

Dual Roles for Coactivator Activator and its Counterbalancing Isoform Coactivator Modulator in Human Kidney Cell Tumorigenesis

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Abstract

Coactivator activator (CoAA) has been reported to be a coactivator that regulates steroid receptor-mediated transcription and alternative RNA splicing. Herein, we show that CoAA is a dual-function coregulator that inhibits G₁-S transition in human kidney cells and suppresses anchorage-independent growth and xenograft tumor formation. Suppression occurs in part by down-regulating *c-myc* and its downstream effectors *cnd1* and *skp2* and causing accumulation of p27/Kip1 protein. In this cellular setting, CoAA directly represses the proto-oncogene *c-myc* by recruiting HDAC3 protein and decreasing both the acetylation of histone H3 and the presence of RNA polymerase II on the *c-myc* promoter. Interestingly, a splicing isoform of CoAA, coactivator modulator (CoAM), antagonizes CoAA-induced G₁-S transition and growth inhibition by negatively regulating the mRNA levels of the endogenous CoAA isoform. In addition, we found that expression of CoAA protein is significantly decreased in human renal cell carcinoma compared with normal kidney. Our study presents evidence that CoAA is a potential tumor suppressor in renal carcinoma and that CoAM is a counterbalancing splice isoform. This is, thus far, the only example of a nuclear receptor coregulator involved in suppression of kidney cancer and suggests potentially significant new roles for coregulators in renal cancer biology. [Cancer Res 2008;68(19):7887–96]

Introduction

Renal carcinoma is the seventh leading cancer in the United States, and its incidence has been increasing at a rate of ~2%/yr for the past six and half decades. The sporadic occurrence of renal cancers with undefined pathogenesis begs for a greater understanding of its mechanisms of tumorigenesis. However, few bona fide tumor suppressor genes, except for VHL, have been shown to contribute to renal cancer.

Recent human genome analyses revealed that up to 70% of human genes have alternative splice forms (1), thereby suggesting that alternative RNA splicing in concert with various posttranslational modifications plays a major role in creating proteome

diversity (2). Under normal physiologic conditions, RNA splicing is tightly modulated in a spatiotemporal manner and errors can cause overexpression or down-regulation of splice variants (3, 4). Such anomalous changes in RNA splicing have been shown to correlate with tumorigenesis by loss of tumor suppressor function and/or gain of tumor promoter function (5, 6). Despite the emerging significance of splicing regulation in various cellular processes, including growth, there have been few examples of specific splice variants in tumor development (3).

Coactivator activator (CoAA; gene symbol *rbm14*) was originally reported as a protein interacting with the proto-oncogene synovial sarcoma translocation (SYT) protein (7). CoAA was later isolated as a secondary coactivator (co-coactivator) for another coactivator called TR-binding protein (TRBP; ref. 8). Its splice variant, termed coactivator modulator (CoAM), serves as a potential dominant-negative inhibitor due to lack of a COOH terminal activation domain. Consistent with the existence of two RNA recognition motifs (RRM), which resemble the RRM of the hnRNP protein family, CoAA is a transcriptional coactivator shown previously to be involved in regulating alternative splicing in addition to steroid hormone-dependent transcription (9).

An emerging body of evidence supports the role of nuclear receptor coregulators as “master regulators,” which integrate diverse physiologic processes. As a result, their abnormal regulations or malfunctions often trigger pathologic processes. With a view to tumorigenesis, over 100 of ~300 known nuclear receptor coregulators are reported to be involved in malignant transformation (10). As a nuclear receptor coregulator of transcription and splicing, CoAA has the potential for regulating both physiologic and pathologic processes. Despite this potential, little is known concerning the biological roles of CoAA. The possibility of CoAA acting as an oncogenic regulator has been raised based on the following structural and functional features: (a) existence of a YQ domain that is also found in the EWS proto-oncoprotein and in the TLS/FUS oncoproteins (11), (b) increased levels of CoAA mRNA in cancer cell lines (8), and (c) interaction with other oncogenic proteins, such as SYT (11) and TRBP (8). TRBP, also known as RAP250/ASC-2/AIB3, is amplified in breast cancer (12). Paradoxically, the possible involvement of CoAA as a tumor suppressor has been indicated by (a) underexpression of *rbm14* gene (CoAA) transcripts in certain cancers⁵ and (b) interactions with p300 and other proteins,⁶ which are often involved in tumor suppression. These observations encouraged us to further

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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⁵ Assembled from data in www.oncomine.org.

⁶ www.nursa.org

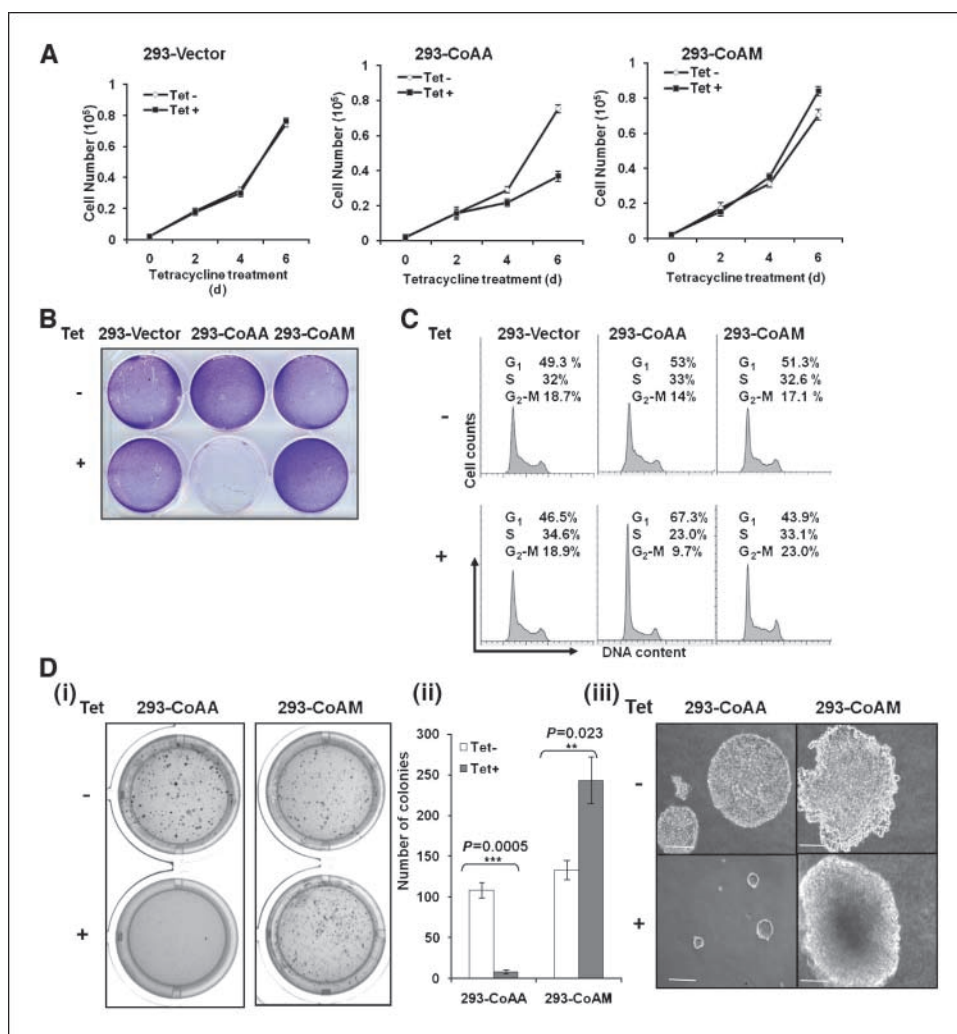


Figure 1. CoAA inhibits, but CoAM facilitates, cell cycle progression, proliferation, and anchorage independent growth. **A**, growth curve showing growth characteristics of the indicated stable cells in the absence (*open symbols*) or the presence (*solid symbols*) of tetracycline. Cell numbers were counted at the indicated time points and presented as the mean \pm SE (*error bar*) from three determinations. Statistical analysis by Student's *t* test was performed (**, $P < 0.01$). **B**, crystal violet staining showing the overall cell growth of the indicated stable cells either in the absence (-, *top*) or the presence (+, *bottom*) of tetracycline after 8 d. **C**, representative histograms of FACS analyses. The relative DNA content of the indicated stable cells was determined by flow cytometry, collecting 10,000 events; x axis, propidium iodide fluorescence intensity for DNA content; y axis, relative cell number. Cells were in the absence (-, *top*) or the presence (+, *bottom*) of tetracycline 72 h before 24-h serum starvation followed by serum release. After harvest, cells were processed as described in Materials and Methods. Histograms were analyzed by Flow Jo software. **D**, *i*, representative plates for soft agar assays with the 293-CoAA (*left*) and 293-CoAM (*right*) in the absence (-, *top*) or the presence (+, *bottom*) of tetracycline. *ii*, Colony numbers were counted as described in Materials and Methods and presented as the mean \pm SE from three determinations. Statistical analysis for cell numbers was performed by Student's *t* test comparing tetracycline-treated (*solid column*) and nontreated (*open column*) 293-CoAA or 293-CoAM cells (***, $P = 0.0005$; **, $P = 0.023$, respectively). *iii*, photos showing the morphology of representative colonies from the plates shown in *E*. Photos were taken at $100\times$.

investigate the functional consequences of modulating the level of CoAA or the ratio of CoAM to CoAA in tumorigenesis.

