

Stearoyl CoA Desaturase (SCD) Facilitates Proliferation of Prostate Cancer Cells through Enhancement of Androgen Receptor Transactivation

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Stearoyl-CoA desaturase (SCD), the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids, is highly expressed in prostate cancer although the SCD protein has been known to be rapidly turned over by proteolytic cleavage. The present data demonstrate that SCD can promote proliferation of androgen receptor (AR)-positive LNCaP prostate cancer cells and enhance dihydrotestosterone (DHT)-induced AR transcriptional activity, resulting in increased expression of prostate-specific antigen (PSA) and kallikrein-related peptidase 2 (KLK2). Interestingly, among the previously reported SCD-derived peptides produced by proteolytic cleavage of SCD, a peptide spanning amino acids 130–162 of SCD (SCD-CoRNR) contained the CoRNR box motif (LFLII) and enhanced AR transcriptional activity. In contrast, a mutant SCD-CoRNR in which Leu¹³⁶ was replaced by Ala had no effect on AR transcriptional activity. Moreover, SCD-CoRNR directly interacted with AR and inhibited RIP140 suppression of AR transactivation. Knockdown of the SCD gene by SCD microRNA suppressed AR transactivation with decreased cell proliferation, suggesting that SCD may regulate the proliferation of LNCaP cells via modulation of AR transcriptional activity. Moreover, ectopic expression of SCD in LNCaP cells facilitated LNCaP tumor formation and growth in nude mice. Together, the data indicate that SCD plays a key role in the regulation of AR transcriptional activity in prostate cancer cells.

INTRODUCTION

Prostate cancer is one of the most commonly diagnosed cancers and the second leading cause of cancer-related deaths in American men (Jemal et al., 2007). It has generally been accepted that androgen receptor (AR) plays an important role in the development and progression of prostate cancer (Suzuki et al., 2003). AR, a member of the nuclear receptor (NR) super-

family, is a ligand-dependent transcription factor that requires many associated proteins for proper function (Chawla et al., 2001). AR coactivators including steroid receptor coactivator 1, the cyclic AMP response element-binding protein-binding protein and ARA70 have been identified and their roles in prostate cancer progression have been studied (Culig et al., 2004). These AR coactivators are recruited to either the ligand binding domain (LBD) or N-terminal activation function-1 domain of liganded AR via their L/FXXLL/F sequences and enhance AR function (He et al., 2002). In contrast, corepressors are recruited to unliganded or antagonist-liganded NRs via a direct interaction between NR LBD and corepressor nuclear receptor (CoRNR) box (L/XXI/VI) motifs present in the repression domain of the corepressors, leading to transcriptional repression of target gene expression (Hu and Lazar, 1999). Recently, corepressors such as receptor interacting protein 140 (RIP140), silencing mediator of retinoic acid and thyroid hormone receptor and nuclear receptor corepressor have been shown to interact with agonist-liganded AR, even in the presence of coactivators, and suppress AR transcriptional activity (Carascossa et al., 2006; Hodgson et al., 2008).

Stearoyl-CoA desaturase (SCD) is a short-lived endoplasmic reticulum membrane protein that catalyzes the delta 9-cis desaturation of acyl-CoA, preferably palmitoyl-CoA and stearoyl-CoA, to produce palmitoleoyl-CoA and oleoyl-CoA, respectively. These products serve as substrates for the synthesis of various kinds of lipids including phospholipids, triglycerides, cholesteryl esters and wax esters (Enoch et al., 1976). Changes in the expression or activity of SCD alter the fatty acid composition of the cell membrane. In addition, the monounsaturated fatty acids (MUFAs), SCD products, are also involved in cell proliferation signaling pathways, suggesting that SCD activity may play a role in cell proliferation and tumorigenesis (Fritz et al., 2010; Leitzmann, 2005). However, the SCD protein is degraded rapidly by proteolytic cleavages, producing multiple different peptides (Heinemann et al., 2003; Man et al., 2006). Although rapid degradation of SCD in different organisms has been reported

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(Mziaut et al., 2000), the physiological significance or consequence of SCD degradation is still not clear. Since AR is a key player in the development and progression of prostate cancer and many cofactors are involved in the regulation of AR activity, it is possible that SCD or its proteolytic products could participate in the initiation and progression of prostate cancer by modulation of AR function. In support of this hypothesis, the present study demonstrates that SCD overexpression in LNCaP cells facilitates prostate cancer cell proliferation and expression of the AR target genes, prostate specific antigen (PSA) and kallikrein-related peptidase 2 (KLK2), by enhancing AR transcriptional activity. In addition, a cytoplasmic fragment of SCD (amino acids 130-162; SCD-CoRNR), which has been reported to be generated by the proteolytic cleavage of SCD, contains the CoRNR box and directly interacts with AR, resulting in enhanced AR transcriptional activity. Moreover, SCD overexpression in LNCaP cells facilitates the formation and growth of xenografted tumors. Taken together, these results demonstrate that SCD plays an important role in prostate cancer progression by regulation of AR transactivation and, thus, serves as a key modulator of prostate cancer initiation and progression.

