Abstract:
The present invention relates to a method and composition for modulating androgen receptor activity. The present invention arises out of studies into the regulation of androgen receptor activity. In the present studies it has been found that the small glutamine-rich tetratricopeptide repeat containing protein alpha (αSGT) modulates androgen receptor activity. Modulating the level and/or activity of αSGT modulates androgen receptor activity.
METHOD AND COMPOSITION FOR MODULATING ANDROGEN RECEPTOR ACTIVITY

This application claims priority from Australian Provisional Patent Application No. 2007905465 filed on 4 October 2007 and Australian Provisional Patent Application No. 2005905468 filed on 5 October 2007, the contents of which are to be taken as incorporated herein by this reference.

Field of the Invention

The present inventions relates to a method and composition for modulating androgen receptor activity.

Background of the Invention

Androgens are hormones most commonly associated with the induction and maintenance of masculine characteristics, and include the testicular hormones testosterone and 5α-dihydrotestosterone (DHT), and the adrenal androgens dehydroepiandrosterone (DHEA) and androstenedione (ASD). In men, androgens play a crucial role in mediating diverse physiological functions.

At a cellular level, the effects of androgens are mediated by the androgen receptor. The androgen receptor is a nuclear transcription factor that activates genes involved in growth, differentiation and homeostasis. Following translation, the androgen receptor undergoes maturation via a stepwise association with chaperones and heat-shock proteins, with the mature ligand-binding competent receptor found stably associating predominantly in the cytoplasm in a complex with the molecular chaperone Hsp90, although the cellular localization varies according to cell type. Conformational changes induced by high affinity androgen binding results in rapid Hsp90-dependent retrograde movement and nuclear import of the receptor, followed by association of the activated androgen receptor with cofactors and the transcription machinery leading to the specific transcriptional regulation of target genes.
Dysregulation of androgen activity and/or an altered response to androgen activity is associated with a number of diseases, condition and states. For example, while virtually all prostate tumors initially respond to androgen ablation therapy, the majority relapse and the disease progresses. It is now recognized that the failure of these hormonal strategies can be explained in many cases by selection of a hyperactive androgen receptor in a low androgen environment. In particular, androgen receptor levels in both animal models and clinical prostate cancer may increase, permissive androgen receptor mutations may be selected during disease progression, and the potential for crosstalk between the androgen receptor and other signaling pathways may also increase.

Whereas these findings suggest that deregulation of androgen receptor signaling is a common phenomenon in prostate cancer progression, they only partially explain how increased androgen signaling is achieved, and particularly how tumors with an apparently intact receptor expressed at levels comparable to that of the normal prostate sustain signaling during androgen ablation therapy.

Androgen receptor expression is not restricted to prostate cells. Indeed the receptor is expressed ubiquitously in human tissue and many malignant tumours in both male and female subjects and as such the androgen receptor has been implicated in a range of different diseases, conditions and states.

For female subjects the combination of synthetic progestins and estrogens in hormone replacement therapy increases the risk of breast cancer compared with estrogen alone. The increased risk of breast cancer is at least in part due to binding of the synthetic progestins to the androgen receptor although the underlying mechanisms are not clear.

Androgen therapy has also been shown to inhibit breast cancer growth by direct interaction with androgen receptors in breast cancer cells. However the advent of other hormonal therapies with lesser side-effects has meant that the mechanisms by which androgen therapy functions to retard breast cancer growth have remained ill defined.

Accordingly, there is continuing need to develop new therapies for preventing and/or treating diseases, conditions and states associated with dysregulation of androgen receptor activity. The present invention relates to the finding that a small molecule
(αSGT) is associated with the folding, localization and function of the androgen receptor, and as such can be used to modulate the activity of androgen receptor in cells.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Summary of the Invention

The present invention arises out of studies into the regulation of androgen receptor activity. In the present studies it has been found that the small glutamine-rich tetratricopeptide repeat containing protein alpha (αSGT) modulates androgen receptor activity. Modulating the level and/or activity of αSGT modulates androgen receptor activity.

In addition, it has been found that there is an increase in the ratio of androgen receptor to αSGT in metastatic prostate tumour samples. Thus, the ratio of androgen receptor to αSGT is a marker for the metastatic potential of prostate cancer cells.

Accordingly the present invention provides a method of modulating androgen receptor activity in a cell, the method including one or more of the following:

(i) modulating the level and/or activity of αSGT in the cell;

(ii) modulating the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor in the cell; and

(iii) modulating the interaction of αSGT with the androgen receptor in the cell.

The present also provides a method of preventing and/or treating a disease, condition or state associated with androgen activity in a subject, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.
The present invention also provides use of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor in the preparation of a medicament for preventing and/or treating a disease, condition or state associated with androgen activity.

The present invention also provides an isolated agent including an amino acid sequence KLQEEGEA, or a functional variant thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

The present invention also provides a pharmaceutical composition including an agent including an amino acid sequence KLQEEGEA, or a functional variant thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

The present invention also provides use of an agent including an amino acid sequence KLQEEGEA, or a functional variant thereof, in the preparation of a medicament for preventing and/or treating a disease, condition or state associated with androgen activity.

The present invention also provides a method of treating a cancer in a subject that has an altered response to androgen therapy, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

The present invention also provides a pharmaceutical composition including an agent that is a microtubule-targetting agent and an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

The present invention also provides a combination product including the following components:
an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor; and

an agent that is a microtubule-targetting agent;

wherein the components are provided in a form for co-administration to a subject or in a form for separate administration to a subject.

The present invention also provides a method of determining the susceptibility of a cancer cell associated with androgen activity to the effect of androgen ablation therapy, the method including determining one or more of following properties of the cell: (i) the level and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor in the cell; and (iii) the interaction of αSGT with the androgen receptor in the cell, wherein a decrease in any one or more of the aforementioned properties is indicative of a cancerous cell with reduced susceptibility to androgen ablation therapy.

The present invention also provides a method of identifying an agent that modulates androgen receptor activity, the method including identifying an agent that modulates one or more of the following:

(i) the level and/or activity of αSGT;

(ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and

(iii) the interaction of αSGT with the androgen receptor.

The present invention also provides a method of identifying an agent that modulates androgen receptor activity, the method including:

(i) providing an agent including an amino acid sequence KLQEEGEA, or a functional variant thereof; and

(ii) determining the ability of the agent to modulate androgen receptor activity.

The present invention also provides a method of diagnosing metastatic prostate cancer, the method including determining the levels of αSGT and androgen receptor in a
prostate cancer sample, wherein a low ratio of αSGT to androgen receptor is indicative of metastatic prostate cancer.

Various terms that will be used throughout the specification have meanings that will be well understood by a skilled addressee. However, for ease of reference, some of these terms will now be defined.

The term "subject" as used throughout the specification is to be understood to mean a human or animal subject. It will be understood that the present invention includes within its scope veterinary applications. For example, the animal subject may be a mammal, a primate, a livestock animal (eg. a horse, a cow, a sheep, a pig, or a goat), a companion animal (eg. a dog, a cat), a laboratory test animal (eg. a mouse, a rat, a guinea pig, a bird), an animal of veterinary significance, or an animal of economic significance.

The term "biological system" as used throughout the specification is to be understood to mean a multi-cellular system and includes isolated groups of cells to whole organisms. For example, the biological system may be a tissue or organ, a tissue or organ in a subject, or an entire human or animal subject.

The term "nucleic acid" as used throughout the specification is to be understood to mean to any oligonucleotide or polynucleotide. The nucleic acid may be DNA or RNA and may be single stranded or double stranded. The nucleic acid may be any type of nucleic acid, including a nucleic acid of genomic origin, cDNA origin (ie derived from a mRNA), derived from a virus, or of synthetic origin.

**Brief Description of the Figures**

Figure 1 shows the interaction of the androgen receptor (AR) with the molecular cochaperone, αSGT. Panel A shows the interaction of AR (amino acids 618-754) and full-length αSGT or the C-terminal region of Hsp90β (amino acids 609-724) in yeast cells. Data represents luminescent β-galactosidase signal and represents mean fold (±sem) activity for 3 individual transformants over AR alone. Panel B shows co-immunoprecipitation of AR and HA tagged αSGT in transfected COS-I cells in the
absence (first panel) or absence and presence (second panel) of 10nM DHT. Immunoprecipitation was performed with AR (N20), αSGT or HA antisera as indicated, and immunoblot with AR-N20 antisera. Panel C shows GST pulldown assays with AR and αSGT from transfected COS-7 cells (immunoblot with αSGT antisera) and vice versa (immunoblot with AR C-19 antisera). Panel D shows the expression of the steroid-receptor interacting Hsp70/Hsp90 TPR cochaperones relative to AR in prostate cancer samples [mean (±sem)] from Affymetrix microarray data. Left panel (ranked by decreasing relative expression) is from microdissected epithelial cells of 23 primary prostate cancers. Right panel (ranked by decreasing significance for a decline) shows the chaperone:AR ratio in metastatic prostate tumors (black) relative to primary samples (grey).

Figure 2 shows that αSGT affects AR basal activity and sensitivity to ligand-dependent activation distinct from classical coregulators. Panel A shows the effect of αSGT overexpression on AR activity. PC-3 cells were transfected with AR (2.5ng), αSGT expression or empty vector control, and ARR3-tk-Luc (10Ong) and treated with vehicle control (ethanol) or DHT as indicated. Data is presented as relative light units (RLU) and represents the mean (±sem) activity from 6-8 independently transfected wells. Boxed inset shows basal activity in presence of vehicle control (ie. in the absence of exogenous ligand). Figures on the graphs show mean (±sem) fold activation (activity divided by basal activity) for the highest concentration of ligand. Immunoblot shows αSGT antisera resolving native and transfected HA-tagged αSGT proteins in a parallel experiment. Panel B shows AR functional assays in C4-2B cells transfected with a specific αSGT siRNA or a non-specific (N.S.) negative control. Results represent the mean (±sem) expression of the androgen-responsive PSA gene as a ratio of mean GAPDH determined by triplicate quantitative real-time PCR analysis 1 day after transfection. Immunoblot analysis of parallel samples demonstrates knockdown of αSGT, but not of AR or actin, by the specific siRNA compared with N.S. control. Panel C shows the effect of increasing amounts of transfected AR on transcriptional activity in PC-3 cells performed as for part A. Immunoblot was performed with AR antisera. The EC50 (ligand concentration required for 50% maximal activity) calculated from transactivation data for each amount of transfected AR is shown below the immunoblot. Panel D shows the effect of αSGT overexpression on ER transcriptional activity in PC-3 cells, essentially as in part A, transfected with an ER expression vector (1-5ng as
indicated), ERE-tk-luc (100ng), and either αSGT expression or empty vector control, and treated with estradiol (E2) as indicated.

Figure 3 shows that αSGT affects AR subcellular distribution. Panel A shows confocal imaging microscopy of AR (green) and αSGT (red) in transfected cells treated with the indicated concentration of DHT or with vehicle control for 24 hours. Nuclei are stained blue with Hoescht dye. Panel B shows transfected cells from three independent experiments (as shown in A) were scored (blinded to category) as exhibiting predominantly nuclear (N>C) or cytoplasmic (C≥N) AR localization by manual counting. Data represents the mean (±sem) percent of transfected cells in each category from three independent experiments.

Figure 4 shows differential expression of AR and αSGT with prostate cancer progression. Panel A shows immunohistochemistry with αSGT and AR U407 antisera. Left panel shows an example of αSGT immunostaining in mouse prostate and competition by a 5x molar excess of specific blocking peptide. Right panels show two examples of immunohistochemistry with αSGT and AR U407 antisera in nonmalignant prostate and in primary and metastatic prostate cancers. Panel B shows a comparison of AR and αSGT immunohistochemistry in 30 primary human prostate tumors. Immunoreactivity of each antisera in epithelial cells (stroma was excluded) was measured by quantitative video image analysis on 20 contiguous fields for each sample. Video image measurements of integrated optical density (IOD) and the total area (TA) analyzed were used to derive the mean integrated optical density (MIOD=IOD/TA), which equates to the mean immunoreactivity per unit area (MIOD) in each sample. The mean MIOD (values in brackets) determined for each antisera is indicated by dashed red lines. The coefficient (R) and probability (p) of correlation between AR and αSGT immunoreactivity (Spearman’s rho test) are shown. Panel C shows a comparison of AR and αSGT levels in 54 metastatic human prostate tumors presented as in part B. Solid black lines represent the mean values in metastatic samples. Dashed red lines (for comparison) are from panel B. Panel D shows quantification of αSGT and AR immunoreactivity in 32 primary prostate cancers, 64 metastatic lesions and 30 non-malignant prostate controls. The ratio of AR:αSGT was calculated for those samples where sufficient informative immunoreactive area was available for both antisera, and represents the data shown in panels B and C.
Figure 5 shows αSGT expression affects AR sensitivity to non-classical ligands. The panels A show the effect of αSGT overexpression on AR transcriptional activity by non-classical ligands. PC-3 cells were transfected with AR (2.5ng), αSGT expression or empty vector control, and ARR3-tk-Luc (100ng) and treated with vehicle control (ethanol) or ligands as indicated [medroxyprogesterone acetate (MPA), androstenedione (ASD), hydroxyflutamide (OHF) or progesterone (PROG)]. Data is presented as relative light units (RLU) and represents the mean (±sem) activity determined from 6-8 independently transfected wells. Boxed insets show basal activity (ie. in the presence of vehicle control only).