The present study reveals that exogenous CoAA expression in tumorigenic human embryonic kidney 293 (HEK 293) cells inhibits cell growth, cell cycle progression, colony formation, and xenograft tumor initiation and growth. CoAA inhibits the transcriptional activation of the potent proto-oncogene *c-myc* by increasing the presence of HDAC3 protein, decreasing the acetylation of histone H3, and decreasing the presence of RNA polymerase II on the *c-myc* promoter. We also show concomitant reductions in mRNA levels of the downstream *ccnd1* (cyclin D1 gene) and *skp2* proto-oncogenes, followed by accumulation of the tumor suppressor protein p27/Kip1 (gene symbol *cdkn1b*). In contrast, knockdown of endogenous CoAA expression up-regulates *c-myc*, *ccnd1*, and *skp2* mRNA levels, resulting in a dramatic decrease in p27/Kip1 protein and an increase of cell growth. CoAM antagonizes CoAA-induced events by decreasing endogenous levels of the CoAA isoform. Our observation of a dramatic decrease of the CoAA protein in human renal cell carcinomas (RCC) supports the pathophysiologic relevance of these findings. This study represents an initial example of how two splicing isoforms of a nuclear receptor coregulator can counterbalance each other to control cell growth and tumorigenesis in human kidney cells.

Materials and Methods

Immunohistochemical analyses. Tumor arrays were purchased from U.S. Biomax (KD991). Immunohistochemical analyses were performed following standard procedures using anti-CoAA antibody (see below). The statistical analysis was performed using total score as an ordinal outcome. A Wilcoxon signed rank test, similar to a paired *t* test, was used to compare the distribution of total score differences (= total Allred score of tumor tissues - total Allred score of normal tissues).

Generation of the TET-inducible stable cell line. Stable cell lines expressing CoAA and CoAM were generated in HEK 293 cells in which the integrated Flp-In-T-Rex system contains a single FRT site and stably expresses Tet repressor for convenient creation of Tet-inducible CoAA (or CoAM)-expressing cell lines. cDNAs were inserted into the pcDNA5/FRT/TO expression vector and transfected into Flp-In-293 host cells following the manufacturer's instructions (Invitrogen).

Cell culture, transient transfection, and reagents. HEK 293 cells were cultured in DMEM with 10% v/v FCS. 293-CoAA, 293-CoAM and 293-Vector cells were maintained in the same medium containing 300 $\mu\text{g}/\text{mL}$ hygromycin and 15 $\mu\text{g}/\text{mL}$ blastacidin. Tetracycline and doxycycline were from Sigma. Tetracycline and doxycycline were in water. Transfection of plasmids was performed using Fugene 6 reagent (Roche Applied Science) following the manufacturer's instructions. The indicated plasmids were transfected into 293-CoAA cells. Tetracycline was added at the time of transfection. At 72 h after transfection, cells were harvested and aliquoted for fluorescence-activated cell sorting (FACS), Western blot, or quantitative

reverse transcription-PCR (qRT-PCR) analyses. All small interfering RNA (siRNA) oligos were purchased from Dharmacon, and all sequences are available upon request. Individual siRNA derived from the SMRT pool (Dharmacon) were used to knockdown CoAA in human kidney cells. Cells were transfected with 30 nmol/L each siRNA with Lipofectamine 2000 (Invitrogen). At 2 d after the first transfection, cells were transfected with 20 nmol/L of each siRNA to prolong knocking down. At 1 d later, cells were harvested and half of the cells were subjected to Western blot analysis and half to qRT-PCR.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) assay was performed using the ChIP assay kit (Millipore). The primer sequences are available upon request.

Repetitions of experiments. Experiments in all figures were repeated at least thrice each except the xenograft experiments.

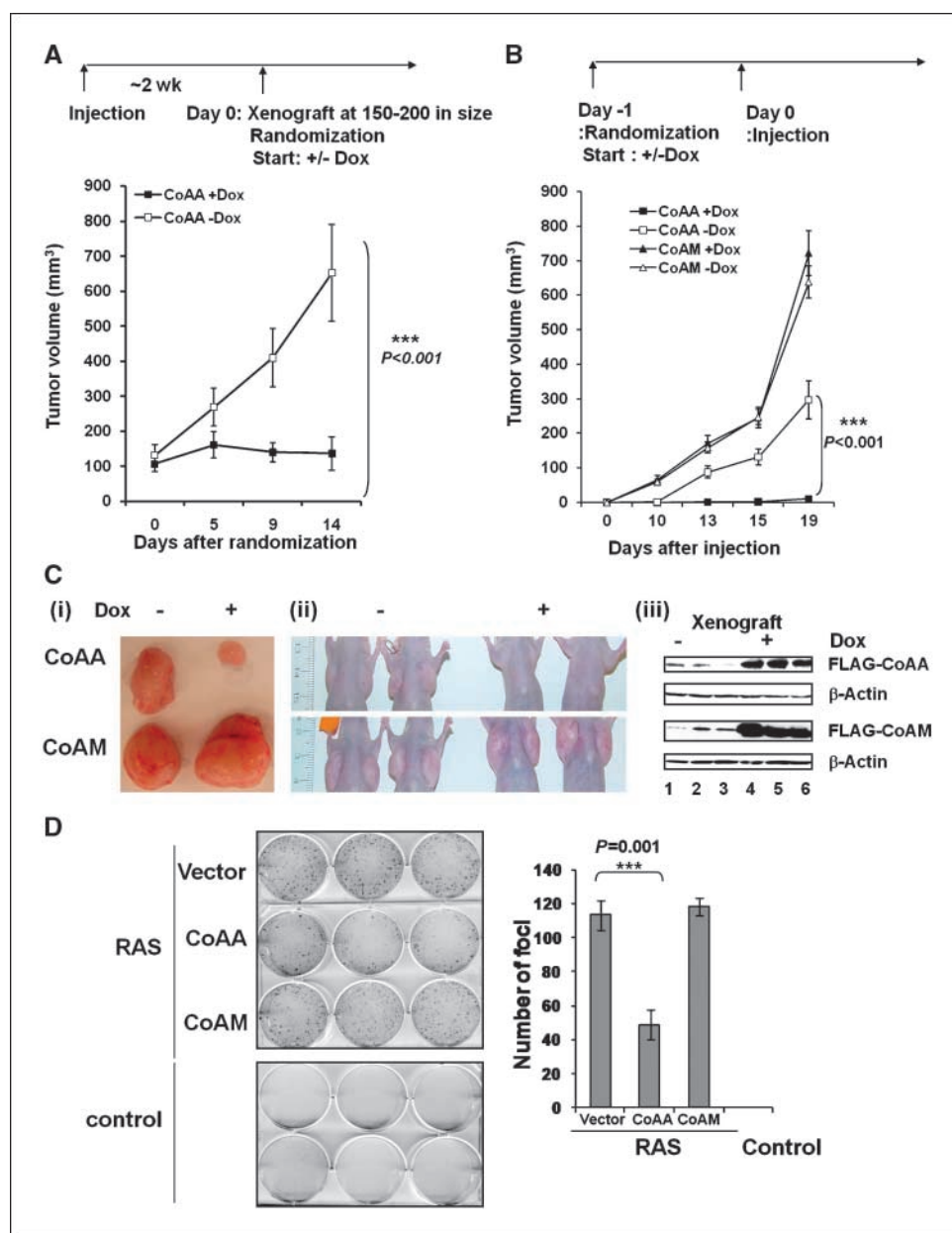
Results

Increased CoAA suppresses cell growth and G₁-S cell cycle progression in HEK cells. To address the function of CoAA and its

splicing isoform (CoAM) in kidney cell tumorigenesis, we first asked whether exogenous expression of CoAA and CoAM could affect cell proliferation in tumorigenic cells. HEK293 FRT-TO cells stably expressing tetracycline-inducible epitope-tagged CoAA (called 293-CoAA) or CoAM (called 293-CoAM), along with the corresponding control cells (called 293-Vector), were generated for this purpose. These cells were chosen for the following reasons: (a) the intrinsic tumorigenic potential of HEK-293 cells (13), (b) their designed isogenic features allowing the convenient generation of stable cells, and (c) gene inducibility, all of which allow us to investigate growth suppressive potentials of an integrated exogenous gene and the kinetics of the molecular events after induction.