MATERIALS AND METHODS

Plasmids

Plasmids pCMX-VP16-AR, pSG5-AR, pEF-RIP140, ARE₄-Luc, and pG5-Luc were described previously (Hu et al., 2004; Kim et al., 2007). Polymerase chain reaction (PCR)-amplified full-length human SCD sequence from HepG2 total RNA was inserted into pcDNA3 (Invitrogen). SCD-CoRNR or mtSCD-CoRNR sequence was amplified from full-length SCD by PCR and cloned into pcDNA3 or pCMX-GAL4. SCD microRNA (miRNA) (SCDmiR) was purchased from Invitrogen and cloned into pcDNATM6.2-GW (Invitrogen).

Cell culture, luciferase assay and stable transfection

Cells were maintained in RPMI-1640 (LNCaP, 22Rv1 and PC-3) or DMEM (DU145 and CV-1) medium supplemented with 10% fetal bovine serum (FBS). RWPE-1 cells were cultured in keratinocyte-SFM (GIBCO) supplemented with defined keratinocyte-SFM growth supplement (GIBCO). Transient transfection and luciferase assays were performed as previously described (Park et al., 2008). Relative luciferase activity was expressed based on the fold-induction relative to transfection of empty vector (set as 1-fold) without ligand and the results were expressed as the mean \pm S.D. of three separate experiments. To establish stable cells, pcDNA3, pcDNA3-SCD, pcDNATM6.2-GW/miR or pcDNATM6.2-GW/SCDmiR were transfected into LNCaP or PC-3 cells and selected using 600 μ g/ml Geneticin or 3 μ g/ml Blastacidin for 2 weeks.

Semi-quantitative reverse transcription PCR (semi-Q RT-PCR)

Total RNA was isolated from cells using Trizol reagent (Invitrogen), and cDNA was synthesized using MMLV-RTase (Promega) and Oligo dT primers (Invitrogen). The mRNA levels of SCD, PSA, KLK2 and 36B4 were determined by semi-Q RT-PCR as described previously (Park et al., 2008). Abundance of SCD, PSA, and KLK2 mRNAs was quantified relative to the transcript of the internal control, 36B4, using a Gel doc XR system (Bio-Rad). RT-PCR was performed three independent times for each experiment. The sequences for the sense and antisense strand PCR primers were: SCD (sense; 5'-CGACGTGGCT TTTTCTTCTC-3') and (antisense; 5'-GGGGGCTAATGTTCT

TGTC-3'), PSA (sense; 5'-CCACGATGGTGCCTTGATC-3') and (antisense; 5'-GGCCAGGTATTTTCAGGTCAG-3'), KLK2 (sense; 5'-GCTGCCATTGCCTAAAGAAG-3') and (antisense; 5'-TGGGAAGCTGTGGCTGACA-3'), 36B4 (sense; 5'-AGATG CAGCAGATCCGCAT-3') and (antisense; 5'-ATATGAGGCAG CAGTTTCTCCAG-3').

Cell viability assay

Stably transfected LNCaP or PC-3 cells were seeded in wells of 12-well plates at a density of 15,000 per well in medium containing 10% FBS. At the indicated times, medium was removed and serum-free medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg/ml; Sigma-Aldrich) was added to each well. Two hours later, the cellular formazan product was dissolved with acidic isopropanol, and the absorbance at 570/650 nm was measured by an ELISA reader. Value of untreated control cells at 0 h was set as 1. The results are the mean \pm S.D. of A₅₇₀ from three independent experiments.

Colony formation assay

The colonogenic assay was performed as previously described (Niu et al., 2008). Briefly, stably transfected LNCaP cells (500 per well) were plated in 100-cm² dish and cultured in RPMI medium with 10% FBS for 2 weeks. The cells were then fixed and stained with 0.25% crystal violet in 80% methanol for 30 min, washed with water, and colonies that contained > 50 cells were counted using a Gel doc XR system (Bio-Rad). Values were expressed as the mean \pm S.D. of three independent experiments.