Figure 6 shows αSGT interactions. In panel A αSGT is predicted to interact with a short peptide sequence in the AR hinge that structurally resembles the C-terminal EEVD peptides from Hsp70 and Hsp90. The AR hinge sequence is shown delineating sites of phosphorylation (P) and acetylation (Ac), and the AR peptide (boxed in green) predicted to mediate interaction with αSGT. The molecular surface model is of the αSGT TPR domain colored according to electrostatic potential; red=negative, blue=positive. Geometric centers (green spheres) of in silico docking solutions of the AR hinge cluster with amino acids 105-127 of αSGT, which encompasses the first of the three TPR repeats. The top 50 docking solutions were clustered to produce a theoretical docked single AR peptide, depicted in ribbon format (inset), demonstrating the predominant role of 638KLQEEGEA645 residues (green). Panel B shows αSGT dimerization. Top panel shows mammalian two-hybrid assay mapping the αSGT dimerization to the first 80 amino acids performed in transfected COS-I cells. Data represents the mean (±sem) activity from 8 independently transfected wells. Bottom panel shows immunoblot analysis performed with αSGT antisera on increasing amounts of COS-I cell lysate resolved by PAGE under non-reducing (native) conditions demonstrating the existence of αSGT dimers. Panel C shows a schematic of αSGT delineating the interaction site (if known) of its client proteins, with a brief description of known effects derived from αSGT interaction. Panel D shows a model detailing the proposed effects of αSGT on AR maturation and transport to the nucleus, (i) The αSGT dimer will facilitate recognition and efficient folding of the nascent AR by Hsp70 by providing both a platform for trimeric interaction between the three proteins and by
enhancing the ATPase activity of Hsp70 and client recognition. (ii) αSGT interaction could allow efficient exchange of Hsp70 for Hsp90 during AR maturation and act to stabilize the aporeceptor/ Hsp90 heterocomplex through trimeric interactions, thereby ensuring greater conformational integrity of apo-receptor pool and limiting passive nuclear transport or inappropriate ligand-mediated signaling, (iii) By facilitating weak association with microtubules, αSGT may further act to limit passive nuclear transport of the apo-receptor and to facilitate efficient tethering of the AR to microtubules following ligand-induced exchange of αSGT for FKBP52.

Figure 7 shows that the AR cochaperone αSGT exhibits homology and structural characteristics with known steroid receptor interacting TPR proteins. Panel A shows an immunoblot with αSGT antisera on lysates of cultured cells (panel 1), resolving endogenous and transfected αSGT in COS-1 cells (panel 2), and blocking of specific signal seen in panel 2 by pre-incubation of the αSGT antisera with 5 fold excess specific peptide (panel 3). Panel B shows a schematic of αSGT delineating its central tetratricopeptide repeat (TPR) and glutamine rich domain (QRD). The percentage homology of αSGT's three TPR motifs to those in known steroid-receptor interacting TRP-containing cochaperones; protein phosphatase 5 (PP5/PP5C), FK506 binding proteins of 51 and 52 kDa (FKBP51/FKBP5 and FKBP52/FKPB4) and cyclophilin-40 (Cyp40/PPID). These proteins exhibit variable specificity for individual steroid receptor-Hsp70/Hsp90 chaperone complexes, with the TPR domains themselves critical in orchestrating their effects. Panel C shows a molecular ribbon model of the αSGT TPR compared to the solved crystal structures of the TPR domains from Cyp40 and PP5.

Figure 8 shows that αSGT affects AR basal activity and sensitivity to ligand-dependent activation distinct from classical coregulators. Panel A shows that AR expression and activation functions are essential for AR-mediated basal activity. PC-3 cells were transfected with 2.5ng wtAR, AR deleted of amino acids 38-360 encompassing activation function 1 (ARΔAF1), or empty vector control and with AR3-tk-Luc (100ng), and treated with vehicle control (ethanol) or DHT (InM) as indicated. Data is presented as relative light units (RLU) and represents the mean (±sem) activity from 6-8 independently transfected wells. Panel B shows the effect of increasing amounts of transfected AR on transcriptional activity in PC-3 cells performed as for panel A. Data was normalized for each amount of transfected AR to percent maximal activity, and
clearly shows a right-shift in the dose-response to DHT for increasing amount of transfected AR. Panel C shows the linear relationship between the amount of transfected AR and the EC50 (ligand concentration required for 50% maximal activity) calculated from data in part B. Panel D shows the effect of overexpressing known AR coregulators, GRIP1/SRC2 or TGFβIII/Hic5/ARA55 on AR transcriptional activity. Transfection was performed as for panel A with 2.5ng of AR and 50ng of either the cofactor expression vector or an equivalent molar amount of empty vector control. Panel E shows endogenous AR functional assays in C4-2B cells transfected with a specific αSGT siRNA or a non-specific (n.s.) negative control. Results represent the mean (±sem) expression of the androgen responsive PSA gene as a ratio of mean GAPDH determined by triplicate quantitative real-time PCR analyses 1 day after siRNA transfection and treatment with vehicle control (ethanol) or 10nM of steroids as indicated [dihydrotestosterone (DHT), progesterone (PROG), medroxyprogesterone acetate (MPA), androstenedione (ASD), estradiol (E2)]. Boxed inset shows basal PSA expression in the presence of vehicle control (i.e. in the absence of any exogenous ligand). Panel F shows deletion of the predicted αSGT binding site from the AR hinge region increases basal receptor activity. Transactivation assays performed as in panel A with wtAR or ARΔ638-646) and ARR3-tk-Luc (10Ong). Figures on the graphs show mean (±sem) fold activation (activity divided by basal activity) for the highest concentration of ligand. Immunoblot analyses were performed on pooled lysates from the same transfection.

Figure 9 shows that αSGT is not recruited to the PSA gene promoter or enhancer. Panel A is a schematic of the human PSA gene enhancer and promoter delineating the position AR responsive elements (AREs). Panels B and C show chromatin immunoprecipitation assays performed with AR, GRIP1 and αSGT antisera in LNCaP cells following 60-minute treatment with or without 10nM DHT. Occupancy of enhancer and promoter elements of the PSA gene (detailed in Panel A) were interrogated in immunoprecipitated lysates by quantitative real-time PCR, with each datapoint representing the mean (±sem) of triplicate PCR reactions.

Figure 10 shows αSGT immunoreactivity in adult mouse tissues. Immunohistochemistry with αSGT antisera in adult mouse tissues were arranged on a single tissue microarray block. Specific immunoreactivity in each tissue could be
competed by a 5x molar excess of specific blocking peptide. Comparatively little αSGT immunoreactivity was evident in the heart.

**General Description of the Invention**

As described above, one embodiment of the invention provides a method of modulating androgen receptor activity in a cell, the method including one or more of the following:

(i) modulating the level and/or activity of αSGT in the cell;

(ii) modulating the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor in the cell; and

(iii) modulating the interaction of αSGT with the androgen receptor in the cell.

As described previously herein, the present invention arises from the finding that modulating the level and/or activity of the small glutamine-rich tetratricopeptide repeat containing protein alpha (αSGT) in a cell expressing the androgen receptor modulates androgen receptor activity.

The cell in the various embodiments of the present invention is a cell including an androgen receptor, including a cell expressing an endogenous androgen receptor and/or a cell expressing an exogenous androgen receptor.

Thus it will be appreciated that cells engineered to express an androgen receptor are also included within the scope of the present invention.

In one embodiment, the cell is a human or animal cell.

The cell type including the androgen receptor in the various embodiments is not particularly limited, and includes a cell type endogenously expressing androgen receptor or a cell engineered to express androgen receptor. In one embodiment, the cell is a prostate cell.

In one embodiment, the cell is a cancerous cell. For example, the cell may be a prostate cancer cell, a breast cancer cell, a colon cancer cell, an endometrial cancer cell or a testicular cancer cell.
The cell may be a cell in vitro, for example a cell in tissue culture, or a cell in a biological system, for example a cell present in a human or animal subject. Thus, it will be understood that the cell may be present in a complex mixture of other cells in vitro or in vivo.

In one embodiment, the cell is present in a human or animal subject. In one specific embodiment, the cell is present in a human or animal subject susceptible to, or suffering from, a disease, condition or state associated with an altered level of androgen activity.

Accordingly, in another embodiment the present invention provides a method of preventing and/or treating a disease, condition or state associated with androgen receptor activity in a subject, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

In one embodiment, the disease, condition or state associated with androgen receptor activity is a disease, condition or state associated with altered androgen receptor activity.

In another embodiment, the disease, condition or state associated with androgen receptor activity is a disease, condition or state associated with altered response to androgen receptor activity.

For example, the disease may be a cancer, such as prostate cancer, including metastatic prostate cancer.

The cancerous cell may be a cancerous cell that is associated with a primary tumour and/or one or more secondary tumours in a subject.

In one embodiment, the cancer is a cancer that is no longer substantially susceptible to androgen ablation therapy.
In another embodiment, the cell is an isolated cell. In this regard, the term "isolated" is to be understood to mean an entity, for example a cell, a nucleic acid or a protein, which is removed and/or purified from its natural environment.

In this regard, the cell may be a primary cell or a cell from a cell line. A primary cell refers to one or more cells taken directly from a living organism, which are not immortalized. A cell line refers to a homogenous population of cells isolated from an animal or human and grown in a laboratory and includes immortalized cell lines. Human prostate specific cell lines include PC3, DU145 and LNCaP cell lines. LNCaP cell lines include androgen sensitive cell line LNCaP-FGC and androgen resistant cell lines LNCaP-r and C4-2B.

The androgen receptor is a nuclear receptor, activated primarily by androgens, including testosterone or dihydrotestosterone. When the androgen receptor is not bound to an androgen, it predominantly resides in the cytoplasm of cells where it is held inactive through association with heat-shock proteins including hsp90, hsp70, and hsp56. Androgen binding to the androgen receptor results in the dissociation of heat-shock proteins, dimerization of the androgen receptor and translocation to the nucleus, wherein the androgen receptor is able to bind to androgen response elements and/or proteins in the nucleus to modulate transcription of androgen responsive genes.

The human androgen receptor is a 100 kDa protein, which contains 919 amino acids and comprises of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal steroid-binding domain. A hinge region is also present between the DNA-binding domain and the C-terminal steroid-binding domain. The nucleotide sequence of the human androgen receptor is available from GenBank and has an accession number of NM_000044.

The androgen receptor shares a high degree of homology between animal species. The sequences of the androgen receptor of different species may be readily identified by a method known in the art by comparison with other available sequences.

The androgen receptor in the various embodiments of the present invention may be an endogenous androgen receptor or an exogenous androgen receptor, and the receptor
may be driven from one or more endogenous or exogenous transcriptional or translation elements. Methods for cloning nucleic acids and introducing nucleic acids into cells are known in the art.

It will also be appreciated that the androgen receptor in the various embodiments may be a natural receptor, a variant of a natural receptor, a chimeric receptor, or a synthetic receptor.

In this regard, the term "variant" as used throughout the specification is to be understood to mean an amino acid sequence of a polypeptide or protein that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties to the replaced amino acid (e.g., replacement of leucine with isoleucine). A variant may also have "non-conservative" changes (e.g., replacement of a glycine with a tryptophan) or a deletion and/or insertion of one or more amino acids. A variant may also be a form of the protein that has one or more deleted amino acids (e.g., a truncated form of the protein), and/or a form of the protein that has one or more additional exogenous amino acids (e.g., a form of the protein fused to another polypeptide sequence). It will be appreciated that a variant will therefore include within its scope a fragment of a protein.