The tetracycline treatment highly induced both genes (Supplementary Fig. S1). Next, we asked how the expression of these two genes influences cell growth. The number of 293-CoAA cells was greatly reduced upon induction of CoAA expression (+Tet) compared with cells without tetracycline (-Tet; Fig. 1A and

Figure 2. CoAA suppresses but CoAM promotes tumor formation in xenografts. **A**, tumor growth curves showing the growth kinetics of established tumors from 293-CoAA xenografts. Mice bearing established 293-CoAA tumors were randomly allocated into two groups at the time (set as day 0) when tumor reached at 150 to 200 mm³. Drinking water was subsequently given to mice with (*n* = 16; *solid symbols*) or without (*n* = 12; *open symbols*) 500 μg/mL doxycycline. The tumor volume was measured at the indicated time points until tumors reached 1,000 mm³. The experiment was carried out during a 14-d period. **B**, tumor growth curves showing the growth kinetics from the initiation of 293-CoAA or 293-CoAM xenograft tumors with (*solid symbols*) or without (*open symbols*) continued doxycycline administration. Mice were randomized 1 d before xenograft injection with or without doxycycline supplementation. Tumor volume was subsequently measured at the indicated time points. **C**, *i*, representative tumors from the experiment in **B** photographed at the 19th day postinjection. *ii*, representative nude mice from the experiment in **B** photographed at the 19th day postinjection. *iii*, Western blot assay showing the expression of FLAG-CoAA or FLAG-CoAM in representative 293-CoAA or 293-CoAM xenograft tumors from the experiments in **B**. A portion of each tumor was homogenized and analyzed by Western blotting with anti-FLAG antibody. β-actin was probed as a control. **D**, foci formation assays showing the effect of CoAA or CoAM on H-ras-induced foci formation in CREFS (*left*). Foci were counted and presented as the mean ± SE (*error bar*) from five independent experiments (*right*). Statistical analysis by Student's *t* test showed significant differences between numbers of foci formation of vector versus CoAA (***, *P* = 0.0015).



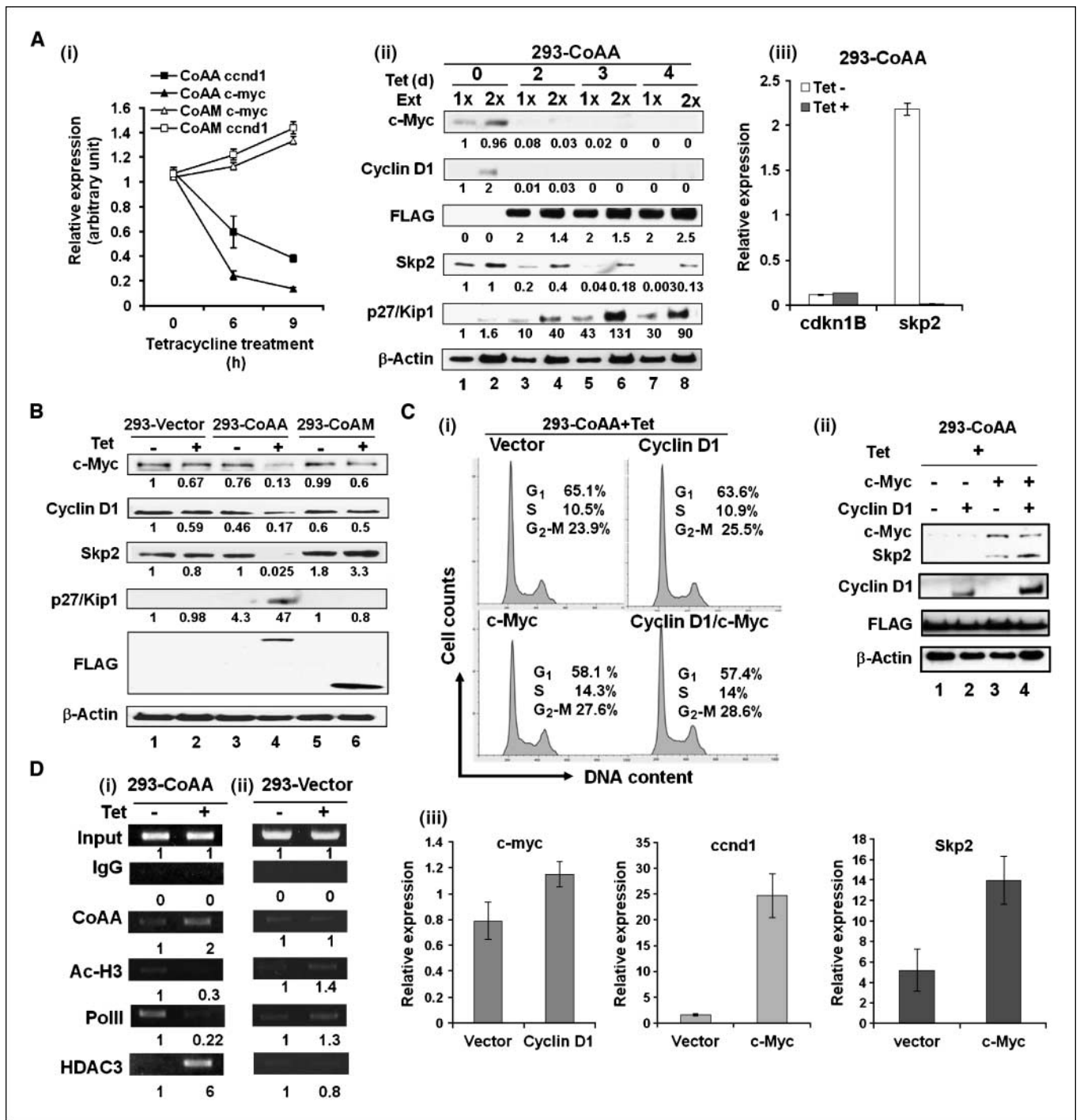


Figure 3. Induction of CoAA and CoAM affects the expression of cell cycle regulatory genes and exogenous expression of c-Myc partly derepresses CoAA-induced cell cycle progression. *A, i*, graph showing relative gene expression in 293-CoAA cells and 293-CoAM cells after tetracycline treatment. Relative expression mRNA levels of *c-myc* (triangle) and *ccnd1* (square) was quantitated by quantitative RT-PCR at the indicated time (h) of tetracycline treatment from 293-CoAA (solid symbols) and 293-CoAM (open symbols) cells. β -Microglobulin was used to normalize mRNA levels. Data represented the mean of relative fold changes by setting the normalized mRNA levels at 0 h as 1. Error bars, SE from three determinations. *ii*, Western blot analyses showing the amount of c-Myc, cyclin D1, Skp2, and p27/Kip1 proteins from the 293-CoAA cells at the indicated time points (d) after tetracycline treatment. Two different amounts (1 \times and 2 \times) of cell extracts from each time point were subjected to analysis. Relative fold changes were presented as described in Materials and Methods. *iii*, qRT-PCR showing the relative mRNA levels of *cdkn1b* and *skp2* in 293-CoAA cells with (+; solid columns) or without (-; open column) tetracycline. *B*, Western blot analysis showing amounts of c-Myc, cyclin D1, Skp2, and p27/Kip1 proteins from 293-Vector (lanes 1 and 2), 293-CoAA (lanes 3 and 4), and 293-CoAM (lanes 5 and 6) cells, with (+) or without (-) tetracycline treatment for 4 d. *C, i*, FACS analysis showing the effect of transiently transfected plasmids encoding the indicated genes on cell cycle progression in 293-CoAA cells in the presence of tetracycline. 1 μ g of each plasmid encoding vector, *ccnd1*, *c-myc*, or *c-myc* with *ccnd1* was transfected into 293-CoAA cells. Total amount of DNA was adjusted with control vector plasmids to 2 μ g/well. *ii*, Western blot analyses showing the changes in the indicated proteins in each transfected cell line used in *i*. *iii*, qRT-PCR showing the amount of *c-myc* mRNA in 293-CoAA cells transiently transfected with vector or *ccnd1*, cyclin D1 mRNA in 293-CoAA cells transiently transfected with vector or c-Myc, and *skp2* mRNA in 293-CoAA cells transiently transfected with vector or c-Myc. *D*, chromatin immunoprecipitation assay showing the presence of the indicated proteins on the *c-myc* promoter in 293-CoAA cells (*i*) and 293-Vector cells (*ii*) with (+) or without (-) tetracycline treatment. Relative fold-changes were calculated by setting the intensity of PCR signal shown in lane 1 to 1.