Tumorigenesis in nude mice

All the animal experiments were conducted in accordance with the protocol approved by Chonnam National University Institutional Animal Care and Use Committee. A suspension of 1×10^6 LN-C or LN-SCD1 cells with Matrigel was injected subcutaneously to the flank of 5-week-old athymic male nude mice (Orient Bio). Mice were then monitored weekly for tumor formation. Tumor volumes were calculated using the formula:

$$V = \pi/6 \times L \times w \times h$$

where V = tumor volume (mm³), L = length (mm), w = width (mm), and h = height (mm) (Torosian and Donoway, 1991).

Western blot analysis

Samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and proteins were transferred from the gel to a polyvinylidene fluoride membrane. The membrane was probed with anti-SCD antibody (GeneTex) and horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology).

RESULTS

SCD enhances proliferation of AR-positive LNCaP prostate cancer cells and AR transactivation

Increased SCD expression in human prostate cancer tissues and cell lines has recently been reported (Fritz et al., 2010). We also found that SCD was highly expressed in prostate cancer cells (LNCaP, PC-3, DU145 and 22Rv1) as compared with normal prostate epithelial cells (RWPE-1) (Fig. 1A). To investigate the biological significance of up-regulated expression of SCD in prostate cancer cells, we stably transfected SCD cDNA into LNCaP cells and three independent LNCaP SCD stable

