SGTA: A New Player in the Molecular Co-Chaperone Game

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Abstract Small glutamine-rich tetratricopeptide repeat-containing protein α (SGTA) is a steroid receptor molecular co-chaperone that may substantially influence hormone action and, consequently, hormone-mediated carcinogenesis. To date, published studies describe SGTA as a protein that is potentially critical in a range of biological processes, including viral infection, cell division, mitosis, and cell cycle checkpoint activation. SGTA interacts with the molecular chaperones, heat shock protein 70 (HSP70) and HSP90, and with steroid receptor complexes, including those containing the androgen receptor. Steroid receptors are critical for maintaining cell growth and differentiation in hormonally regulated tissues, such as male and female reproductive tissues, and also play a role in disease states involving these tissues. There is growing evidence that, through its interactions with chaperones and steroid receptors, SGTA may be a key player in the pathogenesis of hormonally influenced disease states, including prostate cancer and polycystic ovary syndrome. Research into the function of SGTA has been conducted in several model organisms and cell types, with these studies showing that SGTA functionality is cell-specific and tissue-specific. However, very few studies have been replicated in multiple cell types or experimental systems. Although a broad range of functions have been attributed to SGTA, there is a serious lack of mechanistic information to describe how SGTA acts. In this review, published evidence linking SGTA with hormonally regulated disease states is summarized and discussed, highlighting the need for future research to more clearly define the biological function(s) of this potentially important co-chaperone.

Introduction

Small glutamine-rich tetratricopeptide repeat (TPR)-containing protein α (SGTA) was discovered 15 years ago as a novel TPR-containing protein [23] and has been linked to several disparate cellular functions. SGTA remains understudied compared to other TPR-containing proteins and has not yet been the subject of sufficient studies to provide a comprehensive understanding of the full gamut of its functions. Published studies collectively identify SGTA as a potentially important molecule in many pathways. Of particular interest to this readership is the potential ability of SGTA to act as a co-chaperone in the context of steroid hormone receptor signaling. Molecular chaperones are involved in many processes, including steroid receptor signaling, and they modulate diverse functions such as protein folding, receptor stability, subcellular localization, and intracellular trafficking. Co-chaperones are recruited by chaperones or client proteins and assist chaperones. Although there are many published reviews of co-chaperones in steroid receptor maturation [63], some of which focus on TPR-containing proteins as an important subclass of co-chaperones [48], this review is the first to discuss published evidence that SGTA acts as a co-chaperone. Given the potential importance of SGTA in steroid receptor signaling, this review identifies and highlights key functions attributed to SGTA, including the participation of SGTA in holding client proteins in their immature or inactive state or in an intracellular compartment where client proteins are inactive. Furthermore, this review emphasizes which of SGTA's functions have not yet been reproduced or validated or are not yet clearly

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understood at a mechanistic level. Finally and most importantly, this review highlights the importance of SGTA in sex hormone receptor signaling and hormone-mediated carcinogenesis. It is expected that this review will provide a framework to direct effective future study of SGTA, with a particular emphasis not only on hormonally regulated normal and disease physiology but also in other areas of SGTA function.

SGTA was discovered in rat embryonic fibroblast cells as a novel interaction partner of the nonstructural protein 1 (NS1) of parvovirus H-1 [23]. Initial analysis of *Sgta* revealed a 1.3-kb mRNA transcript, encoding a 314-amino-acid, 34-kDa protein [23]. SGTA homologues are found in organisms from eukaryotes to humans [45]. Human SGTA, like rat and mouse SGTA, is expressed in all tissues studied to date [45] (Table 1), although expression levels vary between tissue types and species. Human *SGTA* maps to chromosome 19p13 and encodes a 313-amino-acid protein that not only localizes predominantly in the cytoplasm but has also been detected in the nucleus [23, 33, 45] (Fig. 1a; mouse gene and protein structure shown in Supplementary Fig. 1). Structurally, SGTA exhibits a central tandem array of three TPR motifs, a glutamine-rich C-terminal domain and an N-terminal domain that contains a potential short coiled coil and is capable of self-association through amino acids 1–80 (Fig. 1a) [17, 23, 45, 49, 83]. SGTA interacts with a diverse range of proteins (Fig. 1a), including the human immunodeficiency virus type 1 (HIV-1) viral-encoded protein U (VPU) [14, 30, 37], myostatin [89], the androgen receptor (AR) [8], and the growth hormone receptor (GHR) [71], implicating SGTA in biological processes including viral assembly and release, cell division and apoptosis, intracellular compartmentalization, and molecular co-chaperoning. These diverse functions of SGTA are likely to be regulated in a cell-specific and tissue-specific manner and, in many cases, require further characterization.

Fig. 1 SGTA structural information. **a** The human SGTA gene is located on chromosome 19p13.3. The gene is organized into 12 exons, encoding a 313-amino-acid protein. Schematic of the SGTA protein delineating the interaction site (if known) of its established client proteins. Adapted from Buchanan et al. [8]. **b** TPR-containing chaperone proteins known to associate with steroid receptors. Sourced from Protein Knowledge Base (UniProtKB). **c** Schematic diagram of the alternative isoform of SGTA, SGTB, which has approximately 60 % amino acid sequence identity to SGTA. *Shaded boxes* represent TPR domains; *Q* glutamine-rich domain, *PPIase* peptidylprolyl isomerase, *U-box* E3 ubiquitin ligase, *J domain* DnaJ homologous J domain

Over 50 proteins are known to contain tandem repeats of 3–16 TPR motifs [4]. A TPR motif is characterized by a 34-amino-acid sequence containing 8 loosely conserved amino acid residues which form a helix–turn–helix motif that together create a series of antiparallel α-helical hairpins. The α-helices form an amphipathic groove, which serves as a protein–protein interaction surface [72]. SGTA has structural similarity to protein phosphatase 5 (PP5), cyclophilin 40 (CYP40), FK506-binding protein 51 (FKBP51), FKBP52, carboxy terminus of heat shock protein 70 (HSP70)-interacting protein (CHIP), and tetratricopeptide repeat protein 2 (TPR2) (Fig. 1b); however, SGTA lacks some functional domains (e.g., peptidylprolyl isomerase (PPIase)) found in other TPR-containing co-chaperones. Since proteins in the TPR family are co-chaperones involved in the maturation of steroid hormone receptors and given that the TPR domains of SGTA [30], PP5 [25], and CYP40 [80] are all highly conserved [8], SGTA has been implicated in steroid receptor maturation.

By Western blot analysis, three SGTA protein fractions with apparent molecular weights of 36, 38, and 39 kDa have been identified [92]. It is possible that the SGTA fractions identified by Western blot are different isoforms, which may have differing functions. While there are no specific reports of *SGTA* undergoing alternative mRNA splicing, there are 38

