

Actin monomer enhances supervillin-modulated androgen receptor transactivation

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Abstract

Actin-binding protein, supervillin, has been identified as an androgen receptor (AR) coregulator. Although actin has been suggested to participate in transcription regulation, the mechanism is not clear. Here we demonstrate signals involved in the cytoskeleton dynamic can modulate the coregulator function of supervillin. Three actin isoforms cooperate with supervillin in additive manner to further enhance AR transactivation. Latrunculin B toxin, an actin chelator, reduces the availability of monomer actin and attenuates supervillin function. Rac, the small G-protein kinase, is well studied in reorganization of cytoskeleton. The over-expression of constitutive-active Rac triggers the membrane ruffling site and reduces the coregulator activity of supervillin. Together, the availability of actin monomer affects supervillin-modulated AR transactivation.

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Recent studies demonstrated the existence of actin in chromatin remodeling complex provides the possible role of actin in transcriptional regulation [1]. Furthermore, the signaling cascade of LIM kinase and small G-protein mediating the reorganization of cytoskeleton is reported to modulate the activity of transcription factors [2,3]. The exchange between actin monomer and filament is highly dynamic and frequent in the cell. Therefore, the role of actin in gene transcription demands to be fully discovered.

Supervillin (SV), an actin-binding protein, is characterized as coregulator of androgen receptor (AR) [4]. AR transmits signaling of androgen, testosterone or 5 α -dihydrotestosterone (DHT), into the nuclear and regulates gene transcription [5]. Several AR associated proteins were discovered to modulate its transactivation by recruiting transcriptional machinery, histone de/acetylase, or controlling nuclear translocation of AR [6–10]. The physiological significance of these coregulators is not fully defined. According to their diverse

known functions, other than coregulators, they may therefore play roles in bridging signaling network to AR activity. AR is well characterized in the function of male reproductive organ development, prostate cancer growth, hair follicle formation, and skeletal muscle masculinization [11]. Since SV is abundant in skeletal muscle, AR function in skeletal muscle may depend on its presence [12]. SV is one of the actin-binding proteins with functional nuclear localization signals (NLS) and has been characterized to locate in the nucleus [13]. Its function in actin bundling is suggested and not yet clearly described. The functional coregulator domain of SV is located in amino acid 831–1281 of the bovine origin. In this functional domain, putative actin-binding sites and NLS are predicted [13]. This may provide a potential mechanism for its coregulator function.

Here we study how actin affects AR transactivation and how SV may cooperate with actin. The influence of actin monomer on the coregulator activity of SV is studied by using latrunculin toxin B to chelate the actin monomer. Furthermore, the impact of cytoskeleton reorganization, triggered by Rac signaling, on SV function is characterized.

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Materials and methods

Plasmids. The cDNA encoding actin isoforms were cloned and amplified by RT-PCR and inserted into pSG5. The plasmid, pCGN-HA-Rac1 Q61L, expressing constitutive active Rac protein was a gift from Dr. Jonathan Chernoff (Fox Chase Cancer Center). The plasmids, pEGFP-SV and pEGFP-SV (831–1281), were gifts from Dr. Elizabeth J. Luna (University of Worcester). Latrunculin B toxin was purchased from Calbiochem.

Transfection studies. COS-1 cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 10% fetal calf serum (FCS). Cells were seeded 24 h before transfection. Cells were transfected according to the “SuperFect Transfection” instructions (Qiagen). After 2–3 h incubation, culture medium was refreshed with medium supplemented with 10% charcoal-depleted FCS (CD-FCS). After 20 h, cells were either pretreated with latrunculin B or directly treated with EtOH or DHT as indicated. Cells were further incubated at 37 °C for 16–24 h and harvested. Cell lysates were prepared and used for luciferase (LUC) assay according to the manufacturer’s instructions (Promega). LUC activity was normalized with internal control *Renilla*-LUC activity. The results were obtained from at least three sets of transfection and presented as means ± SD.

Fluorescence microscopy. COS-1 cells were seeded on two-well Lab Tek Chamber slides (Nalge) in DMEM with 10% CD-FCS for 18 h before transfection with 2 µg DNA/10⁵ cells by the FuGENE6 transfection reagent (Boehringer–Mannheim). Transfected cells were incubated for 16 h and then fixed in fixation solution (3% formaldehyde and 10% sucrose in PBS) for 15 min on ice. Slides were then mounted by VECTASHIELD mounting medium with 4’,6-diamidino-2-phenylindole (DAPI) (Vector Lab) and photographed under 40-fold magnification with a Nikon Eclipse E800 microscope.

