Androgens play important roles in the growth of normal prostate and prostate cancer via binding to the androgen receptor (AR). In addition to androgens, AR activity can also be modulated by selective growth factors and/or kinases. Here we report a new kinase signaling pathway by showing that AR transactivation was repressed by wild type glycogen synthase kinase 3β (GSK3β) or constitutively active S9A-GSK3β in a dose-dependent manner. In contrast, the catalytically inactive kinase mutant GSK3β showed little effect on the AR transactivation. The suppression of AR transactivation by GSK3β was abolished by the GSK3β inhibitor lithium chloride. The in vitro kinase assay showed that GSK3β prefers to phosphorylate the amino terminus of AR that may lead to the suppression of activation function 1 (AF1) activity located in the NH2-terminal region of AR. GSK3β interrupted the interaction between the NH2 and COOH termini of AR, and overexpression of the constitutively active form of GSK3β, S9A-GSK3β, reduced the androgen-induced prostate cancer cell growth in stably transfected CWR22R cells. Together, our data demonstrated that GSK3β may function as a repressor to suppress AR-mediated transactivation and cell growth, which may provide a new strategy to modulate the AR-mediated prostate cancer growth.

Androgen exerts its effects via the intracellular androgen receptor (AR), a member of the superfamily of nuclear receptors (1, 2). Like other nuclear receptors, the AR protein is comprised of a nonconserved amino-terminal domain, a highly conserved DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD). Upon androgen binding, AR dissociates from the heat-shock proteins and binds to androgen response elements, resulting in up-regulation or down-regulation of the transcription of AR target genes. Mutations in the AR gene cause a range of androgen insensitivity abnormalities in male sexual development and play a key role in human prostatic carcinogenesis, which is the most common invasive malignancy and second leading cause of cancer deaths in males in the United States (3, 4). In the early stage of this disease, most patients respond favorably to androgen ablation and antiandrogen therapy. However, the effects of androgen ablation are usually transient as cancer cells eventually progress into the androgen-independent phenotype. Several different molecular mechanisms might be responsible for the transition to androgen independence. Many of these involve the mutations in the AR and the altered AR coregulator signaling (5, 6), but they might also include enhanced expression of growth factor receptors and their associated ligands (7).

In addition to responding to ligands, the AR is affected by kinase signaling pathways, which directly or indirectly alter the biological responses to androgens. This phenomenon is mediated by the AR as antiandrogens have been shown to block kinase-induced transcriptional activation (8). Growth factors, cytokines, and neuropeptides have been implicated in various in vitro and in vivo models of human malignancies, including prostate cancers (9). In the absence of androgens, insulin-like growth factor-1, keratinocyte growth factor, and epidermal growth factor are able to activate transcription of AR-regulated genes in prostate cancer cells (10). Mitogen-activated protein kinase and Akt kinase cascades have been shown to be involved in growth factor-mediated AR activation (7, 11–13). Some neuropeptides, such as bombesin and neurotensin, can stimulate AR activation and cancer cell growth in the absence of androgen by activation of tyrosine kinase signaling pathways (14). To date, it is well established that the AR is a phosphoprotein, and its activity is correlated with its phosphorylation status. Prostate cancer cells may progress from androgen dependence to a refractory state resulting from activation of AR by various kinases, thus circumventing the normal growth inhibition caused by androgen ablation.