Table 1 Expression of SGTA in rat [23, 82], mouse [8], and human tissues [8, 45]
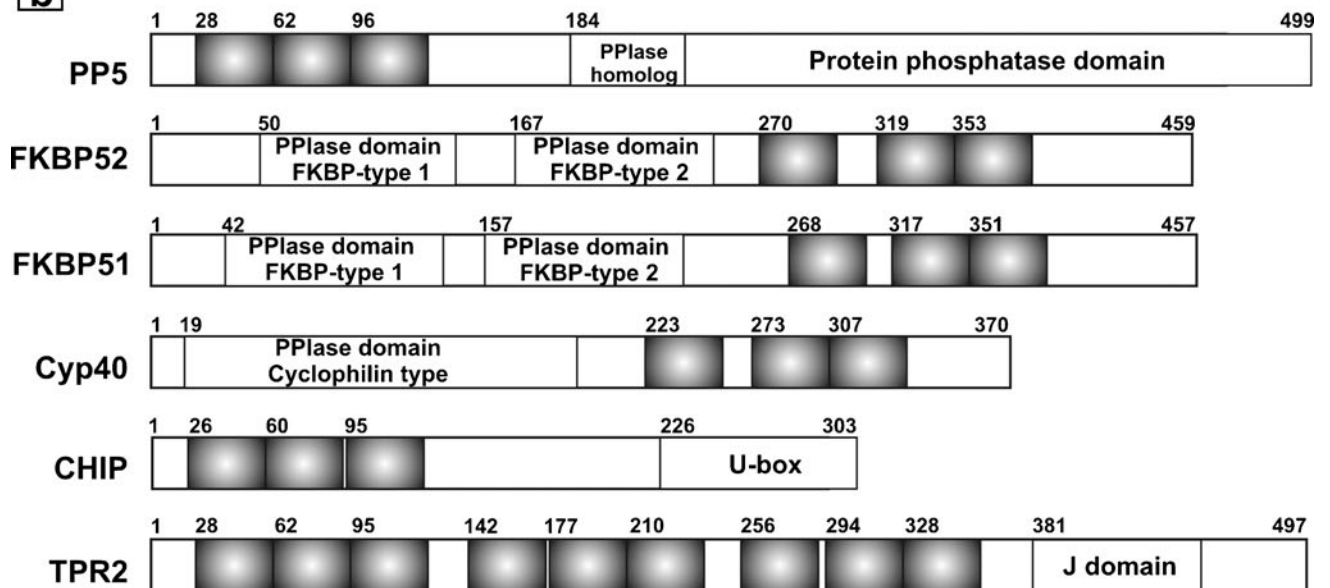
	Rat		Mouse	Human	
	Cziepluch et al. [23], NB	Tobaben et al. [82], WB	Buchanan et al. [8], IHC	Kordes et al. [45], NB	Buchanan et al. [8], mRNA, WB
Brain	++	++		+	
Heart	+	+	−	+++	
Kidney	+++	+	++	+	
Liver	++	+	++	+	
Lung	+	++	++	+	
Pancreas				++	
Placenta				++	
Prostate			++		++
S. muscle	+++	+		++	
Spleen	+	+	++		
Testis	+++	++	++		

IHC immunohistochemistry, *NB* northern blot, *WB* Western blot, *S. muscle* skeletal muscle, − negative expression, + low expression, ++ moderate expression, +++ high expression
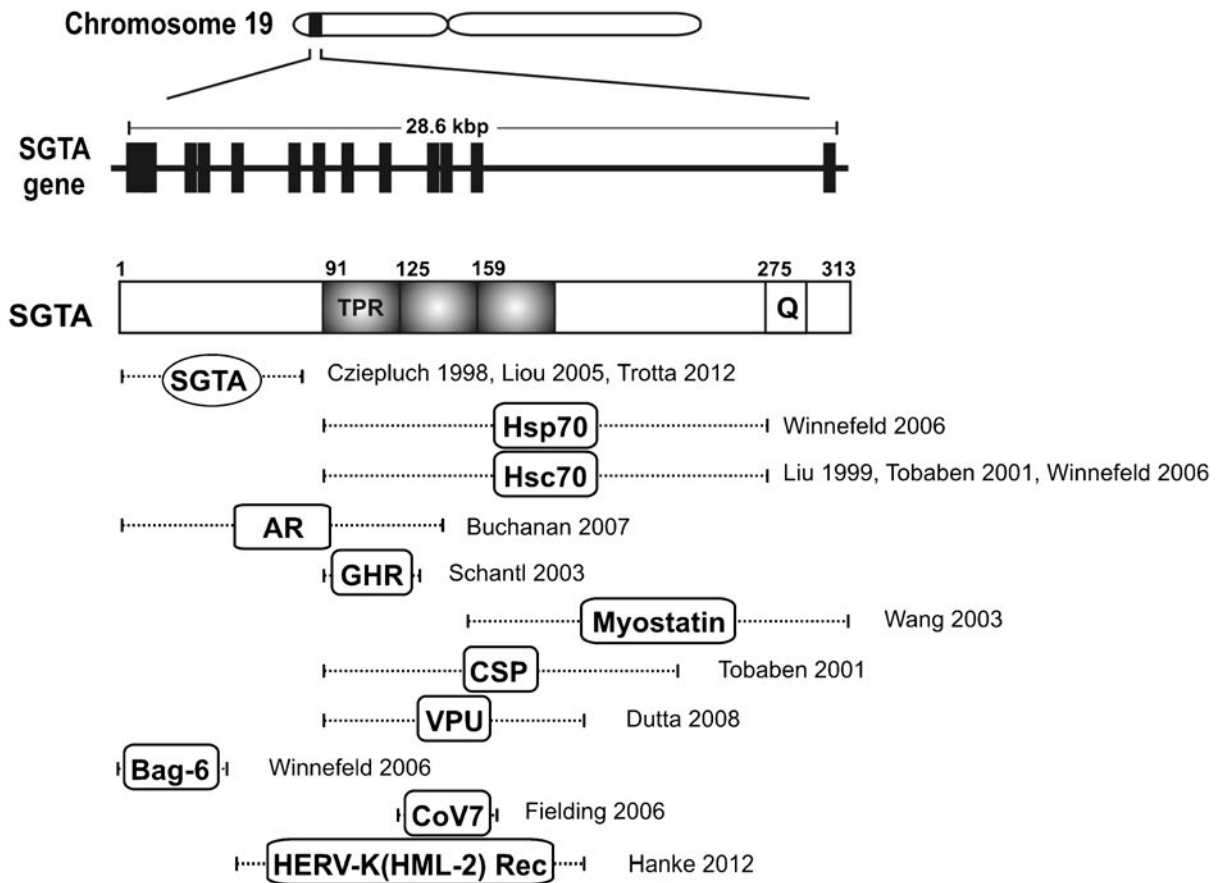
predicted alternatively spliced *SGTA* mRNA species, corresponding to 30 proteins [52]. Functional characterization of potential alternative SGTA isoforms is required as current reports focus only on the wild-type SGTA isoform. Furthermore, since amino acids 1–80 are required for SGTA dimerization, isoforms which do not contain this region may be unable to dimerize, which may substantially influence SGTA function. Likewise, it is also possible that the different-sized SGTA species observed by Western blot are due to post-translational modification. SGTA undergoes phosphorylation within the [121]NPANAVY sequence [94]; however, the functional consequences of this phosphorylation are unknown. Phosphorylation at Ser[305] of SGTA stabilizes its interaction with platelet-derived growth factor receptor α [60]. Conversely, phosphorylation of the TPR-containing immunophilin, FKBP52, inhibits its interaction with HSP90 [58], which regulates steroid receptor activity [22]. It is likely that SGTA phosphorylation may similarly modulate its protein–protein interactions, and if so, this may influence SGTA activity. Proteolytic cleavage can also produce different-sized proteins, and SGTA contains many potential proteolytic cleavage sites. PP5, which exhibits structural similarity to SGTA, is activated by proteolytic cleavage [19, 99], influencing its subcellular compartmentalization [99]. There is, therefore, evidence that the phosphorylation and proteolytic cleavage of TPR proteins closely related to SGTA influence their activity. Coupled with the likely alternative splicing of SGTA, further study of SGTA mRNA and protein species is required to understand its regulation and function.