Results

To examine whether actin affects AR transactivation, we subcloned actin cDNA from human into expression plasmids and transfected into cells. Although they are

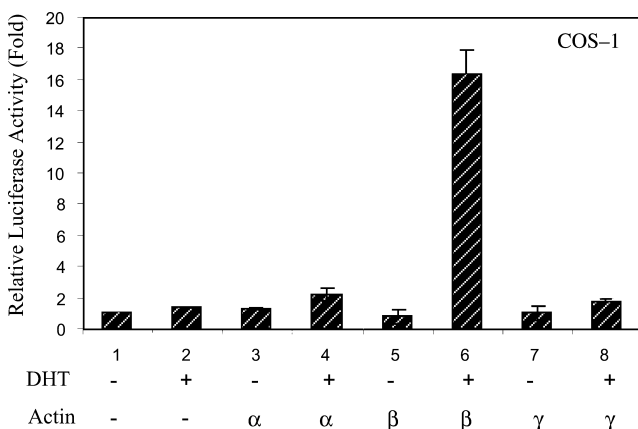


Fig. 1. Overexpression of actin enhances AR transactivation. The plasmids encoding three isoforms of actin (1.5 µg), pSG5-α, β, and γ-actin, were co-transfected with pSG5-AR (30 ng), MMTV-Luc (0.5 µg), and RL-SV40 (1 ng) into COS-1 cell by using SuperFect. After 20 h, cells were treated with EtOH or 10 nM DHT for 24 h before subjected to luciferase assay. After normalized by internal control, relative luciferase activity to lane 1 was calculated. Results are means ± SD of three independent experiments.

very similar to each other with only minor amino acid differences, β-actin exerts a stronger effect in promoting AR transactivation (Fig. 1). The expression efficiency of the three plasmids is similar according to in vitro transcription translation assay. Based on the fact that their expression is cell type specific, it may imply the function of each isoform is specialized and not identical.

SV is one of the actin-binding proteins identified as AR coregulator. To test whether actin affects the coregulator function of supervillin, we co-transfected SV and actin to demonstrate the cooperative effect among them. Actin and SV cooperated in enhancing AR transactivation in an additive manner (Fig. 2). Previous study showed the fragment of SV containing amino acid 831–1281 is sufficient in enhancing AR activity [4]. This fragment of SV contains one putative actin-binding site [13] and can also cooperate with actin to promote AR transactivation. Among the three isoforms, β-actin still exerts the strongest effect.