Glycogen synthase kinase 3β (GSK3β) is a serine/threonine protein kinase that was first described in a metabolic pathway for glycogen synthase regulation (15). It is now clear that GSK3β is a multifunctional kinase that regulates a wide range of cellular processes, ranging from intermediate metabolism and gene expression to cell fate determination, proliferation, and survival (16–19). GSK3β phosphorylates a broad range of substrates, including several transcription factors such as c-Myc, c-Jun, rat glucocorticoid receptor, heat-shock factor-1, nuclear factor of activated T-cells c, and β-catenin (20–25). In contrast to other kinases, GSK3β is highly active in unstimulated cells and becomes inactivated in response to mitogenic stimulation (26). Growth factors down-regulate GSK3β activity through the phosphatidylinositol 3-kinase/Akt signaling cascade and the mitogen-activated protein kinase/p90RSK pathway (27, 28). Consistent with its position downstream of the phosphatidylinositol 3-kinase/Akt and mitogen-activated pro-
tein kinase/p90RSK pathways, GSK3β activity suppresses cell proliferation and induces apoptosis (29, 30). Phosphorylation of serine 9 of GSK3β inhibits its activity by creating an inhibitory pseudosubstrate for the enzyme. Conversely, a mutation that prevents this phosphorylation results in activation of the kinase. GSK3β is also inhibited by Wnt signaling, which may contribute to progression of the prostate cancer (31).

In the present study, the role of GSK3β in AR signaling was assessed. In transfected cell lines, GSK3β inhibited AR-dependent transactivation of several reporter genes as well as endogenous 5α-dihydrotestosterone (DHT)-mediated prostate-specific antigen (PSA) expression. Additionally our data indicated that the effect of GSK3β was mediated through the NH2-terminal activation function 1 (AF-1) of the AR and through suppressing the interaction between the NH2 and COOH termini of AR. Moreover our results suggested that GSK3β can interact directly with the AR and inhibit androgen-stimulated cell growth. These findings suggest that GSK3β might modulate AR signaling and, therefore, may play important roles in the control of the proliferation of normal and malignant androgen-regulated tissues.

EXPERIMENTAL PROCEDURES

Materials and Plasmids—DHT and LiCl were obtained from Sigma. Antibodies to GSK3β and phospho-GSK3β were purchased from New England Biolabs. Purified GSK3β was purchased from Upstate Biotechnology, Lake Placid, NY. The anti-AR polyclonal antibody, NH27, was prepared as described previously (32). The GSK3β plasmids, including wild type (WT), constitutively active, and dominant negative forms, were kindly provided by J. Sadoshima, Pennsylvania State University. Plasmids pCMX-GAL4-AR-DE, pCMX-VP16-AR-N, pCDNA3-AR-N, and pCDNA3-AR-C were constructed as described previously (33). The plasmid pBIG-S9A-GSK3β was constructed by inserting full-length S9A-GSK3β cDNA into pBIG expression vector.

Cell Culture and Transfection Assay—COS-1 and PC-3 cells were maintained in early to midlog phase in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 50 µg/ml streptomycin, and 50 units/ml penicillin, and 50 µg/ml streptomycin in incubators with humidified air and 5% carbon dioxide at 37 °C. LNCaP cells were maintained in RPMI 1640 medium (Invitrogen). Twenty-four hours prior to transfection, cells were washed with Hanks’ buffered saline solution, trypsinized, and seeded to be at a density of 40–60% confluence for transfection. Cells in 24-well plates were refed with fresh medium 2 h before transfection and transfected according to the SuperFect transfection instructions (Qiagen). After 2–3 h of incubation, cells were treated with medium supplemented with charcoal-dextran-treated fetal bovine serum containing either ethanol or ligands. Cells were further incubated at 37 °C for 24 h, washed with phosphate-buffered saline, and harvested. The luciferase results were normalized to Renilla luciferase activity, and the data represent means ± S.D. from duplicate sets of three independent experiments.

In Vitro Kinase Assay—Purified recombinant murine GSK3β (New England Biolabs) was assayed as described previously (7). The kinase buffer contained 25 mM HEPES/NaOH, 7.4, 10 mM MgCl2, and 1 mM dithiothreitol. The kinase reactions were performed for 30 min at 30 °C in the presence of 10 µCi of [γ-32P]ATP, 10 µM ATP, and 0.05 pmol of GSK3β. The time amounts of various AR fragments were purified by Escherichia coli and used as substrates, whereas GST protein was utilized as negative control. The reactions were terminated by addition of 4× SDS sample buffer. The samples were boiled and loaded on 12% SDS-polyacrylamide gels.