SGTA has a related isoform, SGTB (Fig. 1c); collectively, they are known as SGT. It is SGTA which is reviewed here. Although SGTB shows ~60 % amino acid sequence identity to SGTA [82], it is predominantly brain-specific and little is known about its function. Additionally, there is a protein with a similar abbreviated name, SGT1. The SGT1 protein ("suppressor of G2 allele of SKP1 (*Saccharomyces cerevisiae*)") is encoded by the *SUGT1* gene and is located on 13q14.3. Like SGTA, SGT1 is a TPR protein and acts as a cofactor in steroid receptor signaling (reviewed in [64]) but is ultimately unrelated to the SGTA protein discussed herein.

SGTA in Viral Assembly, Replication, and Release

SGTA interacts with viral proteins and has been implicated in viral particle assembly and release. As described previously, in rat, SGTA binds to the NS1 of autonomous parvovirus H-1 [23]. In H-1-infected SV40-transformed human newborn kidney (NBE) cells, NS1 accumulation induces the production of reactive oxygen species, leading to DNA double-strand break formation, cell cycle arrest, and apoptosis [13, 42]. In infected NBE cells, NS1 also co-localizes with viral DNA at sites of viral DNA replication in nuclear bodies, which are important for regulating gene expression and influencing parvoviral replication [24]. Since SGTA also localizes in these nuclear bodies, SGTA may also be implicated in parvoviral replication and/or gene expression [24]. A more recent study in African green monkey kidney epithelial (Vero E6) cells demonstrated that human SGTA co-localizes and interacts with severe acute respiratory syndrome coronavirus (SARS-CoV) protein 7a through SGTA's second TPR domain (Fig. 1a) [33]. SARS-CoV7a also interacts with SARS-CoV structural proteins (nucleocapsid proteins) [33], the membrane and envelope proteins responsible for viral assembly and sufficient for virus-like particle formation. Collectively, these studies provide evidence of a role for SGTA in virus particle release in both the coronavirus and parvovirus families in kidney cell lines.

Human SGTA also interacts with HIV-1 VPU and the viral core protein precursor group-specific antigen (GAG) [14]. VPU facilitates rapid degradation of CD4, the HIV-1 receptor, in the endoplasmic reticulum [20, 90] and enhances virus particle exit from the plasma membrane of infected A3.01 cells (a CD4^{+} lymphocytic cell line) [77, 78]. GAG is important in virus assembly and maturation, is itself sufficient for the production of immature virus particles, and is involved in the recruitment of cellular proteins involved in viral assembly and budding [10, 70]. In HIV-1-infected human epitheloid cervix carcinoma (HeLa) cells, GAG associates with the cytoplasmic side of the plasma membrane, while SGTA relocates from its normal cytoskeletal location to the cell periphery and binds to VPU [37]. This interaction occurs through SGTA's TPR domain and is an inhibitory factor in viral particle release [30]. Consistent with a negative role for SGTA, its overexpression reduced the efficiency of HIV-1 particle release [14]. As VPU-mediated CD4 degradation is required for efficient viral particle release, we speculate that the interaction of SGTA with VPU may sequester VPU and prevent it from binding to CD4. Direct studies are required to test this hypothesis. Similarly, SGTA has recently been shown to bind to human endogenous retrovirus (HERV) protein HERV-K (HML-2) Rec, sequestering SGTA and preventing its interaction with the AR, allowing the AR to exert its transcriptional activity (discussed in detail later) [38]. Overall, SGTA appears to regulate the life cycle of at least three different viruses of the autonomous parvovirus, lentivirus, and coronavirus families [14, 23, 33], but it is clear that further study into the interaction of SGTA and its binding partners is required to delineate the precise role of SGTA in viral infection and to identify key partners important in the viral infection life cycle. However, inhibition of viral particle release is a common theme among the previously mentioned studies, and SGTA may, therefore, be mechanistically involved in self-protection of the cell. This is consistent with the role of co-chaperones in mediating responses to cell stress. SGTA knockout in cells will be a useful tool for continued study into the role of SGTA in viral infection. With the paucity

of published data in the arena of SGTA in viral infection and because few reports to date include direct mechanistic evidence of the role of SGTA in protecting the cell from viral infection, it is difficult to judge how critical SGTA is in viral infection.

SGTA as a Co-Chaperone

Chaperones interact with co-chaperones to ensure appropriate folding, trafficking, and activity of client proteins. Since chaperones such as HSP90 are constitutively expressed, co-chaperones play an important role in determining the specificity of chaperone action. Many TPR-containing proteins, including FKBP51, FKBP52, CYP40, and PP5, possess co-chaperone functionality [66], and all four of these proteins are implicated in steroid receptor signaling, supporting a potential role for SGTA in steroid receptor signaling. To date, the main functional domain in SGTA is the centrally localized TPR domain. We discuss here evidence that SGTA, usually through its TPR domain, acts on several molecules and pathways as a co-chaperone to ensure correct protein trafficking and/or prevent inappropriate movement and activity of signaling molecules. Included in this section are both hormonal and nonhormonal pathways, since understanding broadly how SGTA acts as a co-chaperone may help to delineate how it acts as a co-chaperone in steroid receptor signaling.

SGTA in Cell Division and Apoptosis

Fluorescence-activated cell sorting (FACS) of HeLa cells demonstrated that SGTA is expressed throughout the cell cycle, localizing in the midzone and midbody, two structures critical for cell division [92]. SGTA is localized at the spindle poles in prometaphase and metaphase, relocates to the central spindle where pole microtubules overlap during anaphase, and is detected in the midbody during telophase and early G1 [91], providing evidence for a role in cell division. Furthermore, SGTA exhibits mitosis-specific size differences by Western blot [91], suggesting possible regulation of SGTA function during the cell cycle by splicing and/or post-translational modification. SGTA depletion by SGTA-specific small interfering RNA (siRNA) in NBE and HeLa cells induced mitotic arrest, reduced cell proliferation and cell density, while increasing cell death [92]. Additionally, SGTA knockdown enhanced the formation of binucleated cells and caused an accumulation of cells in G2/M, a reduction of cells in G1 as determined by FACS analysis, and growth retardation [92]. A subsequent study by the same group, also using HeLa cells, confirmed these observations and showed that cells with siRNA-depleted SGTA stayed longer in mitosis and showed delayed prometaphase and

metaphase [91]. In SGTA-depleted cells that underwent mitotic arrest, not all chromosomes correctly aligned on the metaphase plate [91]. This did not occur in cells expressing normal SGTA levels. In HeLa cells, SGTA formed a stable interaction with heat shock cognate protein (HSC70), HSP70, and BAG-6/BAT-3/SCYTHE during prometaphase [91]. Interestingly, BAG-6/BAT-3/SCYTHE depletion resulted in mitotic arrest of cells, with persistence of a few misaligned chromosomes [91], a very similar effect to that observed upon SGTA depletion, implicating these proteins in common functions during this phase of the cell cycle. Together, these results suggest that SGTA and BAG-6/BAT-3/SCYTHE can form complexes during mitosis that are critical for progress through mitosis, but the exact mechanisms involved are not yet clear.

Overexpression and knockdown of SGTA also affects apoptosis, although reports are conflicting with pro-apoptotic and anti-apoptotic outcomes. In cultured human hepatocarcinoma (7721) cells, SGTA knockdown decreased apoptosis independent of cell proliferation or cell cycle, while SGTA overexpression sensitized cells to apoptosis and increased apoptosis-associated caspase activation and cytochrome c release [88]. It was the TPR repeat motif within SGTA that was essential for SGTA's pro-apoptotic function [88]. In viable HeLa cells, SGTA interacted with HSP90β and remained cytoplasmic [96]. During apoptosis, the cytoplasmic SGTA/HSP90β interaction was lost, allowing SGTA to localize in the nucleus [96]. In contrast, Winnefeld and colleagues [92] showed that SGTA depletion induced caspase-independent cell death in human NBE cells. Hence, SGTA appears to have cell type-specific effects on apoptosis. Similar to SGTA, there are examples of other proteins with dual pro-apoptotic and anti-apoptotic roles, such as BCL-X [5]; however, further research is required to clarify the role of SGTA in apoptosis and to identify the context which promotes either a pro-apoptotic or anti-apoptotic outcome. It is possible that the reported pro-apoptotic and anti-apoptotic functions of SGTA are controlled in a cell or tissue type-specific manner or, alternatively, that SGTA promotes caspase-dependent cell death while at the same time inhibiting caspase-independent cell death. In the case of BCL-X, its pro-apoptotic and anti-apoptotic functions occur based on alternative mRNA splicing, which generates two distinct transcripts [5]. The same could be true for SGTA; however, the aforementioned studies did not explore that avenue.