Generally, the variant will be a functional variant, that is, a variant that retains the functional ability of the progenitor protein.

Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Under some circumstances, substitutions within the aliphatic group alanine, valine, leucine and isoleucine are also considered as conservative. Sometimes substitution of glycine for one of these can also be considered conservative. Other conservative interchanges include those within the aliphatic group aspartate and glutamate; within the amide group asparagine and glutamine; within the hydroxyl group serine and threonine; within the aromatic group phenylalanine, tyrosine and tryptophan; within the basic group lysine, arginine and histidine; and within the sulfur-containing
group methionine and cysteine. Sometimes substitution within the group methionine and leucine can also be considered conservative.

The level of activity of the androgen receptor in the various embodiments of the present invention encompasses the various biological activities of the receptor in a cell, including for example the ability of the receptor to regulate transcription. Modulation of activity of the receptor can occur, for example, by alteration in the level of androgen receptor, alteration in the function of the receptor, alteration in the cellular localization of the receptor, alteration in the folding of the androgen receptor, alteration in the rate of the movement of the receptor within a cell, alteration of the dimerisation of the androgen receptor, alteration of the binding of the androgen receptor to androgen response elements (ARE) on DNA, and alteration in the ability of the receptor to associate with other molecules (including co-factors, heat shock proteins and/or molecular chaperones).

In this regard, the level of androgen receptor in a cell may be determined by a suitable method known in the art. The level of androgen receptor may be determined directly or indirectly. Methods for the measurement of androgen receptor protein include for example the use of anti-androgen receptor antibodies for intracellular staining, immunoprecipitation, western blotting or ELISA. Methods to measure androgen receptor mRNA expression are known in the art and include reverse transcription PCR using androgen receptor specific primers.

Androgen receptor activity may be measured for example by determining the level of expression of one or more androgen responsive genes. This may be performed for example by measuring the levels of mRNA expression or protein associated with one or more genes, or alternatively through use of reporter constructs.

In this regard, androgen responsive genes are genes whose expression is modulated as a consequence of androgen receptor activation. The expression of a particular gene may be increased or decreased.

For example, Prostate Specific Antigen (PSA) and KLK2 are genes whose transcription is specifically regulated by androgen receptor activation. Prostate Specific Antigen
(PSA) and KLK2 are secreted kallikrein proteases involved in prostate metabolism. Expression of PSA and KLK2 are increased in response to androgen binding to the androgen receptor. PSA and/or KLK2 expression by a cell can therefore be used to monitor androgen receptor activity either by measuring PSA/KLK2 mRNA or protein expression.

Other genes include FKB15 and TMPRSS2.

In the case of the use of reporter constructs, a suitable construct may be introduced into a cell. For example cells may be transfected with a vector such as ARR3-tk-Luc, which includes an androgen receptor responsive promoter and a reporter gene. Reporter genes may be genes which encode for fluorescent proteins such as green fluorescent protein or derivatives thereof, or enzymes such as luciferase or β-galactosidase, which catalyze detectable reactions under appropriate conditions. Suitable vectors are known in the art. Methods to insert vectors into the cell are known in the art and include transformation using calcium phosphate, viral infection, electroporation, lipofection, and particle bombardment.

Androgen receptor activation may also be determined by immunoblot analysis for phosphorylation of key residues, including ser81, on the androgen receptor, which is an indicator of a transcriptionally active receptor.

In the case of the localization of the androgen receptor, this may be determined for example by intracellular staining using antibodies directed against the androgen receptor. The antibodies may be conjugated to fluorescent molecules to facilitate visualization under a fluorescent microscope. This method may be used to assess how increased or decreased levels of αSGT affect the cellular localization of the androgen receptor and/or the sensitivity and rate of DHT dependent movement of the androgen receptor from the cytoplasm to the nucleus. Intracellular staining may be performed on the cell as a whole after permeabilising the membrane. As an alternative to intracellular staining of intact cells, cells from tissue may be embedded in paraffin or OCT compound, sections cut onto a slide using a cryostat or equivalent machine and stained with the antibodies. Such histological methods are known in the art. Additional stains or antibodies may be used to discriminate the cytoplasm from the nucleus. Nuclear
stains are known in the art and include hematoxylin, propidium iodide, DAPI or carmine alum.

The association of the androgen receptor with other proteins, such as heat shock proteins or other molecular chaperones, may be determined for example by co-immunoprecipitation analysis. Examples of other relevant heat shock proteins and/or molecular chaperones include Hsp70, Hsp90, FKBP4, p23, FKBP5, SYP40, CHIP and HSP27.

Similarly, the binding of androgen to the androgen receptor may be determined by a method known in the art, for example by co-immunoprecipitation as described above.

Dimerisation of the androgen receptor may be determined by a method known in the art. For example, SDS-PAGE and/or immunoprecipitation under non-reducing conditions and assessment of the mass of the isolated protein.

Methods are known in the art for assessing the binding of androgen receptor to an androgen responsive element. For example, chromatin immunoprecipitation may be used to determine the association of androgen receptor with any region of DNA, include AREs (see for example Jariwala et al. (2007) Mol. Cancer 6 (1): 39).

As discussed previously herein, the present invention arises from studies involving overexpression and RNA interference of the small glutamine-rich tetratricopeptide repeat containing protein alpha (αSGT). In particular, it has been found that αSGT acts to (i) promote cytoplasmic compartmentalization of the AR, thereby silencing the receptors basal/ligand-independent transcriptional activity, (ii) regulate the sensitivity of receptor signaling by androgens, and (iii) limit the capacity of non-canonical ligands to induce AR agonist activity. Immunofluorescence, coactivator and chromatin immunoprecipitation analysis indicate that the effects of αSGT on AR function are mediated by interaction in the cytoplasm and are distinct from the receptors response to classic coregulators. The present invention therefore provides a means to modulate androgen receptor activity in a cell by modulating αSGT expression and/or function.
In this regard, αSGT is a 35 kDa protein with 313 amino acids. The protein is also referred to as SGTA. The nucleotide sequence of human αSGT is provided by GenBank accession number NM 003021. αSGT is conserved across a range of species from humans to yeast. The sequences of αSGT of different species may be readily identified by a method known in the art by comparison with other available sequences.

The αSGT protein may be a natural protein, a variant of a natural protein, a chimeric protein, or a synthetic protein. In a cell, the αSGT protein may be an endogenous protein or an exogenous protein.

The level and/or activity of αSGT protein in the various embodiments of the present invention encompasses the various biological activities of the protein associated with directly or indirectly regulating androgen receptor activity, including the ability to dimerize and/or phosphorylation. Methods for modulating the level and/or activity of αSGT may be selected.

The level of αSGT may be determined by a method known in the art. For example, the level of αSGT protein or αSGT mRNA expression in a cell may be determined. Methods for the measurement of αSGT protein include the use of anti αSGT antibodies for intracellular staining, immunoprecipitation, western blotting or ELISA. Methods to measure αSGT mRNA expression include reverse transcription PCR using αSGT specific primers to measure αSGT mRNA levels.

The activity of αSGT may also be determined by a suitable method. For example, the activity of αSGT in one embodiment relates to the ability of the αSGT to interact with the androgen receptor. Methods to assess the interactions between proteins are known in the art, and include for example immunoprecipitation analysis.

In one embodiment, increasing one or more of (i) the level of and/or activity of αSGT in a cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the receptor, decreases the androgen receptor activity in a cell.
Methods for increasing the level of and/or activity of $\alpha$SGT in a cell include for example introducing nucleic acids encoding $\alpha$SGT, or a functional part thereof, into the cells to overexpress $\alpha$SGT, engineering the cell to overexpress the endogenous $\alpha$SGT, or use of an $\alpha$SGT agonist.


In an alternative embodiment, decreasing one or more of (i) the level of and/or activity of $\alpha$SGT in a cell; (ii) the ratio of the level and/or activity of $\alpha$SGT to the level and/or activity of androgen receptor; and (iii) the interaction of $\alpha$SGT with the receptor, increases the androgen receptor activity in a cell.


As can be appreciated, increasing the ratio of the level and/or activity of $\alpha$SGT to the level and/or activity of androgen receptor includes increasing the level and/or activity of
αSGT relative to the level and/or activity of androgen receptor, and/or decreasing the level and/or activity of the androgen receptor in relation to the level and/or activity of αSGT.

In this regard, methods of increasing αSGT are as described above. The level and/or activity of androgen receptor may be decreased for example using antagonists, siRNA, ribozymes, or antisense oligonucleotides. Androgen receptor antagonists include bicalutamide and oxendolone. Human androgen receptor siRNA (catalogue # M-003400), siRNA expression plasmids (catalogue # 62-012 and 62-013) and siRNA kits (catalogue # 60-003) are commercially available from Upstate CA. Messenger RNA hammerhead ribozymes against androgen receptor are described for example in Zegarra-Moro et al. (2002) Cancer Res., 62: 1008-1013. Antisense oligonucleotides directed against human androgen receptor are described for example in Eder et al. (2000) Cancer Gene Therapy, 7(7): 997-1007.

Decreasing the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor includes decreasing the level and/or activity of αSGT relative to the level and/or activity of androgen receptor, and/or increasing the level and/or activity of the androgen receptor in relation to the level and/or activity of αSGT.

In an embodiment, modulating the interactions of αSGT with other proteins may affect androgen receptor activity. For example, as Hsp70 and Hsp90 interact with both the androgen receptor and αSGT, modulation of αSGT binding to Hsp70 and/or Hsp90 can modulate androgen receptor activity. Modulating the interactions includes increasing or decreasing the interactions.

As described previously, in one embodiment the present invention includes modulating androgen activity in a cell as part of a biological system.

In one embodiment the biological system is a human or animal subject.

In one specific embodiment, the biological system is a human or animal subject, wherein the human or animal subject is susceptible to, or suffering from, a disease, condition or state associated with an altered level of androgen receptor activity. An
example of a disease, condition or state associated with androgen receptor activity includes some forms of cancer. In one embodiment, the disease, condition or state is associated with altered androgen receptor activity or an altered response to altered androgen receptor activity.

In one embodiment, the disease, condition or state associated with an altered level of androgen receptor activity or an altered response to androgen receptor activity is a cancer. Examples of cancers include prostate cancer, breast cancer, colon cancer, endometrial cancer, ovarian cancer, testicular cancer and hepatocellular carcinoma.

In this regard, an increased risk of breast cancer has been associated in part with binding of the synthetic progestins to the androgen receptor when synthetic progestins are used in hormone replacement therapies with oestrogen. Androgen therapy has demonstrated some efficacy in inhibiting breast cancer growth. This implies that prevention of synthetic progestin binding to the androgen receptor could restrict the development of breast cancer.

In one embodiment, modulation of the androgen receptor in breast cancer cells involves increasing one or more of the following: (i) the level and/or activity of $\alpha$SGT in the cell; (ii) the ratio of the level and/or activity of $\alpha$SGT to the level and/or activity of the androgen receptor in the cells; and (iii) the interaction of $\alpha$SGT with the androgen receptor in the cell. The increased level and/or activity of $\alpha$SGT in the cells may restrict the responsiveness of the androgen receptor to the non-classical androgen receptor ligand synthetic progestin, thereby reduce the incidence of breast cancer development.

In another embodiment, the cancer is prostate cancer, including metastatic prostate cancer.

In this regard, treatment options for prostate cancer include surgery, radiation therapy, androgen ablation and/or high intensity focused ultrasound. More than one of these options are often used. Androgen ablation is routinely used to treat prostate cancer, as androgens promote the growth of prostate cancer cells. Androgen ablation may target production and/or activity of androgens. Orchiectomy, which is the removal of the testicles, reduces testosterone production and subsequently the level of DHT. The
production of adrenal androgens may be blocked with ketoconazole and aminoglutethimide. Antiandrogens including flutamide, bicalutamide, nilutamide and cyproterone acetate may be used to block the action of testosterone and DHT. Abarelix, leuprolide, goserelin, triptorelin and buserelin may be used to restrict the production of GnRH and/or LH, which can reduce testosterone production.

While androgen ablation therapies are initially effective, prostate cancer cells eventually become resistant to androgen ablation, with the median time for relapse being 12-30 months. As shown in the present studies, the androgen receptor in prostate cancer cells is able to bind non-classical androgen receptor ligands, which may account for the resistance to androgen ablation. Maintaining specificity of the androgen receptor to androgen and DHT by increasing the level and/or activity of αSGT may improve the efficacy of androgen ablation and prevent androgen ablation resistance. Furthermore it has been shown that in recurrent tumours, which are resistant to androgen ablation, high levels of androgen receptor are located in the nucleus. Accordingly, increasing the level and/or activity of αSGT in a prostate cancer cell may promote the cytoplasmic retention of the androgen receptor in the absence of androgen.