B, middle); at the 6th day of tetracycline treatment, the number of 293-CoAA cells was only 40% of that in the absence of tetracycline ($P < 0.01$). In contrast, the 293-CoAM cells displayed only a modest (20%) yet significant increase ($P < 0.01$) in cell number at the same time point (Fig. 1*A* and *B, right*). The 293-Vector cells did not show visible changes in cell proliferation with tetracycline treatment (Fig. 1*A* and *B, left*). Thus, CoAA expression reduced the cell proliferation rate, whereas CoAM modestly increased it.

To further characterize the nature of the alteration in proliferation rate upon induction of exogenous CoAA or CoAM expression, FACS analysis was used to investigate cell cycle progression. In the presence of tetracycline, the 293-CoAA cells showed a significantly greater proportion (14%) of cells accumulated in the G₁ phase (Fig. 1*C, middle top*) and 10% less cells in the S-G₂-M phase compared with the 293-CoAA cells in the absence of tetracycline (Fig. 1*C, middle bottom*). In contrast, the 293-CoAM cells showed a 7.4% decrease in the population of cells in G₁ phase and 7.4% more cells in S-G₂-M phase (Fig. 1*C, right top*) compared with the 293-CoAM cells without treatment (Fig. 1*C, right bottom*). We observed a marginal (2.8%) decrease in the population of cells in G₁ phase in the tetracycline-treated 293-Vector cells (Fig. 1*C, left top*) compared with the untreated 293-Vector cells (Fig. 1*C, left bottom*). These results indicate that induction of CoAA expression retains cells in the G₁ phase, whereas induction of CoAM expression facilitates cell cycle progression.

To extend our observations to the differential effects of CoAA and CoAM to tumorigenesis, we tested the effect of exogenous expression of CoAA and CoAM on the intrinsic transformation activity of HEK 293 cells (13). Soft agar assays were used to examine the effect of tetracycline-induced expression of CoAA or CoAM on the anchorage-independent growth of HEK 293 cells. The 293-CoAA cells, upon tetracycline treatment, formed ~10-fold fewer colonies than the cells without tetracycline (Fig. 1*D, i* and *ii*). In contrast, the 293-CoAM cells in the presence of tetracycline were able to form ~2× more colonies than the cells in the absence of tetracycline (Fig. 1*D, i* and *ii*). With the tetracycline treatment, the colonies of 293-CoAA cells were much smaller than in the absence of tetracycline (Fig. 1*D, iii*). Thus, tetracycline-induced CoAA protein not only suppresses the formation of HEK 293 colonies but also inhibits the growth of the colonies, whereas CoAM further promotes the transformation activity inherent to HEK 293 cells.

Increased CoAA expression suppresses xenograft tumor initiation and growth. To assess the role of CoAA and CoAM in tumor growth *in vivo*, we used xenograft experiments, as previously described (14, 15). The effect of doxycycline-induced exogenous expression of CoAA or CoAM was assessed by measurements of the growth rate of established tumors derived from HEK293 cells. 293-CoAA xenograft tumors with doxycycline treatment began to stabilize or regress after 5 days of doxycycline treatment, whereas the 293-CoAA xenograft tumors without doxycycline grew rapidly, indicating that doxycycline-induced CoAA expression inhibited tumor growth (Fig. 2*A*). During the time course of this experiment and up to the time of euthanization, no observable effect was seen with or without doxycycline from CoAM xenograft tumors likely due to the rapid growth rate of xenograft tumors even without CoAM induction (data not shown).

To evaluate the effect of induced exogenous expression of CoAA or CoAM on tumor initiation, in a second *in vivo* experiment doxycycline treatment was initiated 1 day before injection of cells and continued throughout the experimental time course. The tumor growth curves for all mice in each cohort are shown in

Fig. 2*B*. 293-CoAA xenografts in the presence of doxycycline developed 2.5-fold fewer tumors than untreated 293-CoAA xenografts (Supplementary Table S1). In addition, 293-CoAA xenografts treated with doxycycline produced tumors ~30 times smaller than the untreated 293-CoAA xenografts at the 19th day postinjection (Supplementary Table S1; Fig. 2*B* and *C*). The latency period, as measured from the day of inoculation until tumors were palpable, was delayed for 25 to 30 days when CoAA was overexpressed (Supplementary Table S1). In contrast, 293-CoAM xenografts in the presence of doxycycline showed a modestly (13%) enhanced tumor volume compared with the 293-CoAM xenografts without doxycycline treatment (Supplementary Table S1; Fig. 2*B* and *C*) during the time course of this experiment and up to the time of euthanization. Of note, 293-CoAM xenografts developed more vascularization than 293-CoAA xenografts, as seen in Fig. 2*C, i*. To examine the effect of doxycycline on the growth of 293-CoAA xenografts or 293-CoAM xenografts, the data were statistically analyzed by using linear mixed models with repeated measurements for each of the four time points, for left and right sides, and random effects for each mouse. Cell lines were analyzed separately. The response variable (tumor volume) was transformed by adding 1 and then taking logarithms. Doxycycline, time, and their interaction terms were included in the models. When the interaction term was not significant, it was removed from the models. P values of 5% or less were considered significant. In the analysis for the growth of 293-CoAA xenograft tumors, the results show that the doxycycline effect is significant ($P < 0.001$). The significant interaction increases with time ($P < 0.001$). In the analysis for the growth of the 293-CoAM xenograft tumors, the results are not significant, possibly due to the rapid growth rate of xenograft tumors. Doxycycline-induced CoAA and CoAM expression was confirmed in representative xenograft samples treated with doxycycline (lanes 4, 5, and 6) or without (lanes 1, 2, and 3) using Western blot analysis using anti-FLAG antibody (Fig. 2*C, iii*).

To further define the role of CoAA in transformation *per se*, we determined the effect of CoAA on oncogene-induced transformation in nontumorigenic cells. To this end, we performed focus formation assays by transiently transfecting H-ras along with plasmids encoding either CoAA or CoAM into cloned rat embryonic fibroblasts (CREF), which are immortalized but nontransformed cells. Focus formation was quantified in five separate experiments. Our results showed that expression of H-Ras with empty vector controls induced a significant number of foci (Fig. 2*D, top*; average of 113.3 from five experiments) compared with the vector alone, which did not induce any foci (control). Expression of CoAA along with Ras in CREFs significantly decreased the number of foci (Fig. 2*D, middle*; average of 48.8) but CoAM did not (Fig. 2*D, bottom*; average of 118.6), suggesting that CoAA, but not CoAM, suppresses the oncogenic potential of H-Ras in nontransformed CREFs. Collectively, these results again indicate that CoAA possesses tumor suppressive potential.

Increased CoAA protein expression decreases mRNA levels of *c-myc*, *ccnd1*, and *skp2*. The structural differences between the CoAA and CoAM proteins lie in the CoAA-specific COOH terminus, which is thought to be responsible for its transcriptional activity (8); this led us to consider that the observed differential tumorigenic activities of CoAA and CoAM could result from differential transcriptional activity. To investigate how CoAA modulates accumulation of cells in G₁ phase, we examined the expression levels of cell cycle regulatory genes that are responsible for the G₁-S transition. The *c-myc* and *ccnd1* genes were chosen for analysis

based on their relevance to both the G₁-S transition and tumorigenesis. Total RNAs from each of the stable cells were analyzed by real time qRT-PCR assays after tetracycline treatment for the indicated times. Consistent with earlier results, the mRNA levels of *c-myc* and *ccnd1* genes in 293-CoAA cells were reduced by ~80% and ~60%, respectively, 9 hours after tetracycline treatment (Fig. 3A, *i*); in contrast, the mRNA levels of *c-myc* and *ccnd1* genes rose in 293-CoAM cells by 33% and 44%, respectively, after the same tetracycline treatment (Fig. 3A, *i*).