SGTA and Neurotransmitter Release

In the rat brain, SGTA is a critical component of a trimeric complex involving HSC70 and synaptic vesicle cysteine string protein (CSP), which localizes on the synaptic vesicles and functions as a synaptic chaperone machine [81], aiding in exocytotic release of neurotransmitter, hormones, and enzyme precursors [9, 53]. CSP interacts with the TPR domain of

SGTA (Fig. 1a) [81], which also binds to the C-terminal domain of HSC70 [50, 93]. Binding of CSP to HSC70 completes the trimeric complex [75]. *Csp* knockout mice provide additional evidence for an interaction of CSP and SGTA in vivo as these mice exhibit a concomitant decrease in CSP and SGTA on synaptic vesicles [81], suggesting that CSP is essential for the recruitment of cytoplasmic SGTA to synaptic vesicles. Denatured, unfolded luciferase refolds only when incubated with CSP, SGTA, HSC70, and adenosine triphosphate (ATP), providing further evidence that the trimeric complex acts as a co-chaperone [81]. This suggests that SGTA enhances the efficiency of the CSP/HSC70 system in its refolding reaction through its ability to stabilize the CSP/HSC70 complex and activate HSC70 ATPase activity.

The fate of client proteins may be determined in part by the TPR domain-containing protein that occupies the C terminus of HSC70. In the rat brain, close homologue of L1 (CHL1) can compete with CSP for binding to HSC70, which impedes CSP binding to HSC70 and SGTA [2]. As a result, in the presence of CHL1, the trimeric chaperone complex breaks apart into two components, being CHL1/CSP and CHL1/HSC70/SGTA. The requirement for CHL1 in protein folding has been demonstrated in the brain of *Chl1* knockout mice, which exhibit abnormal protein aggregation and accumulation in lysosomes, suggesting the presence of incorrectly folded proteins [2]. Furthermore, *Chl1* knockout mice, under stress, exhibit decreased chaperone activity in synapses, and the machinery required for vehicle exocytosis is unable to sustain prolonged activity [2]. In particular, SNAP25 and VAMP2, both exocytotic chaperone machine proteins, were susceptible to degradation and showed reduced activity [2]. Both of these proteins required the CHL1/CSP and CHL1/HSC70/SGTA complexes, but not the CSP/HSC70/SGTA complex [2], which suggests that CHL1 enhances the affinity of the SGTA chaperone complex for SNAP25 and VAMP2 to ensure their correct folding and enhance synaptic vehicle exocytosis in central nervous system synapses. This is an example of the fate of client proteins being influenced by the particular TPR-containing protein (in this instance, SGTA) that binds to HSC70.

SGTA and Myostatin

Myostatin is a member of the transforming growth factor-β superfamily and is produced as a precursor protein comprised of an N-terminal secretory signal, a propeptide domain, and a C-terminal cysteine-rich domain, which is the active mature peptide. The mature peptide forms homodimers that interact with the myostatin receptor to control muscle growth [56, 89]. Myostatin is predominantly expressed in skeletal muscle and acts as a negative regulator of skeletal muscle development and growth [36, 56, 57]. The third TPR motif of SGTA binds to the N-terminal signal peptide of myostatin in yeast cells and in

human skeletal muscle [89] (Fig. 1a). Although further functional characterization of the biological effects of this interaction is required, it seems likely that SGTA serves as a molecular co-chaperone to assist in the secretion and activation of myostatin and promote correct muscle growth and development. FKBP52 is a TPR-containing protein with structural similarities to SGTA. Male *Fkbp52* knockout mice are smaller than their wild-type counterparts [97]. Although the cause of their small size has not been investigated, reduced muscle mass could be a factor. We hypothesize that SGTA may be a negative regulator of myostatin. Since SGTA binds to the myostatin signal peptide, it is likely that SGTA regulates myostatin by inhibiting myostatin processing, so that myostatin cannot be processed to its mature, active state. In this case, total or partial deletion of SGTA, such as in a knockout mouse model, would result in a phenotype of reduced muscle mass and lower body weight. Studies of the functional effect of the interaction between SGTA and myostatin should also consider the known redundancy amongst co-chaperone proteins, particularly within the TPR-containing protein family.

SGTA and Growth Hormone Receptor

Growth hormone (GH), acting through the GHR, is involved in somatic growth, cellular differentiation, and metabolism [16]. GH action relies on the presence of circulating GH and the maintenance of GH-binding capacity, which is dependent on the synthesis of new GHR in the endoplasmic reticulum, GHR processing in the Golgi body, and GHR transport to the plasma membrane and, ultimately, removal of GHR from the plasma membrane via endocytosis and lysosomal degradation. Through the first TPR motif, SGTA binds to both precursor and mature forms of the GHR protein (Fig. 1a) [71], suggesting that SGTA may play a role in GHR transport from the endoplasmic reticulum to the plasma membrane. SGTA interacts with the ubiquitin-dependent endocytosis motif of the GHR, suggesting an additional role for SGTA in GHR degradation [71]. A recent report has now shown that SGTA inhibits the degradation of mislocalized proteins that have undergone BAG6-mediated ubiquitination [46], providing these proteins with an opportunity to be correctly localized and folded. It remains to be determined if SGTA physically blocks the binding of alternative co-chaperones or actively promotes GHR deubiquitination. We speculate that the interaction of SGTA with GHR would lead to a larger body size in a model of SGTA overexpression and a smaller body size in a model organism with reduced SGTA.

SGTA and Androgen Receptor Signaling

Steroid receptor signaling pathways are important regulators of gene transcription during normal tissue development and in hormone-related cancers, such as prostate cancer. Androgens

are male sex hormones and include 5α-dihydrotestosterone (DHT) and testosterone. Androgens act as a ligand for the AR, which is a nuclear transcription factor. In the absence of agonist ligand, AR is bound to a cytoplasmic complex of heat shock and other proteins, including HSP40, HSP70, and HSP90 [51], collectively known as the foldosome (Fig. 2). This stabilizes the AR and maintains it in a state competent to bind ligand. Upon agonist ligand binding, AR undergoes a conformational change, which induces cytoplasmic–nuclear shuttling. In the nucleus, ligand-activated AR forms a complex with co-activators and other transcription factors, binds to androgen response elements located in the promoter and/or enhancer

regions of AR target genes, and induces gene transcription. In this way, the AR mediates many cellular processes including differentiation, proliferation, metabolism, and apoptosis. The function of AR and its regulation are vital in the development of many tissues, not just the male sex organs. The AR also plays a role in disease development, including hypogonadism, benign prostatic hyperplasia, male pattern baldness, androgen insensitivity syndrome, and prostate cancer. In prostate cancer, the AR and its signaling pathway are critical in all stages of disease including tumorigenesis, progression, and the development of treatment resistance. Chaperones and co-chaperones are involved at all stages of AR signaling and act to modulate diverse