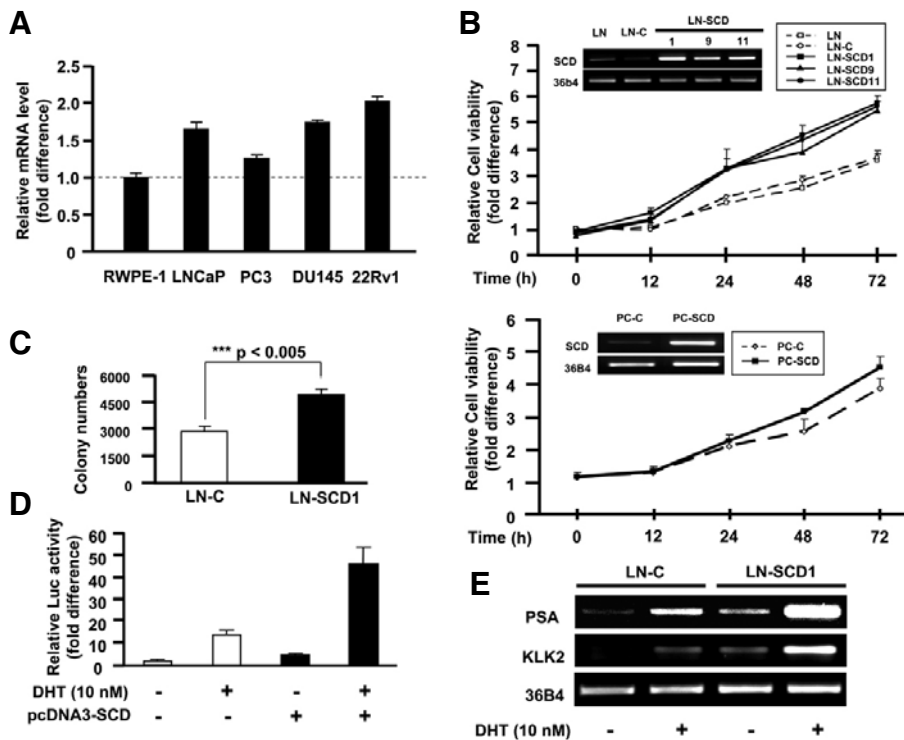


Fig. 1. SCD stimulates AR-positive LNCaP prostate cancer cell growth and enhances AR transcriptional activity. (A) Increased expression of the SCD gene in human prostate cancer cells. The mRNA level of SCD was analyzed in nonmalignant (RWPE-1) and malignant prostate cells (LNCaP, PC-3, DU145 and 22Rv1) by semi-Q RT-PCR analysis. (B) Effect of SCD on the growth rate of LNCaP or PC-3 cells was determined using the MTT viability assay. Cells were cultured in RPMI-1640 with 10% FBS and harvested at the indicated time points. Insets, relative mRNA levels of SCD in SCD stable clones of LNCaP (LN-SCD1, LN-SCD9 and LN-SCD-11) or PC-3 (PC-SCD) were compared with parental LNCaP cells (LN) or empty vector-transfected cells (LN-C or PC-C) using semi-Q RT-PCR. (C) Effect of SCD overexpression on colony formation of LNCaP cells. Two weeks after plating, colonies containing > 50 cells were counted. *** $P < 0.005$. (D) SCD enhances DHT-induced AR transcriptional activity. After 12 h transfection of ARE₄-Luc (300 ng) with pcDNA3 or pcDNA3-SCD (300 ng each), LNCaP cells were treated with vehicle or DHT (10 nM) for 24 h and then harvested for the luciferase assay. (E) SCD increases expression of AR target genes. After treatment with ethanol or 10 nM DHT for 24 h, PSA and KLK2 mRNA levels in LN-SCD1 and LN-C cells were determined by semi-Q RT-PCR.

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clones (LN-SCD1, LN-SCD9 and LN-SCD11) were identified (Fig. 1B, upper panel). In the MTT assay, SCD overexpression in LNCaP cells resulted in significantly increased prostate cancer cell growth on day 3 as compared with parental LNCaP (LN) and empty vector-transfected cells (LN-C). In contrast to the growth promoting effect of SCD on AR-positive LNCaP cells, no significant changes were observed in the proliferation of AR-negative PC-3 cells overexpressing SCD (PC-SCD), suggesting that SCD-mediated prostate cancer cell growth may be AR-dependent (Fig. 1B, upper panel vs. lower panel). To evaluate the *in vitro* tumorigenicity due to SCD expression, LN-C and LN-SCD1 were used as representative cells in a colony formation assay. Consistent with Fig. 1B, overexpression of SCD resulted in a marked increase in colony numbers in LN-SCD1 (167%) compared to LN-C (Fig. 1C; $P < 0.005$). Since AR transcriptional activity is essential for the proliferation of prostate cancer cells (Brinkmann and Trapman, 2000; Chawla et al., 2001; Suzuki et al., 2003), we examined whether SCD could regulate androgen-induced AR transactivation using ARE₄-Luc, a reporter containing four repeats of the AR response element, and 5 α -dihydrotestosterone (DHT), a testosterone metabolite that is a more active androgen than testosterone (Thin et al., 2003). Interestingly, the addition of SCD into LNCaP cells induced AR activity even in the absence of DHT (Fig. 1D; lanes 3 vs. 1). Furthermore, SCD strongly enhanced AR transactivation of ARE₄-Luc induced by 10 nM DHT (Fig. 1D; lane 4 vs. 2) and similar results were observed when ARE₄-Luc was replaced with PSA-Luc containing the PSA promoter (data not shown). PSA and KLK2, members of the kallikrein family of serine proteases, are well-known AR target genes and biochemical markers of prostate cancer. Thus, we tested SCD effect on expression of these genes in LNCaP cells using RT-PCR. Con-

sistent with the reporter gene assays, RT-PCR analysis showed that mRNA levels of the PSA and KLK2 genes in DHT-treated LN-SCD1 cells were increased by 4.8- and 1.67-fold, respectively, compared with DHT-treated LN-C cells (Fig. 1E; lanes 4 vs. 2). Taken together, these data indicate that SCD plays a role as a functional modulator of AR transcriptional activity in prostate cancer cells.

SCD-derived peptide inhibits RIP140 suppression of AR transcriptional activity

SCD is a short-lived multispanning endoplasmic reticulum membrane protein that is rapidly degraded by protease located in the ER membrane. Among the peptides previously shown to be produced by proteolysis of SCD (Mziau et al., 2000), we found that a SCD fragment (amino acids 130-162; SCD-CoRNR) contained the CoRNR box and its proteolytic cleavage sites, and CoRNR box were conserved among human, rat and mouse (Fig. 2A). Since SCD-CoRNR is originally located in the cytoplasmic compartment and CoRNR box motif is known to directly interact with AR, we first tested whether SCD-CoRNR could modulate AR transcriptional activity using ARE₄-Luc. As shown in Fig. 2B, AR was activated by 10 nM DHT and the addition of SCD-CoRNR further enhanced AR transcriptional activity. In contrast, the mutant SCD-CoRNR (mtSCD-CoRNR), in which Leu¹³⁶ was mutated to Ala, was not able to enhance AR transcriptional activity. Using semi-Q RT-PCR, we then determined the mRNA levels of PSA in LNCaP cells transfected with SCD-CoRNR to further confirm its effect on AR transactivation. As expected, the addition of SCD-CoRNR together with 10 nM DHT further increased DHT-induced PSA mRNA levels in LNCaP cells, whereas mtSCD-CoRNR had no effect on DHT-induced PSA expression (Fig. 2C). The mam-