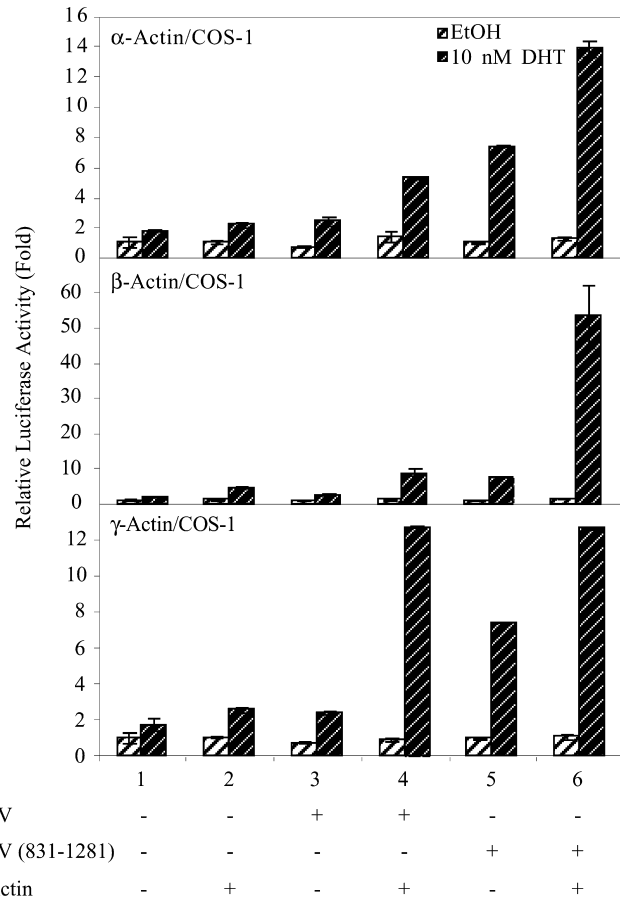


Fig. 2. Actin promotes supervillin enhanced AR transactivation. The plasmids encoding three isoforms of actin (0.75 µg), pSG5-α, β, and γ-actin, were co-transfected with pCMV-AR (30 ng), pEGFP-SV or pEGFP-SV (831–1281) (0.75 µg), MMTV-Luc (0.5 µg), and RL-SV40 (1 ng) into COS-1 cell by using SuperFect. After 20 h, cells were treated with EtOH or 10 nM DHT for 24 h before subjected to luciferase assay. After normalized by internal control, relative luciferase activity to EtOH treated lane 1 was calculated. Results are means ± SD of three independent experiments.

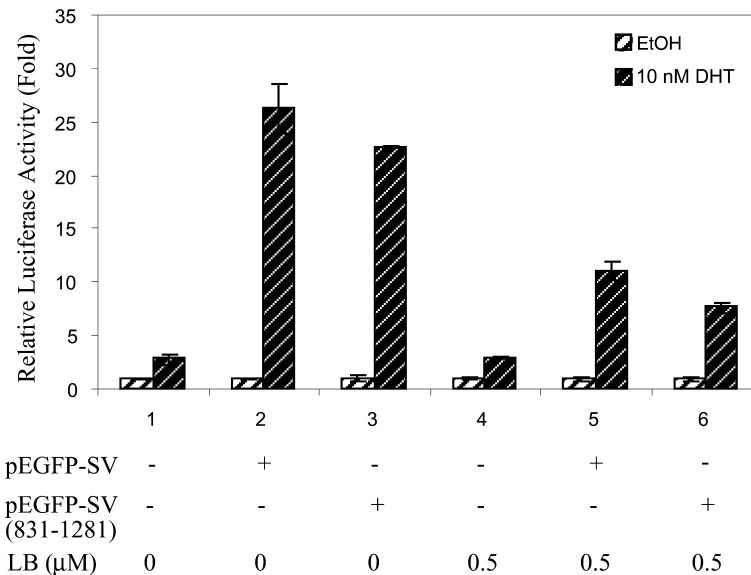


Fig. 3. Latrunculin B suppresses coregulator activity of supervillin. The plasmids, pCMV-AR (30 ng), pEGFP-SV or pEGFP-SV (831–1281) (1.5 μ g), MMTV-Luc (0.5 μ g), and RL-SV40 (1 ng), were co-transfected into COS-1 cells by using SuperFect. After 20 h, cells were pretreated with latrunculin B for 30 min and then combined with EtOH or 10 nM DHT for 16 h before subjected to luciferase assay. After normalized by internal control, relative luciferase activity was calculated using EtOH treatment as 1 for each lane. Results are means \pm SD of three independent experiments.

We further demonstrated the importance of actin in the coregulator function of SV by using latrunculin B toxin (LB) which chelates actin monomer and prevents it from polymerization. Pretreatment of 0.5 μ M LB for 30 min significantly affects actin polymerization that cell morphology dramatically changed. The supervillin enhanced AR transactivation is significantly suppressed after LB pretreatment (Fig. 3). Similarly, SV (831–1281) is also affected by LB though less than full length.

Small G-protein kinase, Rac, has been well studied in its ability of actin reorganization [14]. Recently, its effect in modulating AR transactivation has also been reported [3]. However, the mechanism is not clear. The coregulator activity of SV may involved in this signaling network. We therefore study how Rac might influence SV function. First, the distribution of SV was observed by overexpression of EGFP conjugated SV with or without constitutive active Rac. The signaling activated by Rac results in concentration of SV in membrane ruffling site dramatically (Fig. 4A). Second, the effect of Rac signaling in coregulator activity of SV is assayed. Rac signal significantly suppressed coregulator effect of SV (Fig. 4B). Therefore, the availability of actin monomer abolished by Rac signaling results in the reduction of SV coregulator function.