Real Time Reverse Transcription PCR—Total RNA was isolated using the TRIzol (Invitrogen) reagent according to the manufacturer’s instructions, and 1 µg of RNA was subjected to reverse transcription using Superscript II (Invitrogen). Specific primers for GSK3β, 5’-CTA AGG ATT CCT CAG GAA CAG-3’ (forward) and 5’-TTG AGT GGT GAA GTT GAA GAG-3’ (reverse), were designed according to Bacon Designer2 software. 38B4 primers, 5’-TAC ACC TTC CCA CCT ACT G-3’ (forward) and 5’-GAT TCC TCC GAG GCT TCC-3’ (reverse), were used for GAPDH controls (34). The real time PCR was performed with 1 µl of reverse transcription product, 12.5 µl of 2× SYBR Green PCR Master Mix (Bio-Rad), and 0.5 µl of each primer (10 µM) in a total volume of 25 µl. PCR was performed as follows: 94 °C for 3 min and 40 cycles of 94 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s on an iCycler iQ multicolor real time PCR detection system (Bio-Rad). Each sample was run in triplicate. Data were analyzed by iCycler iQ software (Bio-Rad).

Stable S9A-GSK3β Transfection in CWR22R Cells—The constitutively active form of GSK3β, S9A-GSK3β, was inserted into pBIG vector with hygromycin resistance. The S9A-GSK3β-transfected CWR22R cells were selected and maintained in RPMI 1640 medium containing 50 µg/ml hygromycin (Invitrogen).

Thiazolyl Blue (MTT) Assay—The MTT assay is a quantitative colorimetric assay for mammalian cell survival and proliferation. 5 × 104 CWR22R cells were seeded in 24-well plates and incubated in RPMI 1640 medium with 5% charcoal-dextran-treated fetal calf serum for 48 h. Cells were then treated with ethanol, 10 mM DHT, and/or 2 µg/ml doxycycline for another 5 days. Then 200 µl of MTT (5 mg/ml, Sigma) was added into each well with 1 ml of medium for 3 h at 37 °C. After incubation, 2 ml of 0.04 M HCl in isopropyl alcohol was added into each well. After 5 min of incubation at room temperature, the absorbance was read at a test wavelength of 570 nm.

RESULTS

GSK3β Is Ubiquitously Expressed in Prostate Cancer Cells—Early studies showed that GSK3β mRNA was prominently expressed in testis, thymus, prostate, and ovary (35). We first applied real time PCR to determine the expression of endogenous GSK3β in COS-1 and several prostate cancer cells, including PC-3, LNCaP, and DU145 cells. As shown in Fig. 1A, GSK3β mRNA was detected in all the cell lines tested with the highest level of GSK3β mRNA in LNCaP and the lowest level in COS-1. To further examine the protein expression and activity of endogenous GSK3β in cancer cells, several prostate cancer cell lines, including PC-3, LNCaP, and DU145, were
subjected to Western blotting analysis along with some non-prostate cancer cell lines, including MCF7, C2C12, and COS-1. GSK3β protein was ubiquitously expressed in all cell lines analyzed. GSK3β can be phosphorylated at Ser-9, and phosphorylation at this site has been shown to inhibit kinase activities of GSK3β (36). Therefore, the inactive form of GSK3β with phosphorylation at serine 9 was determined by Western blotting with phosphospecific antibodies. LNCaP cells showed strongly phosphorylated GSK3β compared with PC-3 and DU145 cells (Fig. 1B), suggesting that LNCaP cells may have relatively lower endogenous activity of GSK3β.