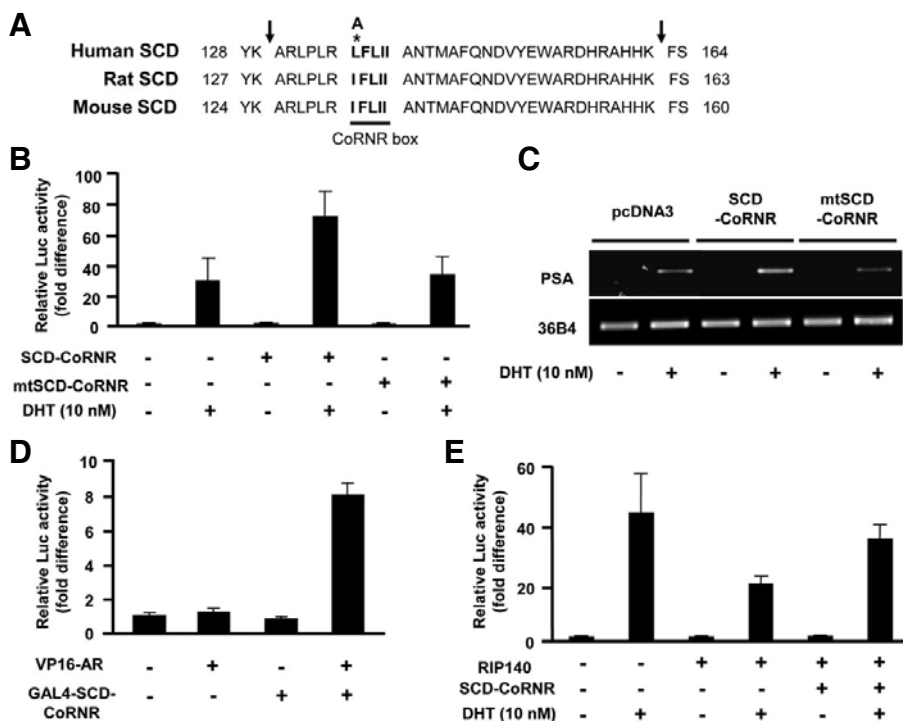


Fig. 2. SCD-derived peptide (SCD-CoRRN) enhances AR transactivation by inhibiting RIP140 suppression of AR transactivation. (A) CORNR box motif and cleavage sites producing SCD-CoRRN are highly conserved among human, rat and mouse SCDs. Cleavage sites are indicated by the arrows. Mutant SCD-CoRRN was constructed by substituting Ala for Leu¹³⁶. (B) SCD-CoRRN enhanced AR transcriptional activity. ARE₄-Luc (300 ng) and pSG5-AR (100 ng) were co-transfected with either pcDNA3, pcDNA3-SCD-CoRRN or pcDNA3-mtSCD-CoRRN (300 ng each) into CV-1 cells as indicated. Cells were treated with 10 nM DHT for 24 h and assayed for luciferase activity. (C) Semi-Q RT-PCR assays were performed to examine the effect of SCD-CoRRN on PSA expression in LNCaP cells. SCD-CoRRN or mtSCD-CoRRN expression vector (6 μ g) was transfected into LNCaP cells as indicated. Cells were treated with 10 nM DHT for 24 h and harvested

for semi-Q RT-PCR. (D) Mammalian two-hybrid assay for the interaction between SCD-CoRRN and AR. CV-1 cells were transiently transfected with 150 ng pG5-Luc and 300 ng of different fusion plasmids, as indicated. The strength of the interaction was expressed as fold-induction relative to that of the GAL4/VP16-transfected sample (set as 1.0-fold). (E) SCD-CoRRN inhibits the suppressive effect of RIP140 on DHT-induced AR transactivation. SCD-CoRRN expression vector (300 ng) was co-transfected with ARE₄-Luc (300 ng), pSG5-AR (100 ng) and pEF-RIP140 (300 ng) into CV-1 cells as indicated. Cells were treated with vehicle or 10 nM DHT for 24 h and harvested for luciferase assay.

malian two-hybrid assay was then performed to test whether SCD-CoRRN could interact with AR. As shown in Fig. 2D, when GAL4-SCD-CoRRN was co-transfected with the pG5 reporter plasmid and VP16-AR into CV-1 cells, significant induction was observed. In contrast, co-transfection of GAL4-SCD-CoRRN or VP16-AR together with either the VP16 or GAL4 empty vector resulted in a low background level of transcriptional activity, suggesting that SCD-CoRRN may interact with AR via the CoRRN box. Furthermore, the addition of SCD-CoRRN into LNCaP cells transfected with pEF-RIP140 and ARE₄-Luc inhibited the suppressive effect of RIP140 on AR transcriptional activity (Fig. 2E).

