however the mechanism controlling the transcriptional regulation of *GSTP1* is unknown [31]. It has been suggested that the down regulation is due to methylation of the promoter region [31]. This epigenetic change in *GSTP1* could predispose normal prostate cells to DNA damage, and possibly epigenetic drift, as a result of inflammation, diet, sex hormones, or even *in utero* exposures contributing to the development of cancer [32-34]. This loss, or down regulation of *GSTP1* is also observed in squamous cell carcinoma of the vulva and esophagus, while an over expression pattern of *GSTP1* is observed in breast, colorectal, and other cancers [35-41]. Two patterns of *GSTP1* expression have been reported depending on the prostate zone [42]. The first pattern of *GSTP1* expression in the transitional zone was similar to normal tissue, where staining was stronger and more diffuse in the basal than the luminal cell layers [42]. The second pattern in the non-transitional zone showed no *GSTP1* expression in secretory cells, but over expression of *GSTP1* in basal cells [42]. Challenging the idea that prostate cancer only develops in luminal cells, basal cells from benign prostate tissue, but not luminal cells, have recently been reported to initiate prostate cancer in mice [43]. Developed cancers in mice showed luminal cell expansion, and no basal cells, indicating the basal cells may be transformed [31]. DNA methylation has also been reported to decrease during prostate cancer progression [44]. Whether over-expression of *GSTP1*in basal cells is common in prostate cancer samples, and if basal cells can initiate cancer growth in humans still needs to be confirmed [42-43]. Studies of related genes *GSTT1* and *GSTM1* were scant, although three studies did show a decrease in *GSTM1* expression in cancerous tissues [26, 30-31, 45-46]. This information suggests that disruption, specifically methylation, of *GSTP1* gene expression in prostate cells contributes to cancer, yet other epigenetic mechanism may contribute to the cancer process as well.

No other gene or receptor showed a consistent pattern of expression in prostate tissues, which may be due to differences in tissue collection, feasibility, laboratory methods, or gene functionality [47-48]. Tissue samples are obtained for diagnostic purposes, so normal tissues are taken adjacent to the tumor, and may result in contamination [47, 49]. Surgical manipulation and devascularization, sample collection, freezing time, and archival storage methods may also differ, influencing results [24, 48, 50-51]. Immunohistochemistry (IHC) and *in situ* hybridization (ISH) measure protein localization, and are labor intensive requiring each sample for each gene individually to be examined by pathologists, limiting the feasibility to examine an entire pathway in a single study. These protein studies may differ by fixative agents and antibodies (direct/indirect) used, natural degredation of protein, and pathologists interpretation [52]. Microarrays developed in the 1990's measure mRNA and are less labor intensive, yet, protein and mRNA do not necessarily have a one-to-one relationship, making these different methods somewhat incomparable [52]. Tissue microarrays (TMAs) have the capacity to investigate many genes in many samples, although there are limitations, including: cost/trained staff, availability of adequate tissue for TMA, differences in archival tissues, tissue oxidation, validated antibody, and lab standardization methods [53-55]. Few studies used compared results from different laboratory methods or used housekeeping genes for validation [27, 56-57]. It is also important to note that expression does not always correspond to enzyme functionality or activity. Given these challenges investigating gene expression alone, research is now moving toward combined studies including genomic profiling studies and epigenetic mapping to examine prostate cancer risk.

While *GSTP1* has been the most widely investigated methylated gene, DNA methylation, the addition of a covalently bonded methyl group to carbon-5 cytosine of the dinucelotide CpG, catalyzed by DNA methyltransferase, is currently an active area of on-going epigenetic research [58-60]. Methylation mapping of the epigenome in fetal fibroblasts and embryonic stem cells has been completed, and mapping of several cancer genomes is underway [59]. Investigating a complete "methylation profile" of prostate cancer, with an emphasis on sex steroid metabolism, may improve detection and diagnosis tools compared to assessing methylation of *GSTP1* alone [59].

Along with the methylation of genes involved in sex steroid metabolism, histone modifications and enzymes involved in these processes may also play a role in prostate cancer etiology [31, 59-60]. Non-covalent histone modifications, nucleosome remodeling and histone variants essentially modify chromatin and change the availability of DNA regulatory sites by sliding and ejection of nucleosomes, which silence and activate genes [31, 61-62]. Little is known about how non-covalent methods may influence sex steroid

metabolism and prostate cancer risk [31]. Covalent modifications acetylation, methylation, phosphorylation, sumoylation, and ubiquitylation, to histones also indirectly influence gene expression [61]. For instance, global levels of histone H3 lysine-18 acetylation (H3K18Ac) and histone H3 lysine-4 dimethylation (H3K4diMe) have been reported to be predictors of prostate cancer relapse-free survival, and other histone modifications have also been implicated in prostate cancer risk as well [44, 60-61]. Changes in enzymes involved in covalent histone modifications, like histone demethylases, which remove methyl group residues on histones, including: *LSD1, GASC1/JMJD2C*, and *PKCβ1* are involved in transcriptional activation of AR [63]. These enzymes are all up-regulated in prostate cancer, and may also influence prostate cancer risk *via* AR activation [63] The National Institutes of Health (NIH) Epigenome roadmap project is currently working towards cataloging several histone modifications in nearly one hundred cell types, and another project is working towards finding unrecognized modifications [59]. These projects may further the understanding of how histone modifications may influence sex steroid metabolism and prostate cancer risk.