Suppression of AR Transactivation by GSK3β—Since growth factors, neuropeptides, and protein kinase A inhibit GSK3β and enhance AR activity concurrently (8, 14, 37–39), we were interested to see whether co-expression of GSK3β might alter AR-dependent transcriptional activity. We took advantage of a dual luciferase assay system (Promega) using reporter and internal control plasmids together. The ARE4-Luc reporter is driven by four androgen-responsive elements (AREs) in the promoter region and functions as a monitor of AR transcriptional activity. We transiently co-transfected GSK3β, AR, and the two reporter plasmids in COS-1 cells, which lack endogenous AR. As shown in Fig. 2A, WT GSK3β reduced the AR-mediated transcription of the luciferase reporter by about 40% (lane 3 versus lane 2). While the KM-GSK3β had only a marginal effect on AR (lane 4), the constitutively active form of the GSK3β (S9A-GSK3β), which prevents serine 9 phosphorylation and inactivation of GSK3β, strongly inhibited AR activity (lane 5), suggesting that the kinase activity of GSK3β is necessary to suppress AR activity.

Since the context of upstream promoter elements may influence transcriptional efficiency, we tested another reporter plasmid, MMTV-Luc, to confirm the suppression effect of GSK3β on AR transcriptional activity. MMTV-Luc is driven by the natural MMTV long terminal repeat promoter that contains several AR response elements. Fig. 2B demonstrates that GSK3β inhibits DHT-mediated AR transcriptional activity in a dose-dependent manner (lanes 2–5). LiCl, a specific inhibitor of GSK3β, not only abolished the inhibitory effect of GSK3β on AR (lanes 8–10) but also slightly enhanced AR transcriptional activity (lane 7 versus lane 2). This result indicates that LiCl may block both endogenously transfected GSK3β as well as the endogenous GSK3β activity in COS-1 cells. Moreover LiCl did not alter luciferase expression in the absence of AR, ensuring that LiCl has nonspecific effect on the MMTV-Luc reporter (data not shown). To rule out the possibility that GSK3β may have nonspecific effects on the general transcription machinery, we also tested its effect on the human glucocorticoid receptor (GR) since early studies reported that GSK3β has little effect on the phosphorylation of human GR. As shown in Fig. 2C (lanes 3 and 4), addition of GSK3β failed to inhibit human GR transcriptional activity.

Fig. 2. Effect of GSK3β on androgen receptor transcriptional activity. A, expression of WT GSK3β, but not the kinase mutant GSK3β, suppressed AR transactivation in COS-1 cells. AR-negative COS-1 cells were transiently transfected using SuperFect transfection reagent (Qiagen) with 3 μg of ARE4-Luc reporter plasmid, 100 ng of pRLtk-Luc as an internal control, 1 μg of pSG5-AR expression plasmid, and 6 μg of WT, S9A, or kinase mutant GSK3β expression plasmids as indicated. The total amount of plasmids was adjusted to 10 μg with vector plasmids. Transfected cells were induced with 10 nM DHT for 18 h before the luciferase activities were measured. Luciferase activity was analyzed following the manufacturer’s instructions (Promega). The results are shown as mean ± S.D. of three independent experiments. B, overexpression of GSK3β inhibits AR transcriptional activity in a dose-dependent manner. COS-1 cells were transfected with increasing amounts of WT GSK3β expression plasmids as indicated. Experiments were performed and analyzed as described in A using MMTV-Luc instead of ARE4-Luc reporter. LiCl, a specific inhibitor of GSK3β, was added into cell medium as indicated 1 h prior to DHT treatment. The results are shown as mean ± S.D. of three independent experiments. C, overexpression of GSK3β has no effect on human GR transcriptional activity. Experiments were performed and analyzed as described in B using GR instead of AR. The results are shown as mean ± S.D. of three independent experiments. Vec, vector.
Inhibition of AR Transactivation and PSA Expression by GSK3β in LNCaP Cells—To examine whether the inhibitory effect of GSK3β on AR transactivation extends to cells that express endogenous AR, LNCaP cells, which have mutated yet functional AR, were co-transfected with the androgen-responsive reporter MMTV-Luc and GSK3β. As shown in Fig. 3A, addition of GSK3β reduced the activity of AR in a dose-dependent manner (lanes 3–5). Moreover, addition of LiCl abrogated the GSK3β-mediated inhibition of AR activity (lane 6). A similar suppression effect also occurred when we replaced MMTV-Luc reporter with the ARE4-Luc reporter system (data not shown).