In the present studies, quantitative immunohistological analysis of αSGT and AR levels in a cohort of 32 primary and 64 metastatic human prostate cancers revealed dysregulation in the level of both proteins during disease progression. In the present studies, the higher AR:αSGT ratio in metastatic samples was consistent with sensitization of prostate tumor cells to androgen signaling with disease progression, particularly in a low-hormone environment. Furthermore, reduction in αSGT levels by siRNA resulted in an increased responsiveness of the androgen receptor in response to non-classical androgen receptor ligands, wherein the non-classical androgen receptor ligands included hydroxyflutamide (OHF), adrenal androgen androstenedione (ASD), progesterone, and the synthetic progestin medroxyprogesterone acetate (MPA). Other molecules which can inappropriately stimulate the androgen receptor in prostate cancer cells include Bisphenol A.

Accordingly, in one embodiment, modulation of the androgen receptor in a prostate cancer cell involves increasing one or more of the following: (i) the level and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or
activity of the androgen receptor in the cells; and (iii) the interaction of αSGT with the androgen receptor in the cell.

In another embodiment, increasing the level and/or ratio of αSGT activity and/or level to the activity and/or level of androgen receptor can maintain the specificity of the androgen receptor to androgen and therein limit the reactivity of the androgen receptor to non-classical androgen receptor ligands. As such, maintaining the specificity of the androgen receptor to androgen may improve the efficacy of androgen ablation therapy and prevent or decrease resistance of prostate cancer cells to androgen ablation therapy.

Androgen ablation therapy is particularly useful for treatment of metastatic prostate cancer as surgical excision of the tumour alone will be ineffective.

In another embodiment, the method of modulating androgen receptor activity in a cell is used to treat cancer that has an altered response to androgen therapy, for example, a cancer no longer susceptible to androgen ablation therapy.

Accordingly, in another embodiment the present invention provides a method of treating a cancer in a subject that has an altered response to androgen therapy, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

In one embodiment, the present invention provides a method of treating a cancer in a subject that has a reduced susceptibility to androgen ablation therapy, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

As previously discussed herein, development of resistance to androgen ablation therapy by cancer cells typically occurs 12 to 30 months after treatment begins and is characterized by the recurrence of tumours, wherein the androgen receptor in the
tumour cells is reactive to non-classical androgen receptor ligands. As a consequence of the reduced specificity of the androgen receptor, the androgen receptor may respond to other steroids, resulting in nuclear translocation and activation of androgen responsive genes. This in turn facilitates the growth of the tumour.

Accordingly, increasing one or more of the following; (i) the level and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of the androgen receptor in the cells; and (iii) the interaction of αSGT with the androgen receptor in the cell, can increase the specificity of the androgen receptor and promote the cytoplasmic localization of the androgen receptor. Such effects may restore the susceptibility of cancer cells to androgen ablation therapy.

In one embodiment, the modulation of androgen receptor activity includes exposing the cell to an agent that modulates one or more of (i) the level and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor in the cell; (iii) the interaction of αSGT with the androgen receptor in the cell.

Examples of agents include a drug, a small molecule, a nucleic acid, an oligonucleotide, a peptide, a polypeptide, a protein, an enzyme, a polysaccharide, a glycoprotein, a lipid, an antibody or a part thereof, and an aptamer.

In one embodiment, the agent includes an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant or mimic thereof.

In one specific embodiment, the agent includes an amino acid acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3), or a functional variant or mimic thereof.

In another embodiment, the agent includes one or more of the following amino acid sequences, or a functional variant or mimic thereof:

<table>
<thead>
<tr>
<th>Sequence</th>
<th>ID No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEG/VD/E</td>
<td>1</td>
</tr>
<tr>
<td>EEGE</td>
<td>2</td>
</tr>
<tr>
<td>EEVD</td>
<td>3</td>
</tr>
<tr>
<td>QEEGE</td>
<td>4</td>
</tr>
</tbody>
</table>
In a further embodiment, any of SEQ. ID NOS. 1 to 23 may further include an alanine residue at the carboxy terminal end.

In one embodiment, the agent increases one or more of (i) the level of and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the receptor and thereby decreases the androgen receptor activity in the cell.

In an alternative embodiment, the agent decreases one or more of (i) the level of and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the receptor and thereby increases the androgen receptor activity in the cell.
In another embodiment, the present invention provides an isolated agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant or mimic thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

In one embodiment the agent is a polypeptide.

In another embodiment, the present invention provides a method of treating a disease, condition or state associated with androgen activity in a subject, the method including administering to the subject an agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant or mimic thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

In one embodiment, the present invention provides a method of treating a disease, condition or state associated with altered levels of androgen activity in a subject, the method including administering to the subject an agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant or mimic thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

In another embodiment, the present invention provides a pharmaceutical composition including an agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant or mimic thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

In another embodiment, the present invention provides an agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant thereof, in the preparation of a medicament for preventing and/or treating a disease, condition or state associated with androgen activity.

The present invention may also be used to determine the susceptibility of a cancer cell to androgen ablation therapy.

Accordingly, in another embodiment the present invention provides a method of determining the susceptibility of a cancer cell associated with androgen activity to
androgen ablation therapy, the method including determining one or more of following properties of the cell: (i) the level and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor in the cell; and (iii) the interaction of αSGT with the androgen receptor in the cell, wherein a decrease in any one or more of the aforementioned properties is indicative of a cancerous cell with reduced susceptibility to androgen ablation therapy.

Methods for determining the properties of the cell are as discussed previously herein.

In one embodiment, the cancer cell is a prostate cancer cell.

The present invention may also be used to diagnose a metastatic prostate cancer in a subject.

Accordingly, in another embodiment the present invention provides a method of diagnosing metastatic prostate cancer, the method including determining the levels of αSGT and androgen receptor in a prostate cancer sample, wherein a low ratio of αSGT to androgen receptor is indicative of metastatic prostate cancer.

A "normal" ratio may be determined by obtaining a biopsy of non-malignant prostate tissue and determining the ratio of αSGT to androgen receptor. The present studies indicate that non-malignant prostate tissue has an average αSGT to androgen receptor ratio of around 1, and that a sample with a αSGT to androgen receptor ratio less than 0.8 is indicative that the tumour may be metastatic.

As described previously herein, the present invention may be used to prevent and/or treat a disease, condition or state associated with androgen receptor activity, including a disease, condition or state associated with altered androgen receptor activity.

Accordingly, in another embodiment the present invention provides a method of preventing and/or treating a disease, condition or state associated with androgen receptor activity in a subject, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity
of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

Examples of agents are as previously described herein.

As discussed previously herein, in one embodiment the disease, condition or state associated with altered androgen receptor activity is a disease, condition or state is associated with an increased level of androgen activity.

For example, the disease may be a cancer, such as prostrate cancer, including metastatic prostrate cancer.

In one embodiment, the cancer is no longer susceptible to androgen ablation therapy.

In one embodiment, the agent increases one or more of (i) the level of and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor and thereby decreases the androgen receptor activity in the cell.

In one embodiment, method further includes administering to the subject an agent that is a microtubule-targeting agent (MTA), such as docetaxel and/or paclitaxel.

In this regard, as cytoplasmic αSGT is associated with microtubules and filaments, the utilisation of a MTA can augment the treatment of the agent used to modulate the androgen receptor activity. MTAs bind to microtubules and prevent cell division. MTAs which bind to taxane domain on the microtubule increase the stability of the microtubule lattice and therein promote irreversible polymerisation of the microtubule. Examples of such MTAs include docetaxel and paclitaxel. Increased stability of the microtubule may maintain the activity of αSGT and thereby influence androgen receptor activity. Other MTAs can inhibit cell division by targeting colchicines or *Vinca* microtubule domains resulting in destabilisation of microtubules and increased amounts of soluble tubulin. Examples of these MTAs include 2-methoxyestradiol and vinblastine.
In one embodiment, the microtubule-targeting agent is administered to the subject in the form of a pharmaceutical composition with the agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

Accordingly, in another embodiment the present invention provides a pharmaceutical composition including an agent that is a microtubule-targeting agent and an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

In another embodiment, the microtubule-targeting agent and an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor are provided in the form of a combination product for co-administration to a subject or in a form for separate administration to a subject.

Accordingly, in another embodiment the present invention provides a combination product including the following components:

an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor;

and

an agent that is a microtubule-targeting agent;

wherein the components are provided in a form for co-administration to a subject or in a form for separate administration to a subject.

In another embodiment the present invention provides use of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor in the preparation of a medicament for
preventing and/or treating a disease, condition or state associated with androgen activity.

Methods are known in the art for exposing cells *in vitro* and *in vivo* to agents that modulate one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

A suitable method for exposing an agent to a cell *in vitro* is by direct exposure of the agent to the cell.

In the case of a cell in a subject, a suitable method of exposing the cell to the agent is by administration of the agent to the subject.

The effective amount of the agent to be exposed to the cell in the various embodiments of the present invention is not particularly limited. Generally an effective concentration of the agent will be in the range from 0.1 μM to 20 μM.

In the case of the use of an agent with other agents, such as a microtubule-targeting agent, the agents may be separately administered to the subject in a suitable form, or alternatively, be co-administered to the subject in a suitable form.

For example, the agent and the microtubule-targeting agent may be included in a combination product for separate or co-administration to a subject.

The components of the combination product may be packaged separately or together in suitably sterilized containers such as ampoules, bottles, or vials, either in multi-dose or in unit dosage forms. The containers are typically hermetically sealed. Methods are known in the art for the packaging of the components.

Co-administration of agents in the various embodiments of the present invention can be sequential or simultaneous and generally means that the agents are present in the subject during a specified time interval. Typically, if a second agent is administered within the half-life of the first agent, the two agents are considered co-administered.
An appropriate dosage regime for the administration of the agent may be chosen by a person skilled in the art. For example, the administration of the agent to the subject may be prior to, concurrently with, or after exposure of the subject to an anti-cancer therapy.

The effective amount of the agent to be administered to the subject is not particularly limited, so long as it is within such an amount and in such a form that generally exhibits a useful or therapeutic effect. The term "therapeutically effective amount" is the quantity which, when administered to a subject in need of treatment, improves the prognosis and/or health state of the subject. The amount to be administered to a subject will depend on a number of factors, including the mode of administration and the characteristics of the subject, such as general health, other diseases, age, sex, genotype, and body weight. A person skilled in the art will be able to determine appropriate dosages depending on these and other factors.

In this regard, details of administration routes, doses, and treatment regimes for treating cancers are known in the art, for example as described in "Cancer Clinical Pharmacology" (2005) ed. By J.H.M. Schellens, H. L. McLeod and D.R. Newell, Oxford University Press; and "Cancer and its management" (2005). Fifth Edition by R. Souhami and J. Tobias, Blackwell Publishing.

As discussed previously herein, administration and delivery of the compositions according to the present invention may be by, for example, intravenous, intraperitoneal, subcutaneous, intramuscular, oral, or topical route, or by direct injection.

The dosage form, frequency and amount of dose will depend on the mode and route of administration. Typically an injectable composition will be administered in an amount of between 5 mg/m² and 500 mg/m², generally between 10 mg/m² and 200 mg/m². Typically an orally administered composition will be administered in an amount of between 5 mg and 5 g, preferably between 50 mg and 1 g.

For example, effective amounts of the agents typically range between about 0.1 mg/kg body weight per day and about 1000 mg/kg body weight per day, and in one form between 1 mg/kg body weight per day and 100 mg/kg body weight per day.
As described above, the administration of a composition may also include the use of one or more pharmaceutically acceptable additives, including pharmaceutically acceptable salts, amino acids, polypeptides, polymers, solvents, buffers, excipients, preservatives and bulking agents, taking into consideration the particular physical, microbiological and chemical characteristics of the agent to be administered.

For example, the agents in the various embodiments of the present invention can be prepared into a variety of pharmaceutical acceptable compositions in the form of, e.g., an aqueous solution, an oily preparation, a fatty emulsion, an emulsion, a lyophilised powder for reconstitution, etc., and can be administered as a sterile and pyrogen free intramuscular or subcutaneous injection or as injection to an organ, or as an embedded preparation or as a transmucosal preparation through nasal cavity, rectum, uterus, vagina, lung, etc. The composition may be administered in the form of oral preparations (for example solid preparations such as tablets, caplets, capsules, granules or powders; liquid preparations such as syrup, emulsions, dispersions or suspensions).