To further characterize the downstream events leading to the CoAA-mediated negative regulation of G₁-S cell cycle progression, we determined the levels of proteins involved in G₁-S cell cycle progression at later time points after CoAA induction. Consistent with the changes in mRNA levels, c-Myc and cyclin D1 proteins were reduced to undetectable levels after 4 days of tetracycline treatment in 293-CoAA cells (Fig. 3A, *ii*). We also found a decrease in endogenous Skp2 protein expression that was followed by p27/Kip1 protein accumulation (Fig. 3A, *ii*). To discriminate whether the changes in the levels of Skp2 and p27/Kip1 proteins were due to increased mRNA levels, qRT-PCR was used to measure their mRNA levels with or without tetracycline treatment in 293-CoAA cells (Fig. 3A, *iii*). Although *skp2* gene mRNA was dramatically reduced by over 10-fold upon administration of tetracycline, changes in the level of *cdkn1b* (p27/Kip1) mRNA were not detected. This indicates that CoAA-mediated induction of p27/Kip1 protein is primarily due to decreased mRNA levels of *skp2*, leading to stabilization of p27/Kip1 protein rather than directly to changes in p27/Kip1 mRNA. Our results raise the possibility that CoAA suppresses c-Myc and cyclin D1 expression to hinder cell cycle progression and cell growth. These findings also suggest that c-Myc and cyclin D1 likely function as positive upstream regulators of *skp2* which, in turn, negatively regulates the stability of p27/Kip1 protein to facilitate the cell cycle progression (16).

In contrast, in tetracycline-treated 293-CoAM cells, we observed a 1.8-fold increase in endogenous Skp2 protein expression compared with the untreated cells (Fig. 3B). However, we did not observe distinct changes in c-Myc, cyclin D1, and p27/Kip1 proteins in 293-CoAM cells upon CoAM induction compared with the uninduced cells. This could be due to the rapid turnover of these proteins during cell cycle progression.

Having shown that repression of *c-myc* and *ccnd1* are among the downstream effects of CoAA, we next asked whether overexpression of *c-myc* and *ccnd1* can rescue cell cycle progression from inhibition by CoAA. We transiently transfected plasmids encoding *c-myc* or *ccnd1* or both into tetracycline-treated 293-CoAA cells and assessed the cell cycle progression in each transfected population. Our results showed that overexpression of *c-myc* partially restored the cell cycle progression inhibited by CoAA (Fig. 3C, *i*). We also observed a modest increase in Skp2 protein level (Fig. 3C, *ii*). This suggested that Skp2-p27/Kip1 pathways could be downstream events of c-Myc. To determine the sequence of molecular events between *c-myc* and *ccnd1* in 293-CoAA cells, we examined the levels of endogenous *c-myc*, *ccnd1*, and *skp2* gene expression in transfected cell populations. The qRT-PCR analyses revealed that exogenous *c-myc* can induce endogenous *ccnd1* and *skp2* gene expression even in the presence of tetracycline in 293-CoAA cells, but exogenous *ccnd1* cannot induce *c-myc* (Fig. 3C, *iii*, left; $P > 0.05$). These results indicate that c-Myc is an upstream regulator of *ccnd1* and *skp2* genes in 293-CoAA cells and suggests that the exogenous expression of CoAA modulates Skp2-p27/Kip1 in part through c-Myc.

To confirm our observations and extend the mechanism, we next used chromatin immunoprecipitation (ChIP) assays. As shown in Fig. 3D, ChIP assays revealed that CoAA indeed is present on the *c-myc* promoter, indicating that the *c-myc* gene is a direct target of the CoAA protein. In addition, we found that, upon induction of CoAA by tetracycline treatment, there is an increased presence of HDAC3 protein, along with a concomitant decreased presence of acetylated histone H3 and RNA polymerase II, on the *c-myc* promoter (Fig. 3D, *i*). However, these changes were not observed in 293-vector cells (Fig. 3D, *ii*). Our results suggest that CoAA functions as a corepressor by recruiting HDAC3 protein, followed by a decreased acetylation of histone H3 and a decreased presence of RNA polymerase II at the *c-myc* gene.

Decreased endogenous CoAA can increase cell growth via up-regulating *c-myc* expression. Given that most renal cancers are derived from proximal tubule, we further examined the role of endogenous CoAA in nontumorigenic human renal proximal tubular epithelial cells (a gift from Dr. L. Racusen). We carried out RNA interference experiments and determined the mRNA levels of *c-myc*, *ccnd1*, and *skp2* genes. Consistent with the exogenous expression results, knocking down CoAA (siCoAA) increased mRNA levels of *c-myc* (Fig. 4A, *i*) and *ccnd1* (Fig. 4A, *ii*) by ~3-fold compared with the cells with control siRNA against GFP (siGFP). *Skp2* mRNA levels were increased by ~2-fold in the cells treated with siCoAA (Fig. 4A, *iii*). Similarly, Skp2 protein levels were increased by 1.85-fold with siCoAA1 and ~3-fold with siCoAA2, whereas p27/Kip1 protein levels were markedly reduced by 93% with siCoAA1 and by 97.5% with siCoAA2 (Fig. 4B). We also confirmed that CoAA protein was dramatically knocked down after 5 days of treatment using two different siRNAs against CoAA, compared with control cells treated with the siRNA against GFP (Fig. 4B). Next, we determined the effect of decreased CoAA protein on the growth of the kidney cells. Significantly, down-regulation of CoAA proteins in kidney cells resulted in an increase of cell growth (Fig. 4C). By the 8th day after siRNA treatment, the number of cells treated with siRNA against CoAA was over 2-fold higher than the cells treated with siRNA against GFP, indicating again that CoAA is a negative regulator for kidney cell proliferation. These results confirm the above exogenous expression studies to indicate and substantiate that CoAA is a cell growth inhibitor and likely a tumor suppressor *in vivo*.

Increased CoAM protein can antagonize CoAA-mediated growth suppression. To this point, our data indicated that CoAA inhibits and CoAM enhances cell growth and colony formation. These results resemble the previously known antagonism of CoAM against the dual activities of CoAA in transcription and alternative splicing and raised a question as to whether CoAM could also antagonize the tumor suppressive activity of CoAA. To this end, plasmids encoding FLAG-CoAM were transfected into HEK293-CoAA cells in the presence of tetracycline, and cell cycle progression was examined by FACS analysis (Fig. 5A). The G₁ population of tetracycline-treated 293-CoAA cells showed a dose-dependent decrease from 62.5% (with vector control) to 52% (with 20× CoAM). This result indicated that CoAA-induced cell cycle arrest could be reversed in part by CoAM expression. BrdUrd incorporation was measured to confirm the effect of CoAM on the cell proliferation rate (Fig. 5B) and showed that ~14.5% more BrdUrd-positive cells were present with additional CoAM expression in tetracycline-induced 293-CoAA cells.

To further validate the antagonism between CoAA and CoAM in anchorage-independent growth, we tested whether exogenous

expression of CoAM could restore colony formation in soft agar in 293-CoAA cells even in the presence of tetracycline (Fig. 5C, *i* and *ii*). Indeed, expression of CoAM in tetracycline-treated 293-CoAA cells facilitated formation of $\sim 5\times$ more colonies (average of 136) than the vector control (average of 26.7). To verify that exogenous CoAM antagonizes the CoAA-mediated molecular events described earlier, we examined the mRNA levels of *c-myc* and *ccnd1* (Fig. 5D, *i*). Consistent with the cell cycle analyses and growth results, CoAM expression in tetracycline-treated 293-CoAA cells increased *c-myc* and *ccnd1* by ~ 4 -fold and ~ 3 -fold, respectively. Western blot analyses revealed that CoAM expression in tetracycline-treated 293-CoAA cells increased levels of Skp2 protein by 2.5-fold and decreased levels of p27/Kip-1 protein by 4-fold (Fig. 5D, *ii*). Overall, these results showed a positive regulatory function for CoAM on cell cycle progression and cell proliferation and suggested that these CoAA and CoAM counterbalance each other's growth modulating activities.