To further demonstrate that GSK3β interacts with AR in mammalian cells, we next used co-immunoprecipitation to examine their interaction by co-transfecting AR and HA-tagged GSK3β into COS-1 cells. The COS-1 cell extracts were immunoprecipitated with an anti-HA antibody. As shown in Fig. 5C, the HA-GSK3β immunocomplexes contained the AR (lane 3), suggesting that AR interacts with GSK3β in the COS-1 cells. HA-tagged GSK3β was also observed in the immunocomplexes pulled down with an anti-AR antibody (data not shown). Next we used LNCaP cells, which express endogenous AR and GSK3β, to examine whether GSK3β interacts with AR physiologically. As demonstrated in Fig. 5D, GSK3β forms a stable complex with AR, suggesting that GSK3β can interact with AR in the same cell and that AR could be a substrate for GSK3β in vivo.

GSK3β Suppresses Androgen/AR-induced Cell Growth—As previous reports revealed that androgen/AR might play important roles in the initiation and progression of prostate cancer, we wanted to know whether the suppression of AR by GSK3β could modulate prostate cancer cell growth. We introduced the inducible constitutively active form of GSK3β (S9A-GSK3β) into the androgen-dependent CWR22R cell line by stable transfection. To distinguish exogenously transfected GSK3β from endogenous GSK3β in CWR22R cells, a Myc-tagged S9A-GSK3β was constructed in the pBIG vector. Doxycycline stimulated the constitutively active S9A-GSK3β expression in CWR22R-S9A-GSK3β cells but not in the vector-transfected CWR22R-pBIG cells (Fig. 6A). Using a luciferase reporter assay, we found that induction of the constitutively active S9A-GSK3β reduced AR transactivation by 30%, while doxycycline had a marginal effect on CWR22R-pBIG cells in the presence of DHT (Fig. 6B). This effect likely represents an underestimate of the total impact of GSK3β on AR activity since CWR22R cells express endogenous GSK3β. To correlate the inhibitory effect of GSK3β on AR with prostate cancer cell growth, the growth of stably transfected CWR22R cells was tested in an MTT assay. The MTT assay (Fig. 6C) shows that addition of DHT induced cell growth in both CWR22R-pBIG and CWR22R-S9A-GSK3β cells. As expected, the doxycycline treatment caused obvious growth arrest in the CWR22R-S9A-GSK3β cells but not in the CWR22R-pBIG cells, suggesting that GSK3β may repress AR-mediated cell proliferation. Taken together, these data indicate that activation of GSK3β inhibits AR transcriptional activity and correlates with the reduced cell growth.

Reduction of the Interaction between the NH₂ and COOH Termini of AR—One potential mechanism through which GSK3β may inhibit AR transactivation is by altering the level of AR expression. To address this issue, AR expression was measured by immunoblot in LNCaP cells transfected with the pCMV vector or with pCMV-GSK3β. As shown in Fig. 7A, little change was seen in the endogenous expression of AR in LNCaP cells. In addition, AR localization was not altered by expressing S9A-GSK3β in LNCaP cells (data not shown). Similar data were observed in transiently transfected COS-1 and in stably transfected CWR22 cells. These data therefore suggest that GSK3β suppression of AR transactivation is not through alteration of endogenous androgen receptor stability or its nucleocytoplasmic distribution.