Compositions containing the agents may also contain one or more excipients including pharmaceutically acceptable preservative, buffering agent, diluent, stabiliser, chelating agent, viscosity-enhancing agent, dispersing agent, pH controller, or isotonic agent. These excipients are well known to those skilled in the art.

Examples of suitable preservatives are benzoic acid esters of para-hydroxybenzoic acid, phenols, phenylethyl alcohol or benzyl alcohol. Examples of suitable buffers are sodium phosphate salts, citric acid, tartaric acid and the like. Examples of suitable stabilisers are antioxidants such as alpha-tocopherol acetate, alpha-thioglycerin, sodium metabisulphite, ascorbic acid, acetylcysteine, 8-hydroxyquinoline, and chelating agents such as disodium edetate. Examples of suitable viscosity enhancing agents, suspending, solubilizing or dispersing agents are substituted cellulose ethers, substituted cellulose esters, polyvinyl alcohol, polyvinylpyrrolidone, polyethylene glycols, caromer, polyoxypropylene glycols, sorbitan monooleate, sorbitan sesquioleate, polyoxyethylene hydrogenated castor oil 60.
Examples of suitable pH controllers include hydrochloric acid, sodium hydroxide, buffers and the like. Examples of suitable isotonic agents are glucose, D-sorbitol or D-mannitol, sodium chloride.

The administration of the agent in the various embodiments of the present invention may also be in the form of a composition containing a pharmaceutically acceptable carrier, diluent, excipient, suspending agent, lubricating agent, adjuvant, vehicle, delivery system, emulsifier, disintegrant, absorbent, preservative, surfactant, colorant, glidant, anti-adherent, binder, flavorant or sweetener, taking into account the physical, chemical and microbiological properties of the agent being administered.

For these purposes, the composition may be administered for example orally, parenterally, by inhalation spray, adsorption, absorption, topically, rectally, nasally, buccally, vaginally, intraventricularly, via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, or by any other convenient dosage form. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, and intracranial injection or infusion techniques.

When administered parenterally, the composition will normally be in a unit dosage, sterile, pyrogen free injectable form (solution, suspension or emulsion, which may have been reconstituted prior to use) which is preferably isotonic with the blood of the recipient with a pharmaceutically acceptable carrier. Examples of such sterile injectable forms are sterile injectable aqueous or oleaginous suspensions. These suspensions may be formulated according to techniques known in the art using suitable vehicles, dispersing or wetting agents, complexing agents, polymers, solubility aids and suspending agents. The sterile injectable forms may also be sterile injectable solutions or suspensions in non-toxic parenterally acceptable diluents or solvents, for example, as solutions in 1,3-butanediol. Among the pharmaceutically acceptable vehicles and solvents that may be employed are water, ethanol, glycerol, saline, dimethylsulphoxide, N-methyl pyrrolidone, dimethylacetamide, Ringer's solution, dextrose solution, isotonic sodium chloride solution, and Hanks' solution. In addition, sterile, fixed oils are conventionally employed as solvents or suspending mediums. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides, corn,
cottonseed, peanut, and sesame oil. Fatty acids such as ethyl oleate, isopropyl myristate,
and oleic acid and its glyceride derivatives, including olive oil and castor oil, especially
in their polyoxyethylated versions, are useful in the preparation of injectables. These oil
solutions or suspensions may also contain long-chain alcohol diluents or dispersants.

The carrier may also contain additives, such as substances that enhance solubility,
isotonicity, and chemical stability, for example anti-oxidants, buffers and preservatives.

In addition, the composition containing the agents may be in a form to be reconstituted
prior to administration. Examples include lyophilisation, spray drying and the like to
produce a suitable solid form for reconstitution with a pharmaceutically acceptable
solvent prior to administration.

Compositions may include one or more buffer, bulking agent, isotonic agent and
cryoprotectant and lyoprotectant. Examples of excipients include, phosphate salts, citric
acid, non-reducing sugars such as sucrose or trehalose, polyhydroxy alcohols, amino
acids, methyamines, and lyotropic salts are preferred to the reducing sugars such as
maltose or lactose.

When administered orally, compositions will usually be formulated into unit dosage
forms such as tablets, caplets, cachets, powder, granules, beads, chewable lozenges,
capsules, liquids, aqueous suspensions or solutions, or similar dosage forms, using
conventional equipment and techniques known in the art. Such formulations typically
include a solid, semisolid, or liquid carrier. Exemplary carriers include excipients such
as lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium
phosphate, mineral oil, cocoa butter, oil of theobroma, alginates, tragacanth, gelatin,
syrup, substituted cellulose ethers, polyoxyethylene sorbitan monolaurate, methyl
hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, and the like.

A tablet may be made by compressing or moulding the agent optionally with one or
more accessory ingredients. Compressed tablets may be prepared by compressing, in a
suitable machine, the active ingredient in a free-flowing form such as a powder or
granules, optionally mixed with a binder, lubricant, inert diluent, surface active, or
dispersing agent. Moulded tablets may be made by moulding in a suitable machine, a
mixture of the powdered active ingredient and a suitable carrier moistened with an inert liquid diluent.

The administration may also utilize controlled release technology.

For topical administration, the composition of the present invention may be in the form of a solution, spray, lotion, cream (for example a non-ionic cream), gel, paste or ointment. Alternatively, the composition may be delivered via a liposome, nanosome, ribosome, or nutri-diffuser vehicle.


Therapeutic delivery of biomolecules is generally as described in Bladon, C. (2002) "Pharmaceutical Chemistry: Therapeutic Aspects of Biomolecules" John Wiley & Sons Ltd.

The present invention may also be used to identify agents that modulate androgen receptor activity.

Accordingly, in another embodiment the present invention provides a method of identifying an agent that modulates androgen receptor activity, the method including identifying an agent that modulates one or more of the following:

(i) the level and/or activity of $\alpha$SGT;
(ii) the ratio of the level and/or activity of $\alpha$SGT to the level and/or activity of androgen receptor; and
(iii) the interaction of $\alpha$SGT with the androgen receptor.

In one embodiment, the method of identifying the agent occurs in a cell.

Methods for screening such agents may be selected, as provided herein.
In another embodiment, the present invention provides a method of identifying an agent that modulates androgen receptor activity, the method including:

(i) providing an agent including an amino acid sequence EEG/VD/E (SEQ ID NO. 1), or a functional variant or mimic thereof; and

(ii) determining the ability of the agent to modulate androgen receptor activity.

In one embodiment, the method of identifying the agent occurs in a cell.

It will also be appreciated that such methods of identifying agents that modulate receptor activity may also include a pre-screen to identify likely agents. For example, the ability of αSGT and chaperones to activate folding of a reporter molecule (e.g. luciferase) may be utilised.

The present invention also provides agents identified according to the above methods. Such agents are candidates for preventing and/or treating diseases, conditions and states as previously discussed herein.

The ability of agents to modulate androgen receptor activity in a cell by modulating one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor, and (iii) the interaction of αSGT with the androgen receptor, may be assessed by a suitable assay, for example by comparing the response of a biological assay to the presence or absence of the agent.

Biological assays include in vitro and in vivo assays. The biological assays may be used to assess any of the aforementioned parameters of androgen receptor activity in the presence of the agent. Such parameters include cellular localization of the androgen receptor, interaction of the androgen receptor with αSGT and expression of androgen sensitive genes. Further assays may assess the specificity of the androgen receptor to non-classical androgen receptor ligands in the presence or absence of the agent.

Agents may be selected that increase or decrease androgen receptor activity and/or increase or decrease αSGT activity.
Alternatively, an in vitro assay may be used to agents that modulate androgen receptor activity.

**Description of Specific Embodiments**

Reference will now be made to experiments that embody the above general principles of the present invention. However, it is to be understood that the following description is not to limit the generality of the above description.

**Example 1**

**Materials and Methods**

i) Cells and tissues

Cell lines were maintained in RPMI-1640 medium with 5% FBS and treated with steroids in phenol red free RPMI-1640 with 5-10% dextran coated charcoal treated FBS. Multitissue blocks of formalin-fixed, paraffin-embedded tissue, consisting of three representative 0.6 mm cores from diagnostic areas of each of 36 primary prostate cancer samples, 79 metastatic prostate cancer lesions and non-neoplastic prostate tissue samples, are described in Holzbeierlein *et al.* (2004) *Am J Pathol* 164: 217-227.

ii) Microarray analysis

Affymetrix U95 microarray data collected as part of our previous studies from 23 prostate cancer samples (manually microdissected for prostate cancer epithelial cells) was analyzed for gene expression as described in Holzbeierlein *et al.* (2004) *Am J Pathol* 164: 217-227.

iii) Immunohistochemistry

Immunohistochemistry/video image analysis was performed as described in Buchanan *et al.* (2005) *Cancer Res.* 65: 8487-8496 on 20 contiguous fields per sample (40x magnification) for serial 5μm formalin fixed paraffin tissue sections stained with rabbit AR (AR-U402, AR-U407; 1:300) or αSGT (αSGT-C18; Zymed Laboratories, Inc., CA; 1:5000). Samples were discounted if they did not contain sufficient informative stained area. Statistical significance was assessed using the Mann-Whitney U test.
iv) Yeast assays

A yeast-two hybrid screen was performed with pAS2-AR(618-754) and a pACT2 pooled-prostate cDNA library (BD Biosciences Clontech, Palo Alto, CA). Specific interactions were demonstrated by retransforming yeast with either empty pAS2, pAS2-AR(618-754) or pAS2-AR(618-917) and positive pACT2 clones by luminescent liquid β-galactosidase assays on triplicate colonies.

v) Transactivation assays

Steroid-receptor negative PC-3 or COS-I cells (10,000-20,000/well of a 96-well plate) were transfected with 0.1-lOng of full-length AR (pCMV-AR; pcDNA3.1AR), AR deleted for amino acids 636-646 (pCMV-ARΔ638-646) or ER (pHEGO) vectors, and 100ng of androgen (probasin ARR3-tk-Luc) or estrogen (ERE-tk-luc) reporter constructs, treated for 24 hours with vehicle control (ethanol) or steroids, and assayed for luciferase activity as previously described in Buchanan et al. (2004) Hum. Mol. Genet. 13; 1677-1692. 5-50ng of pSG5:HA-αSGT, pSG5:HA-Hic5 or pSG5:HA-GRIPl expression vectors were included as appropriate. All transfection mixes were balanced with respect to the molar ratio of expression vectors (with appropriate empty vector) and total plasmid [with pCAT-basic or pBS-sk(-)]. Mammalian two-hybrid assays were performed similarly in COS-I cells with equal molar amounts of vectors expressing GAL4-DBD and VP16-AD fusions of αSGT (maximum 5ng/well), and 25ng of the GAL4 responsive luciferase reporter, pGKl.

vi) Immunoblot and co-immunoprecipitation

Immunoblot analysis was performed using rabbit AR U407 (1:1000), N20 (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), or C-19 (1:1000 Santa Cruz Biotechnology), rabbit αSGT (1:3000, Zymed Laboratories, Inc), and/or goat β-actin (119, Santa Cruz Biotechnology) antisera (14). For immunoprecipitation, lysates of COS-I cells (untransfected or transfected with control, AR and/or αSGT expression vectors) were incubated with 5µg of the appropriate antisera. Antibody bound proteins were collected using Dynal beads (Invitrogen, Mount Waverly, VIC).

vii) GST pull-down
αSGT and AR (amino acids 534-917) were expressed from GST pGEX-4T (Amersham Pharmacia) in the BL21 strain. Purified proteins (5µg) were immobilized with 50% glutathione 4B beads and incubated with 200 µg pre-cleared lysates from COS-7 cells transfected with AR or αSGT expression plasmids with or without 10nM DHT.

viii) Confocal microscopy
Confocal microscopy was performed on PC-3 cells (50,000 cells/well) transfected for 40 hours with AR (50ng/well) and αSGT (500ng/well) expression vectors, or equivalent molar amount of control, and treated with or without 0.1-10nM DHT as described in Jia et al. (2004) Cancer Res. 64, 2619-2626. AR cellular localization was manually scored for each treatment in 25-50 stained cells in three independent experiments.

ix) αSGT knockdown by siRNA
C4-2B cells (100,000 cells/well) were transfected using Oligofectamine™ (Invitrogen) for 72h with 2.86 µg of one of two chemically synthesized 21bp-siRNA duplexes (sense: AGCUCCGGUCACUUGAGUGUTT (SEQ ID NO.24), antisense: ACACUCAAGUGACCGAGCUTT (SEQ ID NO.25), or sense: ACUUUGAAGCUGCCGUGCATT (SEQ ID NO.26), antisense: UGCACGGCAGCUUCAAAGUTT, SEQ ID NO.27) or a non-specific negative control (sense: AGAUCUGGCUAUCGCGGUATT (SEQ ID NO.28), antisense: (UACCGCGAUAGCCAGAUCUTT; SEQ ID NO.29), and treated with or without ligand for 24 hours. RNA was reverse transcribed and assessed for PSA and glyceraldehyde phosphate dehydrogenase (GAPDH) expression by quantitative real-time PCR in triplicate reactions as described in Jia et al. (2004) Cancer Res. 64, 2619-2626.

x) Molecular modeling
Models of αSGT were created using SwissModel (http://swissmodel.expasy.org/SWISSMODEL.html) based on crystrallographic structures of PP5 and Cyp40, and confirmed by WhatIf (http://www.cmbi.kun.nl/whatif/). The AR hinge peptide (amino acids 638-675) was constructed in silico using Chemsite Pro (Pyramid), folded by homology, and energy minimized using AMBER (http://amber.scripps.edu/). Docking simulations were
performed with BiGGER (http://www.cqfb.fct.unl.pt/bioin/chemera/Chemer a/B gg_Algorithm.html), with clustering/scoring according to geometric fit and electrostatic complementarity. Models were rendered with PovRay (http://www.povray.org/).