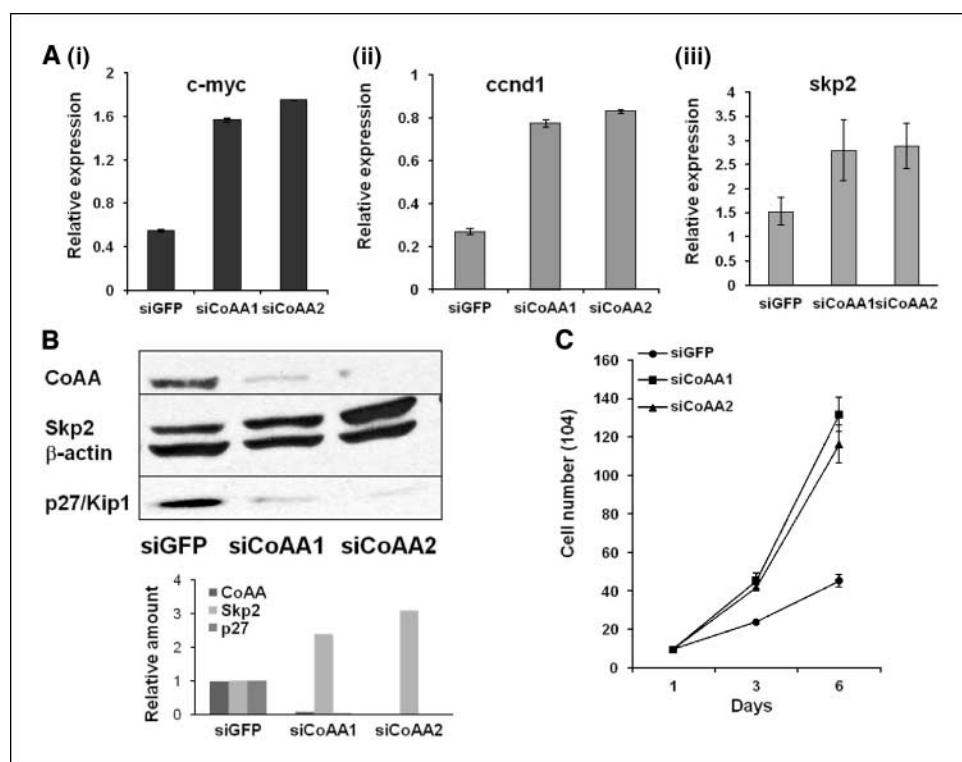
CoAA protein levels are dramatically decreased in human RCCs. Anomalous expression of tumor genes, either up-regulation of oncogenes or down-regulation of tumor suppressors, is a frequent concomitant of tumorigenesis. To determine associations of CoAA in tumorigenesis, we interrogated the data assembled in the Oncomine database (17); results revealed an increase in *rbm14* (CoAA) gene transcripts in certain cancers (leukemia and lung cancer), but a decrease in *rbm14* gene transcripts in other cancers, such as RCC (18). These contradictory results could be due to (a) undistinguished but functional splicing isoforms generated from the *rbm14* gene and/or (b) a dual potential of CoAA in tumorigenesis that is dependent upon specific cellular settings. To substantiate the pathophysiologic significance of the above results, we focused our analyses on RCCs. To confirm that the reduction of CoAA gene transcripts in RCC is due to a decrease of CoAA rather

than other splicing isoforms, the amount of CoAA protein was determined by immunohistochemistry using tissue arrays and an anti-CoAA antibody specific to the CoAA COOH terminus. These tumor arrays contain 1-mm tissue cores of renal cell tumors and adjacent tissues retaining normal histology from kidney cancer patients ($n = 33$). Representative examples of renal cell cancer and normal tissue are shown in Fig. 6B. CoAA immunostaining was present in normal tissues but was decreased or not detected in most tumor tissues (Fig. 6B and C). Among normal specimens, 74% exhibited strong ($T \geq 6$) and 27% showed moderate ($3 \leq T < 6$) CoAA protein expression. In contrast, we observed 52% of tumor samples with weak or undetected ($T < 3$), 24% with moderate ($3 \leq T < 6$) and only 24% with strong ($T \geq 6$) CoAA protein expression (Fig. 6C, *i* and *ii*). There was a statistically significant difference between the expression in tumor and normal tissues for all the cancer samples ($P < 0.001$). Our results show that CoAA protein is significantly decreased in human RCC compared with normal kidney supporting the pathophysiologic relevance of the above shown suppressive activity of CoAA in human RCC. Taken together, results of the present study suggest that CoAA protein and its antagonistic isoform, CoAM, are molecular switches capable of regulating cell growth and tumor formation in kidney cells via modulation of cell cycle regulators, such as *c-myc*, *ccnd1*, and *skp2*-p27/Kip1 (Fig. 6D).

Discussion

The increase in the incidence of sporadic renal cancer, at an annual rate of $\sim 2\%$ for last 65 years, calls for deeper understanding of the molecular basis underlying its onset. Our study reveals a novel function of CoAA as a potential tumor suppressor and suggests a role for CoAM as an antagonistic splice isoform in human kidney cells. The introduction of CoAA in HEK 293 cells is

Figure 4. siRNA knock-down of endogenous CoAA protein increases cell growth by up-regulating *c-myc*, *ccnd1*, and *skp2* expression followed by down-regulating p27/Kip1 protein. **A**, qRT-PCR showing the mRNA levels of *c-myc* (*i*), *ccnd1* (*ii*), and *skp2* (*iii*) in cells treated with each siRNA. **B**, Western blot showing the amounts of the indicated proteins treated with each siRNA. β -Actin was used as a control. The graph shows the relative amounts of each protein normalized to β -actin, compared with siGFP control (designated as 1). **C**, growth curves showing the effect of each siRNA treatment on cell growth.