Fig. 2 A proposed model of the AR signaling pathway depicting the potential role of SGTA in the foldosome complex. In the absence of androgen, the AR is located in the cytoplasm in association with the foldosome complex. Maturation of the nascent AR is a dynamic process that involves three stages: early, intermediate, and mature. At each stage, HSPs and co-chaperones with distinct roles bind to or are released from the foldosome complex in order to stabilize and fold the protein into a conformation that is competent to bind ligand. SGTA binds to HSP70 and can mediate its ATPase activity. In addition, SGTA binds directly to microtubules, thus retaining the AR in the cytoplasm. Androgen binding results in a conformational change of the AR, an exchange of TPR-containing proteins, and initiation of nuclear translocation. In the nucleus, the AR is able to bind to response elements in target genes. Subsequently, cofactors, chaperones, co-chaperones, and other transcriptional machinery are recruited in order to mediate gene transcription. *Darker shading* for SGTA protein indicates better evidence for that particular interaction. *D* dynein, *TM* transcriptional machinery
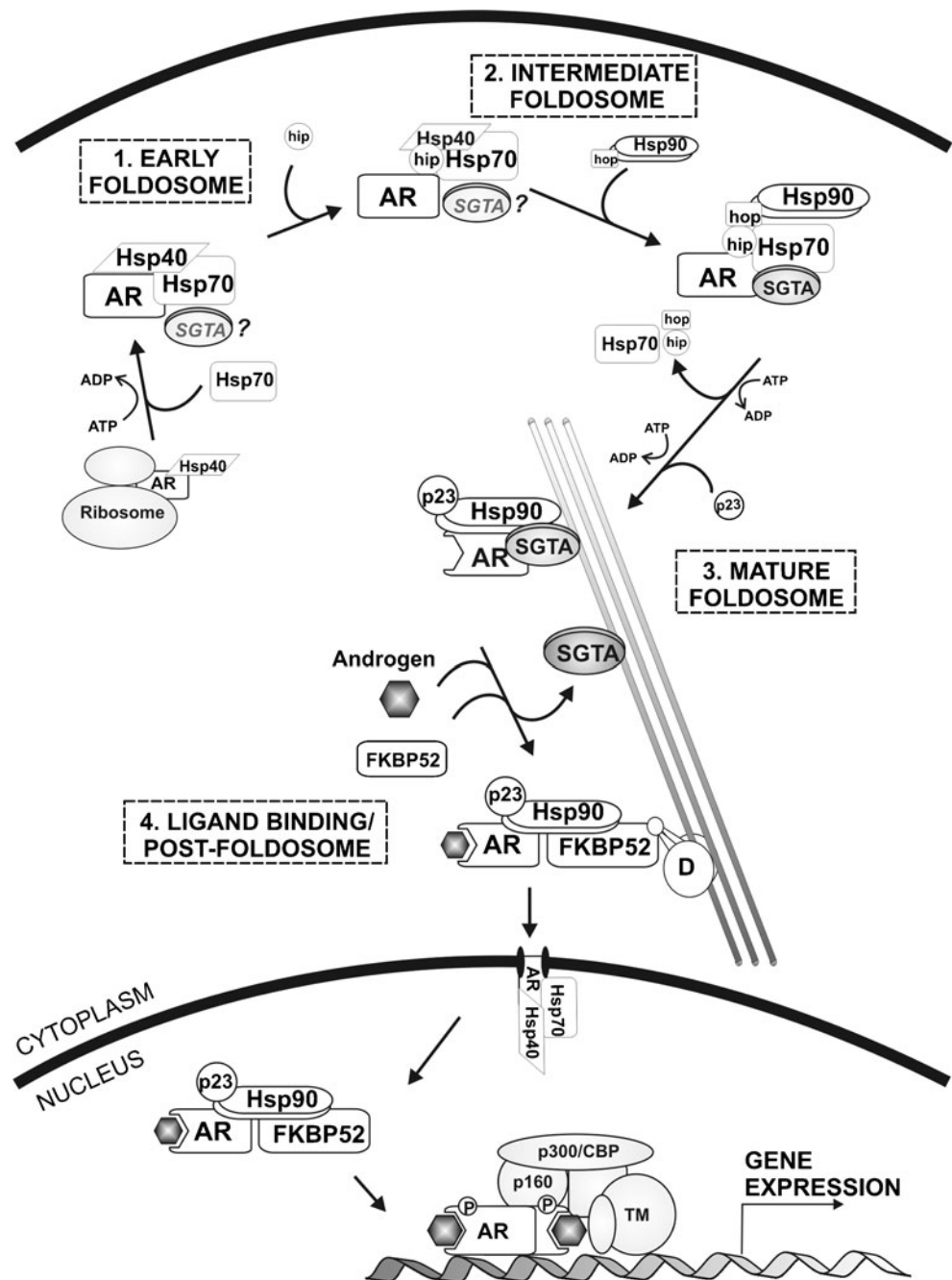
functions such as protein folding, receptor stability, subcellular localization, and intracellular trafficking. SGTA has been implicated as an AR co-chaperone; however, SGTA has been largely overlooked in studies of co-chaperones in steroid receptor maturation. Given the importance of AR signaling in prostate cancer, SGTA may be a novel player implicated in the regulation of hormone signaling in prostate cancer and other hormonally driven diseases, as well as in normal tissue development.

A study conducted in our laboratory was the first to show that human SGTA, acting through its TPR domain, interacts with the AR hinge region in yeast and mammalian cells (Fig. 1a) [8]. Unlike most steroid receptors, un-ligand-bound AR resides in the foldosome complex in the cytoplasm [51]. While a direct role of this complex on AR signaling has yet to be fully demonstrated, a generalized model has been made based on what is known for other steroid receptors. The foldosome is assembled in an ordered, stepwise fashion. During translation, folding of the receptor is induced through its interaction with HSP70 and HSP40 [67], generating the early foldosome (Fig. 2). In order to maintain the un-ligand-bound receptor in a soluble state and prevent aggregation, HSP40 acts to enhance the ability of HSP70 to bind to the receptor. Binding of HSP70 and HSP40 to the un-ligand-bound receptor is followed by binding of HSP-interacting protein (HIP) which stabilizes the HSP70/HSP40/receptor complex, known as the intermediate foldosome (Fig. 2). In the late foldosome, the TPR domain-containing protein, HSP70/HSP90-organizing protein (HOP) binds, which enables a HSP90 dimer to be recruited to the complex. A subsequent ATP-dependent interaction causes a conformational change in HSP90, exposing the receptor ligand binding domain [44]. The small ubiquitous co-chaperone P23 binds to HSP90 to maintain its ATP-dependent conformation and stabilizes the receptor complex [67]. Upon P23 binding, HSP70, HIP, and HOP are released, allowing a TPR co-chaperone protein to bind to HSP90, producing the mature foldosome. Several TPR-containing co-chaperone proteins, including FKBP51, FKBP52, CYP40, PP5, CHIP, and SGTA, have been shown to interact with the mature foldosome complex. In general terms, binding of a TPR-containing protein finalizes maturation of the receptor–foldosome complex, leading to stabilization and ligand binding competence [65]. Upon ligand binding in the cytoplasm, there is an exchange of TPR-containing proteins which allows the receptor to undergo nuclear translocation, where it associates with additional chaperones and transcriptional machinery to bind DNA and modulate expression of target genes (Fig. 2). This exchange of TPR-containing proteins was first demonstrated for FKBP51/FKBP52 on hormone-bound glucocorticoid receptor (GR) [26]. SGTA mediates two major aspects of the AR signaling pathway: (1) HSP70/HSP90 ATPase activity and (2) cytoplasmic–nuclear shuttling of the receptor. Both of these aspects of receptor maturation are discussed in detail in the next section.

