AD_____

Award Number: DAMD17-03-1-0090

TITLE: PTEN Regulates Beta-Catenin in Androgen Signaling:
Implication in Prostate Cancer Progression

PRINCIPAL INVESTIGATOR: Zijie Sun, Ph.D.

CONTRACTING ORGANIZATION: Leland Stanford Junior University
Stanford, California 94305-4125

REPORT DATE: March 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 2006	Annual (1 Mar 05 – 28 Feb 06)

4. TITLE AND SUBTITLE
PTEN Regulates Beta-Catenin in Androgen Signaling: Implication in Prostate Cancer Progression

5. FUNDING NUMBERS
DAMD17-03-1-0090

6. AUTHOR(S)
Zijie Sun, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Leland Stanford Junior University
Stanford, California 94305

E-Mail: zsun@stanford.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The androgen-signaling pathway is essential in male sexual development and in normal and malignant prostate cell growth and survival. PI3K/Akt plays a critical role in prostate cancer cell growth and survival. Recent studies demonstrate that the effect of PI3K/Akt in prostate cells is mediated through androgen signaling. The PI3K inhibitor, LY294002, and a tumor suppressor, PTEN, negatively regulate the PI3K/Akt pathway and repress the androgen receptor (AR) activity. However, the molecular mechanisms whereby PI3K/Akt and PTEN regulate the androgen pathway are currently unclear. During this funding year, we continue examining whether β–catenin is a major downstream effector of the PI3K/Akt and PTEN pathways in androgen-induced cell growth. Several sets of in vivo and in vitro experiments have been performed in this regard. The results suggest that the interactions between PI3K, Wnt, and androgen pathways are the key events in the tumorigenesis of prostate cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
PTEN, Akt, PI3K, androgen receptor, prostate cancer	33
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover……1

SF 298……2

Table of Contents……3

Introduction……4

Body……4-5

Key Research Accomplishments……5

Reportable Outcomes……5

Conclusions……6

References……7

Appendices……8-

INTRODUCTION: Prostate cancer is the most common malignancy in men and the second leading cause of cancer death in the United States (Landis et al., 1999). The androgen signaling pathway, which is mainly mediated through the androgen receptor (AR), is essential for the normal and neoplastic development of prostate cells (Balk, 2002; Gelmann, 2002). However, in contrast to other tumors, the molecular events involved in the development and progression of prostate cancer remain largely unknown. Androgen ablation is an effective treatment for the majority of advanced prostate cancer patients (Kyprianou and Isaacs, 1988). The phosphatidylinositol 3-kinase (PI3K) pathway consists of regulatory (p85) and catalytic (p110) subunits and has been implicated in the androgen-mediated prostate cell growth and survival (Li et al., 2001). PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor gene acts as an inhibitor of the PI3K to hydrolyze the lipid products of PI3K (Cantley and Neel, 1999). Loss of PTEN in prostate cancer cells results in the constitutive activation of enzymes downstream of PI3K, including the Akt protein-Ser/Thr kinase (Li et al., 1997). PI3K/Akt have been shown to promote prostate cancer cell survival and growth via enhancing AR-mediated transcription (Li et al., 2001). Both PTEN and the PI3K inhibitor, LY294002, negatively regulate this process (Li et al., 2001). Although several potential mechanisms have been suggested for this crosstalk, the precise molecular basis by which PI3K/AKT and PTEN regulate AR-mediated transcription is currently unclear. In addition, recent studies have shown that Wnt ligands and their receptors, Frizzled, activate different intracellular cascades through either the 'canonical' or 'non-canonical' pathways in various tumor cells (Nusse, 2003). In the past year, we performed several experiments to further investigate the interaction between PI3K, Wnt, and androgen signaling pathways in the growth of prostate cancer cells. We believe that the results from our current studies should provide fresh insight into the pathogenesis of prostate cancer that will help us identify new pathways that can be targeted for prostate cancer treatment.

BODY: In the past year, we mainly focused on investigating the role of the 'canonical' and 'non-canonical' pathways that are mediated by Wnt growth factors and their interactions with PI3K signaling in prostate cancer cells. As one of the principal physiological substrates of Akt, GSK3 is a ubiquitously expressed protein serine/threonine kinase that was initially identified as an enzyme that regulates glycogen synthesis in response to insulin (Cross et al., 1995; Welsh et al., 1994). It has been shown that GSK3β plays an important role in the Wnt pathway by regulating degradation of β-catenin (Behrens, 2000; Orford et al., 1997). Our previous studies have shown that the repression of AR activity by LY294002 is mediated through phosphorylation and inactivation of GSK3β, a downstream substrate of PI3K/Akt, which results in the nuclear accumulation of β-catenin, suggesting a novel mechanism by which PI3K/Akt modulates androgen signaling. In the past year, we further investigated the potential mechanisms of how Wnt and PI3K/Akt pathways interact with each other and the biological consequence of the regulation in the tumorigenesis of prostate cancer. In the past three years, we have completed Specific Aims 3, and made satisfactory progress in Specific Aims 1 and 2. However, we also encountered some difficulties in experiments that were originally proposed in Specific Aim 1. Particularly, we are facing some challenge in the study of potential roles of the 'non-canonical' pathway in the interaction between Wnt and PI3K/Akt signaling pathway. Therefore, we have been granted a 12-month no cost extension for this award. We hope that results from our study will provide fresh insight into this unclear but very important field.

Objective 1. Determine the role of PI3K pathway in β-catenin mediated cell growth.

As described in our original proposal, one aspect of the hypothesis being examined in the study is whether PI3K/Akt pathway directly regulates β–catenin in the augmentation of AR-mediated cell growth. In the previous years, we have provided several lines of evidence towards this direction. We have generated several LNCaP sublines that were stably transfected with wild type or mutated β–catenin expression vectors. Interestingly, these cells appeared to grow much slower than the cells that were transfected with the pcDNA3 vector, used as the control. In order to confirm our observation, we have generated several tetracycline-inducible LNCaP cell lines, which stably express wild type or mutated β–catenin genes. In our pilot experiments, we observed decreased incorporation in the cells transfected with wild type and mutated β–catenin after the induction, which actually supports our previous observation and suggests a negative role of β–catenin in prostate cancer cell growth. To determine the potential mechanism(s) of the inhibitory role of β-catenin in prostate cancer cells, we repeated the above experiments and examined the expression of the β-catenin in the cells. Interestingly, RT-PCR showed a significant increase of β–catenin transcripts in induced cells but however both western and immunostaining assays only displayed a slight change in the β–catenin protein level. Currently, we are testing the stability of the β-catenin protein in induced LNCaP cells to determine whether other signaling pathways are involved in this regulation. The results from the above analysis should provide very useful information regarding the role of β-catenin in prostate cancer cells.

Objective 2: To determine whether LY294002 and PTEN regulate Tcf/LEF activity.

The canonical pathway mediated by the Wnt growth factors is transduced through β–catenin binding to Tcf/LEF family members to activate the promoters of the downstream target genes. The AR as a β-catenin binding protein can compete with the interaction between Tcf/LEF and β-catenin in prostate cancer cells. Previously, we have shown exogenous β-catenin has little effect on TOPFlash activity, which are consistent with the previous reports (Truica et al., 2001). Currently, we are in the process of confirming the above data.

Objective 3: Determine whether IGF is involved in β-catenin mediated enhancement of AR activity.

In the past years, we have successfully accomplished this objective and most of data were published in our recent manuscript [Meletios V and Sun ZJ. (2005). Beta-catenin Is Involved in Insulin-Like Growth Factor 1-Mediated Transactivation of the Androgen Receptor. *Mol. Endo*., 19:391-98.]

KEY RESEARCH ACCOMPLISHNETS:

1) Generate and establish several LNCaP sublines stably transfected with the wild type and mutated β-catenin constructs.
2) Confirm the negative effect of β-catenin in prostate cancer growth.
3) Further demonstrate that Tcf/LEF can compete with AR for β-catenin in prostate cancer cells.

REPORTABLE OUTCOMES:

Publications:

Verras M and **Sun ZJ** "Roles and regulation of Wnt signaling and beta-catenin in prostate cancer." *Cancer Lett* 2005; [Epub ahead of print]

Huang CY, Beliakoff J, Li X, Lee J, Li X, Sharma M, Lim B, **Sun ZJ** (2005). hZimp7, A Novel PIAS-like Protein, Enhances Androgen Receptor-mediated Transcription and Interacts with SWI/SNF-like BAF Complexes. *Mol Endo,* 19(12): 2915-29.

CONCLUSIONS: Recently, several lines of evidence showed that both Wnt/β-catenin and PI3/Akt pathways play a critical role in cell proliferation and survival. The goal of this study is to determine the molecular mechanisms by which PI3K and PTEN regulate β-catenin in the androgen signaling pathway in prostate cancer cells and the biological consequences of this regulation. In the past year, we performed several sets of experiments in order to achieve our goals. Particularly, we collected several solid lines of evidence showing the effect of β-catenin in the growth of prostate cancer cells. In addition, we also further confirmed the observation by others that TCF/LEF family members are inactive in prostate cancer cells. Based on the observations, we started to investigate the non-canonical pathway in prostate cancer cells. We hope that through these efforts we can gain more information about the interaction between PI3K/Akt, IGF1, and androgen signaling pathways.

REFERENCES:

Balk, S.P. (2002) Androgen receptor as a target in androgen-independent prostate cancer. *Urology*, **60**, 132-138; discussion 138-139.

Behrens, J. (2000) Cross-regulation of the Wnt signalling pathway: a role of MAP kinases. *J Cell Sci*, **113**, 911-919.

Cantley, L.C. and Neel, B.G. (1999) New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci U S A*, **96**, 4240-4245.

Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M. and Hemmings, B.A. (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature*, **378**, 785-789.

Gelmann, E.P. (2002) Molecular biology of the androgen receptor. *J Clin Oncol*, **20**, 3001-3015.

Kyprianou, N. and Isaacs, J.T. (1988) Activation of programmed cell death in the rat ventral prostate after castration. *Endocrinology*, **122**, 552-562.

Landis, S.H., Murray, T., Bolden, S. and Wingo, P.A. (1999) Cancer Statistics. *CA. Cancer J. Clin*, **49**, 8-31.

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S.H., Giovanella, B.C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M.H. and Parsons, R. (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science*, **275**, 1943-1947.

Li, P., Nicosia, S.V. and Bai, W. (2001) Antagonism between PTEN/MMAC1/TEP-1 and Androgen Receptor in Growth and Apoptosis of Prostatic Cancer Cells. *J Biol Chem*, **276**, 20444-20450.

Nusse, R. (2003) Wnts and Hedgehogs: lipid-modified proteins and similarities in signaling mechanisms at the cell surface. *Development*, **130**, 5297-5305.

Orford, K., Crockett, C., Jensen, J.P., Weissman, A.M. and Byers, S.W. (1997) Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. *J Biol Chem*, **272**, 24735-24738.

Truica, C.I., Hsiung, G., Voeller, H.J. and Gelmann, E.P. (2001) beta-catenin mutation are not sufficient to activate Wnt signaling in prostate cells. *AACR Annual Meeting Proceedings*, **42**, 693.

Welsh, G.I., Foulstone, E.J., Young, S.W., Tavare, J.M. and Proud, C.G. (1994) Wortmannin inhibits the effects of insulin and serum on the activities of glycogen synthase kinase-3 and mitogen-activated protein kinase. *Biochem J*, **303**, 15-20.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Cancer Letters xx (2005) 1–11
CANCER Letters
www.elsevier.com/locate/canlet

Mini-Review

Roles and regulation of Wnt signaling and β-catenin in prostate cancer

Meletios Verras, Zijie Sun*

Department of Urology and Department of Genetics, Stanford University School of Medicine, 300 Pasteur Dr, Grant Bldg. S287, Stanford, CA 94305-5328, USA

Received 30 May 2005; accepted 3 June 2005

Abstract

The Wnt signaling pathway and its key component β-catenin play critical roles in embryonic development as well as in human diseases, including various malignancies. Accumulated evidence has demonstrated a significant role for the Wnt pathway in the development and progression of human prostate cancer. The recent discovery of an interaction between β-catenin and the androgen receptor (AR) suggests a possible mechanism of cross talk between Wnt and androgen signaling pathways. In this review, we summarize the recent progresses in this interesting and growing field. Particularly, we focus on the observation that the activation of the Wnt-mediated signal occurs in a different manner in prostate cancer than in colorectal cancer or other human malignancies. Since mutations in Adenomatous polyposis coli (APC), β-catenin, and other components of the β-catenin destruction complex are rare in prostate cancer cells, other regulatory mechanisms appear to play dominant roles in the activation of β-catenin, such as loss or reduction of E-cadherin, a component of cell adhesion complex, and abnormal expression of Wnt ligands, receptors, inhibitors, and other co-regulators. Understanding the role and regulation of the Wnt signaling pathway in prostate cancer cells may help identify new targets for the prostate cancer therapy.

Keywords: Wnt; β-catenin; Frizzled; Androgen receptor; Prostate cancer; E-cadherin; PI3K; Akt

1. Introduction

The long-term ineffectiveness of current treatments for prostate cancer has spurred an increased interest in understanding the mechanisms of prostate cancer tumorigenesis. Androgens are critical for inducing normal differentiation of reproductive organs and for the development and progression of prostate cancer [1]. The effects of androgens are mediated by the androgen receptor (AR), which belongs to the nuclear hormone receptor superfamily [2–4]. Although depletion of androgens via androgen ablation has a temporary repressive effect on tumor growth, most prostate cancer patients eventually develop androgen-insensitive tumors, for which there is no effective therapy [5,6]. Intriguingly, AR is expressed in most prostate cancer samples before and after androgen ablation therapy, which underscores the significance

* Corresponding author. Tel.: +1 650 498 7523; fax: +1 650 723 4200.
E-mail address: zsun@stanford.edu (Z. Sun).

0304-3835/$ - see front matter © 2005 Published by Elsevier Ireland Ltd.
doi:10.1016/j.canlet.2005.06.004

of AR in prostate cancer pathogenesis [7]. Although the mechanisms by which prostate cancer cells become androgen-insensitive are currently unclear, it is believed that the tumor cells must either bypass or adapt the AR pathway in order to survive in a low androgen environment [8]. Several models have been proposed to explain the pathogenesis of androgen-insensitive (AI) prostate cancer, including *AR* gene amplification, AR mutations, aberrant expression or function of AR co-factors, and activation of the receptor by other signaling pathways [8–10].

The Wnt signaling pathway plays significant roles in various processes of early development and the pathogenesis of human diseases, including human malignancies [11–13]. There are 19 closely related Wnt genes that have been identified in humans (for a list see the Wnt Homepage http://www.stanford.edu/~rnusse/wntwindow.html). Their primary receptors are the seven-transmembrane Frizzled proteins, each of which interacts with a single transmembrane LDL receptor-related protein 5/6 (LRP5/6) [14,15]. A number of different secreted proteins, such as secreted frizzled-related proteins (sFRP), Wnt inhibitory factor-1 (WIF1), and Dickkopf (Dkk) prevent ligand-receptor interactions and thus inhibit the Wnt signaling pathway [16]. Wnt proteins activate different intracellular signaling pathways through either the 'canonical' or the 'non-canonical' pathway [17]. In the canonical signaling pathway, secreted Wnt ligands bind to Frizzled proteins and regulate the stability of β-catenin, a key component of Wnt signaling [18]. In the absence of a Wnt signal, β-catenin is constitutively down-regulated by a multicomponent destruction complex containing glycogen synthase kinase 3β (GSK3β), axin, and APC, which promotes phosphorylation on serine and threonine residues in the N-terminal region of β-catenin following 'priming' phosphorylation of Ser45 by Casein Kinase I (CKI), and thereby targeting it for degradation via the ubiquitin proteasome pathway [19–22]. Wnt signaling inhibits this process, leading to the accumulation of β-catenin in the nucleus, in which β-catenin forms transcriptionally active complexes with members of the LEF/TCF family of transcription factors [23]. Non-canonical pathways are alternative modes of Wnt signaling, which do not involve β-catenin. A well characterized example of non-canonical Wnt signaling is the Drosophila planar cell polarity (PCP) pathway [24]. Non-canonical signaling may also be involved in other processes during development [25,26]. Some studies have suggested that either the N-Jun N-terminal kinase (JNK) pathway or modulation of intracellular calcium are potential downstream mediators for non-canonical signaling [27–29]. However, the direct involvement of these pathways remains unclear, since neither has been shown to be consistently activated in response to non-canonical Wnt signals in mammalian cell systems.

The identification of β-catenin, as an AR cofactor, substantiated a possible role of Wnt signaling in prostate cancer development and progression [30–33]. This review centers on the growing evidence implicating the role of the Wnt signaling and β-catenin in the regulation of prostate cancer tumorigenesis, and the interaction between Wnt, androgen and other signaling pathways.

2. Wnt growth factors and frizzled receptors in prostate cells

Intense efforts have been made recently to examine the expression of Wnt growth factors and their receptors in prostate cell lines and prostate tissues. Wnt-1 appears to be expressed at low levels in human primary prostate epithelial cells [34]. However, up-regulation of Wnt-1 expression has been observed in several prostate cancer cell lines and prostate cancer tissues, and particularly in lymph node and bone metastases specimens of prostate cancer patients [34]. The expression of Wnt-1 was positively correlated to Gleason scores, as well as to the cellular level of β-catenin and serum PSA levels. Elevated expression of Wnt-5A and Wnt-2 was observed in prostate cancer tissues over normal samples [35,36], while Wnt-7B and Wnt-5B and Wnt-13 were found to be expressed in normal prostate tissues [37–39]. In a recent study, it was also observed that the majority of Wnt genes are predominantly expressed in epithelial cells, while Wnt-2 is preferentially expressed in stromal cells [40].