Silencing of SCD by miRNA inhibits AR transcriptional activity and cell proliferation in LNCaP cells

We next assessed the effect of SCD knockdown on AR transactivation and cell proliferation in LNCaP cells. To accomplish this, LNCaP cells stably expressing scrambled miRNA (LN-miR) or SCD miRNA (LN-SCDmiR3, LN-SCDmiR5 and LN-SCDmiR8) were first established and suppression of endogenous SCD expression was then confirmed in LN-SCDmiR cells by RT-PCR (Fig. 3A, insert). Silencing of SCD in LNCaP cells (LN-SCDmiRs) significantly decreased cell proliferation by 38% as compared with LN-miR cells in the MTT assay (Fig. 3A). Furthermore, in the colony formation assay, the colony numbers of the LN-SCDmiR8 was drastically decreased by 43.3% compared to LN-miR cells (Fig. 3B). In addition, AR transactivation stimulated by DHT (10 nM) was decreased when endogenous SCD expression was silenced by SCDmiR (Fig. 3C; lanes 2 vs. 4). To further confirm the role of SCD in AR activity, mRNA

level of PSA was compared between LN-SCDmiR8 and LN-miR cells by semi-Q RT-PCR. As was observed above, a loss of SCD reduced the DHT-induced PSA mRNA level by 50% (Fig. 3D). These results suggest that endogenous SCD may play an important role as an activator of cell proliferation in part by upregulation of AR transactivation in LNCaP cells.

SCD promotes prostate tumor growth *in vivo*

The above results showed how SCD enhances AR transactivation and facilitates proliferation of prostate cancer cells *in vitro*. To further determine the role of SCD in tumor growth *in vivo*, LN-C or LN-SCD1 cells were injected subcutaneously in one flank site of 5-week-old male nude mice and then tumor formation and growth rates were monitored for six weeks. As shown in Fig. 4A, the average tumor volume and weight for LN-SCD1 after the sixth week were about 277% and 278% of those from the LN-C group, respectively. To further investigate whether this SCD effect was related to its ability to enhance AR transactivation, we examined the expression levels of PSA. Semi-Q RT-PCR analysis clearly showed that the mRNA levels of PSA in prostate tumor tissues from LN-SCD1 injected mice were higher than those from the control group (Fig. 4B). We also confirmed increased SCD mRNA and protein levels in these tumor tissues by RT-PCR and Western blot analyses. Together, these results show that SCD induces tumor development and progression via up-regulation of AR transcriptional activity.