SGTA and ATPase Activity of Heat Shock Proteins

The ability of HSP70 and HSP90 to bind to and efficiently fold client proteins is ATP-dependent. The N-terminal ATPase domain can be ADP-bound, resulting in a high affinity for proteins and efficient protein folding, or ATP-bound, leading to low binding affinity [54]. For ATP hydrolysis to occur, the C terminus of HSP proteins must bind to a co-chaperone [68]. The co-chaperone TPR domain interacts with the HSP/receptor heterocomplex through the conserved motif, EEVD, within the C terminus of HSC70 and HSP90 [15, 50, 66, 69, 93]. Different TPR-containing proteins (Fig. 1b) can exert opposing effects on HSP ATPase activity. For example, CHIP, HOP, and P23 decrease ATPase activity [43, 55], whereas TPR2 increases ATPase activity [7, 43, 55, 59]. Angeletti and colleagues [3] demonstrated by a luciferase refolding assay that, in vitro, SGTA negatively affects HSP70-mediated ATP hydrolysis, as well as its protein folding capacity. In contrast, Tobaben and colleagues [81] showed that SGTA positively influences HSC70-mediated ATP hydrolysis. Renaturation of unfolded luciferase only occurred with the cooperation of SGTA, HSC70, and CSP, and SGTA increased the efficiency of the CSP/HSC70 system in the refolding reaction, possibly by stabilizing the CSP/HSC70 complex and/or activating the ATPase of HSC70 [81]. As HSC70 is the near-identical counterpart of HSP70, further investigation is required to clarify these contrasting effects of SGTA on ATPase activity. Similarly, despite the fact that HSP70 and HSP90 share a similar TPR binding motif [72], there is no conclusive evidence of SGTA interacting with HSP90. Given that other TPR-containing proteins demonstrate minor individual differences in HSP70 and HSP90 binding motif recognition [6], this is not entirely surprising, but comprehensive computational and experimental analysis of SGTA and HSP90 should be conducted to ascertain if direct binding occurs. In addition, the ability of SGTA to dimerize [8] raises the possibility that SGTA could interact with HSP70 and HSP90 simultaneously; however, this also remains to be shown. Collectively, these observations suggest that SGTA may act predominantly in early protein folding (when steroid receptors are bound to HSP70) and have a weaker effect when bound to HSP90. Kinetic studies should be performed to test the strength of the HSP70–SGTA interaction during protein folding and AR maturation.

Cytoplasmic–Nuclear Shuttling

One of the earliest events in steroid receptor movement toward the nucleus is the exchange of HSP90-bound TPR proteins. While the exact role of TPR-containing proteins remains unclear, there is considerable evidence suggesting that TPR-containing proteins mediate intracellular steroid

receptor shuttling. For example, upon incubation with ligand, GR-bound FKBP51 is exchanged for an alternative TPR-containing protein, FKBP52 [26]. Once bound to GR, the PPIase domain of FKBP52 tethers the receptor complex to the cytoplasmic protein dynein and mediates its movement along microtubules towards the nucleus [66, 73]. Accordingly, FKBP51 overexpression, as observed in New World primates, decreases GR hormone binding affinity [28], whereas FKBP52 overexpression results in increased GR nuclear translocation and a subsequent increase in transcriptional activity of the GR [27].

Unlike FKBP52, SGTA lacks a PPIase domain; therefore, the SGTA–receptor complex is unable to bind to cytoplasmic dynein. As TPR-bound receptors bind to dynein to mediate nuclear translocation, the lack of PPIase domain in SGTA inhibits receptor nuclear translocation and gene transcription. A study conducted by our laboratory discovered that human SGTA interacts with the AR hinge region in both yeast and mammalian cells [8]. The AR hinge region lies between the canonical domains for ligand and DNA binding and has been implicated as a site for chaperone interaction [8]. Under sub-saturating concentrations of ligand, the AR remains cytoplasmic. It is possible that the cytoplasmic retention of AR is mediated by SGTA, binding concurrently to AR and microtubules. This tethers the AR/HSP90 complex to the cytoskeleton or microtubule-associated proteins, prevents the AR from entering the nucleus, and silences the receptor's ligand-independent transcriptional activity [8]. However, under the influence of a saturating concentration of androgen, the cytoplasmic AR/SGTA interaction is lost and AR undergoes nuclear localization [8]. Given the previously reported interaction of SGTA with chaperone proteins, HSP90, HSP70, and HSC70 [49, 50], it is possible that SGTA plays a role as a co-chaperone of the AR, influencing intracellular shuttling of AR. Similar studies have been performed by our laboratory in a female context using ovarian cancer cell lines, in which ablation of SGTA protein by siRNA resulted in increased AR nuclear localization in the absence and presence of androgen [11, 12]. Replication of these observations in different model systems is required to provide a detailed understanding of how SGTA interacts with the AR and cooperates with the co-chaperone machinery to influence AR activity. Studies of AR and hormone receptor signaling in *Sgta* knockout mice would also clarify if SGTA is essential for receptor maturation and nuclear translocation. If SGTA is not essential, this may be due to the known redundancy among TPR-containing proteins, so molecular characterization of the AR foldosome is essential.

Specific evidence for an exchange of SGTA upon ligand binding has been demonstrated by the addition of a saturating concentration of androgen, which enhanced the AR/FKBP52 interaction and reduced the AR/SGTA interaction [8, 21]. In addition, overexpression of SGTA relative to AR decreases DHT-mediated AR transcriptional activity, whereas SGTA knockdown increases the sensitivity of the AR to non-classic ligands, such as progesterone [8]. Trotta and colleagues [83] demonstrated that the interaction of SGTA with AR relies on a conserved sequence in the N-terminal region of SGTA (amino acids 21–40). This was the first study to localize the region of SGTA required for its activity, and surprisingly, the minimal essential region was outside of the TPR domain, which was previously thought to be required for all of SGTA's protein interactions. The interaction, if any, of this minimal essential region in the N terminus of SGTA with the TPR domain, and how this influences AR signaling, remains to be demonstrated. Collectively, these findings suggest that SGTA is a critical part of the receptor complex by acting to maintain the AR in the cytoplasm until adequate ligand binding has occurred, as well as by regulating the sensitivity of the receptor to specific and nonspecific ligands. However, further refinement of the SGTA/AR interaction is required to determine if SGTA is a true co-chaperone of the AR. Also, given the known redundancy of co-chaperones, studies to investigate if the functions of SGTA can be fulfilled by other co-chaperones, such as FKBP52, are essential. Transgenic mice with double knockouts could assist in answering this question.

SGTA and Normal Physiology

SGTA has been detected in the brain, heart, kidney, liver, lung, pancreas, placenta, prostate, skeletal muscle, spleen, and testis (Table 1) [8, 23, 45, 82]. A study conducted in our laboratory was the first to demonstrate that, in the prostate, SGTA expression was restricted to the cytoplasm of epithelial cells [8]. Similarly, a recent study, also from this laboratory, has found SGTA expression to be predominantly cytoplasmic in the surface epithelium and developing follicles of the ovary [12]. Thus, SGTA protein is expressed, predominantly in the cytoplasm, in a subset of hormonally regulated human male and female tissues, including the testis, prostate, endometrium, breast, and ovary (Fig. 3). Additionally, SGTA protein is expressed in the thecal cells of the ovary and the Leydig cells of the testes ([12] and Fig. 3), which are derived from a common mesenchymal origin. As steroid hormone production occurs in both the thecal [39, 98] and Leydig cells [74], it is tempting to speculate that expression of SGTA may be a mechanism developed by these cells to control AR action, and therefore, SGTA may play a role in normal physiology and disease states in these tissues.