Several Wnt ligands, such as Wnt-5a, Wnt-11, can induce non-canonical pathways, which are alternative modes of Wnt signaling and are independent of β-catenin [17]. Recent data have shown an up-regulation of Wnt-11 in prostate cancer samples with Gleason grade 7, or above, in comparison to

nonmalignant or low Gleason grade samples [40]. An increase in Wnt-11 expression was also observed in androgen-independent CWR22 xenograft tumor models [40]. In LNCaP cells, Wnt-11 repressed AR-mediated transcription and reduced androgen-dependent growth. In addition, LNCaP cells cultured in androgen-depleted media showed an increase of Wnt-11 expression, while the addition of the synthetic androgen R1881 repressed the expression in a dose-dependent manner [40]. The regulation of Wnt-11 by androgens appears to involve AR since the effect of R1881 can be blocked by the antiandrogen, Casodex [40].

Characterization of the Frizzled receptors, was also carried out in prostate cell lines and tissues. Transcripts of Frizzled-1, -4, -6, and -10 were detected in normal prostate tissues by conventional Northern blot analyses [41–43]. Using cDNA microarray approaches, Wissmann et al. recently performed a systematic and detailed study to assess the expression of Wnt signaling components and putative Wnt targets in prostate cancer cells. The expression of sFRP4, Frizzled-4, Frizzled-6, Dishevelled-1, TCF4 and MYC was increased, while the expression of Wnt-2, WIF1, β isoform of the catalytic subunit of protein phosphatase 2A (PPP2CB), Cyclin D2 (CCND2) and CD44 was reduced in tumor cells [44]. These changes were further confirmed by immunohistochemistry in prostate cancer samples. Particularly, a reduction of WIF1 protein levels was observed in 23% of prostate cancer samples [44]. However, no significant association was observed between WIF1 down-regulation and the stage or grade of prostate tumors, suggesting that reduction of WIF1 expression may be a very early event in prostate cancer development [44].

Despite the accumulating data identifying expression of Wnt ligands and receptors in prostate cancer cells, the role and regulation of Wnt growth factors and their receptors in normal and malignant prostate cells remains largely unknown. Studies in other tissues and organisms have implicated the critical roles of Wnt signaling in the regulation of various cellular events through the canonical and noncanonical pathways [13]. Recently, using Wnt-3a conditioned medium (Wnt-3a CM) [45], we explored the biological role of Wnt-3a in prostate cancer cells [46]. Wnt-3a CM showed a significant enhancement on AR-mediated transcription of both an ectopically expressed 7Kb PSA promoter reporter and on the endogenous PSA gene in the absence or presence of low concentration of androgens in LNCaP cells. A similar induction by Wnt3a was also observed on the promoter/reporter containing a minimal promoter with two androgen responsive elements (AREs). Knockdown of AR expression by a specific shRNA and inhibition of AR function by an AR antagonist, bicalutamide, significantly reduced the induction of AR-mediated transcription by Wnt-3a CM, suggesting that the AR is the direct target of Wnt-3a mediated induction. Most interestingly, Wnt-3a CM also enhanced the growth of prostate cancer cells in an androgen independent manner. Given the fact that β-catenin is an AR co-activator, we further assessed the possible role of β-catenin in Wnt-3a mediated induction of AR activity. Our findings suggested that β-catenin is involved in the induction of AR-mediated transcription and cell growth by Wnt-3a. Furthermore, purified Wnt-3a, like Wnt-3a CM, also enhanced cell proliferation and colony formation in LNCaP cells [46].

3. Interaction between AR and β-catenin

Although a central role of β-catenin has been described in a variety of human tumors, the biological significance of β-catenin in prostate cancer cells was actually exposed by the discovery of a protein-protein interaction between AR and β-catentin. Truica and colleagues first demonstrated that β-catenin interacts with AR and enhances AR transcriptional activity in LNCaP cells [31]. Several other groups confirmed the interaction using yeast two-hybrid and in vivo and in vitro protein binding assays [30,33,47]. Androgens were shown to enhance the interaction, and the ligand binding domain (LBD) of AR was mapped to be responsible for the binding [33]. An attempt to define the small region and motifs in the AR LBD responsible for this interaction has been made recently [48]. However, since mutations of these motifs within the LBD also diminish the ligand binding activity of the AR, it is difficult to define whether these motifs primarily affect ligand or β-catenin binding. Results from earlier experiments have shown that β-catenin, unlike the steroid hormone receptor 1 (SRC1), preferentially binds AR over several other receptors,

including the estrogen receptor, progesterone receptor, and glucocorticoid receptor [33]. In addition, early mapping experiments suggested that the NH2 terminus and the first six armadillo repeats of β-catenin were involved in the interaction with AR. Particularly, deletion of repeat 6 fully abolished the physical interaction between AR and β-catenin, suggesting a key role of this repeat in the interaction [33]. Post translational modifications of β-catenin may affect its affinity and preference for binding partners, including the AR. It was shown that acetylation of β-catenin on lysine K345, located within armadillo repeat 6, decreases the binding affinity of β-catenin for the AR, but enhances its interaction with Tcf-4 [49]. The central armadillo domain of β-catenin contains five LXXLL motifs, which are responsible for binding to a hydrophobic cleft in the activation domain 2 (AF2) of nuclear receptors [50]. However, structural analysis revealed that the leucine residues in these motifs are buried in the hydrophobic core of the armadillo repeats, which is consistent with previous reports that mutation of these motifs does not affect the binding of β-catenin to the AR [33,48].

The dynamics of the AR-β-catenin interaction were also studied. Subcellular colocalization of the AR and β-catenin has been reported and both endogenous and exogenous AR can enhance the nuclear translocation of β-catenin in the presence of androgens [30]. Although the precise mechanism for the androgen-dependent nuclear translocation of β-catenin is unclear, it seems to be APC-and GSK3β-independent. The biological consequence of the AR-β-catenin interaction and AR-mediated translocation of β-catenin has also been investigated. Several lines of evidence have shown that β-catenin can act as an AR co-activator and enhance its transcriptional activity. In transient transfection assays, overexpression of β-catenin augments AR-mediated transcription of several AR-regulated promoters in both prostate and non-prostate cells [30,33,47,48,51]. Reduction of cellular levels of β-catenin by antisense or shRNA constructs decreases the expression of the *PSA* gene, a downstream target of AR. In addition, β-catenin-mediated enhancement of AR transcriptional activity can be completely abolished by deletion of β-catenin armadillo repeat 6 [33]. All of the above data indicate that through a protein-protein interaction, β-catenin can co-localize with AR in the nucleus and enhance AR-mediated transcription.

The biological significance of the AR-β-catenin interaction in the progression of prostate cancer has been recently explored. It has been shown that the interaction with β-catenin can change the sensitivity and the specificity of the receptor binding to ligands [31]. AR-mediated transcription is enhanced by the expression of β-catenin in the presence of 17β-estradiol or androstenedione, a form of adrenal androgen. Hydroxyflutamide, an AR antagonist, can act as an agonist to enhance the recruitment and transcriptional activity of the T877A AR mutant in LNCaP cells [52,53]. β-catenin can also enhance the transcriptional activity of a W741C AR mutant in a bicalutamide-stimulated LNCaP subline. In contrast, the AR antagonists, L-39 and cyproterone acetate (CPA), which each activate the T877A AR mutant, do not appear to affect the interaction between AR and β-catenin, suggesting that these antagonists may modulate AR activity through different pathways. Mifepristone, an antagonist of steroid receptors, was recently shown to act also as an antiandrogen and inhibit R1881-induced binding of wild type or T877A AR mutant to β-catenin [53].

Although multiple lines of evidence have shown that β-catenin can interact with AR and enhance AR-mediated transcription, it is not clear whether endogenous β-catenin can form a tanscriptional complex with AR on androgen-regulated promoters. Recently, using the chromatin immunoprecipitation assay (ChIP), Li and colleagues demonstrated that β-catenin can be recruited to the endogenous promoter region of *PSA* gene [54]. Although the exact mechanism by which β-catenin enhances the transcriptional activity of AR is not clear, several models have been proposed. The interaction between β-catenin and AR can possibly promote the recruitment of other co-regulators to AR-involved transcriptional complexes. β-catenin has been shown to interact with GRIP1, a nuclear hormone receptor coactivator, and enhances AR-mediated transcription [54]. In addition, CARM1, a histone methyltransferase, β-catenin, and p300 synergistically enhance AR transcriptional activity [55]. Finally, the LIM protein FHL2, an AR coactivator, has also been shown to interact with β-catenin and stimulate AR-mediated transcription in a synergistic manner with p300 [56].

4. Crosstalk between Wnt/β-catenin and other signaling pathways in prostate cells

4.1. IGF-1 signaling pathway in prostate cells

The insulin-like growth factor (IGF) system plays a significant role in regulating cell growth, apoptosis and invasion in different malignancies, including prostate cancer. Previous reports have shown that IGF-1 can enhance AR signaling, but the mechanisms of this regulation are unclear [57]. IGF-1 enhances tyrosine phosphorylation of β-catenin, resulting in dissociation of β-catenin from E-cadherin complexes and an increase in the cytoplasmic level of β-catenin [58]. IGF-1 was previously observed to induce AR-mediated transcription in a ligand-independent manner [57]. Recently, it was shown that both the addition of IGF-1 and overexpression of a constitutively active mutant of the IGF-1 receptor in the LNCaP prostate cancer cell line can enhance endogenous AR-mediated transcription in the presence of a low level of androgens [59]. As observed in other cancer cells, IGF-1 can enhance the stability of the β-catenin protein in prostate cancer cells [59]. These data suggest that β-catenin is involved in IGF-1-induced AR activation, defining an important role of β-catenin in the progression of prostate cancer.

4.2. PI3K/AKT and PTEN

Multiple lines of evidence have shown that PI3K/Akt signaling can promote prostate cancer cell survival and growth, and enhance AR-mediated transcription [60–62]. The tumor suppressor PTEN is frequently mutated in prostate cancer and negatively regulates PI3K/Akt function [63]. Several molecular mechanisms have been proposed to implicate PI3K/Akt regulated AR signaling in prostate cancer cells [60,62,64–66]. Intriguingly, recent studies have shown that PI3K/Akt can modulate the activity of β-catenin through GSK3β [58,62]. One of the principle substrates of Akt, GSK3β, phosphorylates β-catenin and regulates its cellular level [67]. In a PTEN-null prostate cancer cell line, LNCaP, overexpression of PTEN and the addition of the PI3K inhibitor, LY294002, repressed the phosphorylation of Akt and GSK3β, resulting in enhancement of β-catenin phosphorylation, which is known to facilitate ubiquitylation and proteasome-mediated degradation of β-catenin [62]. The above data elucidate a possible link between PI3K/Akt and the Wnt signaling pathway. Another level of interaction between Wnt and PI3K/Akt signaling pathways in prostate cancer cells was shown by the work of Ohigashi et al. [68]. They demonstrated that overexpression of the Wnt inhibitory factor, WIF-1, which is frequently downregulated in prostate cancer, could decrease the amount of phosphorylated GSK3β and the levels of soluble β-catenin in prostate cancer cell lines, PC3 and DU-145 [68]. In the same study, the overexpression of WIF-1 in the PTEN-null PC-3 cells reduced the levels of phosphorylated Akt. GSK3β may also affect the AR in a β-catenin-independent manner. Results from different labs have shown that the expression of GSK3β either enhances or represses AR-mediated transcription depending on the experimental conditions [69–72]. More mechanistic studies are needed to fully understand the regulation of AR-mediated transcription by GSK3β.

4.3. Interaction between AR and TCF/LEF transcriptional factors

Several reports have shown that ligand-bound AR can repress Tcf-mediated transcription in prostate cancer cells [73], neuronal cells [47], and colon cancer cells [74]. These reports suggest that the repression may result from a competition between the AR and TCF for β-catenin and other transcriptional cofactors. The biological significance of this phenomenon is currently not well understood. Although both signaling pathways appear to promote cell growth, the presence of androgen may favor the interaction between β-catenin and AR and enhance AR-mediated transcription and cell growth in prostate epithelial cells [74]. In contrast, androgen ablation may increase the pool of β-catenin available for activation of TCF/LEF target genes, which may lead to relapse.

Another possible route of cross talk between the AR and Wnt/β-catenin signaling was recently identified by the demonstration of a direct, β-catenin-independent, interaction between the AR and Tcf-4 [75]. The DNA binding domain of AR was found to be necessary and sufficient for the interaction with Tcf-4. In LNCaP cells, endogenous AR bound

a Tcf-4 response element in the c-myc promoter, which suggests that AR may be involved in the transcription regulation of certain TCF target genes.

5. The role of β-catenin in the tumorigenesis of prostate cancer

An increase in cellular levels of β-catenin resulting from mutations of β-catenin itself, or of components of the destruction complex, is frequently observed in many cancer cells, including prostate cancer. Detection of β-catenin mutations in prostate cancer samples has been reported previously [76–78]. Approximately 5% of samples revealed mutations at the serine or threonine residues in the NH2-terminal of the β-catenin protein [76–78]. Since the mutations occur focally, it was suggested that alteration of β-catenin may represent a late event in prostate cancer progression. Examination of the β-catenin protein by immunohistochemical assays revealed aberrant localization of the protein in prostate cancer specimens [34,51,79]. In one study, about 20% of hormone-refractory samples showed nuclear localization of β-catenin [51], while a more recent report observed even higher percentage (38.8%) [79]. Alterations of APC and β-TrCP1, which directly affect the degradation of β-catenin, have also been observed in prostate cancer samples [76].

The direct role of β-catenin in promoting prostate cancer cell growth has been examined recently. An LNCaP cell line stably transfected with a stabilized β-catenin mutant showed no significant advantage in cell growth either in the presence or absence of androgens in comparison to the parent line [51], even thought this mutant has been shown to augment AR-mediated transcription. It is unclear why this β-catenin mutant only enhances AR-mediated transcription but not cell growth in LNCaP cells. Thus, more studies in other prostate cancer cell lines are necessary to further determine the effect of β-catenin in androgen-mediated cell growth.

Despite studies of in vitro model systems, multiple lines of evidence collected in vivo implicate a role for β-catenin in prostate cell growth and prostate cancer tumorigenesis. Early studies have shown that castration of animals results in atrophy of prostate, while administration of testosterone to castrated mice restores the growth and prostate gland formation [6,80,81]. Interestingly, increased levels of nuclear β-catenin have been found in proliferating cells in the prostate, suggesting a potential role of β-catenin in androgen-induced prostate cell growth [51]. The biological role of β-catenin in the tumorigenesis of prostate cancer was further characterized using a transgenic mouse model [82]. Specific expression of a mutant β-catenin, lacking exon 3, in prostate tissues results in the development of prostate intraepithelial neoplasia (PIN), a precursor to prostate cancer, in mice ranging between 10-21 weeks of age [82]. However, using similar approaches, a different group observed hyperplasia of the prostate and squamus metaplasia but no PIN lesion in a similar animal model [83]. Although the precise mechanism of β-catenin in the development of prostate cancer is still unclear, the above data have provided a solid link between β-catenin and the pathogenesis of prostate cancer.

6. E-cadherin, β-catenin and prostate cancer

Like most other cancers, prostate carcinogenesis involves a multistep progression from precancerous cells to cells that proliferate locally and then metastasize. Although the majority of early-stage tumors are not life threatening, a subset of these tumors will give rise to metastatic disease and cancer-related death. Recent studies have shown that the regulation of E-cadherin expression may play a critical role in the transition from noninvasive to invasive phenotype in prostate cancer [84,85]. In normal epithelial tissues, E-cadherin complexes with the actin cytoskeleton via cytoplasmic catenins to maintain the functional characteristics of epithelia [86]. Disruption of this complex, primarily due to loss or reduced expression of E-cadherin, is frequently observed in advanced and poorly differentiated prostate cancer [87]. A strong correlation between a lack of E-cadherin and the metastatic and/or invasive potential of prostate cancer was first identified in the Dunning rat model [88]. The observation was corroborated by later studies in prostate cancer patients, which showed reduced or absent expression of E-cadherin in about half of the tumor samples examined [89]. There was a strong association

between aberrant expression of E-cadherin and an invasive and metastatic phenotype of human prostate cancers [89,90]. The interaction of E-cadherin with other proteins, including α-, β-, and γ-catenins, has also been shown to be required in cell-cell adhesion [91]. Moreover, loss of α-catenin expression has been observed in prostate cancer cell line, PC3 [92]. All evidence suggests that E-cadherin and other components of cell junction complexes play a pivotal role in homotypic cell-cell contacts that are directly linked to invasion and metastasis during prostate cancer progression.

Since the cytoplasmic domain of type I cadherins binds to β-catenin, a question has been raised as to whether the cadherin-bound pool of β-catenin can be released and participate in signaling. In order to answer this question, a series of studies have been performed to elucidate the dynamic interaction of β-catenin with cadherins [93]. Phosphorylation of cadherins or catenins affects the structural and functional integrity of the cadherin-catenin complex [94]. A number of receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR) and cytoplasmic tyrosine kinases, such as Fer, Fyn, Yes, and Src, are involved in modulating the cadherin–catenin complexes [58,95–100]. Based on current literature, serine/threonine phosphorylation of β-catenin or E-cadherin appears to increase the stability of the cadherin-catenin complex, whereas tyrosine phosphorylation of β-catenin results in a loss of cadherin-mediated cell-cell adhesion and an increase in the level of cytoplasmic β-catenin [93]. In addition, activation of protein tyrosine phosphatases (PTPases) enhances cell-cell adhesion by stabilizing the cadherin-catenin complex [101–103]. Furthermore, RON receptor tyrosine kinase and the receptor for the hepatocyte growth factor, cMET, can increase Tcf-mediated transcription by promoting tyrosine phosphorylation and accumulation of β-catenin [104].