DISCUSSION

Dysregulated expression of metabolic enzymes has emerged

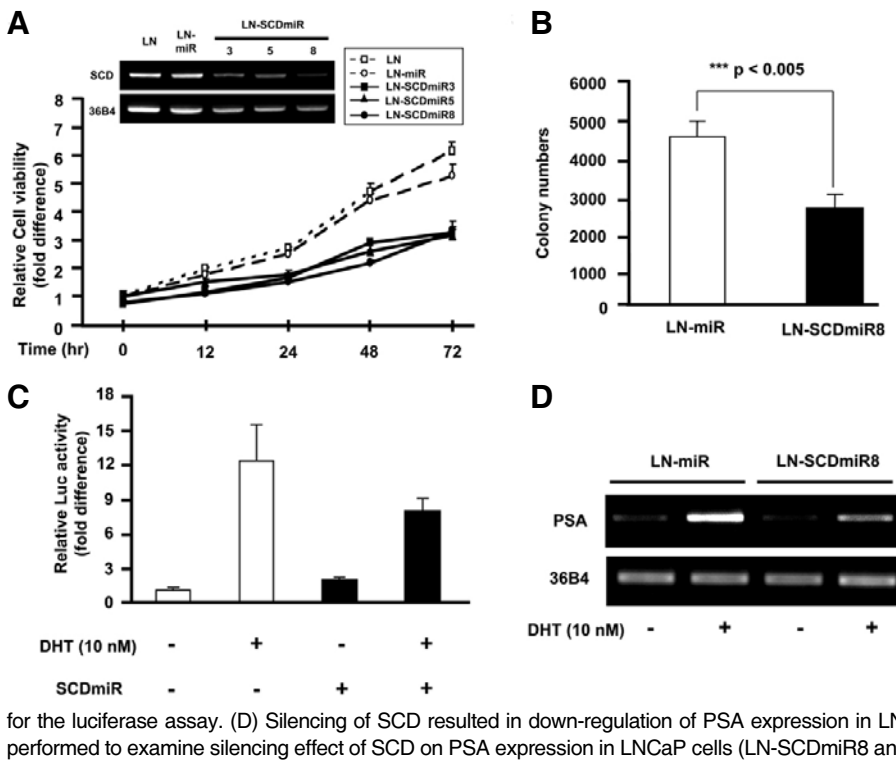


Fig. 3. Knockdown of endogenous SCD inhibits AR transcriptional activity and prostate cancer cell growth. (A) Silencing effect of SCD miRNA on LNCaP cell growth. Relative growth rates of LN, LN-miR, LN-SCDmiR3, LN-SCDmiR5, and LN-SCDmiR8 cells were determined using the MTT assay. Insert: Relative mRNA levels of SCD in LNCaP SCD miRNA stable clones (LN-SCDmiRs) were compared with empty vector-transfected (LN-miR) and parental LNCaP cells (LN) using semi-Q RT-PCR. (B) Reduction in the colony forming units of LNCaP cells by knockdown of the SCD gene. Two weeks after plating, colonies containing > 50 cells were counted. $***P < 0.005$. (C) Effect of SCD knockdown on AR transactivation. LNCaP cells were transfected with ARE₄-Luc (300 ng) in the absence or presence of the SCD miRNA expression vector (100 ng) as indicated. Cells were treated with vehicle or 10 nM DHT for 24 h and harvested for the luciferase assay. (D) Silencing of SCD resulted in down-regulation of PSA expression in LNCaP cells. Semi-Q RT-PCR assays were performed to examine silencing effect of SCD on PSA expression in LNCaP cells (LN-SCDmiR8 and LN-miR) as indicated.

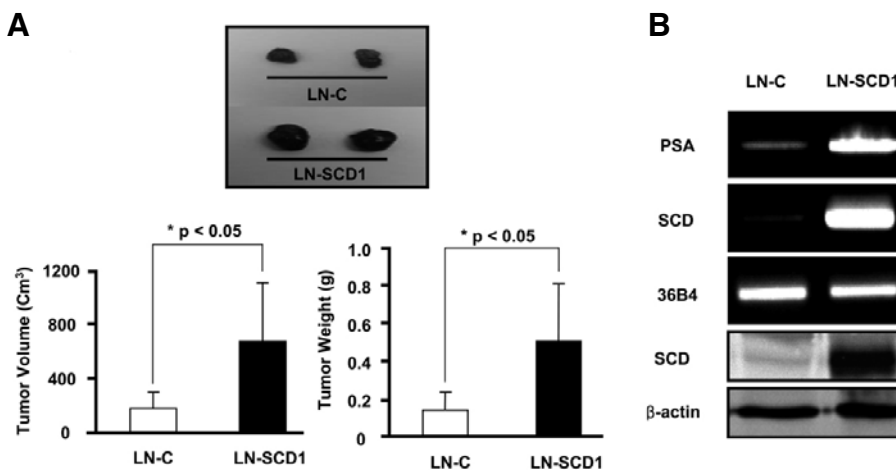


Fig. 4. SCD overexpression stimulates prostate tumor growth in xenograft tumor models. (A) LN-SCD1 or LN-C cells were subcutaneously injected into nude mice. Six weeks after cell injection, tumor volume and weight were measured for LN-SCD1 and LN-C tumors. Two representative tumors excised from LN-SCD1 and LN-C xenografts were shown. Values are given as mean \pm S.D. (n = 7). $*P < 0.05$. (B) Upper panel, PSA and SCD mRNA levels were determined in tumors excised from the LN-SCD1 and LN-C xenografts by semi-Q RT-PCR. Lower panel, Western blot analysis of SCD protein levels in tumors excised from the LN-SCD1

and LN-C xenografts. Representative semi-Q RT-PCR and Western blot data shown here are from three independent experiments with similar results.