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Example 2

Interaction of the AR hinge with a novel molecular chaperone and with Hsp90β

The hinge region of steroid receptors is a short, poorly conserved structure between the canonical domains for ligand and DNA binding.

In order to identify the determinants of AR hinge function and how it relates to prostate cancer, we undertook a yeast two-hybrid screen using a human prostate cDNA library and a fragment of the human AR (amino acids 618-754) encompassing the hinge (amino acids 625-669). The screen yielded 23 independent clones, with the most highly represented encoding full-length αSGT (four clones) and the C-terminal region of sp90β (nine clones), the latter being the classical AR chaperone suspected of interacting with at least amino acids 704-758 of the receptors LBD. A specific interaction of AR with αSGT and Hsp90β was confirmed in yeast cells using luminescent β-galactosidase assays (Figure 1A). Yeast growth on highly selective media determined that the complete AR hinge and LBD (amino acids 618-919) retained interaction with αSGT, whereas interaction with Hsp90β was markedly diminished (data not shown). In yeast, neither interaction was affected by the addition of 5α-dihydrotestosterone (DHT). To analyze the AR/αSGT interaction further, we raised a rabbit polyclonal antibody to the carboxyl-terminal 18 amino acids of αSGT. This antisera demonstrates immunoreactive bands in mammalian cells corresponding to native αSGT and transfected HA-tagged αSGT proteins at molecular weights of approximately 43 and 45kDa respectively, which could be abrogated by a 5-fold excess by weight of specific peptide (Figure 7A).

The AR/αSGT interaction was confirmed in mammalian cells by co-immunoprecipitation of full-length proteins, and by GST pull-down (Figure 1B, C). In the native cellular context, the AR/αSGT interaction is dissociated by DHT (Figure 1B).
There are only two recognizable domains in the $\alpha$SGT protein, a centrally located tetratricopeptide repeat (TPR) protein-protein interaction domain and a small glutamine-rich carboxyl terminus of unknown function (Figure 7B). Consistent with a potential role in AR function, the TPR repeat of $\alpha$SGT exhibits the highest similarity to the analogous regions of the small group of TPR containing proteins previously implicated in chaperoning and/or maturation of steroid hormone receptors (Figure 7B). Importantly, homology modeling of the $\alpha$SGT TPR suggests remarkable structural conservation with the solved crystal TPR domain structures of protein phosphatase 5 (PP5) and cyclophilin-40 (Cyp40) (Figure 7C). The importance of this class of TPR proteins to AR signaling is highlighted by the phenotype of FKBP52 knockout mice, which exhibit defects in male reproductive tissues. Expression of $\alpha$SGT in a cohort of microdissected human prostate cancer epithelial cells is second only to that of FKPB52 amongst this steroidreceptor targeting TPR proteins (Figure ID). Moreover, the level of $\alpha$SGT is the most significantly altered relative to AR in the progression to metastatic disease (Figure ID). Along with the known interaction of $\alpha$SGT with Hsp90 and Hsp70, these findings implicate $\alpha$SGT as a potentially important chaperone of the AR.

Example 3

The relative cellular levels of AR and $\alpha$SGT determine AR transcriptional capacity

Overexpression of $\alpha$SGT relative to AR in PC-3 prostate cancer cells resulted in a 2-fold decrease in DHT-mediated AR transactivation activity (Figure 2A), and reduced basal receptor activity (i.e. activity in the absence of exogenous ligand) by 92.9 (±0.4) percent (Figure 2A, inset). As a consequence of reduced basal activity, the fold induction in receptor activity by 1nM DHT was dramatically increased from 47.6 (+3.6) fold for AR alone to 365.5 (+38.5) fold in the presence of $\alpha$SGT. The basal activity is AR dependent as (i) there is negligible promoter activity in the absence of transfected AR, and (ii) deletion of activation function 1 (AFl), which is essential for AR transcriptional capacity, decreased basal activity by 74.8 (±5.4) percent (Figure 8A). In human C4-2B prostate cancer cells that express both $\alpha$SGT and AR, reducing $\alpha$SGT levels with a specific siRNA resulted in a marked increase in basal and DHT-induced expression from (i) the endogenous androgen-responsive prostate specific antigen (PSA) gene as determined by quantitative real-time PCR (Figure 2C), and (ii) from a
transfected reporter gene (data not shown). Similar results were obtained with LNCaP prostate cancer cells, in both C4-2B and LNCaP cell lines treated with an independent siRNA targeting a different sequence in the αSGT mRNA, and for ectopic AR in transfected PC-3 cells (data not shown). Consistent with the cellular level of αSGT being a determinant of AR function, increasing the level of AR over the endogenous level of αSGT in PC-3 cells resulted in the same outcome as for the siRNA experiments, namely an increase in basal and ligand-induced AR activity and a decrease in fold induction by ligand (Figure 2C). At a distinct threshold level of transfected AR (in this case 5ng) there was a marked (6-fold) increase in basal receptor activity. With increasing AR protein levels there was a 10-fold decrease in the concentration of DHT required to give 50% maximal activity (EC50) (Figure 2C & Figures 8B, 8C). The effects of αSGT on AR are distinct from that of classical receptor coregulators such as the pl60 coactivator glucocorticoid receptor interacting protein 1 (GRIP1), and transforming growth factor β-1 induced 1 (TFGBl/Hic5/ARA55), which increase basal and maximal induction, but have a less dramatic effect on fold and do not alter the sensitivity to ligand (i.e. EC50) (Figure 8D).

In addition, αSGT overexpression did not affect basal ERα activity or the responsiveness of ERα to estradiol (Figure 2D), suggesting that it may exhibit specificity for different steroid receptors as with the other TPR proteins, and arguing against a ubiquitous effect of αSGT on cellular signaling or the Hsp70/Hsp90 chaperone machinery.

**Example 4**

*αSGT acts in the cytoplasm to affect AR subcellular distribution*

Recent evidence suggests that TPR chaperones such as FKBP52 may be involved in shuttling of steroid receptors between the cytoplasm and nucleus. We therefore investigated the effects of αSGT overexpression on AR cellular localization and receptor re-distribution by ligand (Figure 3). In untreated cells, the AR was cytoplasmic with diffuse weak nuclear staining. However, coexpression of αSGT almost completely eliminated nuclear AR in the absence of ligand, and maintained a predominantly cytoplasmic distribution of the receptor even following treatment with 0.1nM DHT. In
Reed, treatment with a saturating concentration of DHT (InM) resulted in nuclear localization of AR in either the absence or presence of exogenous oSGT. Transfected oSGT was exclusively cytoplasmic in all cases. Indeed, chromatin immunoprecipitation failed to detect oSGT at the endogenous AR responsive PSA promoter in C4-2B cells (Figure 9), and had no effect on activity of a constitutively-nuclear and -active AR variant truncated of the LBD at residue 709 (data not shown). These results are consistent with oSGT affecting AR function in the cytoplasm rather than altering the capacity of the receptor per se to generate a competent transcriptional complex.

Example 5

Changes in aSGT and AR levels during the progression of human prostate cancer

Immunohistochemistry demonstrated almost exclusive cytoplasmic staining in a range of normal mouse tissues (Figure 10). In the prostate, oSGT was confined to the cytoplasm of luminal epithelial cells with no evidence of expression in stromal cells (Figure 4A). In contrast, AR is predominantly nuclear in mouse and human prostatic epithelial cells, and is also present in smooth muscle and fibroblast stromal cells (27). To investigate the role of oSGT in AR function in vivo, we undertook quantitative immunohistochemistry of these two proteins in a cohort of human prostate epithelium (Figure 4A) as previously described in Buchanan et al. (2005) Cancer Res. 65: 8487-8496. A correlation between AR and oSGT immunoreactivity was observed in primary tumors (R=0.469, p=0.009; Figure 4B) but not in metastatic disease (R=0.081, p=0.562; Figure 4C), reflecting changes in the expression of one or both proteins with disease progression. Compared with non-malignant prostate samples and primary tumors, the mean and median levels of oSGT immunoreactivity were significantly lower in metastatic disease. In contrast, nuclear AR immuno staining was significantly higher (Figure 4B-D). When immuno staining was considered pairwise, there was a significant increase in the ratio of AR:oSGT immunoreactivity in metastatic [2.1 (±0.4)] compared with primary tumor samples [0.79 (±0.09); p=0.003] and nonmalignant prostate cells [0.99 (±0.12); p=0.040] (Figure 4D). The AR:oSGT ratio was significantly greater (p=0.023) in metastases from patients who received hormone ablation or hormone ablation plus chemotherapy [3.62 (±0.78)] than in metastases from untreated patients.
[1.68 (±0.18)], supporting the notion of further adaptation in a low hormone environment.

Example 6

*aSGT level affects AR sensitivity to non-classical ligands*

The weak-agonist activities of non-classical steroids such as progesterone and estradiol, and of receptor antagonists such as hydroxyflutamide (OHF), have been implicated in maintenance of AR function following androgen ablation. Overexpression of αSGT resulted in a substantial decrease in the capacity of the adrenal androgen androstenedione (ASD) and the synthetic progestin medroxyprogesterone acetate (MPA) to activate the receptor compared with DHT (Figure 5A, B; compare to Figure 2A), and eliminated the capacity of progesterone (PROG) and OHF to induce weak AR activity at comparable concentrations of ligand (Figure 5C, D). These results imply that αSGT acts to maintain fidelity/specificity of AR activation by classical agonist ligands. A mutation in the ligand binding domain of the AR in LNCaP and C4-2B cells allows estradiol, progesterone, MPA and OHF to exhibit full agonist activity on the receptor comparable to DHT (unpublished observations). As expected, reduction in αSGT levels by RNA interference in C4-2B and LNCaP cells resulted in an increase in PSA gene expression with these ligands comparable to that observed with DHT (Figure 8E).

Example 7

*The aSGT TPR interacts with the AR hinge whereas the C-terminal region of the chaperone mediates dimerization*

The TPR domains of steroid receptor associated cochaperones form a conserved amphipathic channel that mediates binding to the C-terminal MEEVD peptide of Hsp90 and/or IEEVD of Hsp70. Using solid phase partial proteolysis and MALDI-TOF peptide mass fingerprint analysis, the AR- αSGT interaction was mapped to the amino-terminal 141 amino acids of αSGT and residues in the AR hinge encompassing amino acids 630-645 (data not shown). In silico docking analysis predicted that binding is mediated primarily through interaction of αSGT residues 105-127, which are contained
within the first two of the TPRs, and AR hinge amino acids 638KLQEEGEA645 (Figure 6A). Significantly, this AR peptide sequence strongly resembles the EEVD-containing peptides of Hsp90 and Hsp70. Deletion of the 638KLQEEGEA645 peptide from the full-length AR (i.e., ARΔ638-646) resulted in increased basal activity and reduced fold activation by DHT compared to wtAR (Figure 8F), but did not alter overall transcriptional activity. Interaction of αSGT with its client proteins appears to be mediated by overlapping binding sites centered on the TPR repeat (see below). The clear exception is the first 80 amino acids, which native page and mammalian two-hybrid analysis suggest form an αSGT dimerization interface (Figure 6B). In this manner, a dimer of αSGT could simultaneously interact with two identical or non-identical client proteins via its TPR domains.