A recent study attempted to determine if increased cytoplasmic and nuclear levels of β-catenin is a consequence of E-cadherin loss in the late stages of prostate cancer cells [105]. Reintroduction of E-cadherin to the E-cadherin negative cell line, TSU.Pr-1, shifted the subcellular localization of β-catenin from the cytoplasm to the cell membrane. The characterization of different truncation mutants of E-cadherin revealed that the extracellular domain is important for the cell–cell contacts, while the cytoplasmic domain is necessary for growth suppression. Previously, we demonstrated that the loss or reduction of E-cadherin expression enhances AR-mediated transcription by increasing the level of the cytoplasmic and nuclear β-catenin in TSU Pr-1. Taken together, the above data suggest that during the process of prostate cancer progression, loss of E-cadherin expression or activation of the Wnt pathway can lead to an increase in the cytoplasmic levels of β-catenin. The excess free β-catenin proteins translocate to the nucleus and specifically interact with the AR to induce androgen-mediated cell growth or survival. As an AR co-activator, β-catenin may also play a critical growth-promoting role by compensating for decreased androgen levels in response to androgen ablation therapy. Interestingly, no effect was observed on a TCF-induced promoter/reporter construct transfected in TSU Pr-1 cells. A similar observation was also reported recently in breast cancer cell lines, in which the E-cadherin gene is transcriptionally silenced [106]. This raises the question as to whether the growth-promoting effect of β-catenin in prostate cancer and other tumor cells is mediated through other partners than the TCF/LEF transcription factors.

7. Conclusion

The Wnt signaling pathway plays a critical role in embryogenesis and tumorigenesis. However, the biological roles of Wnt growth factors have not been fully characterized in prostate development and the pathogenesis of prostate cancer. The Wnt signaling pathway and its key component β-catenin have recently emerged as important players in prostate tumorigenesis. Particularly, recent evidence of a β-catenin-AR interaction provided a direct link between androgen signaling and the Wnt pathway. Since only a small percentage of prostate cancer samples possess mutations in the destruction complex and β-catenin itself, other possible mechanisms may be involved in activating the Wnt signal. E-cadherin forms a protein complex with β-catenin to control cell-cell adhesion and influence cell

migration. Recent data have shown that reduction and loss of E-cadherin, which normally sequesters β-catenin, increases the level of cytoplasmic and nuclear β-catenin, and consequently enhances AR activity, which may play a critical role in augmenting prostate cancer cell proliferation and survival during prostate cancer progression. Moreover, abnormal expression of Wnt ligands, receptors, and other co-regulators may also contribute to the pathogenesis of prostate cancer. Despite accumulating evidence that demonstrates a critical role for Wnt/β-catenin signaling in prostate cancer, many questions need to be further addressed, such as how Wnt signals are transduced in prostate cancer cells to induce cell growth and survival, what is the molecular mechanism by which β-catenin regulates AR in the development and progression of prostate cancer, and how does loss or reduction of E-cadherin affect AR activity in late stages of prostate cancer? Therefore, we anticipate that studying Wnt signaling, β-catenin and the crosstalk with androgen signaling and other pathways will help us to understand the pathogenesis of prostate cancer and to identify new therapeutic strategies for prostate cancer treatment.

Acknowledgements

We thank Jason Beliakoff, Dawn Bravo, Jane Lee, and Gregory Thyssen for critical reading this manuscript. This work was supported by National Institutes of Health Grants CA70297, CA87767, and DK61002, the Department of Army Prostate Cancer grants, and DAMD17-03-1-0090.

References

[1] J.M. Kokontis, S. Liao, Molecular action of androgen in the normal and neoplastic prostate, Vitam. Horm. 55 (1999) 219–307.
[2] C.S. Chang, J. Kokontis, S.T. Liao, Molecular cloning of human and rat complementary DNA encoding androgen receptors, Science 240 (1988) 324–326.
[3] E.P. Gelmann, Molecular biology of the androgen receptor, J. Clin. Oncol. 20 (2002) 3001–3015.
[4] D.B. Lubahn, D.R. Joseph, M. Sar, J. Tan, H.N. Higgs, R. E. Larson, et al., The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate, Mol. Endocrinol. 2 (1988) 1265–1275.
[5] H. Miyamoto, E.M. Messing, C. Chang, Androgen deprivation therapy for prostate cancer: current status and future prospects, Prostate 61 (2004) 332–353.
[6] N. Kyprianou, J.T. Isaacs, Activation of programmed cell death in the rat ventral prostate after castration, Endocrinology 122 (1988) 552–562.
[7] C.A. Heinlein, C. Chang, Androgen receptor in prostate cancer, Endocr. Rev. 25 (2004) 276–308.
[8] B.J. Feldman, D. Feldman, The development of androgen-independent prostate cancer, Nat. Rev. Cancer 1 (2001) 34–45.
[9] G. Jenster, The role of the androgen receptor in the development and progression of prostate cancer, Semin. Oncol. 26 (1999) 407–421.
[10] S.P. Balk, Androgen receptor as a target in androgen-independent prostate cancer, Urology 60 (2002) 132–138 (discussion 1387ndash;139).
[11] P. Polakis, The oncogenic activation of beta-catenin, Curr. Opin. Genet. Dev. 9 (1999) 15–21.
[12] P. Polakis, Wnt signaling and cancer, Genes Dev. 14 (2000) 1837–1851.
[13] C.Y. Logan, R. Nusse, The Wnt signaling pathway in development and disease, Annu. Rev. Cell Dev. Biol. 20 (2004) 781–810.
[14] X. He, M. Semenov, K. Tamai, X. Zeng, LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way, Development 131 (2004) 1663–1677.
[15] J.C. Hsieh, Specificity of WNT-receptor interactions, Front. Biosci. 9 (2004) 1333–1338.
[16] Y. Kawano, R. Kypta, Secreted antagonists of the Wnt signalling pathway, J. Cell Sci. 116 (2003) 2627–2634.
[17] P. Pandur, D. Maurus, M. Kuhl, Increasingly complex: new players enter the Wnt signaling network, Bioessays 24 (2002) 881–884.
[18] R. Nusse, Wnt signaling in disease and in development, Cell Res. 15 (2005) 28–32.
[19] S. Amit, A. Hatzubai, Y. Birman, J.S. Andersen, E. Ben-Shushan, M. Mann, et al., Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway, Genes Dev. 16 (2002) 1066–1076.
[20] M.J. Hart, R. de los Santos, I.N. Albert, B. Rubinfeld, P. Polakis, Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta, Curr. Biol. 8 (1998) 573–581.
[21] E. Latres, D.S. Chiaur, M. Pagano, The human F box protein beta-Trcp associates with the Cul1/Skp1 complex and regulates the stability of beta-catenin, Oncogene 18 (1999) 849–854.
[22] H. Aberle, A. Bauer, J. Stappert, A. Kispert, R. Kemler, Beta-catenin is a target for the ubiquitin-proteasome pathway, Embo J. 16 (1997) 3797–3804.
[23] Q. Eastman, R. Grosschedl, Regulation of LEF-1/TCF transcription factors by Wnt and other signals, Curr. Opin. Cell Biol. 11 (1999) 233–240.

[24] M.T. Veeman, J.D. Axelrod, R.T. Moon, A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling, Dev. Cell 5 (2003) 367–377.
[25] A. Barrallo-Gimeno, J. Holzschuh, W. Driever, E.W. Knapik, Neural crest survival and differentiation in zebrafish depends on mont blanc/tfap2a gene function, Development 131 (2004) 1463–1477.
[26] T.P. Yamaguchi, Heads or tails: Wnts and anterior-posterior patterning, Curr. Biol. 11 (2001) R713–R724.
[27] D.C. Slusarski, J. Yang-Snyder, W.B. Busa, R.T. Moon, Modulation of embryonic intracellular Ca2+ signaling by Wnt-5A, Dev. Biol. 182 (1997) 114–120.
[28] H. Yamanaka, T. Moriguchi, N. Masuyama, M. Kusakabe, H. Hanafusa, R. Takada, et al., JNK functions in the non-canonical Wnt pathway to regulate convergent extension movements in vertebrates, EMBO Rep. 3 (2002) 69–75.
[29] M. Boutros, N. Paricio, D.I. Strutt, M. Mlodzik, Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling, Cell 94 (1998) 109–118.
[30] D.J. Mulholland, H. Cheng, K. Reid, P.S. Rennie, C.C. Nelson, The androgen receptor can promote beta-catenin nuclear translocation independently of adenomatous polyposis coli, J. Biol. Chem. 277 (2002) 17933–17943.
[31] C.I. Truica, S. Byers, E.P. Gelmann, Beta-catenin affects androgen receptor transcriptional activity and ligand specificity, Cancer Res. 60 (2000) 4709–4713.
[32] D.R. Chesire, W.B. Isaacs, Beta-catenin signaling in prostate cancer: an early perspective, Endocr. Relat. Cancer 10 (2003) 537–560.
[33] F. Yang, X. Li, M. Sharma, C.Y. Sasaki, D.L. Longo, B. Lim, et al., Linking beta-catenin to androgen-signaling pathway, J. Biol. Chem. 277 (2002) 11336–11344.
[34] G. Chen, N. Shukeir, A. Potti, K. Sircar, A. Aprikian, D. Goltzman, et al., Up-regulation of Wnt-1 and beta-catenin production in patients with advanced metastatic prostate carcinoma: potential pathogenetic and prognostic implications, Cancer 101 (2004) 1345–1356.
[35] R.V. Iozzo, I. Eichstetter, K.G. Danielson, Aberrant expression of the growth factor Wnt-5A in human malignancy, Cancer Res. 55 (1995) 3495–3499.
[36] M. Katoh, Frequent up-regulation of WNT2 in primary gastric cancer and colorectal cancer, Int. J. Oncol. 19 (2001) 1003–1007.
[37] H. Kirikoshi, H. Sekihara, M. Katoh, Molecular cloning and characterization of human WNT7B, Int. J. Oncol. 19 (2001) 779–783.
[38] T. Saitoh, M. Katoh, Molecular cloning and characterization of human WNT5B on chromosome 12p13.3 region, Int. J. Oncol. 19 (2001) 347–351.
[39] M. Katoh, M. Hirai, T. Sugimura, M. Terada, Cloning, expression and chromosomal localization of Wnt-13, a novel member of the Wnt gene family, Oncogene 13 (1996) 873–876.
[40] H. Zhu, M. Mazor, Y. Kawano, M.M. Walker, H.Y. Leung, K. Armstrong, et al., Analysis of Wnt gene expression in prostate cancer: mutual inhibition by WNT11 and the androgen receptor, Cancer Res. 64 (2004) 7918–7926.
[41] N. Sagara, G. Toda, M. Hirai, M. Terada, M. Katoh, Molecular cloning, differential expression, and chromosomal localization of human frizzled-1, frizzled-2, and frizzled-7, Biochem, Biophys. Res. Commun. 252 (1998) 117–122.
[42] H. Kirikoshi, N. Sagara, J. Koike, K. Tanaka, H. Sekihara, M. Hirai, et al., Molecular cloning and characterization of human Frizzled-4 on chromosome 11q14-q21, Biochem. Biophys. Res. Commun. 264 (1999) 955–961.
[43] M. Tokuhara, M. Hirai, Y. Atomi, M. Terada, M. Katoh, Molecular cloning of human Frizzled-6, Biochem. Biophys. Res. Commun. 243 (1998) 622–627.
[44] C. Wissmann, P.J. Wild, S. Kaiser, S. Roepcke, R. Stoehr, M. Woenckhaus, et al., WIF1, a component of the Wnt pathway, is down-regulated in prostate, breast, lung, and bladder cancer, J. Pathol. 201 (2003) 204–212.
[45] S. Shibamoto, K. Higano, R. Takada, F. Ito, M. Takeichi, S. Takada, Cytoskeletal reorganization by soluble Wnt-3a protein signalling, Genes Cells 3 (1998) 659–670.
[46] M. Verras, J. Brown, X. Li, R. Nusse, Z. Sun, Wnt3a growth factor induces androgen receptor-mediated transcription and enhances cell growth in human prostate cancer cells, Cancer Res. 64 (2004) 8860–8866.
[47] J.E. Pawlowski, J.R. Ertel, M.P. Allen, M. Xu, C. Butler, E. M. Wilson, et al., Liganded androgen receptor interaction with beta-catenin: nuclear co-localization and modulation of transcriptional activity in neuronal cells, J. Biol. Chem. 277 (2002) 20702–20710.
[48] L.N. Song, R. Herrell, S. Byers, S. Shah, E.M. Wilson, E.P. Gelmann, Beta-catenin binds to the activation function 2 region of the androgen receptor and modulates the effects of the N-terminal domain and TIF2 on ligand-dependent transcription, Mol. Cell. Biol. 23 (2003) 1674–1687.
[49] L. Levy, Y. Wei, C. Labalette, Y. Wu, C.A. Renard, M.A. Buendia, et al., Acetylation of beta-catenin by p300 regulates beta-catenin-Tcf4 interaction, Mol. Cell. Biol. 24 (2004) 3404–3414.
[50] A.H. Huber, W.J. Nelson, W.I. Weis, Three-dimensional structure of the armadillo repeat region of beta-catenin, Cell 90 (1997) 871–882.
[51] D.R. Chesire, C.M. Ewing, W.R. Gage, W.B. Isaacs, In vitro evidence for complex modes of nuclear beta-catenin signaling during prostate growth and tumorigenesis, Oncogene 21 (2002) 2679–2694.
[52] D. Masiello, S.Y. Chen, Y. Xu, M.C. Verhoeven, E. Choi, A. N. Hollenberg, et al., Recruitment of beta-catenin by wild-type or mutant androgen receptors correlates with ligand-stimulated growth of prostate cancer cells, Mol. Endocrinol. 18 (2004) 2388–2401.
[53] L.N. Song, M. Coghlan, E.P. Gelmann, Antiandrogen effects of mifepristone on coactivator and corepressor interactions with the androgen receptor, Mol. Endocrinol. 18 (2004) 70–85.
[54] H. Li, J.H. Kim, S.S. Koh, M.R. Stallcup, Synergistic effects of coactivators GRIP1 and beta-catenin on gene activation: cross-talk between androgen receptor and Wnt signaling pathways, J. Biol. Chem. 279 (2004) 4212–4220.

[55] S.S. Koh, H. Li, Y.H. Lee, R.B. Widelitz, C.M. Chuong, M. R. Stallcup, Synergistic coactivator function by coactivator-associated arginine methyltransferase (CARM) 1 and beta-catenin with two different classes of DNA-binding transcriptional activators, J. Biol. Chem. 277 (2002) 26031–26035.

[56] C. Labalette, C.A. Renard, C. Neuveut, M.A. Buendia, Y. Wei, Interaction and functional cooperation between the LIM protein FHL2, CBP/p300, and beta-catenin, Mol. Cell. Biol. 24 (2004) 10689–10702.

[57] Z. Culig, A. Hobisch, M.V. Cronauer, C. Radmayr, J. Trapman, A. Hittmair, et al., Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor, Cancer Res. 54 (1994) 5474–5478.

[58] M.P. Playford, D. Bicknell, W.F. Bodmer, V.M. Macaulay, Insulin-like growth factor 1 regulates the location, stability, and transcriptional activity of beta-catenin, Proc. Natl Acad. Sci. USA 97 (2000) 12103–12108.

[59] M. Verras, Z. Sun, Beta-catenin is involved in insulin-like growth factor 1-mediated transactivation of the androgen receptor, Mol. Endocrinol. 19 (2005) 391–398.

[60] Y. Wen, M.C. Hu, K. Makino, B. Spohn, G. Bartholomeusz, D.H. Yan, et al., HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway, Cancer Res. 60 (2000) 6841–6845.

[61] J.P. Carson, G. Kulik, M.J. Weber, Antiapoptotic signaling in LNCaP prostate cancer cells: a survival signaling pathway independent of phosphatidylinositol 3'-kinase and Akt/protein kinase B, Cancer Res. 59 (1999) 1449–1453.

[62] M. Sharma, W.W. Chuang, Z. Sun, Phosphatidylinositol 3-kinase/Akt stimulates androgen pathway through GSK3beta inhibition and nuclear beta-catenin accumulation, J. Biol. Chem. 277 (2002) 30935–30941.

[63] X. Wu, K. Senechal, M.S. Neshat, Y.E. Whang, C.L. Sawyers, The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway, Proc. Natl Acad. Sci. USA 95 (1998) 15587–15591.

[64] P.M. Ghosh, S. Malik, R. Bedolla, J.I. Kreisberg, Akt in prostate cancer: possible role in androgen-independence, Curr. Drug Metab. 4 (2003) 487–496.

[65] P. Li, S.V. Nicosia, W. Bai, Antagonism between PTEN/MMAC1/TEP-1 and androgen receptor in growth and apoptosis of prostatic cancer cells, J. Biol. Chem. 276 (2001) 20444–20450.

[66] H.K. Lin, S. Yeh, H.Y. Kang, C. Chang, Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor, Proc. Natl Acad. Sci. USA 98 (2001) 7200–7205.

[67] S. Persad, A.A. Troussard, T.R. McPhee, D.J. Mulholland, S. Dedhar, Tumor suppressor PTEN inhibits nuclear accumulation of beta-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation, J. Cell Biol. 153 (2001) 1161–1174.

[68] T. Ohigashi, R. Mizuno, J. Nakashima, K. Marumo, M. Murai, Inhibition of Wnt signaling downregulates Akt activity and induces chemosensitivity in PTEN-mutated prostate cancer cells, Prostate 62 (2005) 61–68.

[69] X. Liao, J.B. Thrasher, J. Holzbeierlein, S. Stanley, B. Li, Glycogen synthase kinase-3beta activity is required for androgen-stimulated gene expression in prostate cancer, Endocrinology 145 (2004) 2941–2949.

[70] M. Mazor, Y. Kawano, H. Zhu, J. Waxman, R.M. Kypta, Inhibition of glycogen synthase kinase-3 represses androgen receptor activity and prostate cancer cell growth, Oncogene 23 (2004) 7882–7892.

[71] T.R. Salas, J. Kim, F. Vakar-Lopez, A.L. Sabichi, P. Troncoso, G. Jenster, et al., Glycogen synthase kinase-3 beta is involved in the phosphorylation and suppression of androgen receptor activity, J. Biol. Chem. 279 (2004) 19191–19200.