We then studied the potential GSK3β effect on the interaction between the NH₂ and COOH termini of AR (AR N-C interaction) as early studies indicated that AR N-C interaction plays important roles for the AR transactivation. We used a mammalian two-hybrid system to study the effects of GSK3β on AR N-C interaction. GALA-AR-LBD and VP16-AR-N plasmids were transfected into COS-1 cells. As shown in Fig. 7B, addition of GSK3β inhibited the interaction of AR NH₂ termi-
FIG. 3. Suppression of AR transactivation and PSA expression by GSK3β in LNCaP cells. A, the LNCaP cells were transfected with WT GSK3β for 3 h followed by DHT treatment for 18 h. Transactivation was measured by luciferase activity using MMTV-Luc as a reporter. B, the LNCaP cells were transfected with WT GSK3β for 3 h followed by DHT treatment for 18 h. PSA expression was measured by luciferase activity using PSA-Luc as a reporter. C, Northern blot analysis of PSA and β-actin expression in LNCaP cells treated with DHT and GSK3β for 18 h. The data are means ± S.D. from three independent experiments. B, overexpression of GSK3β represses PSA promoter activity. Experiments were performed and analyzed as described in A using PSA-Luc instead of MMTV-Luc reporter. C, inhibition of AR target gene PSA expression by GSK3β. LNCaP cells were transfected with WT GSK3β (lanes 3 and 4) or vector (lanes 1 and 2). The cells were treated with ethanol or 10 nM DHT for 18 h as indicated. Total RNA was isolated, and the levels of PSA and β-actin were monitored by Northern blot assay (upper panel). The relative mRNA levels are presented as the ratio of PSA mRNA/β-actin mRNA, and values of bars represent the mean ± S.D. of three similar experiments (upper panel). (*, p = 0.019 versus lane 2, two-tailed Student’s t test).
regarding the stimulation of prostate cancer cell growth by growth factors and cytokines and fit very well with the pro-apoptotic roles of GSK3β in other tissues (16, 44, 45).

Numerous studies have suggested potential links between the androgen/AR and GSK3β signaling pathways. First, testosterone, but not estrogen, prevents the heat shock-induced over-activation of GSK3β, suggesting that androgen may display a neuroprotective effect against Alzheimer’s disease (46). Second, GSK3β plays a pivotal role in the degradation of the free cytoplasmic β-catenin, an AR coregulator, through the ubiquitin proteasome pathway (25). Recent studies indicate that disregulation of β-catenin expression is found in a variety of human malignancies, including prostate cancer in which β-catenin may act as a coactivator of AR (47). Third, GSK3β also phosphorylates c-Myc and cyclin D1, resulting in ubiquitin-mediated degradation (20, 48). This is relevant in that

![Phosphorylation of the NH2 terminus of AR and suppression of AF-1 activity by GSK3β](image-url)

**Fig. 4. Phosphorylation of the NH2 terminus of AR and suppression of AF-1 activity by GSK3β.** A, schematic representation and results of purified AR fragments. GST-ARN, GST-AR-DBD-LBD (GST-AR-DL) and His6-AR-LBD cover various domains of AR as indicated. The GST, GST-ARN, GST-AR-DL, and His-tagged AR-LBD proteins were purified according to the manufacturer’s protocol. B, the in vitro kinase assays were performed as described under “Experimental Procedures.” Purified GSK3β was obtained from Upstate Biotechnology, and reactions were performed for 30 min at 30°C in the presence of 10 μCi of [γ-32P]ATP and 10 μM ATP. The reactions were terminated by addition of 4× SDS sample buffer. The samples were boiled and loaded on 20% SDS-polyacrylamide gels followed by autoradiogram. Arrows indicate the phosphorylated AR fragments. C, COS-1 cells were transfected with the indicated plasmids and pG5-Luc reporter. Transfected cells were cultured for 24 h before the luciferase activities were measured. The data are means ± S.D. from three independent experiments.
elevated cyclin D1 and c-Myc levels may be associated with prostate cancer progression (49–51).