Discussion

Three isoforms of actin differ from each other in amino acid sequence slightly [15]. Among them, α -actin

is abundant in muscle, while β -actin and γ -actin are abundant in non-muscle tissues. In addition to cell type specific distribution, whether they function differently is not well understood. Our study demonstrated the difference among three isoforms in modulating AR activity, which may imply distinct mechanisms in regulating gene transcription exist among three actin isoforms.

Several actin-binding proteins have been identified to modulate nuclear receptor activity, such as filamin, supervillin, and gelsolin [4,9,16]. Among them, the only known mechanism is that filamin is essential for nuclear translocation of AR. Since SV has several putative actin-binding sites and functional NLSs and the minimal functional fragment of SV contains one actin-binding site and locates in the nucleus, recruiting actin into chromatin remodeling complex may be a potential mechanism of coregulators. We pursued this hypothesis by overexpressing actin monomer and squelching actin to observe the impact on coregulator activity of SV. Actin and SV potentiate each other in promoting AR activity in our study. On the other hand, actin chelator, latrunculin B, attenuates coregulator function of both full length and minimal functional fragment of SV.

One report showed Rho guanine nucleotide dissociation inhibitor (Rho GDI) positively regulates AR activity, which suggested small G-protein kinase, Rho, might down-regulate AR transactivation [3]. In contrast, another report showed the coregulator, FHL2, can mediate Rho kinase promoting AR [17]. The conflict resulting from these two studies needs to be

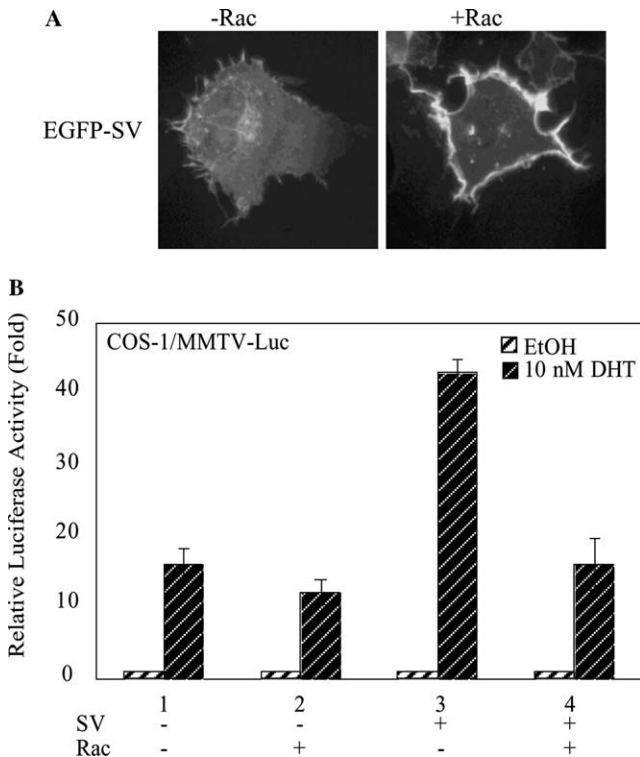


Fig. 4. Constitutive Rac attenuates supervillin enhanced AR transactivation. (A) COS-1 cells were co-transfected with pEGFP-SV and constitutive-active Rac or empty vector by using Fugene6. After 20 h, cells were fixed and observed by fluorescence microscope. (B) The plasmids encoding constitutive Rac (0.1 μ g), pCMV-AR (30 ng), pEGFP-SV (1.4 μ g), MMTV-Luc (0.5 μ g), and RL-SV40 (1 ng) were co-transfected into COS-1 cells by using SuperFect. After 20 h, cells were treated with EtOH or 10 nM DHT for 24 h before subjected to luciferase assay. After normalized by internal control, relative luciferase activity was calculated using EtOH treatment as 1 for each lane. Results are means \pm SD of three independent experiments.

further clarified. In our study, Rac signaling stimulates membrane ruffling that further attenuates coregulator activity of SV. There are two possible explanations for this outcome, one is the accumulation of SV in membrane refraining it from associating with AR, the other is the decrease in the amount of actin monomer affecting SV coregulator activity which demands actin monomers.

Actin highly expresses in cells and exchanges from monomer to filament frequently under the regulation of several extracellular signaling. Therefore, the discovery of actin modulating AR target gene transcription may provide a new angle to study how androgen triggers prostate cancer cell transformation, metastasis, and increased skeletal muscle strength.

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