[72] L. Wang, H.K. Lin, Y.C. Hu, S. Xie, L. Yang, C. Chang, Suppression of androgen receptor-mediated transactivation and cell growth by the glycogen synthase kinase 3 beta in prostate cells, J. Biol. Chem. 279 (2004) 32444–32452.

[73] D.R. Chesire, W.B. Isaacs, Ligand-dependent inhibition of beta-catenin/TCF signaling by androgen receptor, Oncogene 21 (2002) 8453–8469.

[74] D.J. Mulholland, J.T. Read, P.S. Rennie, M.E. Cox, C.C. Nelson, Functional localization and competition between the androgen receptor and T-cell factor for nuclear beta-catenin: a means for inhibition of the Tcf signaling axis, Oncogene 22 (2003) 5602–5613.

[75] A.L. Amir, M. Barua, N.C. McKnight, S. Cheng, X. Yuan, S. P. Balk, A direct beta-catenin-independent interaction between androgen receptor and T cell factor 4, J. Biol. Chem. 278 (2003) 30828–30834.

[76] A.V. Gerstein, T.A. Almeida, G. Zhao, E. Chess, M. Shih Ie, K. Buhler, et al., APC/CTNNB1 (beta-catenin) pathway alterations in human prostate cancers, Genes Chromosomes Cancer 34 (2002) 9–16.

[77] H.J. Voeller, C.I. Truica, E.P. Gelmann, Beta-catenin mutations in human prostate cancer, Cancer Res. 58 (1998) 2520–2523.

[78] D.R. Chesire, C.M. Ewing, J. Sauvageot, G.S. Bova, W.B. Isaacs, Detection and analysis of beta-catenin mutations in prostate cancer, Prostate 45 (2000) 323–334.

[79] A. de la Taille, M.A. Rubin, M.W. Chen, F. Vacherot, S.G. de Medina, M. Burchardt, et al., Beta-catenin-related anomalies in apoptosis-resistant and hormone-refractory prostate cancer cells, Clin. Cancer Res. 9 (2003) 1801–1807.

[80] N.L. Sandford, J.W. Searle, J.F. Kerr, Successive waves of apoptosis in the rat prostate after repeated withdrawal of testosterone stimulation, Pathology 16 (1984) 406–410.

[81] J.F. Kerr, J. Searle, Deletion of cells by apoptosis during castration-induced involution of the rat prostate, Virchows Arch. B Cell Pathol. 13 (1973) 87–102.

[82] F. Gounari, S. Signoretti, R. Bronson, L. Klein, W.R. Sellers, J. Kum, et al., Stabilization of beta-catenin induces lesions reminiscent of prostatic intraepithelial neoplasia, but terminal squamous transdifferentiation of other secretory epithelia, Oncogene 21 (2002) 4099–4107.

[83] B. Bierie, M. Nozawa, J.P. Renou, J.M. Shillingford, F. Morgan, T. Oka, et al., Activation of beta-catenin in prostate epithelium induces hyperplasias and squamous transdifferentiation, Oncogene 22 (2003) 3875–3887.
[84] W.B. Isaacs, G.S. Bova, R.A. Morton, M.J. Bussemakers, J. D. Brooks, C.M. Ewing, Molecular biology of prostate cancer, Semin. Oncol. 21 (1994) 514–521.
[85] W.B. Isaacs, G.S. Bova, R.A. Morton, M.J. Bussemakers, J. D. Brooks, C.M. Ewing, Molecular biology of prostate cancer progression, Cancer Surv. 23 (1995) 19–32.
[86] C. Jamora, E. Fuchs, Intercellular adhesion, signalling and the cytoskeleton, Nat. Cell Biol. 4 (2002) E101–E108.
[87] R. Umbas, J.A. Schalken, T.W. Aalders, B.S. Carter, H.F. Karthaus, H.E. Schaafsma, et al., Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer, Cancer Res. 52 (1992) 5104–5109.
[88] M.J. Bussemakers, R.J. van Moorselaar, L.A. Giroldi, T. Ichikawa, J.T. Isaacs, M. Takeichi, et al., Decreased expression of E-cadherin in the progression of rat prostatic cancer, Cancer Res. 52 (1992) 2916–2922.
[89] P.J. Richmond, A.J. Karayiannakis, A. Nagafuchi, A.V. Kaisary, M. Pignatelli, Aberrant E-cadherin and alpha-catenin expression in prostate cancer: correlation with patient survival, Cancer Res. 57 (1997) 3189–3193.
[90] J. Luo, D.M. Lubaroff, M.J. Hendrix, Suppression of prostate cancer invasive potential and matrix metalloproteinase activity by E-cadherin transfection, Cancer Res. 59 (1999) 3552–3556.
[91] N. Morita, H. Uemura, K. Tsumatani, M. Cho, Y. Hirao, E. Okajima, et al., E-cadherin and alpha-, beta- and gamma-catenin expression in prostate cancers: correlation with tumour invasion, Br, J. Cancer 79 (1999) 1879–1883.
[92] C.M. Ewing, N. Ru, R.A. Morton, J.C. Robinson, M.J. Wheelock, K.R. Johnson, et al., Chromosome 5 suppresses tumorigenicity of PC3 prostate cancer cells: correlation with re-expression of alpha-catenin and restoration of E-cadherin function, Cancer Res. 55 (1995) 4813–4817.
[93] W.J. Nelson, R. Nusse, Convergence of Wnt, beta-catenin, and cadherin pathways, Science 303 (2004) 1483–1487.
[94] J. Lilien, J. Balsamo, C. Arregui, G. Xu, Turn-off, drop-out: functional state switching of cadherins, Dev. Dyn. 224 (2002) 18–29.
[95] P. Bonvini, W.G. An, A. Rosolen, P. Nguyen, J. Trepel, A. Garcia de Herreros, et al., Geldanamycin abrogates ErbB2 association with proteasome-resistant beta-catenin in melanoma cells, increases beta-catenin-E-cadherin association, and decreases beta-catenin-sensitive transcription, Cancer Res. 61 (2001) 1671–1677.
[96] O.G. Morali, V. Delmas, R. Moore, C. Jeanney, J.P. Thiery, L. Larue, IGF-II induces rapid beta-catenin relocation to the nucleus during epithelium to mesenchyme transition, Oncogene 20 (2001) 4942–4950.
[97] J. Piedra, S. Miravet, J. Castano, H.G. Palmer, N. Heisterkamp, A. Garcia de Herreros, et al., p120 Catenin-associated Fer and Fyn tyrosine kinases regulate beta-catenin Tyr-142 phosphorylation and beta-catenin-alpha-catenin interaction, Mol. Cell. Biol. 23 (2003) 2287–2297.
[98] S. Roura, S. Miravet, J. Piedra, A. Garcia de Herreros, M. Dunach, Regulation of E-cadherin/Catenin association by tyrosine phosphorylation, J. Biol. Chem. 274 (1999) 36734–36740.
[99] R. Rosato, J.M. Veltmaat, J. Groffen, N. Heisterkamp, Involvement of the tyrosine kinase fer in cell adhesion, Mol. Cell. Biol. 18 (1998) 5762–5770.
[100] D.W. Owens, G.W. McLean, A.W. Wyke, C. Paraskeva, E. K. Parkinson, M.C. Frame, et al., The catalytic activity of the Src family kinases is required to disrupt cadherin-dependent cell-cell contacts, Mol. Biol. Cell 11 (2000) 51–64.
[101] C.B. Hellberg, S.M. Burden-Gulley, G.E. Pietz, S.M. Brady-Kalnay, Expression of the receptor protein-tyrosine phosphatase, J. Biol. Chem. 277 (1) (2002) 1165–11173.
[102] R. Nawroth, G. Poell, A. Ranft, S. Kloep, U. Samulowitz, G. Fachinger, et al., VE-PTP and VE-cadherin ectodomains interact to facilitate regulation of phosphorylation and cell contacts, Embo J. 21 (2002) 4885–4895.
[103] C.O. Arregui, J. Balsamo, J. Lilien, Regulation of signaling by protein-tyrosine phosphatases: potential roles in the nervous system, Neurochem. Res. 25 (2000) 95–105.
[104] A. Danilkovitch-Miagkova, A. Miagkov, A. Skeel, N. Nakaigawa, B. Zbar, E.J. Leonard, Oncogenic mutants of RON and MET receptor tyrosine kinases cause activation of the beta-catenin pathway, Mol. Cell. Biol. 21 (2001) 5857–5868.
[105] C.Y. Sasaki, H. Lin, P.J. Morin, D.L. Longo, Truncation of the extracellular region abrogrates cell contact but retains the growth-suppressive activity of E-cadherin, Cancer Res. 60 (2000) 7057–7065.
[106] M. van de Wetering, N. Barker, I.C. Harkes, M. van der Heyden, N.J. Dijk, A. Hollestelle, J.G. Klijn, H. Clevers, M. Schutte, Mutant E-cadherin breast cancer cells do not display constitutive Wnt signaling, Cancer Res. 61 (2001) 278–284.

0888-8809/05/$15.00/0
Printed in U.S.A.

Molecular Endocrinology 19(12):2915–2929

doi: 10.1210/me.2005-0097

hZimp7, a Novel PIAS-Like Protein, Enhances Androgen Receptor-Mediated Transcription and Interacts with SWI/SNF-Like BAF Complexes

Chun-Yin Huang, Jason Beliakoff, Xiaoyu Li, Jane Lee, Xiaomeng Li, Manju Sharma, Bing Lim, and Zijie Sun

Departments of Urology and Genetics (C.-Y.H., J.B., J.L., X.L., M.S., Z.S.), Stanford University School of Medicine, Stanford, California 94305-5328; and Division of Hematology/Oncology (X.L., B.L.), Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts 02115

Members of the PIAS (protein inhibitor of activated signal transducer and activator of transcription) family are negative regulators of the Janus family of tyrosine kinase (JAK)-signal transducer and activator of transcription pathway. Recently, PIAS proteins have been shown to interact with multiple signaling pathways in various cellular processes, and it has been demonstrated that PIAS and PIAS-like proteins interact with nuclear hormone receptors. In this study, we have identified a novel human PIAS-like protein, provisionally termed hZimp7, which shares a high degree of sequence similarity with hZimp10 (human zinc finger-containing, Miz1, PIAS-like protein on chromosome 10). hZimp7 (human zinc finger-containing, Miz1, PIAS-like protein on chromosome 7) possesses a molecular mass of approximately 100 kDa and contains a conserved Miz (*m*sx-interacting zinc finger) domain, a nuclear translocation signal sequence, and a C-terminal transactivation domain. Northern blot analysis revealed that hZimp7 is predominately expressed in testis, heart, brain, prostate, and ovary. Moreover, immunohistochemical staining of prostate tissues revealed that endogenous hZimp7 protein localizes to the nuclei of prostate epithelial cells and costains with the androgen receptor (AR). Further analysis of hZimp7 subcellular localization revealed that hZimp7 and the AR colocalize within the nucleus and form a protein complex at replication foci. Transient transfection experiments showed that hZimp7 augments the transcriptional activity of the AR and other nuclear hormone receptors. In contrast, reduction of endogenous hZimp7 protein expression by RNA interference decreased AR-mediated transcription. Finally, we determined that hZimp7 physically associates with Brg1 and BAF57, components of the ATP-dependent mammalian SWI/SNF-like BAF chromatin-remodeling complexes. The above data illustrate a potential role for hZimp7 in modulation of AR and/or other nuclear receptor-mediated transcription, possibly through alteration of chromatin structure by SWI/SNF-like BAF complexes. (*Molecular Endocrinology* 19: 2915–2929, 2005)

THE PIAS PROTEINS [protein inhibitor of activated signal transducer and activator of transcription (STAT)] were first identified as transcriptional coregulators of the Janus family of tyrosine kinase (JAK)-STAT pathway (2). The binding of cytokines to cell surface receptors activates the Janus, or JAK, family of tyrosine kinases, which phosphorylate a family of at least seven cytoplasmic transcription factors termed STATs. PIAS1 and PIAS3 have been shown to inhibit the activity of STAT1 and STAT3, respectively, by blocking their abilities to bind DNA (3–5). However, a recent study has shown that the PIAS proteins may play a more general role in regulating chromatin structure (6, 7). Cross talk between PIAS proteins and other signaling pathways has also been demonstrated in various cellular processes, including signaling through the tumor suppressor p53, Smad proteins, and steroid hormone receptors (7–9).

PIAS and PIAS-like proteins share a zinc finger domain, termed Miz (*m*sx-interacting zinc finger) (10). This domain appears to be important for protein-protein interactions and was recently shown to mediate the interaction between the homeobox protein Msx2 and PIASxβ. An increasing number of proteins from invertebrates have been found to contain the Miz do-

First Published Online July 28, 2005

Abbreviations: AR, Androgen receptor; ARE, androgen-responsive element; BrdU, bromodeoxyuridine; DBD, DNA-binding domain; DHT, dihydrotestosterone; DTT, dithiothreitol; ERα, estrogen receptor α; FBS, fetal bovine serum; GR, glucocorticoid receptor; hZimp10, human zinc finger containing, Miz1, PIAS-like protein on chromosome 10; Miz, *m*sx-*i*nteracting *z*inc finger; NLS, nuclear localization signal; PCNA, proliferating cell nuclear antigen; PRβ, progesterone receptor β; PSA, prostate-specific antigen; β-gal, β-galactosidase; PIAS, protein inhibitor of activated STAT; RACE, rapid amplification of cDNA ends; RLU, relative light units; shRNA, short hairpin RNA; STAT, signal transducer and activator of transcription; SUMO, small ubiquitin-like modifier; TAD, transcription activation domain; TR, thyroid hormone receptor; VDR, vitamin D receptor.

***Molecular Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

main, suggesting a conserved and biologically important role for PIAS proteins throughout evolution. Recently, an increased interest has been focused on the role of PIAS proteins in sumoylation (11). It has been shown that the small ubiquitin-like modifier (SUMO) E3 ligase RING domain shares significant homology with the PIAS proteins (12). Moreover, PIASxα, xβ, 1, and 3 have been found to interact with SUMO-1 and Ubc9 and to mediate the sumoylation of a variety of cellular proteins (13–15).

The androgen receptor (AR) belongs to the nuclear receptor superfamily (16, 17). The AR and other receptors in this family possess identifiable activation domains that confer transactivation potential when fused to a heterologous DNA-binding domain (DBD). However, an important feature of the AR and other nuclear receptors that distinguish them from other transcription factors is that they are activated through their ligand-binding domains. The unbound AR forms a complex with heat-shock proteins, which holds the AR in a conformation capable of high-affinity ligand binding (18, 19). Upon binding to ligand, the AR dissociates from the heat-shock proteins and translocates into the nucleus, where it binds to androgen response elements (AREs) and recruits cofactors to regulate the transcription of target genes (20).

Like other steroid hormone receptors, the AR can bind to different cofactors through its distinct functional domains (21). Through such interactions, these cofactors can modulate AR activity. Several members of the PIAS family have been implicated in the regulation of several nuclear hormone receptors, including the AR (3, 9, 22). Indeed, PIASxα was originally isolated as an AR-interacting protein 3, and it binds to AR and modulates AR-mediated transcription (23).

Recently, we identified a novel PIAS-like protein, hZimp10 (human zinc finger-containing, Miz1, PIAS-like protein on chromosome 10), which physically interacts with the AR and augments AR-mediated, ligand-dependent transactivation in prostate cells (1). Using specific antibodies, both endogenous AR and hZimp10 proteins were costained in the nuclei of prostate epithelial cells from normal and malignant human tissue samples. A conserved Miz domain and a strong intrinsic transactivation domain (TAD) were identified in the central and C-terminal regions of hZimp10, respectively. Transfection of hZimp10 into human prostate cancer cells showed augmentation of AR-mediated ligand-dependent transcription. A novel *Drosophila* gene, termed tonalli (tna), was identified recently and is the *Drosophila* ortholog of hZimp10 (24). The protein encoded by *tna* genetically interacts with the chromatin-remodeling complexes SWI2/SNF2 and Mediator, suggesting that it may play a role in transcription.

In the process of searching for potential homologs of hZimp10, we found a nucleotide sequence located on human chromosome 7 that shares a high degree of sequence similarity with hZimp10. Using 5′-RACE (rapid amplification of cDNA ends), we cloned the full-length protein. Like hZimp10 and other PIAS proteins, this novel protein contains a conserved Miz domain. Thus, we named the protein hZimp7 (human zinc finger containing, Miz1, PIAS-like protein on chromosome 7). hZimp7 is predominately expressed in testis, heart, brain, prostate, and ovary tissues. Part of the AR TAD (amino acids 243–333) and the central region of hZimp7 (amino acids 392–527) were found to be responsible for the interaction. Through fusion of hZimp7 to a heterologous DBD, it was determined that a strong TAD exists in the C terminus of the protein. Moreover, we demonstrated that hZimp7 colocalizes with the AR in the nucleus of prostate cells and prostate tissues and forms a protein complex at replication foci. Furthermore, we identified an interaction between hZimp7 and Brg1 and BAF57, components of the mammalian SWI/SNF-like BAF complexes, suggesting a possible role for hZimp7 in chromatin remodeling.

RESULTS

Isolation of a Novel PIAS-Like Protein, hZimp7

In searching for the homolog of hZimp10, we identified a KIAA clone, KIAA1886, which shares significant similarity with hZimp10. Because KIAA1886 is a truncated fragment, we performed 5′-RACE to isolate the full-length cDNA. Sequence analysis of the full-length clone, created by combining the 5′-RACE fragments and the KIAA1886 clone, revealed a methionine initiation codon at nucleotide 28 followed by an open reading frame encoding an 892-amino acid protein with a predicted molecular mass of approximately 100 kDa (GenBank accession no. AY426594). Using an *in vitro* transcription and translation assay, an approximately 100-kDa protein was generated by the full-length clone (data not shown), confirming the identity of the predicted initiation codon.

A BLAST search of the human genome database showed that this full-length sequence is located on human chromosome 7 at 7p13 and is comprised of 17 putative exons. Comparison of this protein with hZimp10 showed that they share more than 71% sequence similarity, particularly in the C-terminal region (Fig. 1A). Further analysis of the protein sequence revealed that it contains several functional domains, including a Miz zinc finger, a nuclear localization signal (NLS), and a proline-rich region (see Fig. 3C). A high degree of sequence similarity was observed when this clone was aligned with the Miz domains of other PIAS proteins (Fig. 1B). Based on these features, we named this protein hZimp7 (human zinc finger containing, Miz1, PIAS-like protein on chromosome 7).

hZimp7 Is Selectively Expressed in Human Testis, Heart, Brain, Pancreas, Prostate, and Ovary

Northern blot analysis was carried out to examine the expression of hZimp7 in human tissues. A cDNA frag-

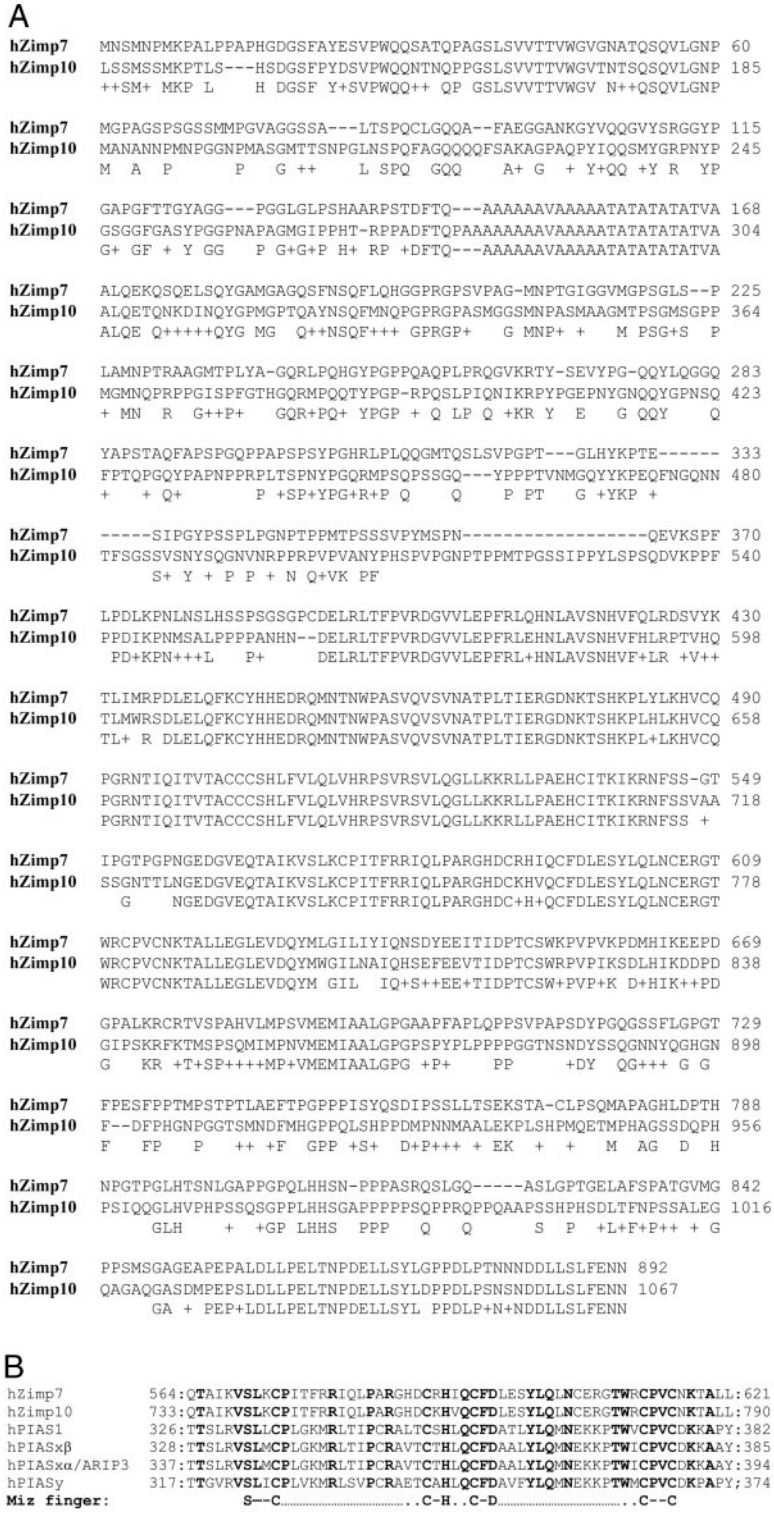

Fig. 1. Alignment of hZimp7, hZimp10, and Other PIAS Proteins

A, Alignment of hZimp7 and hZimp10 amino acid sequences, GenBank accession nos AY426594 and AY235683, respectively. Both identical and similar amino acids are marked. B, Alignment of the Miz zinc finger domain of hZimp7 with those of other PIAS and PIAS-like proteins. *Letters in bold* correspond to identical amino acids. The consensus sequence of Miz finger is shown.

ment encoding the N-terminal region (amino acids 1–316) of hZimp7 was used as the probe, and a β-actin probe was used to control for RNA loading. An approximately 4.2-kb transcript was detected by the hZimp7 probe in various human tissues (Fig. 2A). To precisely assess the expression of hZimp7, we measured the mean intensity of the hZimp7 and β-actin transcripts by densitometry (Fig. 2B). The transcript of hZimp7 was detected most abundantly in testis and at modest levels in heart, brain, pancreas, prostate, and ovary. There was little or no detectable signal in other human tissues. Interestingly, the expression profile of hZimp7 in human tissues is different from our previous observation with hZimp10 (1).

hZimp7 Is an AR-Interacting Protein

Because hZimp10, a homolog of hZimp7, was previously identified as an AR-interacting protein (1), we performed yeast two-hybrid assays to assess a possible interaction between the AR and hZimp7. We cotransformed full-length hZimp7 in a VP16-containing vector (pACT2) with various constructs containing either a GAL4 DBD alone or with various fusions of different fragments of AR into the modified yeast strain PJ69–4A (25) (Fig. 3A). A liquid β-galactosidase (β-gal) assay was performed to quantify the interactions. The AR/pTAD1 construct containing the partial TAD (amino acids 1–333) showed an approximately 23-fold induction compared with pVP16 alone. However, the AR/TAD2 (amino acids 1–243) showed virtually no interaction with hZimp7, suggesting that the region between amino acids 243–333 is critical for the interaction. In addition, in the presence of 100 nM dihydrotestosterone (DHT), the ligand-binding domain of AR showed approximately 4-fold induction compared with samples in which no DHT was added. No significant production of β-gal was observed in samples cotransformed with hZimp7 and the AR-DBD. As observed in our previous experiments with hZimp10, we found that the region between amino acids 243–333 in the TAD of the AR is required for the interaction with hZimp7.

The central region of hZimp10 (amino acids 556–790) has been shown to be responsible for binding to AR (1). This region shares significant sequence similarity with hZimp7 between amino acids 386–621(Fig. 1B). Based on this feature, we made a series of deletion mutants to determine whether the region between amino acids 386–621 is required for interacting with the AR (Fig. 3C). No interaction was observed between the AR and the truncated mutants of hZimp7 (1–435, 506–643, and 581–892) (Fig. 3C). In contrast, full-length hZimp7 and two deletion mutants, hZimp7 (310–892) and hZimp7 (310–700), which possess the entire region between amino acids 386–621, showed interactions with the AR. An additional mutant that contains the central region of the protein (amino acids 392–527) was generated and used to further map the precise interaction region of hZimp7. As expected, this mutant showed the highest β-gal activity, indicating that the central region between amino acids 435–506 may be the primary binding region for AR (Fig. 3C).

To confirm the interaction between hZimp7 and the AR *in vivo*, we tagged hZimp7 at its amino terminus with a FLAG epitope and expressed the tagged protein together with the AR in CV-1 cells. Both AR and FLAG-hZimp7 proteins were detected in the transfected cells (Fig. 3D, *top panels*). Whole cell lysates containing equal amounts of overexpressed proteins were immunoprecipitated with normal mouse IgG or an anti-FLAG monoclonal antibody. As shown in Fig. 3D, the AR protein was detected only in immunoprecipitates in which the FLAG antibody was used, indicating that the AR protein forms a protein complex with FLAG-

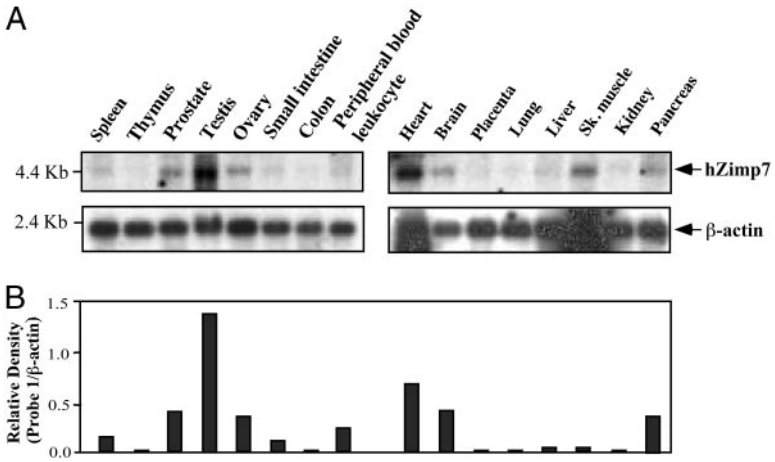

Fig. 2. Expression of hZimp7 in Human Tissues

A, Multiple human tissue blots were hybridized with an hZimp7 probe covering the N-terminal region between amino acids 1–316. The blots were reprobed for β-actin to control for equal loading. B, Relative densities (signals of the hZimp7 probe divided by those of β-actin), were used to measure the expression levels.

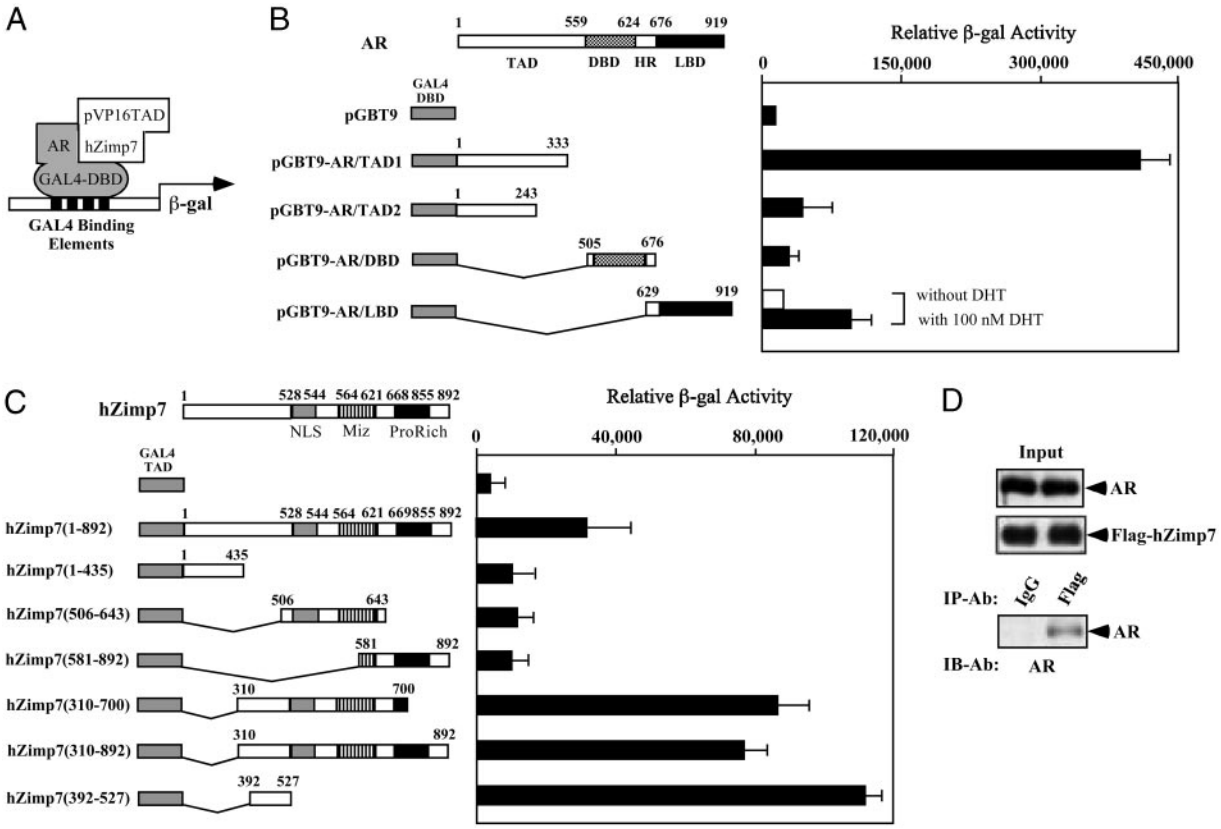

Fig. 3. Specific Interaction between hZimp7 and AR

A, A schematic representation of the yeast two-hybrid assay for mapping the interaction between the AR and hZimp7 proteins. B, The cDNA fragments containing different portions of the human AR were fused to the GAL4-DBD in the pGBT9 vector. *Numbers* correspond to amino acid residues. The pACT2-hZimp7 containing the fusion protein of VP16-TAD and hZimp7 was cotransformed with pGBT9 vector alone or different pGBT9-AR constructs. Transformed cells were plated on SD-Ade-Leu-Trp plates and SD-Leu-Trp plates to monitor transformation efficiency. Three independent colonies were inoculated from each transformation experiment for subsequent liquid β-gal assays. The data for the liquid β-gal assays are shown as the mean $\pm$ SD. C, Different truncation mutants of hZimp7 were generated by fusing portions of the hZimp7 sequence to the TAD of VP16 in the pACT2 vector and were cotransformed with the pGBT9-AR/pTAD1. Transformants were selected and analyzed as described in the above experiments. D, CV-1 cells were transiently cotransfected with AR and FLAG-tagged hZimp7. Equal amounts of whole-cell lysates were blotted with AR or FLAG antibody to detect expression levels of the two proteins (input) or subjected to immunoprecipitation (IP) with normal mouse IgG or anti-FLAG monoclonal antibody. The precipitated fractions were then resolved by SDS-PAGE and analyzed by Western blot (IB) using anti-FLAG or anti-AR antibody (Ab). HR, Hinge region; LBD, ligand-binding domain.

hZimp7. These data suggest that the AR and hZimp7 interact in mammalian cells.

hZimp7 Protein Is Expressed in the Nuclei of Prostate Epithelial Cells and Colocalizes with the AR Protein

To further explore the potential biological role of hZimp7, we examined the expression of hZimp7 in human prostate tissues by immunohistochemistry. The human prostate tissues used in our experiments were collected from normal prostate, benign prostatic hyperplasia, and prostate cancer samples obtained by radical prostatectomy. Two adjacent sections from three individual tissue samples were stained with either an anti-AR or anti-hZimp7 antibody directed against the N terminus of the protein. As reported previously, AR was found exclusively in the nuclei of prostate epithelial cells (Fig. 4, A, C, and E). hZimp7 protein also showed a strong nuclear staining pattern in normal and malignant prostate epithelial cells (Fig. 4, B, D, and F). There was no, or very weak, staining in the stromal elements with either antibody in all samples examined. Similar results were also obtained using another hZimp7 antibody directed against the C-terminal region (data not shown). As shown in Fig. 4, a clear costaining of AR and hZimp7 proteins was found in the nucleus of prostate epithelial cells. The above data demonstrate that endogenous AR and hZimp7 proteins are both expressed in the nuclei of human prostate cells, suggesting that they may interact *in vivo*. Consistent with our immunohistochemical staining results, endogenous hZimp7 was also detected in

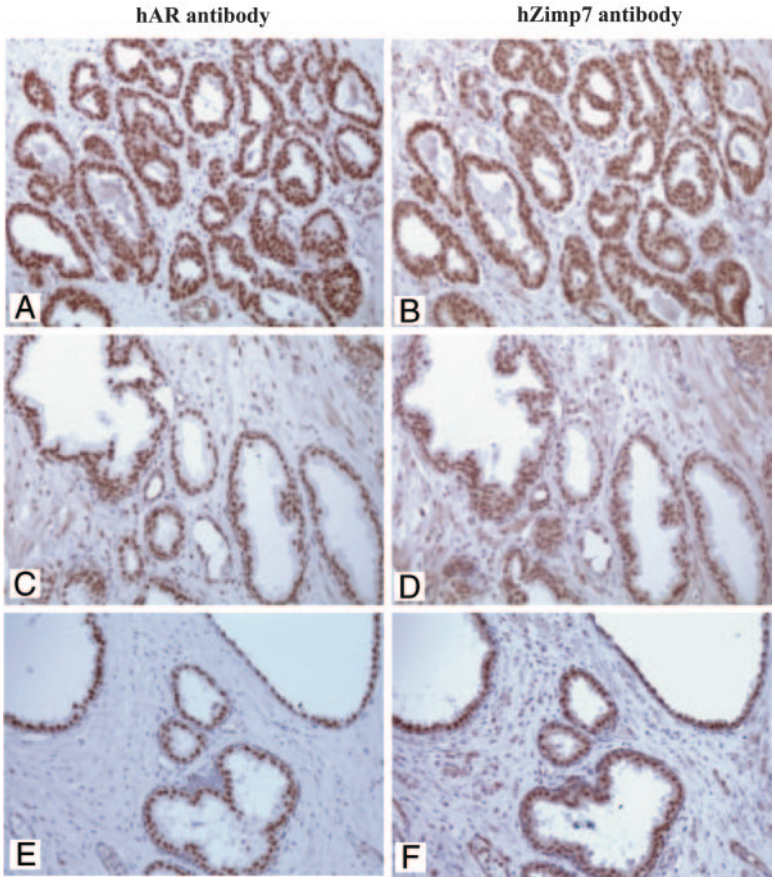

Fig. 4. hZimp7 and the AR Colocalize in Prostate Epithelial Cells

Three pairs of paraffin-embedded human prostate tissue samples (A–F) were stained with either anti-AR (*left panel*) or anti-hZimp7 (*right panel*) antibodies. *Color* was developed with DAB in PBS. All sections used for immunohistochemistry were lightly counterstained with 5% (wt/vol) Harris hematoxylin.

the nuclei of LNCaP prostate cancer cells using immunofluorescent staining (data not shown).

hZimp7 Contains a Strong TAD

The C-terminal sequences of hZimp7 and hZimp10 are very similar (Fig. 1B), and a strong TAD has been identified at the C-terminal region of hZimp10 (1). Based on these observations, we investigated a possible role for hZimp7 in transcription. Fragments containing full-length or various N-terminal truncation mutants of hZimp7 were targeted to DNA by fusion with the GAL4 DBD. These constructs were then tested for their abilities to modulate transcription from a minimal promoter, derived from the chicken myelomonocytic growth factor gene (−41 to +61), driving transcription of a luciferase reporter (26).

Fusion of the GAL4 DBD to full-length hZimp7 showed an approximately 4-fold induction compared with the GAL4 DBD alone, and deletions of the N-terminal region between amino acids 1–377 did not significantly affect the activity (Fig. 5). However, removal of amino acids 377–512 elevated the activity. The truncated mutants containing the C-terminal region (amino acids 512–892) showed 80-fold more transcriptional activity than that of the full-length hZimp7 construct, and deletion of the NLS and Miz domains significantly reduced the transcriptional activity. Moreover, the N-terminal fragment (amino acids 1–619) showed no transcriptional activity. Taken together, these results suggest that the C terminus of hZimp7 containing the NLS, Miz domain, and proline-rich region possesses strong transcription activity.

hZimp7 Functions as a Transcriptional Coactivator

Next, we investigated whether hZimp7 enhances AR-mediated transcription. Transient transfection experiments were first carried out in LNCaP, an AR-positive cell line. A luciferase reporter driven by the 7-kb promoter of the prostate-specific antigen (PSA) gene (27) was cotransfected with hZimp7, or hZimp10 as a control. In the presence of 1 or 10 nM DHT, ligand-dependent transactivation mediated by endogenous AR was observed (Fig. 6A). Cotransfection with hZimp7 or hZimp10 expression constructs further augmented AR activity (Fig. 6A). Of note, cells transfected with

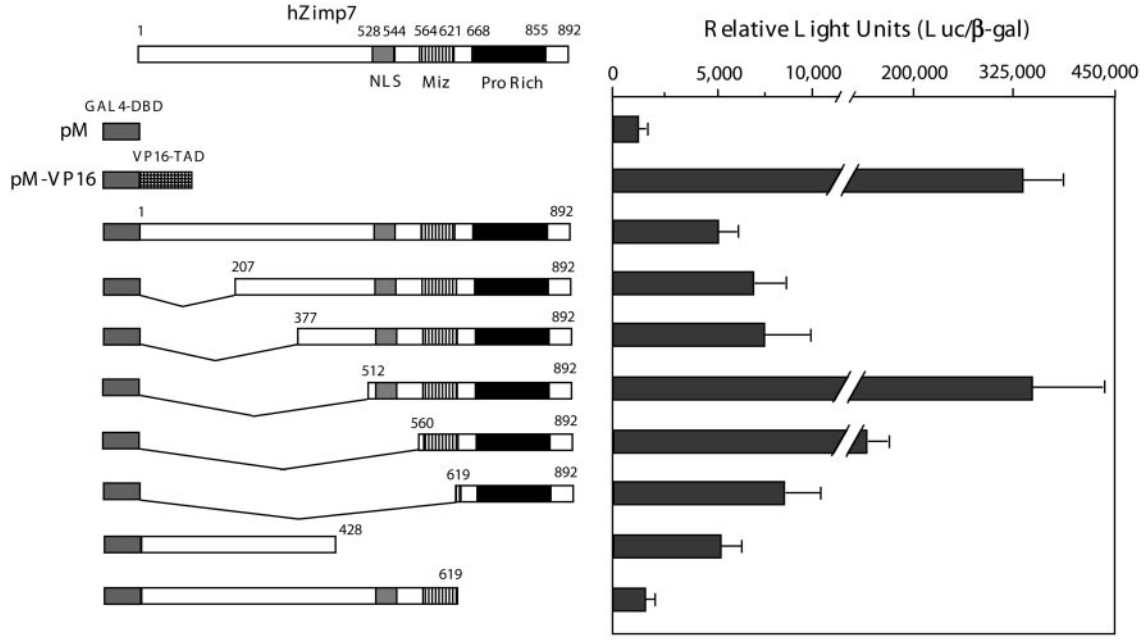

Fig. 5. Detection of Intrinsic Transcriptional Activity of hZimp7

Full-length or truncated fragments of hZimp7 were fused to the GAL4 DBD in the pM expression vector. *Numbers* correspond to amino acid residues. CV-1 cells were cotransfected with the pM constructs, luciferase reporter constructs containing the chicken myelomonocytic growth factor gene minimal promoter (−41 to +61) containing GAL4 binding sites, and a constitutive β-gal reporter. Data are presented in RLUs, which were obtained by normalizing the activities of luciferase to those of β-gal. Individual transfection experiments were done in triplicate, and the results are reported as the mean ± SD from representative experiments.

hZimp7 showed approximately 35–45% more luciferase activity than those transfected with hZimp10. These results provide the first line of evidence that hZimp7 functions as a coactivator of AR and is able to enhance AR-mediated transcription.

The specificity of hZimp7-mediated augmentation was further investigated with other nuclear receptors, including the glucocorticoid receptor (GR), progesterone receptor β (PRβ), estrogen receptor α (ERα), thyroid receptor β (TRβ), and vitamin D receptor (VDR). We first examined whether hZimp7 could enhance the activity of the AR, GR, and PRβ. To limit experimental variation, we used a luciferase promoter containing the mouse mammary tumor virus promoter, which can be activated by all three receptors (28, 29). In CV-1 cells, we observed that hZimp7 augments AR-mediated transcription on the mouse mammary tumor virus promoter, but has no effect on GR or PRβ activity (Fig. 6B). The effect of hZimp7 on transcription was further investigated with other nuclear receptors. As shown in Fig. 6C, hZimp7 enhances AR-mediated transcription from a luciferase reporter driven by a minimal promoter containing two AREs. However, hZimp7 also augments VDR, ERα, and TRβ-mediated transcription from the promoters driven by their corresponding responsive elements. These results suggest that hZimp7 functions as a transcriptional coactivator to augment AR and other nuclear hormone receptor-mediated transcription.

To further examine the enhancement of hZimp7 in a biologically relevant manner, we knocked down endogenous hZimp7 expression in LNCaP cells with a lentivirus containing an hZimp7-specific small hairpin RNA (shRNA). Because the lentiviral vector also expressed a blasticidin resistance gene, cells infected with the hZimp7 shRNA or vector alone were selected with blasticidin, and AR-mediated transcription was then assessed using the PSA-luciferase reporter. As shown in Fig. 6D, DHT-stimulated reporter activity was reduced approximately 50% in cells infected with the hZimp7 shRNA virus when compared with vector control. These data provide an additional line of evidence to demonstrate the biological role of hZimp7 in AR-mediated transcription.

To further study whether the effect of hZimp7 on AR is through an interaction between the two proteins, we made several truncated mutants of hZimp7 and tested their abilities to augment AR transcriptional activity (Fig. 6E). As observed previously, hZimp7 showed an enhancement on AR-mediated transcription (Fig. 6F). Cotransfection of the truncated hZimp7 constructs with the AR and full-length hZimp7 expression plasmids showed that the mutant, hZ7D2 (amino acids 392–527), covering the binding region for AR, inhibits the enhancement of AR activity by full-length hZimp7 (Fig. 6F). These data demonstrate a dominantly negative effect of hZ7D2 mutant in hZimp7-mediated enhancement and provide an additional line of evidence

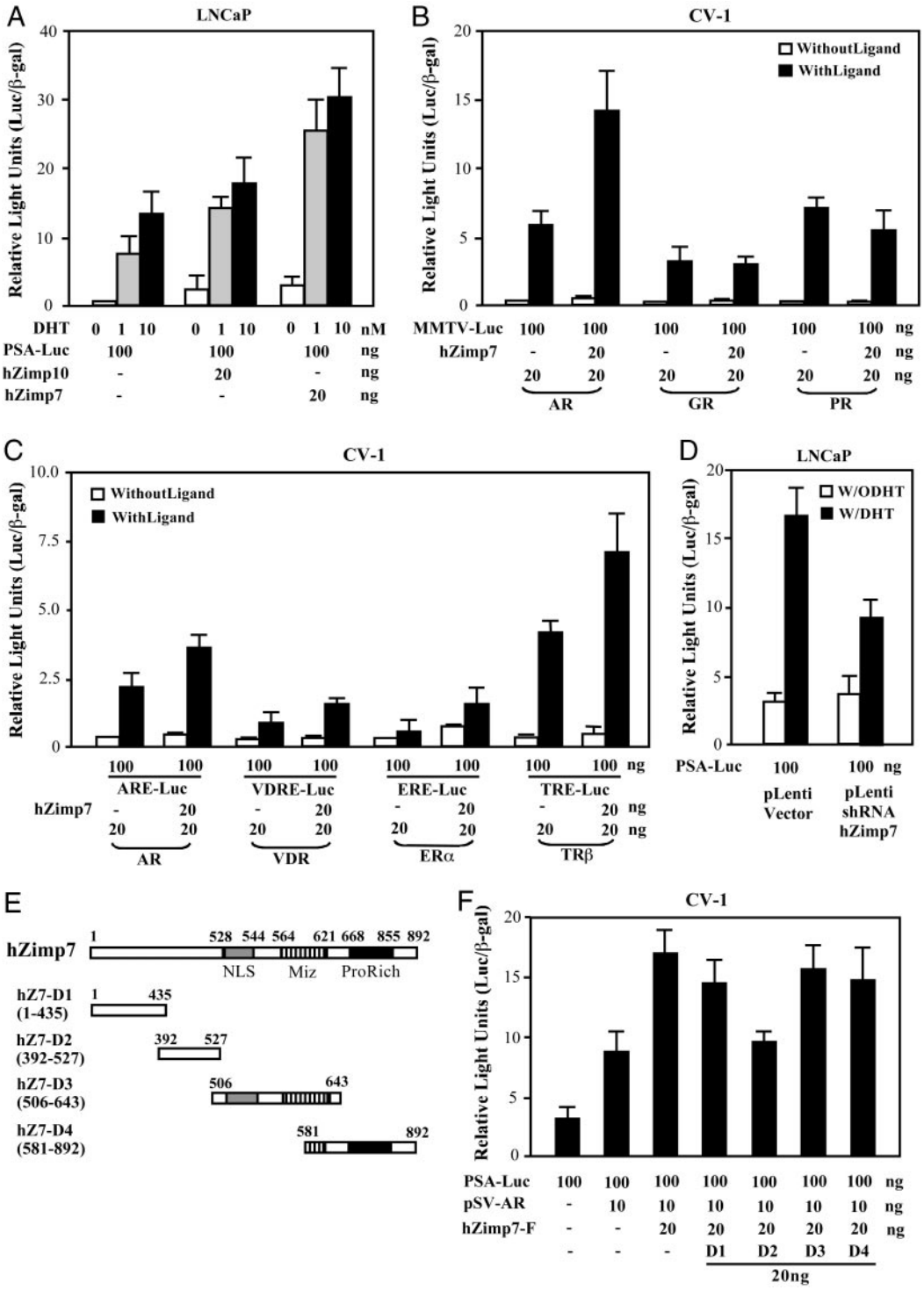

Fig. 6. hZimp7 Enhances AR and Other Nuclear Hormone Receptor-Mediated Transcription

A, LNCaP cells were transfected with a luciferase reporter driven by the human PSA promoter (100 ng), pcDNA3-β-gal (25 ng), and different amounts of pcDNA3-FLAG-hZimp7 or -hZimp10 as indicated. Cells were incubated 24 h after transfection in the presence or absence of DHT for 24 h. Cell lysates were then prepared for assessment of luciferase and β-gal activities. B, MMTV-Luc (100 ng) and expression vectors for different steroid hormone receptors were transfected with or without hZimp7 into CV-1 cells. Cells were cultured for 24 h in the presence or absence of specific ligands for each receptor, including 10 nM DHT, dexamethasone, and progesterone. C, Luciferase reporters (100 ng) driven by different response elements, as labeled in the figure, were cotransfected with 50 ng of pSV40-β-gal, 10 ng of different receptors, and 0 or 20 ng of pcDNA3-FLAG-hZimp7 into CV-1 cells. Cells were cultured in the presence or absence of the specific ligands to each receptor, including 10 nM DHT, 10 nM 1α25-dihydroxyvitamin D_3, 100 nM β-estradiol, and 10 nM triiodothyronine. Cell lysates were measured for luciferase and β-gal

that the interaction between hZimp7 and AR through this region is responsible for enhancement of AR activity.

hZimp7 Is Found at Cell Cycle-Regulated DNA Replication Foci throughout S Phase

Previous data have shown that hZimp10 is found at sites of DNA synthesis throughout all phases of DNA replication (1). Therefore, we systematically probed the nuclear distribution of hZimp7 during DNA replication by using immunofluorescence imaging. Cells were transfected with FLAG-hZimp7, synchronized in late G_1 phase with mimosine, and then allowed to enter S phase (30). Newly synthesized DNA was detected by bromodeoxyuridine (BrdU) labeling and staining with a fluorescein isothiocyanate-conjugated anti-BrdU antibody (Fig. 7A, *left panel*), and hZimp7 was detected using an anti-FLAG monoclonal antibody and a rhodamine-conjugated secondary antibody. A pattern of replication foci in synchronized cells was observed for hZimp7 during S-phase progression (Fig. 7A, *middle panel*). Replication foci changed from numerous small, punctate structures in early S-phase cells to large, toroidal structures in late S-phase cells. Intriguingly, hZimp7 displayed a similar pattern of nuclear distribution as the BrdU-labeled DNA and colocalized with BrdU throughout S phase (Fig. 7A, *right panel*). Next, we costained hZimp7 with PCNA (proliferating cell nuclear antigen) to confirm the localization of hZimp7 at DNA replication foci. As shown in Fig. 7B, we observed similar staining patterns for PCNA and hZimp7 throughout S phase. Taken together, our results demonstrate that like hZimp10, hZimp7 localizes to sites of DNA synthesis throughout all phases of DNA replication, implying that hZimp7 may play a role in DNA synthesis and chromatin modification.

hZimp7 Colocalizes with the AR during Cell Cycle Progression

Next, we examined whether hZimp7 colocalized with the AR during cell cycle progression. As described previously, cells were synchronized in late G_1 phase with mimosine and then allowed to progress through S phase. In cells synchronized in late G_1 phase, both the AR and FLAG-hZimp7 showed diffuse nuclear staining (Fig. 7C). When merged, these staining patterns showed a considerable amount of overlap (*yellow*) throughout the nucleus, suggesting that the proteins colocalize during G_1 phase. When the cells were allowed to progress into S phase, FLAG-hZimp7 became associated with the distinctive small punctate structures of early S phase replication foci, whereas a portion of AR protein retained a diffuse nuclear staining pattern (Fig. 7C; 0 and 4 h). When the cells progressed further into S phase, FLAG-hZimp7 displayed the slightly larger punctate staining indicative of mid-S phase (Fig. 7C; 8 and 12 h), and then the large, toroidal replication foci characteristic of late S phase (Fig. 7C; 18 h). A significant amount of overlap between FLAG-hZimp7 and AR was observed throughout S phase. Next, we costained both endogenous AR and hZimp7 proteins in LNCaP. As shown in Fig. 7D, both AR and hZimp7 proteins are localized in the nuclei, and a significant amount of overlay between these two proteins was observed. These data are consistent with our previous observation in CV-1 cells and suggest that hZimp7 colocalizes with the AR at replication foci.

hZimp7 Interacts with the Mammalian SWI/SNF-Like BAF Complexes

One of the mechanisms by which coregulators modulate nuclear hormone receptors is through modification of chromatin. The *Drosophila* ortholog of hZimp7 and 10, *tonalli* (*tna*), has been shown to genetically interact with the SWI2/SNF and Mediator chromatin-remodeling complexes (24). Thus, we performed immunoprecipitation experiments to assess the potential interaction between hZimp7 and mammalian SWI/SNF-like BAF complexes (31). Expression constructs of FLAG-hZimp7 and Brg1, a component of SWI/SNF-like BAF complexes, were cotransfected into CV-1 cells. Nuclear extracts containing equal amounts of hZimp7 protein were immunoprecipitated with normal mouse IgG or an anti-FLAG monoclonal antibody. As shown in Fig. 8A, FLAG-hZimp7 protein was detected in immunoprecipitates where the FLAG antibody was used. Importantly, the Brg1 protein was also detected in the same immunoprecipitates, suggesting a protein-protein interaction between hZimp7 and Brg1. An interaction between hZimp7 and BAF57, a Brg1-associated protein, was also demonstrated using the same procedure (Fig. 8B). These data provide the first line of evidence that hZimp7 interacts with Brg1 and BAF57, members of the mammalian SWI/SNF-like BAF chromatin-remodeling complexes.

activities as described above. D, LNCaP cells were infected with lentivirus containing hZimp7 shRNA or vector control and selected with blasticidin for stable integrants. Cells were then transfected with PSA-luc (100 ng) and pcDNA3-β-gal (25 ng). Cells were stimulated 24 h after transfection with 10 nM DHT for 24 h. Lysates were collected and luciferase and β-gal activities were determined as described above. E, A schematic representation of the truncated hZimp7 constructs was shown. *Numbers* correspond to amino acid residues. F, PSA luciferase reporters (100 ng) were cotransfected with 25 ng of pSV40-β-gal, 5 ng of AR expression vector (pSVAR), and 20 ng of pcDNA3-FLAG-hZimp7 in the presence of 20 ng of different truncated hZimp7 plasmids into CV-1 cells. Cell lysates were collected and measured for luciferase and β-gal activities as described above. ERE, Estrogen response element; MMTV, mouse mammary tumor virus; TRE, thyroid response element; VDRE, vitamin D response element.

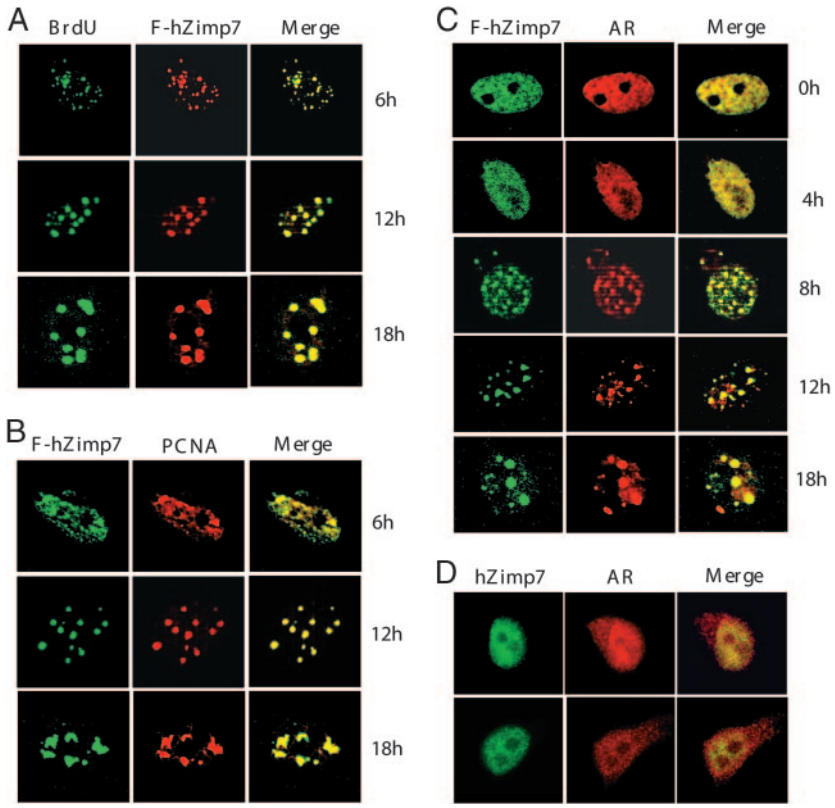

Fig. 7. Immunofluorescent Colocalization of hZimp7 and AR at Sites of DNA Replication throughout S Phase

A, pcDNA3-FLAG-hZimp7 (F-hZimp7) was transfected into CV-1 cells. Cells were then synchronized with 0.5 mM mimosine (see *Materials and Methods*). Representative confocal laser scanning microscopy images from cells expressing FLAG-tagged hZimp7 proteins and pulsed with BrdU are shown. Cells were immunostained with either a fluorescein isothiocyanate (FITC)-conjugated monoclonal anti-BrdU antibody (*green*) or an anti-FLAG primary antibody followed by a rhodamine-conjugated secondary antibody (*red*). Merge (*right panel*) of *left and middle panels* indicates areas of colocalization (*yellow*). B, The above experiments were repeated. Overexpressed hZimp7 or endogenous PCNA proteins were detected by anti-FLAG or PCNA antibody followed by FITC or rhodamine-conjugated secondary antibodies as labeled in the figure. C, CV-1 cells cotransfected with pcDNA3-FLAG-hZimp7 and pSVARo were synchronized with mimosine. Double immunostaining was conducted with anti-FLAG monoclonal and anti-AR polyclonal antibodies, followed by secondary antibodies conjugated with FITC (*green*) or rhodamine (*red*), respectively. Merged images demonstrating colocalization of proteins are shown in the right panels (*yellow*). D, As described above, LNCaP cells growing on chamber slides were fixed with 4% paraformaldehyde, permeabilized, and incubated with antibodies against hZimp7 and the AR. Species-specific Alexafluor 488 (*green*) and 594 (*red*) antibodies were used to detect hZimp7 and AR, respectively. *Left panels* indicate hZimp7 localization, *middle panels* indicate AR localization, and *right panels* are merged images showing areas of hZimp7-AR overlap.

Finally, we tested whether hZimp7 is involved in Brg1 and hBAF57-mediated enhancement of AR activity. Transfection of the AR expression construct with a luciferase reporter driven by the 7-kb PSA promoter showed a clear ligand-dependent enhancement of reporter activity (Fig. 8C), and overexpression of hZimp7 further enhanced AR-mediated transcription. Importantly, cotransfection of either human Brg1 or BAF57 augmented the activity of AR significantly in the presence of hZimp7 but showed no effect in the absence of hZimp7, suggesting an involvement of hZimp7 in hBrg1- and BAF57-mediated enhancement of AR activity. Next, we further examined the involvement of endogenous hZimp7 in hBrg1 and BAF57 using a RNA interference approach. As shown in Fig. 8D, knockdown of endogenous hZimp7 using an shRNA construct of hZimp7 reduces the enhancement of Brg1 and BAF57 on AR-mediated transcription in LNCaP cells. Taken together, these data demonstrate that BRG1 and BAF57 may cooperate with hZimp7 to enhance AR-mediated transcription.

DISCUSSION

hZimp7 is a novel PIAS-like protein that shares high sequence similarity with hZimp10. Because hZimp10 has been suggested to be an AR coactivator, in this study we first tested whether hZimp7 also interacts with the AR and augments AR-mediated transcription. Using immunoprecipitation assays, we demonstrated that the AR interacts with hZimp7 to form a protein complex in cells. In addition, we showed that the re-

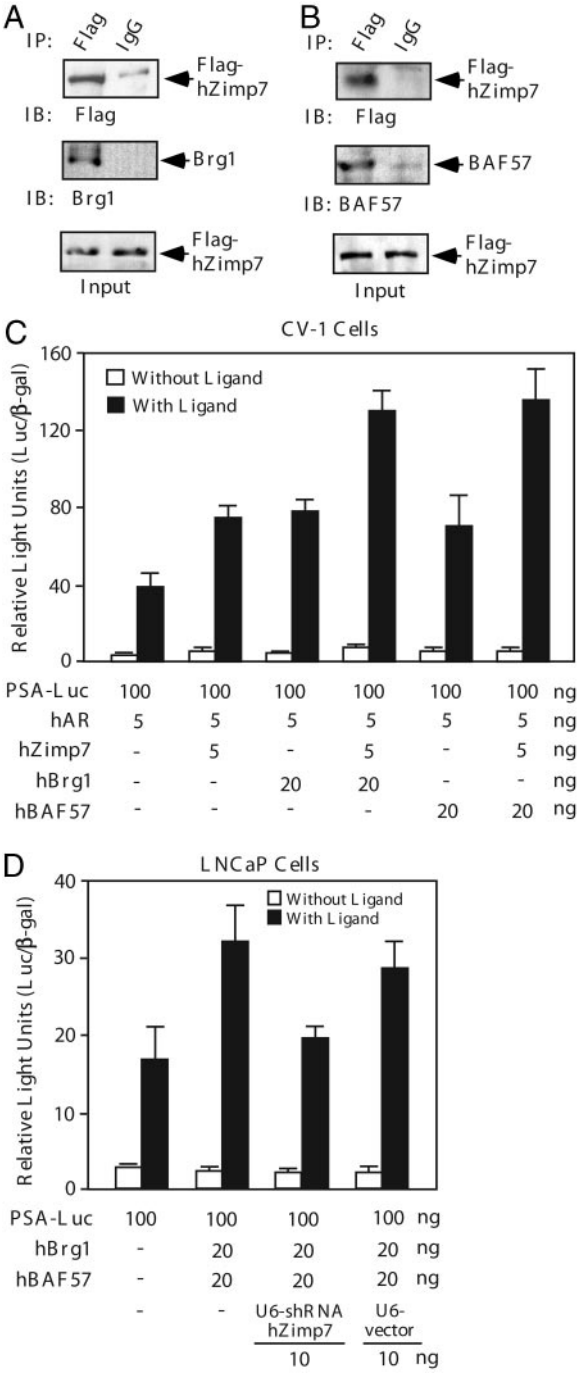

Fig. 8. hZimp7 Interacts with Components of the SWI/SNF Protein Complex

A and B, CV-1 cells were cotransfected with pcDNA3-FLAG-hZimp7 and Brg-1 or BAF57 expression constructs. After 48 h incubation, nuclear extracts were prepared. Equal amounts of nuclear extracts were subjected to immunoprecipitation with equal amounts of normal mouse IgG or anti-FLAG antibody. Proteins were resolved by SDS-PAGE and immunoblotted with antibodies to FLAG, Brg-1 (panel A), or BAF57 (panel B). C, CV-1 cells were grown in 48-well plates and cotransfected with 100 ng of PSA-luc reporter, 50 ng of pSV40-β-gal, 10 ng of hAR, and 5 ng of pcDNA3-FLAG-hZimp7, with 20 ng of either Brg-1 or BAF-57 expression plasmid. After a 48-h incubation, cells were harvested and luciferase and β-gal activities were measured, and RLUs were calculated. Individual transfection experiments were carried out in triplicate, and the results from representative experiments are reported as the mean $\pm$ SD. D, LNCaP cells were transfected with PSA-luc (100 ng), pcDNA3-β-gal (25 ng), and 20 ng of hBrg1 and BAF57 in the presence or absence of an hZimp7 shRNA construct. Cells were stimulated 24 h after transfection with 1 nM DHT for 24 h. Lysates were collected, and luciferase and β-gal activities were determined as described above. IB, Immunoblot; IP, immunoprecipitation.

gion between amino acids 243 and 333 within the TAD of the AR and the central region of hZimp7 between amino acids 435 and 506 are necessary and sufficient for the interaction using yeast two-hybrid analysis. To investigate the functional consequence of the hZimp7-AR interaction, we performed transient transfection assays and demonstrated that hZimp7 augments the ligand-dependent activity of the AR on both the natural AR-dependent promoter from the PSA gene and on a minimal promoter containing only two AREs. Consistent with the idea that hZimp7 is an AR coactivator, reduction of hZimp7 expression reduced endogenous AR-mediated transcription by approximately 50% in the human prostate cancer cell line, LNCaP. Taken together, these data show that, like hZimp10, hZimp7 interacts with the AR and enhances AR-mediated transactivation.

Sequence analysis showed that hZimp7 shares high sequence similarity with hZimp10. Both of these proteins contain a central Miz finger and a C-terminal proline-rich domain. Fusing the full-length protein and a series of truncation mutants of hZimp7 to the DBD of GAL4, we demonstrated that the C terminus of hZimp7, which contains the NLS, Miz, and proline-rich domains, possesses significant, intrinsic transcriptional activity. Intriguingly, the transactivation activity of hZimp7 is much higher than that of other transcription factors, such as p53, Smad, ER, and AR, and is even comparable to transactivation by the TAD of VP16. These results, combined with our previous observations with hZimp10, suggest that hZimp7 and hZimp10 may function as transcriptional coactivators through their intrinsic TADs. Identification of the intrinsic transcriptional activation domains in hZimp7 and hZimp10 also suggests a unique and distinctive role of these two proteins from other PIAS proteins in transcriptional regulation. In this study, we also examined whether hZimp7 enhances or represses the activity of other nuclear hormone receptors. Interestingly, aside from acting as an AR coactivator, hZimp7 was also shown to augment TRβ, ERα, and VDR-mediated transcription to varying degrees. This result suggests that hZimp7 may act more broadly than hZimp10 in modulating nuclear hormone receptor-mediated transcription.

As we observed previously with hZimp10 (1), the full-length hZimp7 displays very limited activity

compared with the truncated mutants containing the C-terminal proline-rich domain. Using a series of deletion mutants, we identified that the N terminus of hZimp7 (amino acids 1–512) significantly inhibits the activity of the C-terminal proline-rich region. Currently, the molecular mechanism(s) of this autoinhibition is unknown. The autoinhibitory effects in both hZimp7 and hZimp10 suggest that a similar mechanism may be involved in the regulation of these two PIAS-like proteins. Further investigation into the mechanism(s) by which hZimp7 and hZimp10 are released from this inhibition will be extremely important for understanding the *in vivo* function of these hZimp proteins.

Using the N-terminal fragment of hZimp7, the nucleotide sequence of which is very distinct from hZimp10 and other PIAS, we assessed the expression of hZimp7 in human tissues by Northern blotting. Interestingly, hZimp7 and hZimp10 show different tissue distribution profiles (1). hZimp7 is highly expressed in testis, whereas hZimp10 was detected most abundantly in ovary. The different expression profiles of hZimp7 and hZimp10 may implicate specific roles for these proteins in different human tissues. Identification of targets for hZimp10 and hZimp7 may help us to better elucidate the functional differences between these two related proteins.

Multiple members of the PIAS protein family have been shown to be capable of interacting with the AR and other nuclear hormone receptors and to regulate their activities (3). PIASxα was originally identified as an AR-interacting protein, named AR-interacting protein 3 (23). PIAS and PIAS-like proteins share a conserved Miz domain in their central regions important for interactions with target proteins (10). Previously, we demonstrated that the central region of hZimp10, which contains the Miz finger, is involved in the interaction with the AR (1). In this study, we used several truncation mutants of hZimp7 to map the interaction region for binding to the AR. We showed that the region between amino acids 392–527, rather than the Miz region, is required for the interaction. Interestingly, this region is highly conserved between both hZimp7 and hZimp10. Recently, using a comparable construct, we further confirmed that a similar region in hZimp10 displayed strong AR binding (data not shown).

In this study, we show that hZimp7 colocalizes with newly synthesized DNA and PCNA at replication foci throughout S phase. In eukaryotic cells, newly synthesized DNA must be rapidly assembled into the proper chromatin configuration to form transcriptionally active (euchromatin) and inactive domains (heterochromatin), respectively (32, 33). These data suggest a possible role of hZimp7 in both chromatin assembly and maintenance of chromatin. A homolog of hZimp7 and hZimp10, termed *tonalli* (*tna*), was identified recently in *Drosophila* (24). Intriguingly, the protein encoded by *tna* was shown to interact with SWI2/SNF2 and the Mediator complex. In this study, we examined the possible interaction between hZimp7 and the mammalian SWI/SNF-like BAF complexes (31). Using immunoprecipitation assays, we demonstrated that hZimp7 interacts with both Brg1 and BAF57, the DNA-binding subunits of the above complexes (34). Moreover, cotransfection of Brg1 or BAF57 with hZimp7 enhanced AR-mediated transcription to a greater extent than with either protein alone. Furthermore, knockdown of endogenous hZimp7 reduced the augmentation of Brg1 and BAF57 on AR-meditated transcription. These data provide the first link between hZimp7 and the human SWI/SNF-like BAF complexes. Unlike the yeast SWI/SNF complex, which is monomorphic, the mammalian BAF complexes contain several subunits that are coexpressed in the same cell, thus leading to their combinatorial assembly and the generation of perhaps hundreds of complexes (35). Identification of the interaction between hZimp7 and the components of BAF complexes suggests a role for hZimp7 in BAF complex-modulated transcription, which may further contribute to the heterogeneity of these complexes.

Modification of chromatin by different mechanisms, such as acetylation, methylation, phosphorylation, and ubiquitination, plays important roles in the regulation of chromatin structure to either foster or inhibit transcription (36). Recently, PIAS proteins-mediated sumoylation was also implicated in this regulatory process (37, 38). Although the precise role of PIAS proteins in the modulation of chromatin is unclear, it has been shown that PIAS proteins can recruit SUMO and Ubc 9 onto chromatin (37). Previously, we also demonstrated the colocalization of hZimp10 and SUMO-1 at replication foci (1). In this study, we observed that hZimp7 colocalizes with AR and SUMO-1 at replication foci (data not shown). However, hZimp7 does not directly affect AR sumoylation. Our observations suggest that hZimp7 not only directly enhances AR-mediated transcription but also participates other regulatory processes, possibly through modulating chromatin and/or recruiting other transcriptional factors such as AR onto chromatin. In both regards, it will be very interesting and worthwhile to further characterize the interaction between AR and hZimp7 at replication foci.

In conclusion, we have identified another novel PIAS-like protein, hZimp7. Multiple lines of evidence provided in this study suggest that hZimp7, like hZimp10, functions as a transcriptional coregulator to modify the activity of the AR and, probably, other nuclear hormone receptors. Intriguingly, we have also demonstrated that hZimp7 interacts with the mammalian SWI/SNF-like BAF complexes, suggesting a potential important role for hZimp7 in chromatin modification and transcriptional regulation. Further studies on the role of hZimp7 in transcription should provide new insight into the biology of PIAS and PIAS-like proteins.

MATERIALS AND METHODS

Plasmid Construction

Full-length hZimp7 cDNA was created by combining the cDNA fragment isolated by 5′-RACE (1–237 amino acids) and the KIAA 1886 fragment (238–892 amino acids) in the pcDNA3 vector either with or without an amino-terminal FLAG epitope tag (39). Subsequently, truncated mutants of hZimp7 were generated from the full-length clone in the pM vector containing a GAL4 DBD, the pVP16 vector containing the transcriptional activation domain of VP16, and the pcDNA3 expression vector.