Recent studies also demonstrate that GSK3β may regulate AR activity through an AR coactivator, β-catenin. Our study raises the possibility that GSK3β directly influences AR activity independent of the β-catenin-mediated pathway. The interaction between AR and β-catenin is DHT-dependent, and our data demonstrate that the inhibition of GSK3β by LiCl
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Fig. 7. Effect of GSK3β on the AR N-C interaction. A, GSK3β does not change AR protein amount. COS-1 cells were transfected with WT GSK3β or mock vector as indicated. After a 24-h transfection, 50 μg of whole cell extract was immunoblotted with AR antibody (NH27). B, inhibition of the interaction between the NH2 and COOH termini of AR by GSK3β. The COS-1 cells were transfected with GAL4-AR-LBD (amino acids 624–918) and VP16-AR-N (amino acids 1–556). The interactions between the COOH and NH2 termini of AR were determined by luciferase assay by using pG5-Luc as a reporter. C, the reconstituted AR transcription assays were performed to determine the association of the AR NH2 terminus with the AR-DBD-LBD. COS-1 cells were transfected with MMTV-Luc, pCDNA3-AR-N (amino acids 1–556), pCDNA3-AR-DBD-LBD (amino acids 556–919), and GSK3β as indicated. After transfection, cells were treated with 10 nM DHT or 25 mM LiCl for 24 h before harvesting. The LUC activity relative to lane I was calculated, and results are the mean ± S.D. of three independent experiments.

increases AR transcriptional activity in the absence of DHT. Also, several factors that inhibit GSK3β, such as insulin-like growth factor-1 and insulin, do not stabilize β-catenin or stimulate β-catenin-dependent gene transcription (32). This observation argues for the direct effect of GSK3β on AR. Moreover, β-catenin enhances AR activity through interaction with the AR-LBD, which contains the AF-2 domain. Our data suggest that AF-1 activity, but not that of AF-2, is reduced by GSK3β (Fig. 4). Furthermore, GSK3β directly phosphorylates the NH2-terminal region of AR. Our GST pull-down assay and co-immunoprecipitation assay indicate the interaction between GSK3β and AR (Fig. 5A). Together, these lines of evidence indicate that GSK3β and β-catenin may affect the AR at different levels and that the inhibition of GSK3β followed by elevated β-catenin levels may cooperate to enhance AR activity and lower the requirement for androgen in prostate cancer cells.

Although we favor the hypothesis that AR phosphorylation and the resulting inhibition of AR activity contribute to the blockade of DHT-induced cell growth induced by activated GSK3β (Fig. 6), we cannot rule out the possibility that the phosphorylation of a variety of other substrates by GSK3β might influence cell growth. For example, by inhibiting GSK3β, growth factors might promote the dephosphorylation and stabilization of cyclin D1 and c-Myc (20, 48, 53). Elevated cyclin D1 might enhance the activities of cyclin-dependent protein kinases CDK4 and CDK6, resulting in the inactivation of the retinoblastoma gene and entry into the S phase of the cell cycle. c-Myc is known to stimulate prostate cancer cell proliferation and survival as shown in many reports (54, 55). GSK3β is also known to phosphorylate c-Jun, resulting in inhibition of the DNA binding of this transcription factor that has been implicated in cell growth, differentiation, and development (56, 57). Active GSK3β, therefore, is implicated as a key factor in maintenance of the basal states of several important signaling pathways, and disregulation of GSK3β may lead to transformation to malignancy.

In summary, our data demonstrate that AR might be a substrate for GSK3β, and that GSK3β negatively regulates AR-mediated gene transcription to modulate androgen AR-mediated cell growth. Our study raises the possibility that this pathway may function in the progression of prostate cancer to androgen independence. These findings suggest potential approaches for the development of pharmacological inhibitors designed to increase GSK3β activity, an effect that may be useful for prostate cancer therapy.

Acknowledgments—We thank Dr. Junichiro Sadoshima for the generous gifts of plasmids. We also thank Loretta L. Collins and Karen Wolf for grammar and vocabulary corrections.

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J. Biol. Chem. 2004, 279:32444-32452. doi: 10.1074/jbc.M313963200 originally published online June 3, 2004

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