Example 8

The AR cochaperone αSGT exhibits homology and structural characteristics with known steroid receptor interacting TPR proteins.

Figure 7 shows that the AR cochaperone αSGT exhibits homology and structural characteristics with known steroid receptor interacting TPR proteins. Panel A shows an immunoblot with αSGT antisera on lysates of cultured cells (panel 1), resolving endogenous and transfected αSGT in COS-I cells (panel 2), and blocking of specific signal seen in panel 2 by pre-incubation of the αSGT antisera with 5 fold excess specific peptide (panel 3). Panel B shows a schematic of αSGT delineating its central tetratricopeptide repeat (TPR) and glutamine rich domain (QRD). The percentage homology of αSGT’s three TPR motifs to those in known steroid-receptor interacting TRP-containing cochaperones; protein phosphatase 5 (PP5/PP5C), FK506 binding proteins of 51 and 52 kDa (FKBP51/FKBP5 and FKBP52/FKPB4) and cyclophilin-40 (Cyp40/PPID). These proteins exhibit variable specificity for individual steroid receptor-Hsp70/Hsp90 chaperone complexes, with the TPR domains themselves critical in orchestrating their effects. Panel C shows a molecular ribbon model of the αSGT TPR compared to the solved crystal structures of the TPR domains fromCyp40 and PP5.

Example 9
aSGT affects AR basal activity and sensitivity to ligand-dependent activation distinct from classical coregulators.

Figure 8 shows that aSGT affects AR basal activity and sensitivity to ligand-dependent activation distinct from classical coregulators. Panel A shows that AR expression and activation functions are essential for AR-mediated basal activity. PC-3 cells were transfected with 2.5ng wtAR, AR deleted of amino acids 38-360 encompassing activation function 1 (ARΔAF1), or empty vector control and with ARR3-tk-Luc (100ng), and treated with vehicle control (ethanol) or DHT (1nM) as indicated. Data is presented as relative light units (RLU) and represents the mean (±sem) activity from 6-8 independently transfected wells. Panel B shows the effect of increasing amounts of transfected AR on transcriptional activity in PC-3 cells performed as for panel A. Data was normalized for each amount of transfected AR to percent maximal activity, and clearly shows a right-shift in the dose-response to DHT for increasing amount of transfected AR. Panel C shows the linear relationship between the amount of transfected AR and the EC50 (ligand concentration required for 50% maximal activity) calculated from data in part B. Panel D shows the effect of overexpressing known AR coregulators, GRIP1/SRC2 or TGFβIII/Hic5/ARA55 on AR transcriptional activity. Transfection was performed as for panel A with 2.5ng of AR and 50ng of either the cofactor expression vector or an equivalent molar amount of empty vector control. Panel E shows endogenous AR functional assays in C4-2B cells transfected with a specific aSGT siRNA or a non-specific (n.s.) negative control. Results represent the mean (±sem) expression of the androgen responsive PSA gene as a ratio of mean GAPDH determined by triplicate quantitative real-time PCR analyses 1 day after siRNA transfection and treatment with vehicle control (ethanol) or 10nM of steroids as indicated [dihydrotestosterone (DHT), progesterone (PROG), medroxyprogesterone acetate (MPA), androstenedione (ASD), estradiol (E2)]. Boxed inset shows basal PSA expression in the presence of vehicle control (ie. in the absence of any exogenous ligand). Panel F shows deletion of the predicted aSGT binding site from the AR hinge region increases basal receptor activity. Transactivation assays performed as in panel A with wtAR or ARΔ638-646) and ARR3-tk-Luc (100ng). Figures on the graphs show mean (±sem) fold activation (activity divided by basal activity) for the highest concentration of ligand. Immunoblot analyses were performed on pooled lysates from the same transfection.
Example 10

*aSGT is not recruited to the PSA gene promoter or enhancer*

Figure 9 shows that *aSGT* is not recruited to the PSA gene promoter or enhancer. Panel A is a schematic of the human PSA gene enhancer and promoter delineating the position of AR responsive elements (AREs). Panels B and C show chromatin immunoprecipitation assays performed with AR, GRIPI and *aSGT* antiserum in LNCaP cells following 60-minute treatment with or without 10nM DHT. Occupancy of enhancer and promoter elements of the PSA gene (detailed in Panel A) were interrogated in immunoprecipitated lysates by quantitative real-time PCR, with each datapoint representing the mean (±sem) of triplicate PCR reactions.

Example 11

*aSGT immunoreactivity in adult mouse tissues.*

Figure 10 shows *aSGT* immunoreactivity in adult mouse tissues. Immunohistochemistry with *aSGT* antisera in adult mouse tissues were arranged on a single tissue microarray block. Specific immunoreactivity in each tissue could be competed by a 5x molar excess of specific blocking peptide. Comparatively little *aSGT* immunoreactivity was evident in the heart.

Discussion

Little is known about the cellular role and function of *aSGT*. This is surprising given that *aSGT* has been highly conserved in evolution from yeast to humans, implicated in cell division and apoptosis and has the potential to affect the movement, localization and functional response of a broad range of important signaling molecules of mammalian and viral origin (Figure 6C).

Although the four known steroid-receptor associated TPR proteins, FKBP51, FKBP52, PP5 and Cyp40 exhibit a similar affinity for Hsp90 in vitro, their presence and function
in a mature Hsp90/steroid receptor heterocomplex varies according to their relative abundance, the particular receptor, cellular location, and whether the receptor resides in a ligand-bound state. For example, the relative cellular levels of FKBP51 and FKBP52 affect the affinity of GR for ligand both in vitro and in vivo (38), yet FKBP52 overexpression does not appreciably effect binding affinity of the AR, instead causing a leftshift in the dose response curve and an increase in receptor transactivation activity. Conversely, we observed that overexpression of αSGT results in a right-shift in the dose response and a decrease in receptor transactivation activity. Whereas the AR/FKBP52 interaction is enhanced by the addition of ligand, we have shown that DHT decreases interaction of AR with αSGT, suggesting that these two cochaperones may act at different points in AR signaling. Importantly, αSGT did not affect the ligand responsiveness of ERα arguing against a ubiquitous effect of αSGT on the Hsp70/Hsp90 chaperone system.

Whereas αSGT is unique amongst the related TPR proteins in that it lacks a peptidyl-prolyl isomerase (PPIase) domain implicated in protein folding, its cochaperone functions could nonetheless affect the capacity of the AR to bind and respond to ligand. The refolding capacity and weak ATPase activity of Hsp70, which provides the free energy for chaperone function and the cyclic association and disassociation of client proteins, are enhanced by interaction with αSGT leading to a higher affinity of Hsp70 for substrate proteins and more efficient protein folding. Consequently, yeast that lack αSGT exhibit a 50-fold reduction in recovery from heat shock compared to the native yeast strain. By enhancing the ATPase activity of Hsp70, and/or Hsp90, which favors their ADP-dependent association with client molecules, αSGT could affect ligand-binding capacity of the AR via the maturation pathway, stabilize the receptors interaction with Hsp90, or limit the pool of misfolded receptors with inappropriate activities. By ensuring the folding quality and control of cytoplasmic AR, this may explain how αSGT overexpression limits receptor activity and nuclear transport in the absence of ligand, and prevents inappropriate responses to weak androgens and non-classical ligands. Conversely, our data suggests that when αSGT is limiting, as is the case when AR levels are increased, misfolded receptors can exhibit aberrant responses.

Preferential interaction of the GR/Hsp90 heterocomplex with FKBP52 in the cytoplasm following dexamethasone binding is one of the earliest events in receptor activation. By
stabilizing the GR/Hsp90 interaction and tethering the receptor via its PPIAse domain to cytoplasmic dynein, the motor protein that mediates retrograde movement of proteins along microtubules, FKBP52 promotes nuclear transport and enhances receptor transactivation. Considering the androgen-insensitive phenotype of FKBP52 knockout mice, a similar mechanism likely exists for the AR, and importantly the AR nuclear targeting sequence lies directly adjacent to or overlaps the αSGT binding site identified in the current study. It is possible that by acting to retain apo-AR in a ‘persistent’ complex with Hsp90, the PPIase-less αSGT will uncouple the receptor from the dynein transport machinery mediated by the related TPR proteins, thereby preventing inappropriate cytoplasmic aggregation and movement of the receptor to the nucleus in the absence of a specific hormonal signal. That deletion of the αSGT binding site recapitulates only one effect of αSGT knockdown (i.e. increased basal but not an overall increase in receptor function), supports the notion of common or overlapping binding sites in the AR hinge for different TPR proteins, each contributing to distinct aspects of receptor signaling. We have shown that ligand causes the disassociation of αSGT from the AR, and conversely that αSGT overexpression decreases the capacity of ligand to mediate receptor transport to the nucleus. In support of this hypothesis, mutations in the AR nuclear targeting sequence have been shown to result in delayed ligand-dependent nuclear transport, cytoplasmic aggregation of the receptor in a complex with Hsp70 and other chaperones, and nuclear-clearing of the apo-AR similar to that seen in the current study with overexpression of αSGT.

Cytoplasmic αSGT is associated with microtubules and actin filaments, but does not form an integral part of the cytoskeleton. Microtubule-targeting by αSGT could contribute to cytoplasmic retention of apo-AR, and may facilitate efficient exchange of αSGT for dynein-linked TPR proteins following ligand binding and the subsequent receptor redistribution to the nucleus. Importantly, the redistribution of αSGT observed with the collapse of the microtubule network during cell division and by chemical means, suggests that microtubule-targeting agents will disrupt αSGT regulation of its clients. This may explain the efficacy of the chemotherapeutic agent, docetaxel, in prostate cancer patients following failure of conventional androgen ablation.

Collectively, αSGT may act to ensure the quality of Hsp70/Hsp90 dependent receptor maturation, prevent inappropriate cytoplasmic aggregation, stabilize the apo-AR in a
'persistent' chaperone heterocomplex in the cytoplasm in order to prevent inappropriate movement of the receptor to the nucleus in the absence of a specific hormonal signal, and/or mediate the efficient exchange of TPR proteins following agonist binding (Figure 6D). Evolutionarily, the maintenance of a cytoplasmic AR may stem from the receptors capacity to weakly activate androgen regulated genes in the absence of ligand, or in response to extraneous steroids or low concentrations of cognate ligands, which could be undesirable depending on the cellular and/or developmental context.

In summary, the current study indicates that the equilibrium between cytoplasmic and nuclear localization of the AR in prostate cancer cells, and the sensitivity of the receptor to activation by ligand, will depend in part on the relative cellular levels of both AR and αSGT. αSGT can therefore be considered a molecular rheostat of androgen signaling in prostate epithelial cells, and thus a contributor to the homeostatic control of androgen action. The increased AR level often observed in metastatic prostate cancer, and recently shown to be predictive of progression in localized disease, may overwhelm the capacity of limiting cellular αSGT to (i) ensure AR conformational quality and appropriate cellular localization, (ii) buffer basal and ligand-independent receptor transactivation and/or (iii) limit AR’s response to androgenic and non-steroidogenic ligands. Equally, decreased levels of αSGT could mimic these effects without any identifiable change in AR expression in an individual tumor. In either case, our findings identify a novel mechanism to explain how how the AR can continue to signal in prostate cancer cells in a low hormonal environment.

Finally, it will be appreciated that various modifications and variations of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are apparent to those skilled in the art are intended to be within the scope of the present invention.
Claims

1. A method of modulating androgen receptor activity in a cell, the method including one or more of the following:
   (i) modulating the level and/or activity of αSGT in the cell;
   (ii) modulating the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor in the cell; and
   (iii) modulating the interaction of αSGT with the androgen receptor in the cell.

2. A method according to claim 1, wherein increasing one or more of (i) the level of and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the receptor, decreases the androgen receptor activity in the cell.

3. A method according to claim 1, wherein decreasing one or more of (i) the level of and/or activity of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the receptor, increases the androgen receptor activity in the cell.