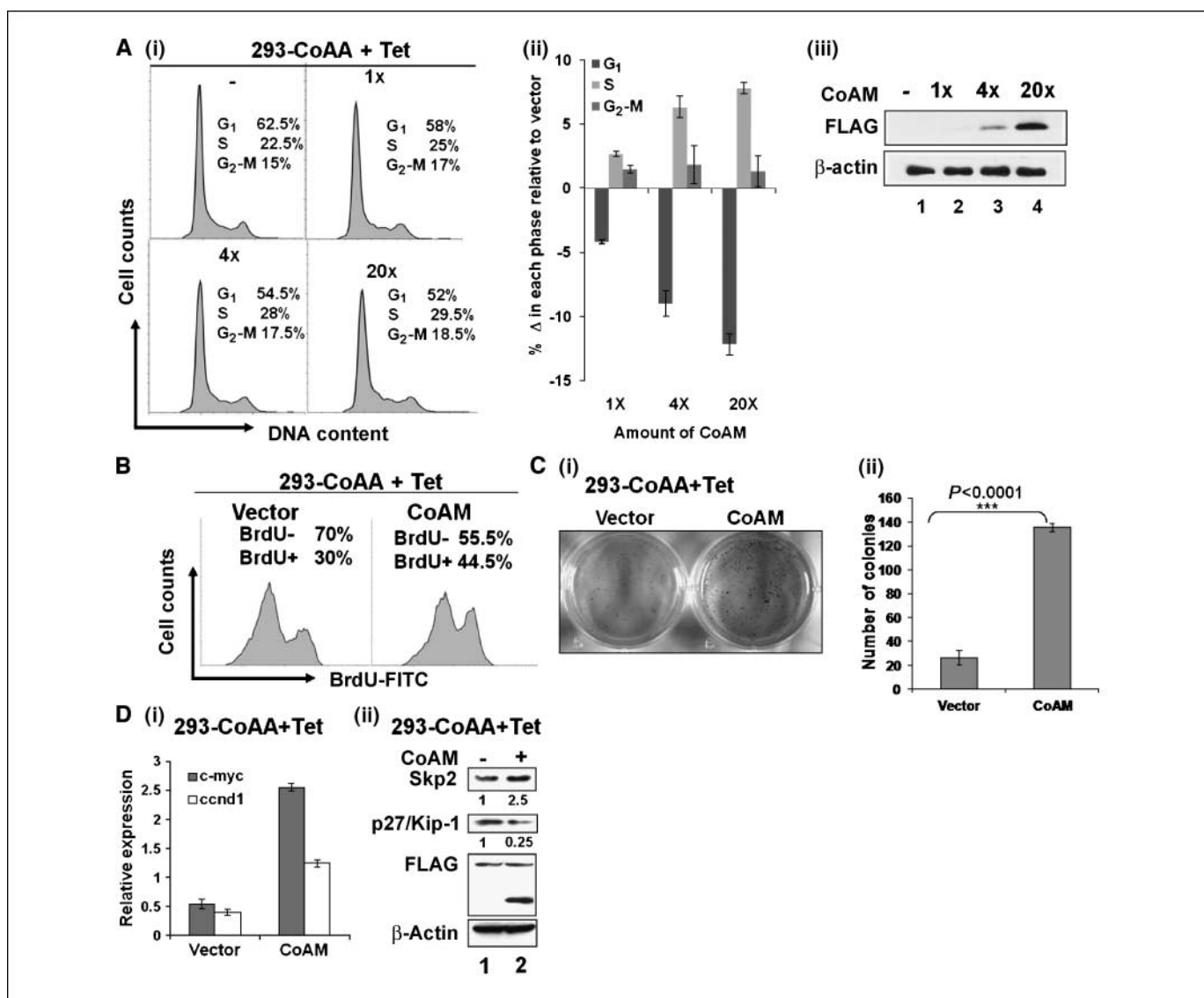


Figure 5. CoAM antagonizes CoAA-induced inhibition of cell cycle progression and cell growth. *A, i*, histogram of FACS analyses showing the effect of increasing amounts of CoAM on cell cycle progression in tetracycline-treated 293-CoAA. 100 ng (1×), 400 ng (4×), and 2 μg (20×) of FLAG-CoAM expression plasmids were transfected into 293-CoAA cells. Total amounts of DNA were adjusted with control vector plasmids to 2 μg/well. *ii*, the graph showing the relative proportions of each phase of the CoAM-transfected cells compared with vector-transfected cells. *iii*, Western blot analysis showed the expression of CoAM protein. *B*, histograms of FACS analysis showing the 293-CoAA cells transfected with 2 μg of either vector (*left*) or CoAM (*right*) in the presence of tetracycline. At the time of transfection, cells were treated with tetracycline. At 72 h after transfection, transfected cells were labeled with BrdUrd and analyzed by FACS to determine the fraction of BrdUrd-positive cells. *C*, CoAM facilitates colony formation in soft agar in tetracycline-treated 293-CoAA cells. *i*, representative plate for soft agar assay with the 293-CoAA. *ii*, colony numbers were counted as described in Materials and Methods and presented as the mean. Error bars, SE from three determinations. Statistical analysis for cell numbers was performed by Student's *t* test comparing vector control and CoAM expression (***, $P = 0.00006$). *D, i*, qRT-PCR showing the amount of *c-myc* (solid column) and *ccnd1* (open column) mRNA in tetracycline-treated 293-CoAA cells transfected with vector or CoAM. *ii*, Western blot analyses showing the changes in the indicated proteins in tetracycline-treated 293-CoAA cells with or without CoAM.

sufficient to inhibit their intrinsic oncogenic growth capacity by repression of strong oncogenes, such as *c-myc*, *ccnd1*, and *skp2*, and significant induction of the potent tumor suppressor p27Kip1; those changes in gene expression are accompanied by attenuation of G₁-S transition. Exogenous CoAA expression is also able to suppress Ras-mediated oncogenic transformation in nontumorigenic cells. Reduced expression of endogenous CoAA increases *c-myc*, *ccnd1*, and *skp2* expressions and cell growth, indicating an intrinsic ability of the CoAA protein to suppress tumorigenic machinery (Fig. 6D). Consistent with prior demonstrations of the dominant-negative function of CoAM against the transcription and splicing activity of CoAA (8), we showed that the antagonistic

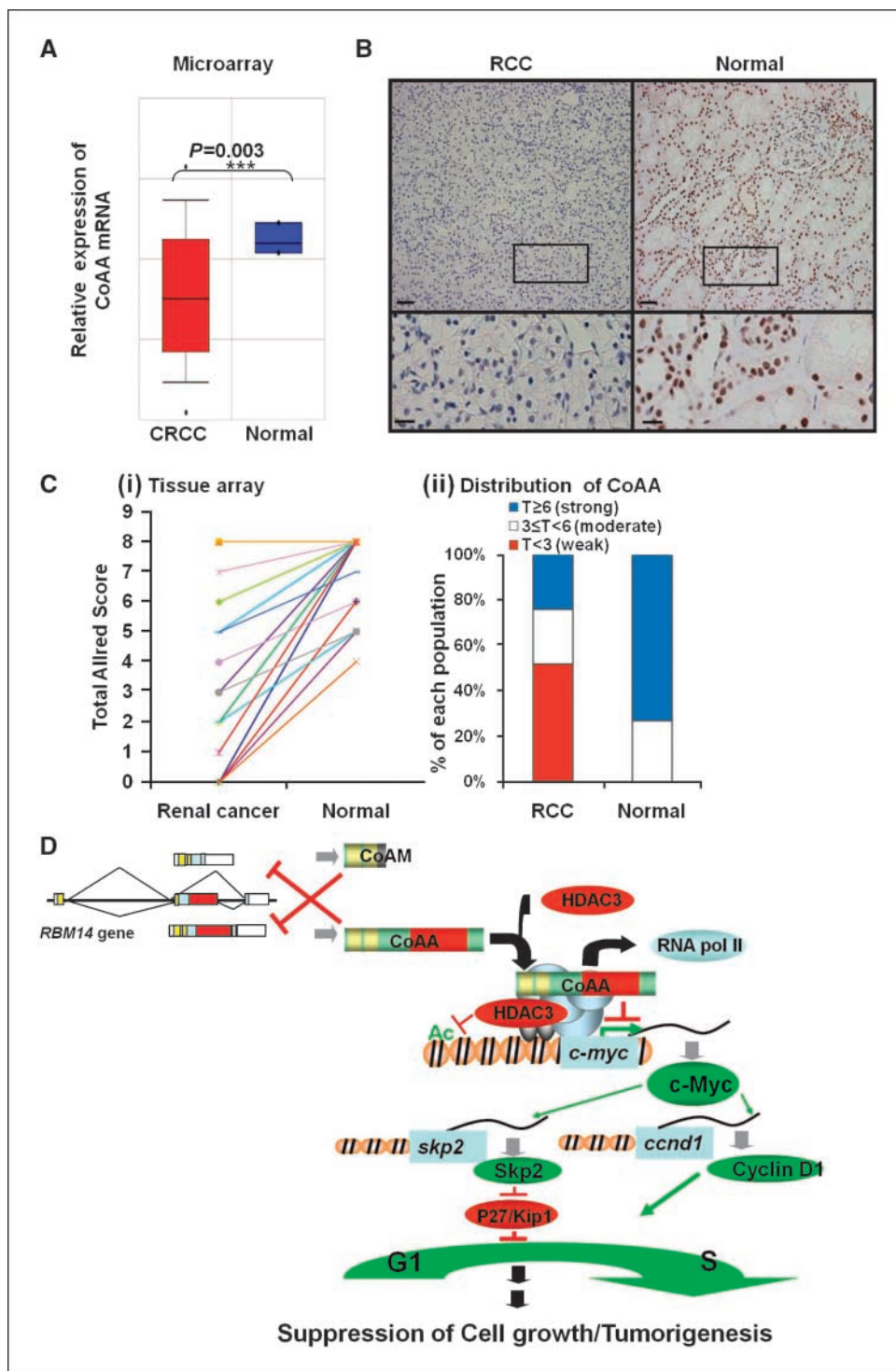
potential of CoAM can occur through activation of the *c-myc* and *ccnd1* genes and facilitation of cell cycle progression through repression of endogenous CoAA mRNAs. This provides an initial example of two splicing isoforms of a nuclear receptor coregulator that direct opposite decisions on tumorigenic cell growth. This contradictory growth modulation by CoAA/CoAM splicing isoforms supports the functional importance of alternative splicing in tumorigenesis and highlights a novel function of a coregulator(s) in renal cancer pathogenesis.

Despite the molecular functions of CoAA and CoAM that have been characterized mainly by transient transfection assays with synthetic reporters, such as luciferase and minigenes, the cellular

functions of CoAA and CoAM, including their endogenous target genes, have not been known until this study. We show that, in kidney cells, *c-myc* is a direct target of CoAA, which plays an unexpected negative coregulator role on *c-myc* gene expression by increasing the presence of HDAC3 protein and reducing presence of RNA polymerase II at the *c-myc* promoter. During revision of our manuscript, p300/CBP has been shown to directly repress *c-myc* gene through YY1 binding site by recruiting HDAC3 protein (19). This, together with known interaction between CoAA and p300/CBP (8),

suggests that CoAA protein may cooperate with p300/CBP in this mechanism. Given the established significance of *c-myc* in renal cancers (20–23), our demonstration of *c-myc* as a direct downstream target gene of CoAA is intriguing and substantiates the potential significance of CoAA in renal cancers. Moreover, our data suggest that the *skp2*-p27/Kip1 axis, another downstream target of CoAA, has physiologic and clinical relevance to renal cancers (refs. 24, 25; Fig. 6D). Because CoAM can antagonize the cellular functions of CoAA, our results suggest that regulating the ratio of CoAA versus

Figure 6. CoAA mRNA and protein levels are decreased in human RCC compared with normal kidney. **A**, box plot showing the microarray results (ref. 18, reported in the Oncomine database) comparing clear RCC (CRCC) to normal kidney (Normal). The box is the interquartile range. The whiskers are the 10th to 90th percentage range. The asterisks are the minimum and maximum. **B**, photomicrographs showing the immunostaining of CoAA in the representative RCC sample (left; hematoxylin counterstained) with weak or absent staining and in the adjacent normal kidney tissue (right; hematoxylin counterstained) with strong nuclear staining. (This antibody does not detect CoAM.) Photos were taken at 100 \times . Scale bar, 50 μ m. Bottom panels of the photos were enlarged thrice from the top ones. Scale bar, 20 μ m. **C**, *i*, graph plotting the total Allred score [T(0–8) = proportion score (scale of 0–5) + intensity score (scale of 0–3); ref. 32] for positive CoAA staining in individual samples. The total Allred scores for each tumor paired with that of its normal counterpart are paired by an individual line. Average of total score differences (= total score in RCC – total score in Normal) is –3.69, and their SD is 2.74 ($n = 33$). *ii*, graph showing the distribution of total Allred scores of CoAA immunostaining in RCC ($n = 33$) and normal kidney samples ($n = 33$). **D**, a schematic model to depict antagonistic control of the cell growth by CoAA and CoAM.