The significance of SGTA in male and female physiology, specifically concerning normal development and disease states mediated by sex steroid receptor signaling, is largely unknown and requires more study. There are no studies of *Sgta* knockout mice reported in the literature; however, we can speculate on a possible function for SGTA in normal

Fig. 3 Immunohistochemical protein expression of SGTA in human male and female reproductive tissues. **a** Cytoplasmic SGTA was present in the epithelial cells (*E*) but not the stroma (*St*) of the prostate. **b** In the testes, SGTA was expressed within the cytoplasm of Leydig (*L*) cells. SGTA was also strongly expressed in cells (including Sertoli, spermatid, and spermatocytes) that make up the seminiferous tubule. **c** SGTA was present in the granulosa (*G*) and germ cells (*Oo*) of ovarian follicles with little or no expression in the ovarian stroma. **d** Granulosa and theca (*T*) cells of large antral follicles also exhibited cytoplasmic SGTA expression. **e** SGTA was expressed in the epithelial lining of the fallopian tube with little or no expression in the stroma. **f** SGTA was also expressed in the epithelial glands of the breast. Cytoplasmic SGTA was observed in the glandular epithelium (*E*) of the endometrium at the **g** proliferative and **h** secretory phase of menstruation. However, in both phases, cytoplasmic and nuclear SGTA expression was observed in the stroma. Immunohistochemistry was performed using a standard heat-mediated antigen retrieval protocol with overnight incubation of the SGTA (Proteintech Group Inc., Chicago, IL, USA) antibody. Visualization was achieved with a standard immunoperoxidase reaction using biotinylated anti-rabbit antibody, streptavidin–horseradish peroxidase complex, and diaminobenzidine tetrahydrochloride
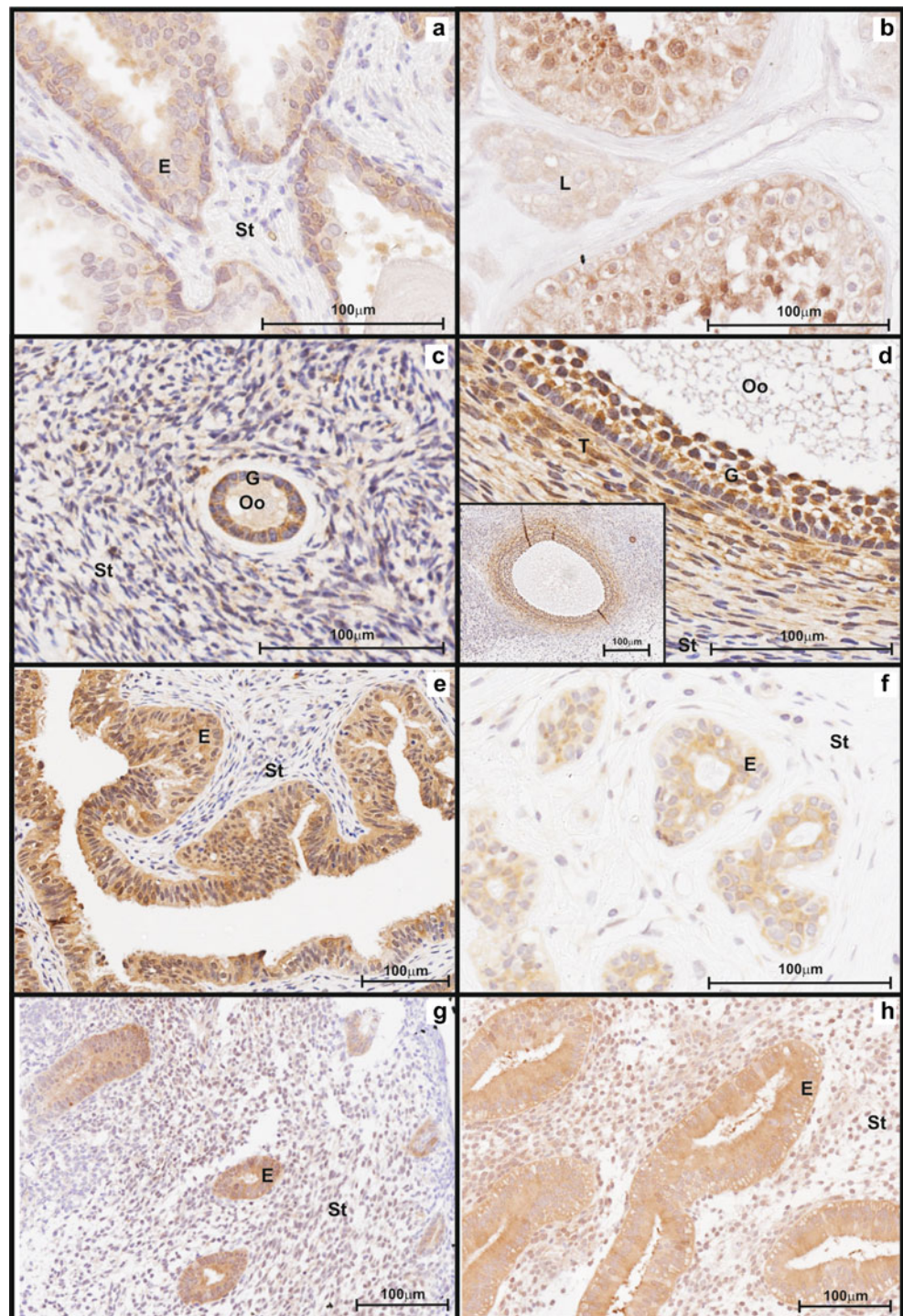
physiology based on mouse knockout studies of the closely related TPR protein, FKBP52. For example, in vitro in human cells, the interaction between AR and FKBP52 is enhanced by the presence of ligand, FKBP52 overexpression enhances AR-mediated transactivation, and FKBP52 knockdown inhibits AR transcriptional activity without affecting ligand binding or nuclear transport [21]. FKBP52 knockdown causes a significant physiological effect on AR-responsive tissues. Given that SGTA is similar to FKBP52, depleting SGTA may

also affect the physiology of AR-responsive tissues. Indeed, *Fkbp52*-deficient male mice are infertile due to defects in the reproductive organs that require AR activity for their development or function [18, 21, 97]. In contrast, although female *Fkbp52*-deficient mice are also infertile, this is not due to defects in the reproductive organs that exhibit AR activity, but a failure of uterine implantation due to a disruption in progesterone receptor A activity [95]. This highlights the potential importance of SGTA in all sex steroid receptor

signaling, not just in AR signaling. In comparison, FKBP51, which in vitro enhances AR-dependent transcription and ligand responsiveness [61], does not produce an apparent male or female physiological phenotype in a knockout mouse model [97]. Collectively, these knockout studies draw attention to the complex and redundant nature of TPR-containing protein function in steroid receptor signaling and their impact on hormone-mediated physiological processes. Despite this complexity, we speculate that SGTA is likely to play a critical role in steroid signaling in a tissue-specific and steroid receptor-specific manner. It is necessary to delineate which steroid receptors and in which tissues SGTA exerts its predominant co-chaperone effects and also to identify which functions of SGTA can be replaced by alternative TPR-containing proteins with a similar structure and expression pattern. Additionally, it is possible that SGTA function may be modified by interaction with other co-chaperone molecules.

SGTA and Disease

There is increasing evidence for a role of SGTA in disease states. Inhibition of the putative ortholog of human SGTA in *Caenorhabditis elegans* results in the suppression of toxicity associated with β-amyloid peptide, a primary constituent of extracellular senile plaques typical of Alzheimer's disease [34], implicating SGTA as a possible future therapeutic target for Alzheimer's disease. However, this is not well-studied, and additional research is required to clarify the exact role of SGTA and to identify if alterations in SGTA expression or activity are a driver of disease or a consequence of the pathogenic process.