4. A method according to any one of claims 1 to 3, wherein the cell is part of a biological system.

5. A method according to claim 4, wherein the biological system is a human or animal subject.

6. A method according to claim 5, wherein the human or animal subject is susceptible to, or suffering from, a disease, condition or state associated with an altered level of androgen receptor activity.

7. A method according to claim 6, wherein the disease is cancer.

8. A method according to claim 7, wherein the cancer is prostate cancer.
9. A method according to claim 8, wherein the cancer is metastatic prostate
cancer.

10. A method according to any one of claims 7 to 9, wherein the cancer is no
longer susceptible to androgen ablation therapy.

11. A method according to claims 1 to 10, wherein the modulation of androgen
receptor activity includes exposing the cell to an agent that modulates one or more of (i)
the level and/or activity of $\alpha$SGT in the cell; (ii) the ratio of the level and/or activity of
$\alpha$SGT to the level and/or activity of androgen receptor in the cell; (iii) the interaction of
$\alpha$SGT with the androgen receptor in the cell.

12. A method according to claim 11, wherein the agent includes an amino acid
sequence EEG/VD/E (SEQ ID NO.1) or a functional variant thereof.

13. A method according to claim 12, wherein the agent includes an amino acid
sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3).

14. A method according to claim 11, wherein the agent increases one or more of (i)
the level of and/or activity of $\alpha$SGT in the cell; (ii) the ratio of the level and/or activity
of $\alpha$SGT to the level and/or activity of androgen receptor; and (iii) the interaction of
$\alpha$SGT with the receptor and thereby decreases the androgen receptor activity in the cell.

15. A method according to claim 11, wherein the agent decreases one or more of
(i) the level of and/or activity of $\alpha$SGT in the cell; (ii) the ratio of the level and/or activity
of $\alpha$SGT to the level and/or activity of androgen receptor; and (iii) the interaction of $\alpha$SGT with the receptor and thereby increases the androgen receptor activity in the cell.

16. A method of preventing and/or treating a disease, condition or state associated
with androgen receptor activity in a subject, the method including administering to the
subject an effective amount of an agent that modulates one or more of (i) the level
and/or activity of $\alpha$SGT; (ii) the ratio of the level and/or activity of $\alpha$SGT to the level
and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

17. A method according to claim 16, wherein the disease, condition or state with androgen receptor activity is a disease, condition or state is associated with an increased level of androgen activity.

18. A method according to claim 16 or 17, wherein the disease is a cancer.

19. A method according to any one of claims 16 to 18, wherein the disease is prostate cancer.

20. A method according to claim 19, wherein the prostate cancer is metastatic prostate cancer.

21. A method according to any one of claims 18 to 20, wherein the cancer is no longer susceptible to androgen ablation therapy.

22. A method according to any one of claims 17 to 21, wherein the agent increases one or more of (i) the level of and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor and thereby decreases the androgen receptor activity in the cell.

23. A method according to any one of claims 16 to 22, wherein the agent includes an amino acid sequence EEG/VD/E (SEQ ID NO.1) or a functional variant thereof.

24. A method according to any one of claims 16 to 23, wherein the agent includes an amino acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3).

25. A method according to any one of claims 16 to 24, wherein the method further includes administering to the subject an agent that is a microtubule-targeting agent.
26. A method according to claim 25, wherein the microtubule-targeting agent is docetaxel and/or paclitaxel.

27. Use of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor in the preparation of a medicament for preventing and/or treating a disease, condition or state associated with androgen activity.

28. Use according to claim 27, wherein the agent includes an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant thereof.

29. A method according to any one of claims 27 or 28, wherein the agent includes an amino acid acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3).

30. An isolated agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

31. An isolated agent including an amino acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3), wherein the agent modulates the interaction of αSGT with the androgen receptor.

32. An agent according to any one of claims 30 or 31, wherein the agent is a polypeptide.

33. A method of treating a disease, condition or state associated with androgen activity in a subject, the method including administering to the subject an agent according to any one of claims 30 to 32.

34. A pharmaceutical composition including an agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant thereof, wherein the agent modulates the interaction of interaction of αSGT with the androgen receptor.
35. A pharmaceutical composition including an agent including an amino acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3), or a functional variant thereof, wherein the agent modulates the interaction of αSGT with the androgen receptor.

36. Use of an agent including an amino acid sequence EEG/VD/E (SEQ ID NO.1), or a functional variant thereof, in the preparation of a medicament for preventing and/or treating a disease, condition or state associated with androgen activity.

37. Use of an agent including an amino acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3), or a functional variant thereof, in the preparation of a medicament for preventing and/or treating a disease, condition or state associated with androgen activity.

38. A method of treating a cancer in a subject that has an altered response to androgen therapy, the method including administering to the subject an effective amount of an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

39. A method according to claim 38, wherein the method further includes administering to the subject an agent that is a microtubule-targeting agent.

40. A method according to claim 39, wherein the agent is docetaxel and/or paclitaxel.

41. A pharmaceutical composition including an agent that that is a microtubule-targetting agent and an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of androgen receptor; and (iii) the interaction of αSGT with the androgen receptor.

42. A combination product including the following components:
   an agent that modulates one or more of (i) the level and/or activity of αSGT; (ii) the ratio of the level and/or activity of αSGT to the level and/or activity of
androgen receptor; and (iii) the interaction of αSGT with the androgen receptor;
and
an agent that is a microtubule-targetting agent;
wherein the components are provided in a form for co-administration to a subject or in a
form for separate administration to a subject.

43. A method of determining the susceptibility of a cancer cell associated with
androgen activity to the effect of androgen ablation therapy, the method including
determining one or more of following properties of the cell: (i) the level and/or activity
of αSGT in the cell; (ii) the ratio of the level and/or activity of αSGT to the level and/or
activity of androgen receptor in the cell; and (iii) the interaction of αSGT with the
androgen receptor in the cell, wherein a decrease in any one or more of the
aforementioned properties is indicative of a cancerous cell with reduced susceptibility to
androgen ablation therapy.

44. A method according to claim 43, wherein the cancer cell is a prostate cancer
cell.

45. A method of identifying an agent that modulates androgen receptor activity,
the method including identifying an agent that modulates one or more of the following:
(i) the level and/or activity of αSGT;
(ii) the ratio of the level and/or activity of αSGT to the level and/or activity of
androgen receptor; and
(iii) the interaction of αSGT with the androgen receptor.

46. A method of identifying an agent that modulates androgen receptor activity,
the method including:
(i) providing an agent including an amino acid sequence EEG/VD/E (SEQ ID
NO.1), or a functional variant thereof; and
(ii) determining the ability of the agent to modulate androgen receptor activity.

47. A method of identifying an agent that modulates androgen receptor activity,
the method including:
(i) providing an agent including an amino acid sequence EEGE (SEQ ID NO.2) and/or EEVD (SEQ ID NO.3), or a functional variant thereof; and
(ii) determining the ability of the agent to modulate androgen receptor activity.

48. A method of diagnosing metastatic prostate cancer, the method including determining the levels of αSGT and androgen receptor in a prostate cancer sample, wherein a low ratio of αSGT to androgen receptor is indicative of metastatic prostate cancer.
Fig. 1

A

β-gal activity (fold)

AR(618-754)
pACT
EMPTY
αSGT
C#1
αSGT
C#2
Hsp90β

B

IP Ab. 10% input

rlgG αSGT HA AR

AR

IgG

IB: AR antibody

C

GST
GST-AR
DHT
αSGT

10% input

IB: αSGT antibody

D

Mean expression relative to AR in primary PCa (arbitrary units)

p53
FRKB52
P53
Cyp40

Normalized expression relative to AR in metastatic PCa (arbitrary units)

p53
FRKB52
P53
Cyp40

p53
n.s.
Fig. 2
Fig. 3
**Fig. 4**

**A**  
Normal mouse prostate  
\[ \text{SGT (1:4000)} \]  
\[ \text{SGT (1:4000) + peptide} \]

**Human prostate samples**  
\[ \text{αSGT Primary Metastatic} \]  
\[ \text{NM Primary Metastatic} \]  
Androgen receptor (U407)

**B**  
**PRIMARY**  
\[ (17.36) \]  
\[ R=0.469 \]  
\[ p=0.009 \]

**C**  
**METASTATIC**  
\[ (11.48) \]  
\[ R=0.081 \]  
\[ p=0.562 \]

**D**  
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<td>non-malignant</td>
<td>(n=28) *</td>
<td>(n=30)</td>
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<tr>
<td>Mean (±SEM)</td>
<td>11.92 (±0.95)</td>
<td>14.45 (±1.16)</td>
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<td>Median (range)</td>
<td>12.43 (0.54-21.37)</td>
<td>13.58 (4.29-30.71)</td>
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<tr>
<td>primary</td>
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<td>(n=32)</td>
</tr>
<tr>
<td>Mean (±SEM)</td>
<td>12.15 (±1.34)</td>
<td>17.36 (±1.25)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>10.81 (1.84-36.10)</td>
<td>16.96 (4.36-40.38)</td>
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<td>metastatic</td>
<td>(n=64)</td>
<td>(n=64)</td>
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<td>Mean (±SEM)</td>
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<td>11.48 (±9.81)</td>
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<td>p (primary)</td>
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<td>&lt;0.0001</td>
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* MIOD: Mean integrated optical density (average intensity of immunoreactivity per unit area; arbitrary units).  
\* n: number of samples with sufficient informative immunoreactive area.  
\& Significant differences between groups assessed by the Mann Whitney U test. Significance was set at p<0.05. n.s.: not significant.
**Fig. 6**

A. [Image of a molecular structure or diagram related to protein or gene expression.]

B. Table or figure showing pM-αSGT constructs with corresponding luciferase activity data. (Luciferase activity (x10⁶ RLU) and COS-1 lysate data are presented in a bar graph format.)

C. Diagram illustrating the effects of αSGT on protein folding and functional integrity. Key points include:
- Enhances ATPase activity and folding capacity
- Synergy with Hsp70 to inhibit synaptic transmission
- Aldose transport to plasma membrane
- Stabilization of client proteins
- Enhancement of aggregation
- Post-translational modification of αSGT
- Redistribution of dystroglycan to the plasma membrane and viral particle release

D. Diagram showing the interaction of αSGT with receptors and associated processes such as assembly, maturation, and retrograde movement. Key points include:
- Microtubule
- Maturation
- Primed and stabilized AR complex
- Ligand-induced transformation
- Retrograde movement
- Dynemin
- HC
- FKBP52
- p20
- hsp90
- AR

Native PAGE data showing the separation of αSGT and αSGT variants.
Fig. 7

![Diagram showing protein expression patterns and domain structures]
Fig. 9

A

PSA Gene

ENHANCER

AREs

VI
V
IV
III
IIIA
IIIB

-4366

-4148

-3874

PROMOTER

ARE II
ARE I

-383

-370

+42

ATG...

B

C

Percentage input

0
0.2
0.4
0.6
0.8
1.0

DHT
Ab
IgG
AR
GRIP1

ENHANCER (ARE III)

PROMOTER (ARE I)

Percentage input

0
0.2
0.4
0.6
0.8
1.0

DHT
Ab
IgG
AR
GSGT

ENHANCER (ARE III)

PROMOTER (ARE I)
**Fig. 10**

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<td><strong>SGT (1:4000) + peptide</strong></td>
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INTERNATIONAL SEARCH REPORT

International application No. PCT/AU2008/001296

A CLASSIFICATION OF SUBJECT MATTER

Int C1

GOIN 33/68 (2006.01) A61K 38/17 (2006.01)

According to International Patent Classification (IPC) or both national classification and IPC

B FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPDOC, WPI, MEDLINE, CAPLUS, BIOSIS, GENOMEQUEST (SEQ ID NOs: 1, 2 and 3, alphaSGT, SMALL GLUTAMINE RICH TETRARICICOPERIDE)

C DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents

'A' document defining the general state of the art which is not considered to be of particular relevance

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'Date of the actual completion of the international search 20 October 2008

Date of mailing of the international search report 13 NOV 2008

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AUSTRALIAN PATENT OFFICE

PO BOX 200, WODEN ACT 2606, AUSTRALIA

E-mail address pct@ipaustral ta gov au

Facsimile No +61 2 6283 7999

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<td>PX</td>
<td>BUCHANAN, G. et al Cancer Research (15 October 2007), Vol 67 (20) pp 10087-96 &quot;Control of Androgen Receptor signalling in prostrate cancer by the co-chaperone small glutamine-rich tetratricopeptide repeat containing protein α.&quot; (Whole document)</td>
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX