The single-macro domain protein LRP16 is an essential cofactor of androgen receptor

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Abstract

LRP16 is a special member of the macro domain superfamily, containing only a stand-alone macro domain functional module. Previous study demonstrated that the estrogenically regulated LRP16 cooperates with the estrogen receptor α and enhances the receptor's transcriptional activity in an estrogen-dependent manner. Here, we discovered that LRP16 binds to androgen receptor (AR) via its macro domain and amplifies the transactivation function of AR in response to androgen. Similarly, we also discovered that LRP16 acts as a potential coactivator to amplify the transactivation of at least other four nuclear receptors (NRs). Importantly, we show that the single macro domain in LRP16 can serve as the AR coactivator. RNA interference knockdown of LRP16 leads to impaired AR function and greatly attenuates the coactivation of AR by other AR coactivators such as ART-27 and steroid receptor coactivator-1. This interference also markedly inhibits the androgen-stimulated proliferation of androgen-sensitive LNCaP prostate cancer cells. However, LRP16 knockdown did not significantly affect the growth rate of AR-negative PC-3 prostate cancer cells. Furthermore, we observed the induction effect of LRP16 expression by androgen and established a feedforward mechanism that activated AR transactivation. Our results suggest that the macro domain protein LRP16 represents a novel type of cofactor of NR. They also indicate that LRP16 plays an essential role in AR transactivation.

Endocrine-Related Cancer (2009) 16 139–153

Introduction

The androgen receptor (AR) is a ligand-dependent transcription factor of the nuclear receptor (NR) superfamily. Like other NRs, AR is composed of a N-terminal transactivation domain (NTD) harboring one or more hormone-independent activation function collectively named AF1, a highly conserved central DNA binding domain (DBD), and a C-terminal domain (CTD) that contains both the AR ligand-binding domain (LBD) and harbors a ligand-dependent activation function (AF2; Brinkmann *et al.* 1989). In the absence of androgen, AR is inactive and unable to influence the transcription rate of its target gene promoters. Androgen-bound AR engages with androgen response elements in the promoter and enhancer regions of target genes and regulates transcription (White & Parker 1998).

AR-mediated transcription is a highly complex process involving multiple of coregulatory factors (McKenna *et al.* 1999, McKenna & O'Malley 2002, Rosenfeld *et al.* 2006). The AR LBD is the major protein–protein interaction surface used to recruit coactivators (Chang & McDonnell 2005). The last decade has seen the identification of an overwhelming number of AR coregulators, including ART-27 (Markus *et al.* 2002, Heemers & Tindall 2007). Most coactivator proteins contain different activation domains or enzyme activity modules such as classical histone acetylase (HAT), bromo, chromo, SET (Su(var) 3–9, enhancer-of-zeste, Trithorax) and ATPase domains, by which coactivators facilitate AR transactivation (Heemers & Tindall 2007). The AR activity is not only critical for male sex determination but also plays an essential role for normal prostate development and malignant growth (Lee & Chang 2003, Dehm & Tindall 2006, 2007).

The macro domain is an evolutionary conserved sequence (130–190 amino acids) that can be found in all organisms (Karras *et al.* 2005). This domain was initially described as the non-histone region of the variant histone macroH2A (Pehrson & Fried 1992). In vertebrates, macro domains are sometimes found physically associated with proteins involved in ADP-ribosylation or poly(ADP-ribose) polymerization, as well as ATP-dependent chromatin remodeling (Aguilar *et al.* 2005, Goenka & Boothby 2006). Recent biochemical and structural analyses have revealed that macro domains are robust ADP-ribose and poly(ADP-ribose) binding modules (Karras *et al.* 2005, Egloff *et al.* 2006). However, the macro domain of histone macroH2A does not bind poly (ADP-ribose), but does bind the monomeric SirT1 metabolite *O*-acetyl-ADP-ribose with high affinity through its macro domain (Kustatscher *et al.* 2005). Emerging evidence indicates that macro domains modulate transcription by two models that are mutually exclusive. First, macro domains repress transcription when the domains are positioned to a promoter (Ladurner 2003); for instance, the macro domain of histone macroH2A has been implicated in the direct silencing of transcription by interfering with NF- κ B binding to its cognate sequence (Angelov *et al.* 2003) and that of the BAL family proteins represses transcription when tethered to a promoter (Aguilar *et al.* 2005). Second, macro domains activate transcription by functioning as a coactivator of some specific transcription factors. For example, a triplicated macro domain embedded within CoaSt6 protein can serve as a cofactor significantly enhancing IL4-induced Stat6 transactivation (Goenka & Boothby 2006).

LRP16 is a special member of macro domain superfamily, the structure of which is simple in contrast to other macro domain protein members, composed of only a stand-alone macro module at its C-terminal region (Han *et al.* 2002, Aguilar *et al.* 2005). Given the unicity of its structural module, LRP16 should become an ideal model gene for clarifying and extending the biological function of macro domains. In a previous report, we demonstrated the potential of the macro domain acting as a coactivator by providing evidence that LRP16 cooperates with estrogen receptor α (ER α) and amplifies the receptor's transactivation (Han *et al.* 2007).

The *LRP16* gene was originally isolated from human lymphocyte cells and predominantly localizes in the nucleus (Han *et al.* 2001, 2002). Although the expression pattern of LRP16 is ubiquitous in multiple human tissues and tumor cells (Han *et al.* 2001, Imagama *et al.* 2007), the expression of LRP16 strongly

depends on the estrogen actions in ER α -positive breast cancer cell lines (Zhao *et al.* 2005, Han *et al.* 2007, 2008). Testosterone can also activate the responsiveness of the *LRP16* gene promoter via AR mediation in COS-7 cells (Han *et al.* 2003), which suggests that LRP16 is an AR response target in AR-positive prostate cancer cells. The expression level of LRP16 was positively linked to the proliferation and invasion of the ER α -positive breast cancer and endometrial cancer cell lines and to the progression of primary breast cancers (Han *et al.* 2003, 2007, Liao *et al.* 2006, Meng *et al.* 2007). Furthermore, we have demonstrated that the estrogenically regulated LRP16 can interact with ER α and enhance the receptor's transcriptional activity in a ligand-dependent manner, thus establishing a positive feedback regulatory loop between LRP16 and ER α signal transduction (Han *et al.* 2007). Recently, a RUNX1-LRP16 fusion has been identified in a patient with acute monocytic leukemia (Imagama *et al.* 2007), which also suggests the transcriptional regulatory role of LRP16 when it is engaged with a transcription factor.

To further identify the transcription factors that potentially interact with LRP16, we herein performed a yeast two-hybrid screen with the full-length human LRP16 bait and a MCF-7 cDNA library. The well-known AR coactivator ART-27 was identified to physically interact with LRP16. We further provided evidence that LRP16 is able to interact with AR independently of ART-27 mediation. The molecular determinants of LRP16-AR interaction and the functional significance were further characterized. We first uncovered a direct link between the coregulation of AR and the single macro domain of LRP16. Finally, we investigated the induction effect of LRP16 expression by androgen and established the existence of a feedforward mechanism that activated AR transactivation.

Materials and methods

Chemicals, cell lines and siRNA

Testosterone (T), 17 β -estradiol (E2), dexamethasone, and fenofibrate were purchased from Sigma. Troglitazone was obtained from Sankyo Pharmaceuticals (Tokyo, Japan). Steroid-deprived serum was prepared as described previously (Han *et al.* 2008). MCF-7, COS-7, LNCaP, PC-3, DU145, HeLa, and 293T cells were originally obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured according to the instructions. The duplexes of LRP16 specific siRNA 374, 668, and the

control-siRNA were synthesized as in a previous report (Han *et al.* 2007).

Plasmids

The pcDNA3.1-LRP16 was described previously (Han *et al.* 2003). The luciferase reporter pGL3-S₀ and pGL3-S₂, driven by an LRP16 promoter region, were described previously (Zhao *et al.* 2005). The reporter 3×ERE-TATA-*Luc* was provided by Prof. Donald P McDonnell (Duke University Medical Center, Durham, NC, USA). The reporters MMTV-*Luc* and PPRE-TK-*Luc* were provided by Dr Masatoshi Nomura (Kyushu University, Fukuoka, Japan) and Dr Jianzhong Zhang (Peiking Medical University, Beijing, China) respectively. The plasmids Gal4-*Luc* and Gal4-VP16 were obtained from Dr Guangchun Chen (Kyushu University). The steroid receptor coactivator 1 (SRC-1) expression vector pSG5-SRC-1 was described previously (Chauchereau *et al.* 2000). The pcDNA3-Flag plasmid was provided by Jie Liu (Beijing Institute of Biotechnology, Beijing, China). The full-length cDNA of AR, estrogen receptor β (ER β), glucocorticoid receptor (GR), and peroxisome proliferator-activated receptor α (PPAR α) and γ (PPAR γ) were PCR-amplified from their expression plasmids as previously described (Han *et al.* 2003), then cloned into pcDNA3.1 by using appropriate sites. The GAL4-VP16 was PCR-amplified from pACT plasmid and inserted into pcDNA3.1 at *Bam*H I and *Kpn* I sites.

The yeast expression plasmid pGBKT7-LRP16 (Gal4 BD:bait gene fusion) was generated by inserting the full-length LRP16 cDNA in-frame at *Eco*R I sites of pGBKT7. For generating the glutathione S-transferase (GST)-LRP16 fusion plasmid and its mutants GST-LRP16-N (1–160) and GST-LRP16-C (161–324), the corresponding fragments were PCR-amplified and inserted at the *Eco*R I/*Hind* III sites of pGEX-6p-1 (Amersham Biosciences). For generating AR deletion mutants Flag-AR-N1 (1–266), AR-N2 (1–557), AR-N3 (1–621), and AR-C (622–920), the corresponding fragments were PCR-amplified and inserted at the *Xho* I/*Xba* I sites of pcDNA3-Flag. The full-length coding region of human ART-27 was amplified from GAL4 AD:ART-27 (pGADT7-ART-27) and then cloned into pcDNA3.1 at *Bam*H I/*Eco*R I sites.

Generation of cDNA library and yeast two-hybrid screening

Total RNA of MCF-7 cells was extracted by use of TRIzol reagent (Invitrogen). The MCF-7 cDNA library was generated by using the BD SMART kit from

Clontech according to the manufacturer's instructions. Yeast two-hybrid screening used to identify LRP16 interacting proteins involved the Matchmaker two-hybrid system 3 kit from Clontech according to the manufacturer's instructions.

Cell transfection and cell proliferation assays

pcDNA3.1-LRP16 or empty vector was stably or transiently transfected into LNCaP cells by use of Superfect reagent (Qiagen) according to the manufacturer's instructions. For stable transfection, cells were selected with 0.8 μ g/ml G418 (Invitrogen) for 2 weeks after 48-h transfection. All of the LNCaP parental cells were killed by G418 within this period. The selected cells were maintained as stable LRP16-expressing cells in media supplemented with 0.4 μ g/ml G418.

For cell proliferation assay, cells were plated onto 24-well plates at 1×10^4 cells/well in culture medium supplemented with 1 or 5% fetal bovine serum (Hyclone, Logon, UT, USA) and the medium was changed every 2 days. Cell proliferation rate was quantified by the use of CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega). Each experiment was performed in triplicate and repeated on three occasions.

GST pull-down assay

GST and GST fusion proteins were prepared as described previously (Han *et al.* 2007). [³⁵S]methionine-labeled proteins were produced by the use of Promega's TNT coupled *in vitro* transcription and translation (IVT) system, with the expression vectors for ER β , GR, PPAR α , PPAR γ , ART-27, VP16, AR, and its derivatives in pcDNA3.1 or pcDNA3-Flag. An amount of 20 μ l of the IVT reactions was incubated with GST or GST-fusion proteins bound to glutathione-sepharose beads. The binding reaction was performed for 1 h at room temperature, and the beads were washed four times and resuspended in 20 μ l of 2×SDS-PAGE loading buffer, boiled for 5 min, and resolved on a SDS-12% PAGE gel followed by radiophotography.

Immunoprecipitation (Co-IP) and immunoblotting

Cells were cultured in 10-cm dishes and transfected with expression vectors. Forty-eight hours after transfection, cells were harvested and lysed in 500 μ l lysis buffer (20 mM Tris (pH 7.4), 50 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.5% SDS, 0.5% deoxycholate, and protease inhibitors). An amount of 500 μ g of lysate (1 μ g/ μ l) was precleared with 50 μ l protein A-Sepharose beads (Upstate Biotechnology, Lake

Placid, NY, USA) for 2 h at 4 °C. An appropriate amount of rabbit anti-AR antibody (Abcam, Cambridge, UK), rabbit anti-LRP16 antibody, rabbit anti-Flag antibody (Sigma) or rabbit non-specific immunoglobulin G (IgG) (Clontech) was then added and incubated overnight at 4 °C. An amount of 100 µl of preblocked agarose beads was added to the antibody/lysate mixture for another 2 h at 4 °C, and the beads were pelleted and washed thrice with lysis buffer. Bound proteins were eluted in SDS sample buffer, subjected to SDS-PAGE and analyzed by immunoblotting. The rabbit anti-LRP16 antibody was described previously (Han *et al.* 2007). Rabbit anti-ART-27 (Aviva Systems Biology, Beijing, China), mouse monoclonal anti-Flag (Sigma), mouse monoclonal anti-PSA, mouse monoclonal anti-Gal4, rabbit anti-ERβ, rabbit anti-GR, rabbit anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-PPARα and anti-RRARγ (Aviva Systems Biology) were used for immunoblotting analyses.

Luciferase assays

Cells that had reached a 50% confluency rate in 35 mm dishes were cotransfected using Superfect (Qiagen). An amount of 0.25 µg reporter, 0.25 µg AR or other NR expression vectors or GAL4-VP16, and 0.5 µg ART-27 and/or LRP16 expression vector were used to cotransfect cells. pRL-SV40 was cotransfected (1 ng/per well) with reporters to normalize the transfection efficiency. The total DNA was adjusted to 2 µg per well with pcDNA3.1 empty vector. Cell extracts were prepared 42 h after transfection, and the relative luciferase activities were measured as described previously (Han *et al.* 2007).

An amount of 1 µg of siRNA duplexes, 0.5 µg MMTV-*Luc*, 0.25 µg AR, 0.5 µg ART-27 or SRC-1 expression vector, and 1 ng pRL-SV40 were cotransfected using Lipofectamine 2000 according to the manufacturer's recommendations (Invitrogen). The total amount of nucleotides was adjusted to 4 µg per well with pcDNA3.1 empty vector. Then, 48 h later, cells were harvested and the relative luciferase activities were measured as described previously (Han *et al.* 2007).

Northern blot and quantitative real-time PCR

Total RNA was extracted by TRIzol reagent (Invitrogen) and underwent northern blot analysis as previously described (Han *et al.* 2007). The membranes were probed with a 550 bp fragment of LRP16, a 621 bp fragment of AR, 243 bp of PSA and a 515 bp of β-actin fragment labeled with [α -³²P]dCTP by

random priming. For quantitative real-time PCR (qPCR) experiment, cDNA was prepared using Superscript II RNase H⁻ reverse transcriptase (Invitrogen) and 1 to 2 µg total RNA. cDNA was subjected to qPCR using the SYBR Green PCR Reagents kit (Bio-Rad). PCR primers were as follows: *PSA* sense, 5'-ACCCTCAGAAGGTGACCAAGT-3'; *PSA* antisense, 5'-TGAAGCACACCATTACAGACAA-3'. *HPRT* sense, 5'-TTGCTCGAGATGTGATGAAAGGA-3'; *HPRT* antisense, 5'-TTCCAGTTAAAGTTGAGAGATCA-3'. Reactions were run on a LightCycler (Roche). Experiments were performed in triplicate for each data point.

Statistical analysis

Experimental results were expressed as the mean ± S.E.M. Statistical analysis was performed using Chess software. All data were evaluated for paired variables to compare two groups. *P* < 0.05 was considered to be statistically significant.

Results

LRP16 interacts with AR coactivator ART-27 and AR

To identify new interactors of LRP16, we performed yeast two-hybrid screening from a human breast cancer MCF-7 cDNA library using LRP16 as bait. This screening yielded 9 validated cDNA clones corresponding to 12 different LRP16-associated proteins. Among them, two clones code for the α-class prefoldin family protein ART-27. Sequence analysis of the two ART-27 clones revealed that both clones retained a full-length coding fragment of ART-27 (amino acids 1–157).

ART-27 was previously identified to directly interact with AR and act as its coactivator (Markus *et al.* 2002). In addition, ART-27 is involved in other steroid and thyroid hormone receptor-mediated transcriptional activation (Markus *et al.* 2002). Therefore, we assessed the interaction among LRP16, ART-27, and AR in pull-down assays with the *in vitro*-translated, [³⁵S]methionine-labeled full-length ART-27 alone, AR alone or their combination incubated with GST-LRP16 or GST alone. ART-27 and AR were pulled down by GST-LRP16 but not by GST (Fig. 1A). Both ART-27 and AR were simultaneously pulled down by GST-LRP16 (Fig. 1A). These results suggested the reciprocal interaction among LRP16, ART-27, and AR proteins in a cell-free system.

To identify which domain in AR is necessary for AR-LRP16 association, we used a series of

AR deletion mutants in GST pull-down assays. GST-LRP16 did not bind to AR-N1, intensively bound to AR-C, and weakly bound to AR-N3 and AR-N2 (Fig. 1B). As negative controls, the deletion mutants of AR did not associate with GST alone. Next, we tested the N- and C-terminal deletion mutants of LRP16 for their ability to interact with AR. The full-length AR was pulled down by GST-LRP16-C but not by GST-LRP16-N (Fig. 1C). Collectively, these results indicated that the interaction between AR and LRP16 is primarily mediated by the CTD of AR and the single macro domain of LRP16.

To confirm that LRP16 can interact with ART-27 or AR in cells, we performed Co-IP experiments. COS-7 cells were transiently cotransfected with LRP16 and Flag-ART-27 or LRP16 and flag empty vector. Cell lysates were precipitated with a flag antibody or LRP16 antibody, followed by immunoblotting analyses with their reciprocal antibody. An intense band corresponding to LRP16 was detected only in immunoprecipitate containing Flag-ART-27 (Fig. 1D, upper panel). The presence of LRP16 in the ART-27-containing immune complexes is specific, because a non-specific antibody failed to immunoprecipitate LRP16. A reciprocal Co-IP experiment with LRP16 antibody revealed the presence of ART-27 in the immune complexes containing LRP16 as well (Fig. 1D, bottom panel). LNCaP is an AR-positive and androgen-sensitive

Figure 1 Interaction of LRP16 with ART-27 and AR in cell-free and cell systems. (A) Interaction of LRP16 with both ART-27 and AR on pull down assays. Coomassie blue-stained GST and GST-LRP16 are shown in the left panel. [³⁵S]methionine-labeled ART-27 and AR were incubated with GST or GST-LRP16 as indicated in the right panel. Input reflects 10% of the protein incubated with the beads. (B) Mapping of AR domains that interact with LRP16 by GST pull-down assay. The left panel shows the schematic illustration of AR and its mutants. GST pull-down assays were performed with various [³⁵S]-labeled AR mutants and GST-LRP16 or GST alone (right panel). (C) Mapping of LRP16 domains that interact with AR by GST pull-down assay. The left panel shows the schematic illustration of LRP16 and its mutants. The middle panel shows Coomassie blue-stained GST, GST-LRP16-N and GST-LRP16-C. GST pull-down assays were performed with [³⁵S]-labeled AR and GST-LRP16-N, GST-LRP16-C or GST alone (right panel). (D) Interaction of ART-27 and LRP16 in cells. COS-7 cells were transfected with the indicated plasmids. Forty-eight hours after transfection, cell lysates were immunoprecipitated and immunoblotted with the indicated antibodies. (E) Interaction of LRP16 with AR in LNCaP cells. Cells were transfected with or without LRP16. Forty-eight hours after transfection, whole cell lysates were immunoprecipitated and immunoblotted with the indicated antibodies. (F) Interaction of the CTD of AR with LRP16 in 393T cells. Cells were transfected with the indicated plasmids. Forty-eight hours after transfection, cells were lysed and underwent Co-IP experiments using the indicated antibodies. The asterisk reflects non-specific band. NsIgG: non-specific IgG.

prostate cancer cell line. The interaction between LRP16 and AR was determined by Co-IP experiments in LNCaP cells with or without LRP16 transfection in the absence of androgen treatment. Cell lysates were immunoprecipitated with a LRP16 antibody, and then underwent immunoblotting analysis with an anti-AR antibody. The endogenous AR was revealed in the immune complexes containing overexpressed or only endogenous LRP16 (Fig. 1E, left panel). Next, the same experiments performed in the reciprocal manner revealed both the exogenous and endogenous LRP16 detected in the AR antibody immunoprecipitated complex (Fig. 1E, right panel). To confirm whether the C-terminal region of AR is the primary region for AR-LRP16 association, we performed Co-IP assays using LRP16 and Flag-AR-N3 or LRP16 and Flag-AR-C cotransfected 293T cells. Immunoblotting results showed that the AR-C and LRP16 were intensively and reciprocally coprecipitated (Fig. 1F). Although AR-N3 appeared to be coprecipitated in LRP16 antibody immunoprecipitated complex, its expression was very weak when compared with that of AR-C. Collectively, these results confirmed the interaction of LRP16 with ART-27 and AR in cells in an androgen-independent manner.

Analysis of LRP16 interaction with other NR

Given the high homology among NR family members, we presumed that LRP16 might be able to interact with other members of NR family proteins. To test this presumption, we performed GST pull-down assays using a panel of *in vitro*-translated, [³⁵S]methionine-labeled transcription factors and GST-fused LRP16 protein. The full-length ERβ, GR, PPARα, and PPARγ but not VP16 could be pulled down by LRP16 (Fig. 2). Thus, these preliminary results suggested that LRP16 can interact not only with AR and ERα but also with at least four other NRs.

Figure 2 Interaction of LRP16 with other nuclear receptors in GST pull-down assays. GST pull-down assays were carried out with [³⁵S]methionine-labeled ERβ, GR, PPARα, PPARγ, or VP16 in the presence of GST-LRP16. GST protein was used as a control. Input reflects 10% of the protein incubated with the beads.

Overexpression of LRP16 enhances the transcriptional activation of its interacting NR

To explore the possible functional significance of the interaction of LRP16 with ART-27 and AR, we examined the effect of LRP16 on AR-mediated transcription activity. AR-deficient HeLa cells were cotransfected with AR, AR-responsive reporter construct MMTV-*Luc*, LRP16, and/or ART-27. Cotransfection with LRP16 or ART-27 did not affect the AR-mediated transcription in the absence of testosterone stimulation (Fig. 3A). In the presence of testosterone (10^{-7} M/l), AR up-regulated the reporter gene activity, which was further increased about threefold by either ART-27 or LRP16 cotransfection. Although ART-27 and LRP16 showed an enhanced effect on AR-mediated transcription, no additive or synergetic effect was observed on cotransfection of ART-27 and LRP16. Similar results were observed in AR-positive LNCaP human prostate cancer cells (Fig. 3B). These results indicate that LRP16 enhances AR-mediated transcriptional activation in a ligand-dependent manner.

The ability of LRP16 to affect transactivation by other NRs was also tested using transient transfection assays. Similar to the effect of LRP16 on AR transcriptional activation, LRP16 significantly enhanced reporter gene activities mediated by ERβ, GR, PPARα, and PPARγ on stimulation with their corresponding ligands (Fig. 3C–F). Recall that VP16 did not interact with LRP16 in a cell-free system (Fig. 2A) and in mammalian two-hybrid hybridization (Han *et al.* 2007). We next tested the effect of LRP16 on VP16-dependent transcription. Consistent with this lack of interaction, LRP16 expression had no effect on GAL4-VP16 activity from a reporter plasmid containing five GAL4-binding sites upstream of the TK promoter (Fig. 3G). These results suggest that LRP16 might enhance the functional activity of its interacting NRs in a ligand-dependent manner.

The macro domain of LRP16 enhances ligand-induced AR transactivation

Two of the most salient features of the LRP16 open reading frame (ORF) are its lack of modules represented in conventional transcriptional coregulators (e.g., HAT, bromo, chromo, SET, or ATPase domains) and its membership in a small family of proteins containing macro domains. As a special member of the macro domain protein family, LRP16 exclusively contains a stand-alone macro domain, which mediates its interaction with AR as illustrated in Fig. 1C. A mutant of LRP16 consisting exclusively

of a C-terminal region was compared with the full-length LRP16. The macro domain of LRP16 increased AR-mediated reporter gene activity nearly as high as did the full-length LRP16 in transfection assays; however, the N-terminal region of LRP16 failed to enhance reporter gene activity relative to the background (Fig. 4A). To test whether the single macro domain of LRP16 can induce AR-mediated gene expression, we performed qPCR to measure the mRNA level of the well-known AR target gene *PSA*. Transduction experiments using endogenous AR-positive LNCaP cells confirmed that both the LRP16-C and the full-length LRP16 significantly amplified AR-mediated induction of *PSA* mRNA by testosterone (10^{-8} M/l), when compared with the empty vector and the LRP16-N (Fig. 4B). As negative controls, neither the LRP16-C nor the full-length LRP16 changed the mRNA level of *HPRT* gene (Fig. 4B). Collectively, these findings demonstrate that the potentiation of ligand-induced AR transactivation by LRP16 relies on the presence of the macro domain.

Knockdown of LRP16 sharply diminishes ligand-induced AR transactivation and hampers coactivation of the AR by ART-27 and SRC-1

To further test whether LRP16 is required for AR transactivation, we used the MMTV-*Luc* reporter gene to evaluate the effect of LRP16 knockdown on AR activation status. Compared with the control siRNA, both LRP16-siRNA374 and LRP16-siRNA668 caused a specific reduction of LRP16 expression at both mRNA and protein levels in LNCaP cells but no change in expression of *AR* and β -*actin* genes (Fig. 5A). Consistent with our previous report (Han *et al.* 2007), LRP16-siRNA374 was reproducibly better than LRP16-siRNA668 and therefore was used more frequently in later experiments. Luciferase assays revealed that the decrease of endogenous LRP16 markedly inhibited testosterone-induced AR transactivation (Fig. 5B). To determine whether knockdown of LRP16 affected the AR transactivation enhanced by ART-27 and other coactivators, we also cotransfected AR, MMTV-*Luc*, ART-27, or SRC-1 in LRP16-inhibited LNCaP cells. The AR transcriptional response to ART-27 or SRC-1 was markedly attenuated by LRP16-siRNA374 relative to the control-siRNA and LRP16-siRNA668 (Fig. 5B). These results indicate that LRP16 is essential for ligand-induced AR transactivation and suggested that LRP16 is required for the potentiation of AR transactivation by some other coactivators.

To address the necessity for LRP16 in regulation of AR target genes, we used *PSA* to detect its expression in response to LRP16 knockdown. *PSA* expression at both mRNA and protein levels was significantly decreased by LRP16-siRNA374 and was partially decreased by LRP16-siRNA668 in LNCaP cells (Fig. 5A).

Knockdown of LRP16 markedly inhibits the growth of androgen-sensitive LNCaP prostate cancer cells

To determine the effect of the LRP16-AR association on the growth of androgen-dependent prostate cancer cells, we stably transfected LRP16 or empty vector into LNCaP cells for cell proliferation assays. However, ectopic LRP16 expression did not markedly promote cell growth when cells were cultured in normal or testosterone (10^{-9} M/l)-supplemented medium (data not shown). Next, we transiently transfected LRP16-siRNA374 or control-siRNA into LNCaP cells for cell proliferation assays. Cells carrying LRP16-siRNA374 or control-siRNA showed no significant growth difference on culture in steroid-deprived medium (Fig. 6A). Testosterone (10^{-12} M/l and 10^{-9} M/l) stimulated proliferation of LNCaP cells carrying either control siRNA or LRP16-siRNA 374, but the degree of testosterone-stimulated proliferation of LRP16-siRNA374 expressing cells was much less than that of control-siRNA expressing cells (Fig. 6B and C). To determine whether LRP16 is required for cell growth independent of its effect on AR activity, we transiently transfected LRP16-siRNA374 or control-siRNA into AR-deficient PC-3 prostate cancer cells and performed cell proliferation assays. As shown in Fig. 6D, knockdown of the endogenous LRP16 did not significantly inhibit growth of PC-3 cells. Immunoblotting analysis revealed that the endogenous LRP16 in LNCaP and PC-3 cells was still partially inhibited by LRP16-siRNA374 at days 9 and 6 respectively after transfection (Fig. 6E). These findings suggest that inhibition of LRP16 expression blocks the proliferation of androgen-dependent prostate cancer cells through suppressing AR functional activity.

Androgen stimulates LRP16 promoter response and upregulates LRP16 expression in AR-positive prostate cancer cells

Previously, we demonstrated that the synthesis of LRP16 depends on estrogen activities in breast cancer cells, and in turn, LRP16 enhances ER α functional activity by cooperating with ER α (Han *et al.* 2007). We also previously observed that androgen can upregulate

Figure 3 LRP16 enhances the transactivation of its interacting nuclear receptors in a ligand-dependent manner. (A) HeLa cells were transiently transfected with the indicated plasmids. Thirty hours after transfection, cells were stimulated with or without 10⁻⁷ M/l testosterone for 12 h before undergoing luciferase assay (left panel). Cell lysates were immunoblotted with the indicated antibodies to show the protein levels of the transfected genes (right panel). (B) LNCaP cells were transiently transfected with the indicated plasmids and were assayed as in A (left panel). Cell lysates were immunoblotted with the indicated antibodies to show the protein levels of the transfected genes (right panel). (C–G) The indicated plasmids were transfected into the indicated cells. Thirty-six hour after transfection, cells were treated with or without the indicated agonist for an additional 12 h and underwent luciferase assay (left panel). Cell lysates were immunoblotted with the indicated antibodies to show the protein levels of the transfected genes (right panel). Relative luciferase activity levels were normalized in all cases by luciferase reporter, pRL-SV40 and mock effector cotransfection and arbitrarily assigned a value of 1. All experiments were performed in triplicate and were repeated at least thrice; the results are expressed as mean ± S.E.M.

LRP16 promoter activity via AR mediation in COS-7 cells (Han *et al.* 2003). On the basis of these findings and the fact that LRP16 serves as a coactivator of AR, we hypothesized the existence of a feedback loop between AR function and LRP16 expression. To evaluate this hypothesis, we first examined the androgen responsiveness of LRP16 promoter-driven reporter genes pGL3-S₀ and pGL3-S₂ in LNCaP cells. Treatment with 10^{−10} M/l testosterone resulted in a 1.2- to 1.3-fold higher pGL3-S₀ and pGL3-S₂ reporter gene activities than without testosterone stimulation (Fig. 7A). The testosterone induction of the reporter activity of pGL3-S₀ and pGL3-S₂ was further enhanced (five- to sixfold) by their cotransfection with AR. These findings indicated that LRP16 is an androgen-responsive target gene in AR-positive prostate cancer cells. Next, we examined the induction effect of androgen on LRP16 expression at the protein level in LNCaP cells. LNCaP cells were treated with various concentrations of testosterone (10^{−12}–10^{−6} M/l) for 24 h, and then underwent western blot analyses. Testosterone, from 10^{−10} to 10^{−6} M/l, significantly increased LRP16 expression, with the low concentration (10^{−10} M/l) producing maximal induction (Fig. 7B). We further investigated the effect of 10^{−10} M/l testosterone on the expression level of LRP16 over 48 h (Fig. 7B). A significant increase of LRP16 expression was observed as early as 3 h after the addition of testosterone, which indicates that LRP16 is a testosterone-induced early response gene. The testosterone induction of LRP16 expression reached to the peak at 6 h and remained for at least 48 h. To further confirm the regulatory effect of AR on LRP16 expression, we measured the expression of LRP16 protein in AR-positive and AR-negative human prostate cancer cell lines. LRP16 protein level was much lower in AR-negative PC-3 and DU145 cells than in AR-positive LNCaP cells (Fig. 7C). Finally, we overexpressed AR in PC-3 cells and observed a marked increase of LRP16 protein level (Fig. 7D). The induction of LRP16 in AR-overexpressed PC-3 cells indicated that the culture medium containing non-stripped serum provided the source of ligand for AR. Altogether, these data confirmed the induction effect of LRP16 expression by androgen via AR mediation and established a feedforward mechanism that activated the AR in human prostate cancer cells.

Discussion

The transactivation potential of an NR depends on the cofactors it recruits, a key mechanism by which NRs regulate transcriptional programs (McKenna *et al.*

1999, McKenna & O'Malley 2002, Heemers & Tindall 2007). A central finding of the present study is that the protein LRP16 serves as a coactivator that amplifies the transactivation function of AR in response to androgen. Similarly, LRP16 was suggested to amplify the transactivation of other NRs, including ERβ, GR, PPARα, and PPARγ, in the presence of their corresponding agonists, by functioning as a potential coactivator. Importantly, we have uncovered a direct link between the coregulation of AR and the macro domain of LRP16. Knockdown of the endogenous LRP16 in cells significantly inhibited androgen-induced AR transactivation and diminished the coactivation of AR by other AR coactivators such as ART-27 and SRC-1. These findings suggest that the single macro domain protein LRP16 represents a novel type of cofactor of NRs and underscore a critical role of the macro domain in transcription modulation of multiple NRs.

Protein ADP-ribosylation modification is emerging as a regulatory process of protein function in prokaryotes and eukaryotes and may act on the level of signaling transduction, modulation of chromatin structure and epigenetic histone code (Hassa *et al.* 2006). The increased negative charge of ADP-ribosylated proteins may alter their spatial conformation and biological properties (Corda & Girolamo 2003, Hassa *et al.* 2006). Previously identified as displaying an Appr-1^{''}-p (ADP-ribose-1^{''}-monophosphate) processing activity and robust ADP-ribose binding activity (Culver *et al.* 1994, Karras *et al.* 2005, Kumaran *et al.* 2005, Egloff *et al.* 2006), the macro domain may play roles in distinct ADP-ribose pathways, such as the ADP-ribosylation of proteins, an important post-translational modification (Kraus & Lis 2003, Amé *et al.* 2004). In this study, we validated that a single macro domain within LRP16 is able to act as a coactivator of AR. Considering the biochemical characterization of macro domains binding to ADP-ribose, we proposed that LRP16 may act as a donor protein of ADP-ribose for ADP-ribosylation modification of NRs. Although the capacity of macro domains to transfer poly(ADP-ribose) to its partners is not yet authenticated, this hypothesis appears to be reasonable in that the poly(ADP-ribose) was indeed found to be transferred from its binding protein to another partner protein. For example, tankyrase, a member of PARP superfamily proteins, was found to confer poly(ADP-ribose) to its interacting protein TRF1, then ADP-ribosylation of TRF1 diminished its ability to bind to telomeric DNA (Smith *et al.* 1998). In general, because of the well-known function of the macro domain and the dependence of AR

Figure 4 The macro domain of LRP16 enhances AR-mediated transcription. (A) Segments of LRP16 were transfected into LNCaP cells along with an AR-dependent MMTV-Luc reporter and AR. Thirty hour after transfection, cells were stimulated with 10^{-10} M/I testosterone for 12 h before luciferase assay. The relative normalized luciferase activity level for empty vector transfection was arbitrarily assigned a value of 1. Shown are mean $\pm$ S.E.M. from at least three independent experiments. (B) LNCaP cells were transfected with the indicated segments of LRP16 or empty vector. Twelve or 32 h after transfection, cells were stimulated with 10^{-10} M/I testosterone for an additional 12 h. Endogenous mRNA expression of PSA and HPRT were measured by qPCR. Data represent mean $\pm$ S.E.M. from independent three experiments.

Figure 5 Knockdown of LRP16 sharply diminishes AR-mediated transcription. (A) LNCaP cells were transfected with the indicated siRNA. Forty-eight hour after transfection, the endogenous expression of LRP16, AR, PSA, and β -actin was monitored by northern blot and western blot analysis. (B) The indicated siRNA oligonucleotides were cotransfected with MMTV-Luc and the indicated plasmids into LNCaP cells. Thirty-six hour after transfection, cells were treated with 10^{-10} M/I testosterone or dimethyl sulfoxide (DMSO) for 12 h before luciferase assay. The relative luciferase activity levels were normalized by the use of mock effector transfection and arbitrarily assigned a value of 1. All experiments were performed in triplicate and were repeated at least thrice; the results are expressed as mean $\pm$ S.E.M. (# $P < 0.01$; * $P < 0.05$; N $P > 0.05$).

transactivation on LRP16 as we show, we propose that the ADP-ribose binding/processing activity may be required for NR activation and might depend on interaction with LRP16.

Several areas of high sequence conservation are observed in macro domains. Structure analysis also revealed a conserved ADP-ribose binding pocket in

different macro domains (Karras *et al.* 2005, Egloff *et al.* 2006). However, only about 30–40% sequence homology is shared between any two macro domains (Aguilar *et al.* 2005, Egloff *et al.* 2006). A significant degree of sequence variation exists among different macro domains. These variations should be the determinants of predisposing the selectivity of macro domain binding partners. Through binding to different transcription factors, different macro domains are disposed to affect different cellular signaling transduction and transcription regulation. CoaSt6 was previously identified to cooperate with Stat6 but not Stat1 and amplify IL4-induced transactivation (Goenka & Boothby 2006). In this study, several NRs were affected by LRP16. Further identification of LRP16-interacting transcription factors will help clarify the partner selectivity of a single macro domain in LRP16 and the importance of the macro domain in transcription regulation.

GST pull-down and CoIP assay has shown to primarily interact with the AR CTD and probably the DBD and AF1b regions. By contrast, LRP16 was previously shown to primarily interact with the ER α N terminus by the two mammalian two-hybrid assays (Han *et al.* 2007). On the basis of the homologous sequence derived from the AR C terminus and ER α N terminus, we analyzed homology of NR along with the other receptors affected by LRP16; however, this search failed to reveal a definite LRP16 interaction motif. One plausible explanation is that LRP16 may associate with NRs through multiple interactions with different affinity. In addition, the AR differs from other

Figure 6 Knockdown of LRP16 blunts the androgen-responsive proliferation ability of LNCaP cells but does not inhibit proliferation of PC-3 cells. (A–C) LNCaP cells were cultured in medium supplemented with steroid-stripped FBS (5%) for 3 days and then transiently transfected with LRP16-siRNA374 or control-siRNA. Forty-eight hour after transfection, cells were treated with testosterone at different concentrations for the indicated times. Cell proliferation rate was quantified by CellTiter 96 Aqueous assay. Each data point represents the mean $\pm$ S.E.M. of at least three independent experiments. (* $P < 0.05$, # $P < 0.01$). (D) PC-3 cells were transfected with either LRP16-siRNA374 or control-siRNA. Forty-eight hour after transfection, cells were plated onto 24-well plates at 1×10^4 cells/well in the culture medium supplemented with 5% FBS. Cell proliferation rate was quantified by CellTiter 96 Aqueous assay. Each data point represents the mean $\pm$ S.E.M. of at least three independent experiments. (E) LNCaP cells transfected with the indicated siRNAs were treated as in C. 9 d after siRNA transfection, cells were cultured as in C and the endogenous expression of LRP16 and β -actin was monitored by immunoblotting (left panel). PC-3 cells transfected with the indicated siRNAs were treated as in D. 2 d and 6 d after transfection, the endogenous expression of LRP16 and β -actin was monitored by immunoblotting (right panel).

Figure 7 Androgen stimulates LRP16 promoter activity via AR and up-regulates LRP16 expression. (A) LNCaP cells were cotransfected with the indicated plasmids. Thirty-six hour after transfection, cells were treated with or without 10⁻¹⁰ M/l testosterone for an additional 12 h and underwent luciferase assay. The relative luciferase activity levels were normalized in all cases by mock effector transfection and arbitrarily assigned a value of 1. All experiments were performed in triplicate and were repeated at least three times; results are expressed as mean ± S.E.M. (B) LNCaP cells were cultured in medium supplemented with steroid-stripped FBS (5%) for 3 days, then treated with testosterone at the indicated concentration for the indicated times. Immunoblots were probed for LRP16 and β-actin. (C) Total protein from the indicated cell lines was extracted and used for monitoring the expression of AR, LRP16, and β-actin by immunoblotting assays. (D) PC-3 cells were transfected with AR or the empty vector and cultured in a medium containing non-stripped serum. Immunoblots were probed for AR, LRP16, and β-actin.

NRs in that AR-mediated transcription requires the interaction of the AR NTD with the AR LBD in a hormone-dependent manner when the AR is not bound to DNA (He *et al.* 2000, van Royen *et al.* 2007), thus possibly generating a new conformation that is

important for recruitment of at least some of AR coactivators (Toumazou *et al.* 2008). So, another explanation for differential LRP16-binding motifs between AR and ERα is that the interaction of LRP16 with the AR C terminus may be more suitable for the special AR configuration rather than ERα.

ART-27 is a well-known AR coactivator that interacts with the AR N terminus and activates AR activity in a ligand-dependent manner (Markus *et al.* 2002, Taneja *et al.* 2004). LRP16 also interacts with ART-27, which indicates that LRP16 communicates with at least one other transcriptional regulatory cofactor of AR. SRC-1 interacts directly and ligand dependently with the AR to enhance AR-mediated transcription (Powell *et al.* 2004). Although SRC-1 has been shown to interact with AR AF-2, it interacts primarily with the AR N-terminus and possibly the DBD (Bevan *et al.* 1999, Powell *et al.* 2004, Heemers & Tindall 2007). Moreover, SRC-1 functions as a scaffold protein that attracts additional coactivator proteins such as p300, the p300 homolog CREB-binding protein (CBP), and p300/CBP-associated factor (P/CAF; Chen *et al.* 1997, Spencer *et al.* 1997, Heemers & Tindall 2007). Despite the lack of binding competition for the AR motif between LRP16 and ART-17 or SRC-1, we found the AR-induced transcriptional activation facilitated by both ART-27 and SRC-1 markedly diminished in LRP16-inhibited LNCaP cells (Fig. 5B), which suggests that LRP16 may play an essential role in AR transcription complex assembly after AR is exposed to ligand. Overexpression of LRP16 cannot amplify the coactivation of AR by ART-27 (Fig. 3A and B), which suggests that the endogenous LRP16 in cells is enough to support the excessive activities of other AR coactivators.

By promoter analyses and expression measurement of the *LRP16* gene in androgen-sensitive LNCaP prostate cancer cells (Fig. 7), we confirmed the induction effect of LRP16 expression by androgen via AR mediation. In a 2.6-kb region of LRP16 upstream regulatory sequence (pGL3-S₀), which confers the androgen responsiveness as illustrated in Fig. 7A, we failed to find the canonical androgen response element (ARE) (imperfect palindrome GGTACAnnnTGTCT) or either of the perfect half-sites. However, three sites containing imperfect AR-binding sequence (nGTACnnnnnGnnCn) were found across a 1.6-kb region of LRP16 upstream regulatory sequence. In addition, four imperfect half-sites across a 2.0-kb region of LRP16 upstream regulatory sequence were also observed. We arbitrarily proposed that one of the imperfect AREs or ARE half-sites may mainly contribute to the androgen responsiveness through AR binding to it, because it has been reported that there is a wide range of sequences that AR

can bind, typically containing the core requirement of three out of four guanines contacts at GGTA-CAnnnTGTTCT (Nelson *et al.* 1999). Certainly, we cannot exclude the possibility that *LRP16* is a secondary response gene of androgen. The molecular mechanism by which androgen upregulates *LRP16* expression is being investigated in our laboratory.

Considering the essential roles of the AR activity in normal and cancerous prostate tissues, a precise understanding of the mechanisms of AR regulation is of utmost importance. The expression of most AR coactivators is out of control by AR activity. Yet, emerging evidence has demonstrated that some of the AR coactivators such as SRG3 and FHL2 are AR-regulated targets (Hong *et al.* 2005, Heemers *et al.* 2007). This positive regulatory mechanism of AR transactivation by its response targets may be important for the rapid proliferation of cells during prostate development and may be essential for AR activation during prostate cancer progression (Hong *et al.* 2005, Heemers *et al.* 2007). In this study, we established the existence of a feedforward mechanism whereby the androgen-responsive protein *LRP16* activates the AR in human prostate cancer cells, which is similar to the *LRP16*-ER α feedforward regulation in estrogen-dependent breast cancer cells (Han *et al.* 2007). Furthermore, we also demonstrated that the inhibition of the endogenous *LRP16* in androgen-sensitive prostate cancer cells markedly diminished androgen-stimulated cell growth (Fig. 6), although its overexpression did not significantly promote cell proliferation. These results indicated that the AR-*LRP16* feedback pathway may play an essential role in maintaining androgen-induced AR activation. Appropriate expression of *LRP16* in androgen-sensitive prostate cancer cells is critical for cell proliferation. The breakdown of this feedforward signaling might be beneficial in AR-targeting therapy of androgen-dependent prostate cancer.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This study was supported by the National Natural Science Foundation of China (grants 30670809 and 30870968) and partially supported by a grant from the Ministry of Science and Technology of China (2005CB522603).

Acknowledgements

We thank Prof. Donald P McDonnell from Duke University Medical Center for providing 3 $\times$ ERE-TATA-*Luc* plasmid and Dr Masatoshi Nomura from Kyushu University for providing MMTV-*Luc* and other plasmids.

References

- Aguiar RCT, Takeyama K, He C, Kreinbrink K & Shipp MA 2005 B-aggressive lymphoma family protein have unique domains that modulate transcription and exhibit poly (ADP-ribose) polymerase activity. *Journal of Biological Chemistry* **280** 33756–33765.
- Amé JC, Spenlehauer C & de Murcia G 2004 The PARP superfamily. *BioEssays* **26** 882–893.
- Angelov D, Molla A, Perche PY, Hans F, Cote J, Khochbin S, Bouvet P & Dimitrov S 2003 The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling. *Molecular Cell* **11** 1033–1041.
- Bevan CL, Hoare S, Claessens F, Heery DM & Parker MG 1999 The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC-1. *Molecular and Cellular Biology* **19** 8383–8392.
- Brinkmann AO, Faber PW, van Rooij HCJ, Kuiper GGJM, Ris C, Klaassen P, van der Korput JAHM, Voorhorst MM, van Laar JH, Mulder E *et al.* 1989 The human androgen receptor: domain structure, genomic organization and regulation of expression. *Journal of Steroid Biochemistry* **34** 307–310.
- Chang CY & McDonnell DP 2005 Androgen receptor–cofactor interactions as targets for new drug discovery. *Trends in Pharmacological Sciences* **26** 225–228.
- Chauchereau A, Georgiakaki M, Perrin-Wolff M, Milgrom E & Loosfelt H 2000 JAB1 interacts with both the progesterone receptor and SRC-1. *Journal of Biological Chemistry* **275** 8540–8548.
- Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y & Evans RM 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90** 569–580.
- Corda D & Girolamo MD 2003 Functional aspects of protein mono-ADP-ribosylation. *EMBO Journal* **22** 1953–1958.
- Culver GM, Consaul SA, Tycowski KT, Filipowicz W & Phizicky EM 1994 tRNA splicing in yeast and wheat germ. A cyclic phosphodiesterase implicated in the metabolism of ADP-ribose 1'', 2''-cyclic phosphate. *Journal of Biological Chemistry* **269** 24928–24934.
- Dehm SM & Tindall DJ 2006 Molecular regulation of androgen action in prostate cancer. *Journal of Cellular Biochemistry* **99** 333–344.
- Dehm SM & Tindall DJ 2007 Androgen receptor structural and functional elements: role and regulation in prostate cancer. *Molecular Endocrinology* **21** 2855–2863.
- Egloff MP, Malet H, Putics Á, Heinonen M, Dutartre H, Frangeul A, Gruez A, Campanacci V, Cambillau C,

- Ziebuhr J et al. 2006 Structural and functional basis for ADP-ribose and poly(ADP-ribose) binding by viral macro domains. *Journal of Virology* **80** 8493–8502.
- Goenka S & Boothby M 2006 Selective potentiation of Stat-dependent gene expression by collaborator of Stat6 (CoaSt6), a transcriptional cofactor. *PNAS* **103** 4210–4215.
- Han WD, Yu L, Lou FD, Wang QS, Zhao Y, Shi ZJ, Jiao HY & Zhou JJ 2001 Cloning and expression characteristics of the full length cDNA for a novel leukemia-associated gene LRP16. *Academic Journal of PLA Postgraduate Medical School* **17** 209–214.
- Han WD, Lou FD, Yu L, Han XP, Wang QS, Li N & Zhou CX 2002 Bioinformatic analysis and subcellular distribution of LRP16 protein. *Academic Journal of PLA Postgraduate Medical School* **23** 277–279.
- Han WD, Mu YM, Lu XC, Xu ZM, Li XJ, Yu L, Song HJ, Li M, Lu JM, Zhao YL et al. 2003 Up-regulation of LRP16 mRNA by 17 β -estradiol through activation of estrogen receptor α . ER α , but not estrogen receptor β ER β and promotes human breast cancer MCF-7 cell proliferation: a preliminary report. *Endocrine-Related Cancer* **10** 217–224.
- Han WD, Zhao YL, Meng YG, Zang L, Wu ZQ, Li Q, Si YL, Huang K, Ba JM, Morinaga H et al. 2007 Estrogenically regulated ER α target gene LRP16 interacts with ER α and enhances the receptor's transcriptional activity. *Endocrine-Related Cancer* **14** 741–753.
- Han WD, Si YL, Zhao YL, Li Q, Wu ZQ, Hao HJ & Song HJ 2008 GC-rich promoter elements maximally confer estrogen-induced transactivation of LRP16 gene through ER α /Sp1 interaction in MCF-7 cells. *Journal of Steroid Biochemistry and Molecular Biology* **109** 47–56.
- Hassa PO, Haenni SS, Elser M & Hottiger MO 2006 Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? *Microbiology and Molecular Biology Reviews* **70** 789–829.
- He B, Kempainen JA & Wilson EM 2000 FXXLF and WXXLF sequences mediate the NH2-terminal interaction with the ligand binding domain of the androgen receptor. *Journal of Biological Chemistry* **275** 22986–22994.
- Heemers HV & Tindall DJ 2007 Androgen receptor coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. *Endocrine Reviews* **28** 778–808.
- Heemers HV, Regan KM, Dehm SM & Tindall DJ 2007 Androgen induction of the androgen coactivator four and a half LIM domain protein-2: evidence for a role for serum response factor in prostate cancer. *Cancer Research* **67** 10592–10599.
- Hong CY, Suh JH, Kim K, Gong EY, Jeon SH, Ko M, Seong RH, Kwon HB & Lee K 2005 Modulation of androgen receptor transactivation by the SWI3-related gene product (SRG3) in multiple ways. *Molecular and Cellular Biology* **25** 4841–4852.
- Imagama S, Abe A, Suzuki M, Hayakawa F, Katsumi A, Emi N, Kiyoi H & Naoe T 2007 LRP16 is fused to RUNX1 in monocytic leukemia cell line with t(11;21)(q13;q22). *European Journal of Haematology* **79** 25–31.
- Karras GI, Kustatscher G, Buhecha HR, Allen MD, Pugieux C, Sait F, Bycroft M & Ladurner AG 2005 The macro domain is an ADP-ribose module. *EMBO Journal* **24** 1911–1920.
- Kraus WL & Lis JT 2003 PARP goes transcription. *Cell* **113** 677–683.
- Kumaran D, Eswaramoorthy S, Studier FW & Swaminathan S 2005 Structure and mechanism of ADP-ribose-1''-monophosphatase (Appr-1''-pase), a ubiquitous cellular processing enzyme. *Protein Science* **14** 719–726.
- Kustatscher G, Hothorn M, Pugieux C, Scheffzek K & Ladurner AG 2005 Splicing regulation NAD metabolite binding to histone macroH2A. *Nature Structural and Molecular Biology* **12** 624–625.
- Ladurner AG 2003 Inactivating chromosomes: a macro domain that minimizes transcription. *Molecular Cell* **12** 1–4.
- Lee HJ & Chang C 2003 Recent advances in androgen receptor action. *Cellular and Molecular Life Sciences* **60** 1613–1622.
- Liao DX, Han WD, Zhao YL, Pu YD, Mu YM, Luo CH & Li XH 2006 The expression and clinical significance of LRP16 gene in human breast cancer. *Ai Zheng* **25** 866–870.
- Markus SM, Taneja SS, Logan SK, Li WH, Ha S, Hittelman AB, Rogatsky I & Garabedian MJ 2002 Identification and characterization of ART-27, a novel coactivator for the androgen receptor N terminus. *Molecular and Cellular Biology* **13** 670–682.
- McKenna NJ & O'Malley BW 2002 Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell* **108** 465–474.
- McKenna NJ, Lanz RB & O'Malley BW 1999 Nuclear receptor coregulators: cellular and molecular biology. *Endocrine Reviews* **20** 321–344.
- Meng YG, Han WD, Zhao YL, Huang K, Si YL, Wu ZQ & Mu YM 2007 Induction of LRP16 gene by estrogen promotes the invasive growth of Ishikawa human endometrial cancer cells through down-regulation of E-cadherin. *Cell Research* **17** 869–880.
- Nelson CC, Hendy SC, Shukin RJ, Cheng H, Bruchovsky N, Koop BF & Rennie PS 1999 Determinants of DNA sequence specificity of the androgen, progesterone, and glucocorticoid receptors: evidence for differential steroid receptor response elements. *Molecular Endocrinology* **13** 2090–2107.
- Pehrson JR & Fried VA 1992 MacroH2A, a core histone containing a large nonhistone region. *Science* **257** 1398–1400.
- Powell SM, Christiaens V, Voulgaraki D, Waxman J, Claessens F & Bevan CL 2004 Mechanisms of androgen receptor signaling via steroid receptor coactivator-1 in prostate. *Endocrine-Related Cancer* **11** 117–130.
- Rosenfeld MG, Lunyak VV & Glass CK 2006 Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes and Development* **20** 1405–1428.

- van Royen ME, Cunha SM, Brink MC, Mattern KA, Nigg AL, Dubbink HJ, Verschure PJ, Trapman J & Houtsmuller AB 2007 Compartmentalization of androgen receptor protein–protein interactions in living cells. *Journal of Cell Biology* **177** 63–72.
- Smith S, Giriati I, Schmitt A & de Lange T 1998 Tankyrase, a poly(ADP-ribose) polymerase at human telomeres. *Science* **282** 1484–1487.
- Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ *et al.* 1997 Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature* **389** 194–198.
- Taneja SS, Ha S, Swenson NK, Torra IP, Rome S, Walden PD, Huang HY, Shapiro E, Garabedian MJ & Logan SK 2004 ART-27, an androgen receptor coactivator regulated in prostate development and cancer. *Journal of Biological Chemistry* **279** 13944–13952.
- Toumazou C, Li J & Wong J 2008 Cofactor restriction by androgen receptor N-terminal and C-terminal interaction *Molecular Endocrinology* [in press].
- White R & Parker MG 1998 Molecular mechanisms of steroid hormone action. *Endocrine-Related Cancer* **5** 1–14.
- Zhao YL, Han WD, Li Q, Mu YM, Lu XC, Yu L, Song HJ, Lu JM & Pan CY 2005 Mechanism of transcriptional regulation of LRP16 gene expression by 17- β estradiol in MCF-7 human breast cancer cells. *Journal of Molecular Endocrinology* **34** 77–89.