as a risk factor for the development and progression of prostate cancer (Menendez and Lupu, 2007). In line with this notion, an increased expression level of SCD has been reported in prostate and other cancers (Scaglia and Igal, 2005). Here, we report that SCD facilitates proliferation of prostate cancer cells by enhancing AR transcriptional activity. SCD protein experiences rapid degradation by endoplasmic reticulum-resident protease, resulting in the production of a variety of peptides and this characteristic of SCD has been conserved during evolution (Heinemann et al., 2003; Man et al., 2006). Considering the high turn-over rate of SCD, it is possible that in addition to supplying new lipid components for membrane biogenesis in proliferating cancer cells, peptides derived from proteolytic cleavages of SCD may play an independent role in the initiation and

development of prostate cancer. A previous report demonstrated that the C-36 peptide, which is a degradation product of a serum protease inhibitor α_1 -antitrypsin, inhibited the α_1 -fetoprotein transcription factor transcriptional activity by inhibiting α_1 -fetoprotein transcription factor from binding to its target gene promoters (Gerbod-Giannone et al., 2002). This finding indicates that peptides derived from protein degradation may play a role in the transcriptional regulation of gene expression. In accordance with this notion, we found that SCD-CoRNR was able to enhance AR transcriptional activity, resulting in a further increase in PSA expression. Corepressor-NR interaction is required for corepressor-dependent suppression of NR activity. Peptides containing LXXLL-like motifs, which are important coactivator motifs for interaction with NRs, play a role as a pep-

tide antagonist of NRs via targeting coactivator binding surfaces in AR and estrogen receptor (Chang et al., 1999; He et al., 2004; Heldring et al., 2007). Thus, it is possible that the CoRNR box present in the SCD-CoRNR plays a key role in SCD-mediated enhancement of AR transactivation. In support of this hypothesis, SCD-CoRNR directly interacted with the LBD of AR in the presence or absence of DHT. Moreover, overexpression of the SCD-CoRNR inhibited RIP140 suppression of DHT-mediated AR transactivation. The LYYML sequence located in the carboxyl terminal region of the corepressor RIP140 plays an essential role in the interaction between RIP140 and retinoic acid receptor/retinoid X receptor (Chen et al., 2002). Interestingly, in this study, mutant carboxyl terminal peptide (amino acids 1063-1076) of RIP140, in which LYYML motif was mutated to a CORNR box, competitively inhibited RIP140 interaction with the retinoic acid receptor/retinoid X receptor. Thus, it is plausible that SCD-CoRNR may displace corepressors such as RIP140 from DHT-liganded AR and the CoRNR box sequence present in this peptide is critical for enhancing effect of SCD on AR transcriptional activity. The significance of the CoRNR box of SCD-CoRNR on AR activity was further supported by mutating the CoRNR box in SCD-CoRNR. Although SCD-CoRNR was able to enhance androgen-induced AR transcriptional activity, the effect of SCD-CoRNR on AR transactivation was lower than that of full-length SCD. In addition, a reporter gene assay revealed that SCD could slightly induce AR activity even in the absence of DHT, suggesting that in addition to an AR coactivator role of the SCD-CoRNR, intact SCD may play an additional role in AR transcriptional activity. However, how SCD also participates in AR transcriptional activity is unclear. One possibility is that MUFAs produced by SCD may also contribute to enhancing effect of SCD on AR activity. Previous studies showed that MUFAs such as oleic acid can activate mitogen-activated protein kinase (MAPK), which functions in prostate cancer progression by activation of AR activity (Askari et al., 2002; Lu et al., 1996; Paine et al., 2000; Werz et al., 2002; Yeh et al., 1999). Furthermore, chemical inhibition of SCD in LNCaP cells reduces phosphorylation of MAPK (Fritz et al., 2010). However, additional studies will be required to define the mechanism by which SCD promotes AR activity independent of direct interaction of SCD-CoRNR with AR. We also investigated whether SCD could affect prostate tumorigenesis and cancer progression. Consistent with *in vitro* results, the xenograft model revealed that overexpression of SCD strongly stimulated growth of tumors derived from AR positive LNCaP cells together with increased PSA expression. Consistent with these *in vitro* results, the xenograft model revealed that overexpression of SCD strongly stimulated growth of tumors derived from AR-positive LNCaP cells together with increased PSA expression.

In summary, our data demonstrate that SCD promotes prostate cancer cell growth via the up-regulation of the AR transcriptional activity *in vitro* and *in vivo*, implying that SCD may be a new target for developing more efficient therapeutic strategies to treat prostate cancer.

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