CoAM isoforms could be a pivotal strategy to influence cellular physiologies. Thus, a disturbance in the homeostatic equilibrium of CoAA and CoAM in normal cells represents a likely molecular mechanism for promoting oncogenesis. Consistent with this hypothesis, we show a significant decrease of the CoAA protein in clinical RCC. This, together with its suppressive activity via repressing proto-oncogenes in HEK cells, supports the role of CoAA protein as a potential tumor suppressor in human RCC. Only six specific amino acids in CoAM impede the development of CoAM-specific antibody, as well as analysis of CoAM proteins levels in RCC samples. Nevertheless, the reduced CoAA amount, together with counterregulation of each other's expression, will lead to the alterations of the ratio of CoAA versus CoAM isoforms.

During the course of our study, a report appeared suggesting the involvement of CoAA in oncogenic processes similar to the EWS or TLS proteins (26). That study showed an increase in fluorescence *in situ* hybridization (FISH) signals using a probe containing the CoAA (*rbm14*) gene and a correlation of certain cancers with increased CoAA mRNA and protein levels (26). The discrepancy between our data and that presented by Sui and colleagues possibly is confounded by the use of a probe for Northern dot blot analysis and FISH analysis that contains the full-length ORF of CoAA and, thus, could detect both CoAA and CoAM. However, the observation by Sui and colleagues is important because it verifies that the gene encoding CoAA, as well as its splice variant CoAM, is amplified in certain cancers. The best resolution of the two data sets would be that both are correct and that the contrasting results are due either to (a) alternations of ratio among splicing isoforms generated from the *rbm14* gene and/or (b) cancer-specific Yin-Yang activities

of the CoAA proteins in tumorigenesis. We favor the latter hypothesis because it is consistent with an example from our previous publications on SRC-3, which acts as an oncogene in breast cancers but as a tumor suppressor in lymphomas (27). Furthermore, a growing body of evidence corroborates the emerging notion of ambidexterity for several tumor relevant genes, such as E1A (28), NF- κ B (29), REST (30), and SRC-3 (31). Such examples of contrasting pathologic outcomes are likely a result of unpredictable genetic outcomes where coactivators can act as corepressors and vice versa under certain signaling and cell contexts. The emerging documentation of dual potencies for tumorigenic regulators substantiates an important concept and remains a focus for future exploration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

- Modrek B, Lee C. A genomic view of alternative splicing. *Nat Genet* 2002;30:13-9.
- Maniatis T, Tasic B. Alternative pre-mRNA splicing and proteome expansion in metazoans. *Nature* 2002;418:236-43.
- Srebrow A, Kornblihtt AR. The connection between splicing and cancer. *J Cell Sci* 2006;119:2635-41.
- Faustino NA, Cooper TA. Pre-mRNA splicing and human disease. *Genes Dev* 2003;17:419-37.
- Julian P, Venables. Unbalanced alternative splicing and its significance in cancer. *Bioessays* 2006;28:378-86.
- Brinkman BMN. Splice variants as cancer biomarkers. *Clin Biochem Spec Issue: Recent Adv Cancer Biomarkers* 2004;37:584-94.
- Brett D, Whitehouse S, Antonson P, Shipley J, Cooper C, Goodwin G. The SYT protein involved in the t(X;18) synovial sarcoma translocation is a transcriptional activator localised in nuclear bodies. *Hum Mol Genet* 1997;6:1559-64.
- Iwasaki T, Chin WW, Ko L. Identification and characterization of RRM-containing coactivator activator (CoAA) as TRBP-interacting protein, and its splice variant as a coactivator modulator (CoAM). *J Biol Chem* 2001;276:33375-83.
- Auboeuf D, Dowhan DH, Li X, et al. CoAA, a nuclear receptor coactivator protein at the interface of transcriptional coactivation and RNA splicing. *Mol Cell Biol* 2004;24:442-53.
- Lonard DM, Lanz RB, O'Malley BW. Nuclear receptor coregulators and human disease. *Endocr Rev* 2007;28:575-87.
- Perani M, Antonson P, Hamoudi R, et al. The proto-oncoprotein SYT interacts with SYT-interacting protein/co-activator activator (SIP/CoAA), a human nuclear receptor co-activator with similarity to EWS and TLS/FUS family of proteins. *J Biol Chem* 2005;280:42863-76.
- Lee SK, Anzick SL, Choi JE, et al. A nuclear factor, ASC-2, as a cancer-amplified transcriptional coactivator essential for ligand-dependent transactivation by nuclear receptors *in vivo*. *J Biol Chem* 1999;274:34283-93.
- Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 1977;36:59-74.
- Cheng JD, Dunbrack RL, Jr., Valianou M, Rogatko A, Alpaugh RK, Weiner LM. Promotion of tumor growth by murine fibroblast activation protein, a serine protease, in an animal model. *Cancer Res* 2002;62:4767-72.
- Ho DH, Vu H, Brown SAN, Donohue PJ, Hanscom HN, Winkles JA. Soluble tumor necrosis factor-like weak inducer of apoptosis overexpression in HEK293 cells promotes tumor growth and angiogenesis in athymic nude mice. *Cancer Res* 2004;64:8968-72.
- Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol* 1999;1:193-9.
- Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. *Neoplasia* 2004;6:1-6.
- Higgins JPT, Shinghal R, Gill H, et al. Gene expression patterns in renal cell carcinoma assessed by complementary DNA microarray. *Am J Pathol* 2003;162:925-32.
- Sankar N, Baluchamy S, Kadeppagari RK, Singhal G, Weitzman S, Thimmapaya B. p300 provides a corepressor function by cooperating with YY1 and HDAC3 to repress c-Myc. *Oncogene*. Epub 2008 Jun 9.
- Gordan JD, Bertout JA, Hu CJ, Diehl JA, Simon MC. HIF-2 α promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. *Cancer Cell* 2007;11:335-47.
- Yao M, Shuin T, Misaki H, Kubota Y. Enhanced expression of c-myc and epidermal growth factor receptor (C-erbB-1) genes in primary human renal cancer. *Cancer Res* 1988;48:6753-7.
- Trudel M, D'Agati V, Costantini F. C-myc as an inducer of polycystic kidney disease in transgenic mice. *Kidney Int* 1991;39:665-71.
- Zhang H, Gao P, Fukuda R, et al. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. *Cancer Cell* 2007;11:407-20.
- Wolf G, Schanze A, Stahl RA, Shankland SJ, Amann K. p27(Kip1) Knockout mice are protected from diabetic nephropathy: evidence for p27(Kip1) haplotype insufficiency. *Kidney Int* 2005;68:1583-9.
- Pertia A, Nikoleishvili D, Trsintadzhe O, Gogokhia N, Managadze L, Chkhotua A. Loss of p27(Kip1) CDK1 is a predictor of poor recurrence-free and cancer-specific survival in patients with renal cancer. *Int Urol Nephrol* 2007;39:381-7.
- Sui Y, Yang Z, Xiong S, et al. Gene amplification and associated loss of 5' regulatory sequences of CoAA in human cancers. *Oncogene* 2007;26:822-35.
- Coste A, Antal MC, Chan S, et al. Absence of the steroid receptor coactivator-3 induces B-cell lymphoma. *EMBO J* 2006;25:2453-64.
- Frisch SM, Mymryk JS. Adenovirus-5 E1A: paradox and paradigm. *Nat Rev Mol Cell Biol* 2002;3:441-52.
- Perkins ND. NF- κ B: tumor promoter or suppressor? *Trends Cell Biol* 2004;14:64-9.
- Majumder S. REST in good times and bad: roles in tumor suppressor and oncogenic activities. *Cell Cycle* 2006;5:1929-35.
- Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley BW. The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. *Proc Natl Acad Sci U S A* 2000;97:6379-84.
- Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999;17:1474-81.

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Dual Roles for Coactivator Activator and its Counterbalancing Isoform Coactivator Modulator in Human Kidney Cell Tumorigenesis

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