The human AR expression vector, pSV-hAR, was kindly provided by Dr. Albert Brinkmann (Erasmus University, Rotterdam, The Netherlands). A simian virus 40-driven β-gal reporter plasmid (pSV-β-GAL) was purchased from Promega Corp. (Madison, WI). The human ERα expression construct and pERE-luc plasmid were generously supplied by Dr. Myles Brown (Dana-Farber Cancer Institute, Boston, MA). A human PRβ construct and the PRE-luc reporter were provided by Dr. Kathryn B. Horwitz (University of Colorado, Boulder, CO). The expression constructs of human GR and VDR and the pV-DRE-luc reporter plasmid were the kind gifts of Dr. David Feldman (Stanford University, Stanford, CA). The pARE-luc reporter was the kind gift of Dr. Chawnshang Chang (40). The pPSA7kb-luc was kindly provided by Dr. Jan Trapman (41). The human Brg1 and BAF57 expression vectors were gifts from Dr. Gerald Crabtree (Stanford University). The lentiviral construct of hZimp7 shRNA was generated as described previously (42). A 22-mer sequence (GGGCAGCAGCAG-CAGTTCTCAA) for the hZimp7 transcript was introduced into the pBS/U6 vector to generate the hZimp7 shRNA (43). Subsequently, the U6 promoter and the hZimp7 shRNA were PCR amplified and transferred into the pLentiSuper vector (Invitrogen, Carlsbad, CA). The viral vector was cotransfected with other packaging plasmids into human embryonic kidney 293T cells to produce the hZimp7 lentivirus (42).

Yeast Two-Hybrid System

Yeast two-hybrid experiments were performed as described previously (44). The DNA fragments containing the various truncation mutants of AR were fused in frame to the GAL4 DBD in the pGBT9 vector (CLONTECH Laboratories, Inc., Palo Alto, CA). Different hZimp7 mutants were fused to the GAL4 TAD in the pACT2 vector (CLONTECH). The constructs were transformed into the modified yeast strain PJ69–4A (25). Transformants were selected on Sabouraud Dextrose medium lacking tryptophan, leucine, and/or adenine. The specificity of interaction with the AR was measured by a liquid β-gal assay as described previously (44).

Cell Culture and Transfection

The monkey kidney cell line, CV-1, was maintained in DMEM supplemented with 5% fetal bovine serum (HyClone Laboratories, Inc., South Logan, UT). An AR-positive prostate cancer cell line, LNCaP, was maintained in T medium (Life Technologies, Inc., Gaithersburg, MD) with 5% fetal bovine serum (FBS). A LNCaP variant stably expressing hZimp7 shRNA was generated by infecting with a hZimp7shRNA-containing lentivirus and selecting for infected cells with 10 μg/ml blasticidin. A cell line expressing the lentiviral vector alone was generated as a negative control. Transient transfections were carried out using LipofectAMINE for CV-1 cells, and LipofectAMINE 2000 for LNCaP cells (Invitrogen, Carlsbad, CA). For reporter assays, approximately 1.5–2 $\times$ 10^4 cells were plated in a 48-well plate 16 h before transfection. Twelve to sixteen hours after transfection, the cells were washed and fed medium containing 5% charcoal-stripped FBS (HyClone) in the presence or absence of ligands. Cells were incubated for another 24 h, and luciferase activity was measured as relative light units (RLUs) (45). The RLUs from individual transfections were normalized by measuring the activity of a cotransfected constitutive β-gal reporter in the same samples. Individual transfection experiments were done in triplicate, and the results were reported as mean RLU/β-gal ($\pm$SD).

Northern Blot Analysis

Blots with RNA from multiple human tissues were obtained from CLONTECH Laboratories, Inc., and hybridized to DNA fragments specific for the N-terminal region (amino acids 1–316) of hZimp7. β-Actin was used to normalize loading.

Preparation of Whole-Cell Lysates and Nuclear Extracts

To make the whole-cell lysates, cells were washed with PBS and resuspended in RIPA buffer [1% Nonidet P-40, 0.1% sodium dodecyl sulfate, 50 mM NaF, 0.2 mM Na_3VO_4, 0.5 mM dithiothreitol (DTT), 150 mM NaCl, 2 mM EDTA, 10 mM sodium phosphate buffer (pH 7.2)]. Nuclear extracts were prepared according to the method of Dignam *et al.* (46) with minor modifications. Briefly, cells were washed with PBS, mechanically disrupted by scraping into homogenization buffer A (10 mM HEPES, pH 7.9; 10 mM KCl; 1.5 mM $MgCl_2$; 0.5 mM DTT; and 0.5 mM phenylmethylsulfonylfluoride), and incubated on ice for 10 min. Cells were further disrupted by 10 strokes with a homogenizer and centrifuged at 15,000 rpm for 20 min. The pellet was resuspended in buffer containing 20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.5 mM phenylmethylsulfonylfluoride, and 25% glycerol, and then homogenized with 10 strokes. The lysate was incubated on ice for 30 min and centrifuged for 10 min at 15,000 rpm. The supernatant was saved and analyzed as the nuclear fraction.

Immunoprecipitation and Western Blotting

Whole-cell lysates or nuclear extracts were first precleared with Protein A Sepharose beads for 1 h and then incubated with mouse or rabbit normal IgG or specific antibodies conjugated with preequilibrated Protein A Sepharose beads at 4 C for 2 h. The beads were collected by centrifugation and gently washed three times with the same buffer as described above. Proteins were eluted by boiling in sodium dodecyl sulfate-sample buffer, resolved on 10% polyacrylamide gels, and transferred onto nitrocellulose membranes. Membranes were then blocked with 5% milk in Tris-buffered saline-Tween 20 for 1 h, and then probed with anti-AR (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-Brg-1, or anti-BAF57 specific antibody (provided by G. Crabtree, Stanford University), followed by incubation with species-specific horseradish peroxidase-conjugated antibodies.

Antibody Production

The N-terminal region (amino acids 277–363) or C-terminal region (amino acids 714–824) of hZimp7 was cloned into the pGEX-4T1 vector, and glutathione-*S*-transferase fusion proteins were generated as described previously (1). These glutathione-*S*-transferase fusions were then used as a source of antigen for antibody production. Rabbit polyclonal, affinity-purified antibodies were produced by Proteintech Group, Inc. (Chicago, IL). Antibody specificity was confirmed by Western blot and ELISA assay.

Cell Synchronization, BrdU Labeling, and Immunofluoresence

Experiments were performed as described previously (1). A FLAG-tagged hZimp7 cDNA-containing vector was trans-

fected with pSV-hAR into CV-1 cells using the LipofectAMINE-plus reagent (Invitrogen). Synchronization was carried out as described by Krude (30). Briefly, at 18 h posttransfection, cells were treated with 0.5 mM mimosine (Sigma Chemical Co., St. Louis, MO) in DMEM supplemented with 10% FBS for 24 h to arrest cells in late G_1 phase. Cells were released from the mimosine block by washing three times with PBS and incubating in fresh DMEM containing 10% FBS at 37 C, which allowed the growth-arrested cells to progress synchronously through S phase.

For detection of DNA replication, cells were pulsed with 10 μM 5-BrdU (Sigma) and 1 μM fluorodeoxyuridine (Sigma) for 15min in the dark at 37 C to inhibit thymidylate synthetase. Cells were then washed twice with cold PBS and fixed with 3% formaldehyde for 30 min at room temperature. To visualize the newly synthesized DNA labeled with BrdU, the cells were treated with 4 N hydrochloric acid for 30 min at room temperature to denature the DNA, rinsed several times in Tris-buffered saline-Tween 20, and incubated at 37 C for 1 h with fluorescein isothiocyanate-conjugated monoclonal anti-BrdU antibody (PharMingen, San Diego, CA). For the cells cotransfected with different plasmids, specific primary antibodies and fluorescein isothiocyanate-conjugated antimouse or rhodamine-conjugated antirabbit secondary antibody were used (Molecular Probes, Inc., Eugene, OR). Images were acquired using a confocal microscope.

Immunohistochemical Staining

Human prostate tissues were fixed in 10% neutral-buffered formalin and processed to paraffin. Samples were cut into 3- to 5-μm sections, deparaffinized in xylene, and rehydrated using a decreasing ethanol gradient followed by PBS. Tissues were then blocked with 3% hydrogen peroxide in methanol and protein block for 60 min each to inhibit endogenous peroxidase activity and nonspecific antibody binding, respectively. Samples were exposed to a 1:500 dilution of rabbit polyclonal anti-hZimp7 antibody or anti-AR antibody (clone 441; Santa Cruz Biotechnology) in 1% goat serum overnight at 4 C. Slides were then incubated with biotinylated antirabbit/antimouse antibody solution (Biogenex, San Ramon, CA) and streptavidin peroxidase (Lab Vision, Fremont, CA) for 30 min each. Between each antibody step, slides were washed three times with PBS. Antibody staining was visualized with 3,3′-diaminobenzidine substrate solution (DAKO Corp., Carpinteria, CA) in PBS containing 0.3% hydrogen peroxide. Slides were subsequently counterstained with 5% (wt/vol) Harris hematoxylin.

Acknowledgments

We thank Drs. Albert Brinkmann, Kathryn B. Horwitz, Myles Brown, Chawnshang Chang, Jan Trapman, David Feldman, Donald Ayer, and Gerald Crabtree for the various reagents received. We thank Dr. Jorma Palvimo for critical reading of our manuscript.

Received February 22, 2005. Accepted July 20, 2005.

Address all correspondence and requests for reprints to: Zijie Sun, Ph.D., Departments of Urology and Genetics, S287, Grant Building, Stanford University School of Medicine, Stanford, California 94305-5328. E-mail: zsun@stanford.edu.

This work was supported by National Institute of Health Grants CA70297, CA87767, and DK61002 (to Z.S.) and DK 47636 and 54417 (to B.L.), and the Department of Army Prostate Cancer Grant DAMD17-03-1-0090 (to Z.S.).

REFERENCES

1. Sharma M, Li X, Wang Y, Zamegar M, Huang CY, Palvimo JJ, Lim B, Sun Z 2003 hZimp10 is an androgen receptor co-activator and forms a complex with SUMO-1 at replication foci. EMBO J 22:6101–6114
2. Shuai K 2000 Modulation of STAT signaling by STAT-interacting proteins. Oncogene 19:2638–2644
3. Tan J, Hall SH, Hamil KG, Grossman G, Petrusz P, Liao J, Shuaki K, French FS 2000 Protein inhibitor of activated STAT-1 (signal transducer and activator of transcription-1) is a nuclear receptor coregulator expressed in human testis. Mol Endocrinol 14:14–26
4. Chung CD, Liao J, Liu B, Rao X, Jay P, Berta P, Shuai K 1997 Specific inhibition of Stat3 signal transduction by PIAS3. Science 278:1803–1805
5. Liu B, Liao J, Rao X, Kushner SA, Chung CD, Chang DD, Shuai K 1998 Inhibition of Stat1-mediated gene activation by PIAS1. Proc Natl Acad Sci USA 95:10626–10631
6. Dobreva G, Dambacher J, Grosschedl R 2003 SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin μ gene expression. Genes Dev 17:3048–3061
7. Jackson PK 2001 A new RING for SUMO: wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. Genes Dev 15:3053–3058
8. Megidish T, Xu JH, Xu CW 2002 Activation of p53 by protein inhibitor of activated Stat1 (PIAS1). J Biol Chem 277:8255–8259
9. Kotaja N, Vihinen M, Palvimo JJ, Janne OA 2002 Androgen receptor-interacting protein 3 and other PIAS proteins cooperate with glucocorticoid receptor-interacting protein 1 in steroid receptor-dependent signaling. J Biol Chem 277:17781–17788
10. Wu L, Wu H, Ma L, Sangiorgi F, Wu N, Bell JR, Lyons GE, Maxson R 1997 Miz1, a novel zinc finger transcription factor that interacts with Msx2 and enhances its affinity for DNA. Mech Dev 65:3–17
11. Schmidt D, Muller S 2003 PIAS/SUMO: new partners in transcriptional regulation. Cell Mol Life Sci 60:2561–2574
12. Hochstrasser M 2001 SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. Cell 107:5–8
13. Kotaja N, Karvonen U, Janne OA, Palvimo JJ 2002 PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol Cell Biol 22:5222–5234
14. Nishida T, Yasuda H 2002 PIAS1 and PIASxα function as SUMO-E3 ligases toward androgen receptor and repress androgen receptor-dependent transcription. J Biol Chem 277:41311–41317
15. Kahyo T, Nishida T, Yasuda H 2001 Involvement of PIAS1 in the sumoylation of tumor suppressor p53. Mol Cell 8:713–718
16. Tsai MJ, O'Malley BW 1994 Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63:451–486
17. Horwitz KB, Jackson TA, Bain DL, Richer JK, Takimoto GS, Tung L 1996 Nuclear receptor coactivators and corepressors. Mol Endocrinol 10:1167–1177
18. Sanchez ER, Faber LE, Henzel WJ, Pratt WB 1990 The 56–59-kilodalton protein identified in untransformed steroid receptor complexes is a unique protein that exists in cytosol in a complex with both the 70- and 90-kilodalton heat shock proteins. Biochemistry 29:5145–5152
19. Sullivan WP, Vroman BT, Bauer VJ 1992 Isolation of steroid receptor binding protein from chicken oviduct and production of monoclonal antibodies. J Steroid Biochem Mol Biol 43:37–41
20. Jenster G 1999 The role of the androgen receptor in the development and progression of prostate cancer. Semin Oncol 26:407–421

21. Heinlein CA, Chang C 2002 The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 16:2181–2187
22. Tan JA, Hall SH, Hamil KG, Grossman G, Petrusz P, French FS 2002 Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J Biol Chem 277:16993–17001
23. Moilanen AM, Karvonen U, Poukka H, Yan W, Toppari J, Janne OA, Palvimo JJ 1999 A testis-specific androgen receptor coregulator that belongs to a novel family of nuclear proteins. J Biol Chem 274:3700–3704
24. Gutierrez L, Zurita M, Kennison JA, Vazquez M 2003 The *Drosophila* trithorax group gene tonally (tna) interacts genetically with the Brahma remodeling complex and encodes an SP-RING finger protein. Development 130: 343–354
25. James P, Halladay J, Craig EA 1996 Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics 144:1425–1436
26. Sterneck E, Muller C, Katz S, Leutz A 1992 Autocrine growth induced by kinase type oncogenes in myeloid cells requires AP-1 and NF-M, a myeloid specific, C/EBP-like factor. EMBO 11:115–126
27. Pang S, Dannull J, Kaboo R, Xie Y, Tso CL, Michel K, deKernion JB, Belldegrun AS 1997 Identification of a positive regulatory element responsible for tissue-specific expression of prostate-specific antigen. Cancer Res 57:495–499
28. Hoeck W, Hofer P, Groner B 1992 Overexpression of the glucocorticoid receptor represses transcription from hormone responsive and non-responsive promoters. J Steroid Biochem Mol Biol 41:283–289
29. Mink S, Ponta H, Cato AC 1990 The long terminal repeat region of the mouse mammary tumour virus contains multiple regulatory elements. Nucleic Acids Res 18: 2017–2024
30. Krude T 1999 Mimosine arrests proliferating human cells before onset of DNA replication in a dose-dependent manner. Exp Cell Res 247:148–159
31. Wang W, Chi T, Xue Y, Zhou S, Kuo A, Crabtree GR 1998 Architectural DNA binding by a high-mobility-group/kinesin-like subunit in mammalian SWI/SNF-related complexes. Proc Natl Acad Sci USA 95:492–498
32. Lewin B 1994 Chromatin and gene expression: constant questions, but changing answers. Cell 79:397–406
33. Berger SL, Felsenfeld G 2001 Chromatin goes global. Mol Cell 8:263–268
34. Chi TH, Wan M, Zhao K, Taniuchi I, Chen L, Littman DR, Crabtree GR 2002 Reciprocal regulation of CD4/CD8 expression by SWI/SNF-like BAF complexes. Nature 418:195–199
35. Wang W, Cote J, Xue Y, Zhou S, Khavari PA, Biggar SR, Muchardt C, Kalpana GV, Goff SP, Yaniv M, Workman JL, Crabtree GR 1996 Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J 15:5370–5382
36. Wolffe AP 1997 Histones, nucleosomes and the roles of chromatin structure in transcriptional control. Biochem Soc Trans 25:354–358
37. Vigodner M, Morris PL 2005 Testicular expression of small ubiquitin-related modifier-1 (SUMO-1) supports multiple roles in spermatogenesis: silencing of sex chromosomes in spermatocytes, spermatid microtubule nucleation, and nuclear reshaping. Dev Biol 282:480–492
38. Hannich JT, Lewis A, Kroetz MB, Li SJ, Heide H, Emili A, Hochstrasser M 2005 Defining the SUMO-modified proteome by multiple approaches in *Saccharomyces cerevisiae*. J Biol Chem 280:4102–4110
39. Kikuno R, Nagase T, Nakayama M, Koga H, Okazaki N, Nakajima D, Ohara O 2004 HUGE: a database for human KIAA proteins, a 2004 update integrating HUGEppi and ROUGE. Nucleic Acids Res 32(Database issue): D502–D504
40. Yeh S, Chang C 1996 Cloning and characterization of a specific coactivator, ARA_{70}, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 93: 5517–5521
41. Cleutjens KB, van Eekelen CC, van der Korput HA, Brinkman AO, Trapman J 1996 Two androgen response regions cooperate in steroid hormone regulated activity of the prostate-specfic antigen promoter. J Biol Chem 271: 6379–6388
42. Delenda C 2004 Lentiviral vectors: optimization of packaging, transduction and gene expression. J Gene Med 6 (Suppl 1):S125–S138
43. Sui G, Soohoo C, Affar el B, Gay F, Shi Y, Forrester WC 2002 A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc Natl Acad Sci USA 99:5515–5520
44. Yang F, Li X, Sharma M, Zarnegar M, Lim B, Sun Z 2001 Androgen receptor specifically interacts with a novel p21-activated kinase, PAK6. J Biol Chem 276: 15345–15353
45. Sharma M, Zarnegar M, Li X, Lim B, Sun Z 2000 Androgen receptor interacts with a novel MYST protein, HBO1. J Biol Chem 275:35200–35208
46. Dignam JD, Lebovitz RM, Roeder RG 1983 Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 11:1475–1489

Molecular Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**