To date, SGTA has been associated with two androgen-associated disorders, namely, prostate cancer and polycystic ovary syndrome (PCOS), reiterating the need for future studies to focus on more clearly defining the role of SGTA in androgen signaling. Prostate growth and development is reliant on a functioning androgen-signaling axis. While the AR is acknowledged as the key determinant of prostate cancer survival, the mechanisms by which AR sustains genomic signaling during androgen deprivation therapy are the subject of considerable research efforts. Our laboratory has shown significant changes in SGTA and AR protein levels during human prostate cancer progression, with a reduction in SGTA immunoreactivity in metastatic disease compared with nonmalignant prostate samples and primary tumors [8]. In contrast, nuclear AR immuno-staining significantly increases with disease progression, resulting in an increased AR/SGTA ratio in metastatic samples [8]. Since SGTA may restrain AR in the cytoplasm and thereby minimize gene transactivation by ligand-bound AR, an increased AR/SGTA ratio in metastatic prostate cancer is consistent with the sensitization of prostate tumor cells to androgen signaling with disease progression. This study, therefore, implicates SGTA in the control of androgen action in the prostate

as prostate cancer progresses [8]. In support of this concept, a recent study has suggested that HERVs, especially the HERV-K (HML-2) subfamily, may interfere with SGTA/AR binding, leading to enhanced AR activity by allowing increased AR nuclear translocation [38]. This enhanced AR activity may lead to increased transcription of not only AR-dependent genes but also to HERV-K (HML-2) transcription and particle production [38]. Since HERVs display oncogenic properties and are induced in some cancers, this may represent a vicious cycle in which increased cell proliferation and reduced apoptosis may contribute to cancer development and progression. Direct testing of this hypothesis is necessary.

PCOS is an endocrine disorder which affects 6–8 % of women of reproductive age and is the major cause of anovulatory infertility [62]. The etiology of PCOS remains unknown; however, familial clustering suggests the involvement of genetic factors [1, 31]. SGTA is a compelling candidate gene for PCOS based on several lines of evidence. Firstly, the *SGTA* gene is located near the dinucleotide repeat polymorphism D19S884 on chromosome 19p13.2 that possesses the strongest evidence to date for a PCOS susceptibility locus [76, 84–86]. Secondly, two independent studies have reported that a specific haplotype of single nucleotide polymorphisms within the *SGTA* gene is associated with an increased risk of PCOS [32, 35]. Thirdly, variation in the *SGTA* gene in women with PCOS was also associated with increased insulin resistance, leading to increased β-cell function and insulin secretion [35]. Consequently, SGTA may be a link between hormone action and metabolic signaling pathways in the pathogenesis of PCOS. While knowledge of AR action in female reproductive tissues remains unclear, knockout mouse models and the association of abnormal androgen levels with fertility disorders implicate AR signaling as an essential feature of normal reproductive function [47, 62, 87]. SGTA is expressed in epithelial cells in the ovary, fallopian tube, and endometrium (Fig. 3). Since epithelial cells of these female tissues also express AR [40, 41, 79], SGTA has the potential to function as a mediator of AR action in these cells, which may influence androgen-associated disorders of these tissues.

Recently, our laboratory explored this concept in ovarian cancer tissues [11]. SGTA restrained nuclear entry of the AR in an AR-positive serous ovarian cancer cell line, similar to what has been observed in a prostate cancer and the previously mentioned ovarian granulosa tumor cell line [8, 12]. Cytoplasmic SGTA was observed in the ovarian epithelia of benign and malignant disease states. While AR expression was reduced in serous carcinoma compared to benign and borderline disease, levels of SGTA protein were not different among disease states. Consequently, the ratio of AR/SGTA was lower in ovarian cancers compared to nonmalignant ovarian tissues, the opposite to what is reported in a prostate context [11]. Therefore, this study suggests that the in vivo influence of SGTA depends on the relative level of AR in the

tissue. Whether a reduced AR/SGTA ratio attenuates AR transcriptional activity in ovarian cancer tissues remains to be determined.

Future Work

While this review has discussed what is currently known about SGTA, we have also highlighted significant gaps in the knowledge. We have included here prospects for future study which would greatly add to the body of knowledge of how SGTA functions. Future studies should focus on demonstrating a direct role of SGTA in AR maturation and signaling to support the inferred evidence discussed previously. A number of groups have generated *SGTA* deletion constructs to map the domains of SGTA critical for interaction with client proteins [71, 83]. These constructs provide a valuable resource to more broadly and systematically study SGTA interaction with client proteins, which should enable a more detailed mapping of the functional consequence of modulating SGTA activity. Critically, since SGTA is a small molecule with a partially known crystal structure [29] and multiple well-defined interactions, it represents a target which would be highly amenable to disruption by rationally designed drugs. However, an important consideration in targeting SGTA would be identifying the specific interactions which would be disrupted. Given that SGTA binds to many proteins, there is a high potential for unwanted side effects. Further research aimed at clarifying the role of SGTA in different disease states and mapping the protein–protein interaction surface prior to undertaking any drug design project should ensure that the drug specifically targets the desired interaction. It should be noted that, in some disease states, such as prostate cancer, SGTA acts to restrain the AR in the cytoplasm, with an overall effect of decreasing AR-mediated gene transcription. Blocking this interaction is unlikely to be desirable for prostate cancer therapy. Similarly, in PCOS, SGTA may play a protective role to minimize the transcriptional activity of excessive androgens, acting through the AR. However, the role of SGTA in PCOS has not been directly studied and SGTA may exert an opposite effect on AR, thereby making it a potentially attractive therapeutic target in this disease. Since many co-chaperones act on multiple steroid receptors, it is also possible that, in female tissues which express a range of steroid receptors, SGTA may play a role in the maturation and signaling of multiple receptors, and this also remains to be defined.

Conclusions

In summary, SGTA acts as a co-chaperone of numerous proteins, including steroid receptors, such as the AR. Our analysis

of the published data on SGTA suggests that the most common function of SGTA is to act as a co-chaperone to maintain client proteins in their immature or inactive state which regulates protein activity. Myostatin, GHR, VPU, and AR have been demonstrated experimentally to be SGTA client proteins. SGTA is expressed in hormonally responsive tissues, in which appropriate hormone signaling is required for their correct tissue development. In normal physiology, there is evidence to suggest that SGTA is involved in regulating the growth and development of reproductive organs. We have identified that future studies, for example, using SGTA knockout models, are required to confirm this. In disease, we presented evidence for the involvement of SGTA in the pathogenesis of both prostate cancer and PCOS, two important diseases of the male and female reproductive organs, respectively, both of which require improved therapeutic options. Critical future studies are essential to define how or, in fact, if SGTA contributes to prostate cancer, PCOS, or other disease states. Once it is known if changes in SGTA expression or activity occur as an initiating factor or later in the pathogenic process, this will assist in determining if therapeutic modulation of SGTA is a viable option for treating disease.

The information presented here gives an exciting overview of the potentially critical role for SGTA as a co-chaperone in the development of normal and diseased states in humans. The study of co-chaperone molecules is complex because they act in multiprotein complexes and can have high levels of redundancy. Despite this, in light of the current literature describing SGTA and its functions, we have proposed a series of studies which will be critical for developing a solid understanding of how SGTA functions and how this is controlled in a sex-specific and context-specific manner. We believe that SGTA is an important co-chaperone protein which warrants further investigation, particularly with regard to its role in androgen-associated disorders of male and female tissues.

Conflict of Interest The authors declare no conflict of interest.

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