Micro-RNAs, or miRNAs, are small non-coding RNAs which may influence the functional expression of sex steroid metabolism by binding to target mRNA sequences resulting in mRNA degradation or translational repression [64-65]. A recent review of miRNAs in prostate cancer indicates that up to 50 miRNAs have been identified in prostate cancer, while only a few targets are known [66] miRNAs are thought to act as oncogenes or tumor suppressor genes depending on the target, and androgen and AR signaling have been implicated in miRNA regulation [66]. For instance, androgen induced AR has been reported to bind to mi-RNA 21, leading to mi-RNA 21 over expression, and high levels of mi-RNA 21 in androgen independent cells [65-66]. mi-RNA 31 has only been identified in AR positive cells [66]. miR-221/-222 is thought to play a role in castration resistant prostate cancer *via* AR signaling in androgen independent cells, although results are not consistent [66]. Since mi-RNAs are particularly stable in serum and plasma, miRNAs are currently being explored as possible biomarkers for prostate cancer screening and detection [66].

Prostate specific antigen (PSA) is currently widely used as a biomarker for prostate cancer, but its utility is heavily debated because it can be elevated due to infection, prostatitis, or BPH yielding a sensitivity of 90% and specificity of 10-31%, and thus producing a high frequency of false positive and negative results [31]. Therefore, new biomarkers are necessary to improve prostate cancer detection, particularly if blood and urine tests can be used, and prostate cancer biopsy can be avoided [31]. Hypermethylation of *GSTP1* has been widely investigated as a possible prostate cancer biomarker [31, 60]. Yet, the specificity of blood and urine tests varies depending on the region of the CpG island and are not tissue-specific, so it is unclear if using methylation of one gene would improve patient care [31, 60]. *GSTP1* has consistently been found to be down regulated and methylated in prostate cancer, yet a complete 'methylation profile' is currently underway, and may be more important to use a complete profile to diagnose and treat prostate cancers [31, 61]. The Food and Drug Administration (FDA) has approved several drugs that target DNA methyltransferase (DNMTs) and histone deacetylases (HDACs) for other cancers, and it is likely that a combination of epigenetic drugs will be necessary to treat prostate cancers [31, 60-61]. Micro-RNAs have been implicated in AR signaling, most mRNA targets are still under investigation, and several mi-RNAs are being considered as prostate cancer biomarkers and may also be targets for drug therapy [66]. Epigenetic studies may explain how sex steroid metabolism contributes to prostate cancer etiology, bridging the gap between clinical, laboratory and epidemiologic data. Epigenetic changes which influence gene expression in prostate cancer have the potential to improve screening, prognosis and treatment, since these events are thought to happen early in cancer development and may be reversible by drug treatment [59, 67].

In order to overcome the limitation of past studies focusing on one or few genes, there is a strong need for epigenetic studies, along with genomic profiling, genome wide association studies, and proteomic studies. These studies may explain how sex steroid metabolism contributes to prostate cancer etiology, bridging the gap between clinical, laboratory, and epidemiologic data. Given that cell and tissue sample collection requires less preservation for the assessment of methylation compared to the measurement of mRNA, epigenetic changes have the potential to improve screening, prognosis, and treatment since these events are thought to happen early in cancer development and may be reversible by drug treatment [59, 67].

It is well established that *GSTP1* is methylated in prostate cancer tissues, and our results from this review of gene expression studies indicates that gene expression studies agree with these findings [22-28, 30]. However, the role of histone modifications and miRNAs is less clear [61, 65-66]. Given that technology is now available and cost-effective to examine methylation patterns in prostate cancer, these alterations are now being studied on a large scale as part of the NIH Epigenome roadmap project [59]. In the coming decade, prostate cancer methylation patterns involving the sex steroid hormone pathway are going to be uncovered. Newly determined methylation profiles may lead to the development of more accurate cancer screening methods, and methylation based prostate cancer treatments.

Epigenetic modifications are thought to leave a chronicle of endogenous and exogenous exposures that lead to diseases like prostate cancer, which are currently inadequately captured in epidemiologic data assessing exposure [68]. To date the androgen hypothesis is still the most compelling hypothesis in prostate cancer research despite mixed epidemiologic results [9-10]. Determining if epigenetic alteration is due to differing tissue-specific levels of sex steroid hormones may provide additional insights into cancer etiology and progression [22-28, 30]. How methylation and other epigenetic alterations relate to gene expression studies and SNP studies of genes involved in the sex steroid hormone pathway should be explored in future studies in relation to prostate cancer risk.

SUPPLEMENTAL MATERIALS

The supplemental materials can be downloaded from the journal website along with the article.

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