Serine/Arginine Protein–Specific Kinase 2 Promotes Leukemia Cell Proliferation by Phosphorylating Acinus and Regulating Cyclin A1

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Abstract

Serine/arginine (SR) protein–specific kinase (SRPK), a family of cell cycle–regulated protein kinases, phosphorylate SR domain–containing proteins in nuclear speckles and mediate the pre-mRNA splicing. However, the physiologic roles of this event in cell cycle are incompletely understood. Here, we show that SRPK2 binds and phosphorylates acinus, an SR protein essential for RNA splicing, and redistributes it from the nuclear speckles to the nucleoplasm, resulting in cyclin A1 but not A2 up-regulation. Acinus S422D, an SRPK2 phosphorylation mimetic, enhances cyclin A1 transcription, whereas acinus S422A, an unphosphorylatable mutant, blocks the stimulatory effect of SRPK2. Ablation of acinus or SRPK2 abrogates cyclin A1 expression in leukemia cells and arrest cells at G_1 phase. Overexpression of acinus or SRPK2 increases leukemia cell proliferation. Furthermore, both SRPK2 and acinus are overexpressed in some human acute myelogenous leukemia patients and correlate with elevated cyclin A1 expression levels, fitting with the oncogenic activity of cyclin A1 in leukemia. Thus, our findings establish a molecular mechanism by which SR splicing machinery regulates cell cycle and contributes to leukemia tumorigenesis. [Cancer Res 2008;68(12):4559–70]

Introduction

Pre-mRNA splicing is essential for the process of eukaryotic protein-encoded genes. It occurs in the splicesome complex, which contains two classes of splicing factors: small nuclear ribonucleoprotein (snRNP) particles and non-snRNP splicing factors consisting of a serine/arginine (SR)–rich domain (SR proteins). Splicing machinery concentrates in the nuclear speckles, which act as storage sites for splicing factors while splicing occurs on nascent transcripts. Splicing factors redistribute in response to transcription inhibition or viral infection, and nuclear speckles break down in metaphase and reassemble as cells progress through mitosis (1, 2). SR protein–specific kinase 1 (SRPK1) and SRPK2 are regulated by the cell cycle and are specific for SR proteins (3). Two families of kinases, SRPK and Clk/Sty, have been identified that phosphorylate SR domain–containing splicing factors. Clk/Sty was initially cloned as a cyclin-dependent kinase (CDK)–like kinase by PCR (4, 5), as well as a dual specificity kinase in an expression screening (6–8). SR splicing factors activated by its upstream kinases is essential for the alternative splicing machinery. For instance, HIV expression is significantly increased when one of SR proteins, Srp75, is phosphorylated by SRPK2 (9). The SRPK family of kinases, containing bipartite kinase domains separated by a unique spacer, is mainly localized in the cytoplasm, which is critical for nuclear import of SR proteins in a phosphorylation-dependent manner. Removal of the spacer in SRPK1 has little effect on the kinase activity, but triggers the nuclear translocation of kinases and consequently induces aggregation of splicing factors in the nucleus. Moreover, cell cycle signal induces nuclear translocation of the kinases at the G_2-M boundary, indicating that SRPKs play a role in cell cycle progression (10). In agreement with this observation, cdc2 kinase, a cdc2/cyclin B complex essential for G_2-M phase transition, phosphorylates SF2/ASF (11). Thus, SRPKs and SR splicing factor phosphorylation implicate in cell cycle regulation.

SR proteins, such as SF2/ASF, 9G8, and acinus, constitute a highly conserved family of splicing factors that play a role in selection at 5′ splice sites. SR proteins usually contain RNA-binding domain and a COOH terminal region enriched in repeating SR dipeptide (SR domains). Acinus resides in the nuclear speckles and induces apoptotic chromatin condensation after cleavage by caspases. Acinus is cleaved by caspases on both its NH_2 and COOH termini, generating a p17 active form (amino acids 228–335), which triggers chromatin condensation in the absence of caspase-3 (12). Acinus contains a region similar to the RNA recognition motif (RRM) of *Drosophila* splicing regulator Sxl, suggesting that it is implicated in RNA metabolism. Indeed, acinus is a component of functional splicesomes (13). It consists of three SR dipeptide repeat domains in the COOH terminus. Moreover, different acinus isoforms are found in the apoptosis-associated and splicing-associated protein (ASAP) complex, which is composed of the proteins SAP18, RNPS1, and distinct isoforms of Acinus. The complex inhibits RNA processing and accelerates the progress of cell death after induction of apoptosis (14, 15). Acinus is also a component of exon junction complex, which is deposited on mRNAs upstream of exon-exon junctions as a consequence of pre-mRNA splicing, and stimulates gene expression at the RNA level (16). Recently, we show that acinus is a physiologic substrate of nuclear Akt, which phosphorylates acinus on serine 422 and 573 and leads to its resistance to caspase cleavage and the inhibition of acinus-dependent chromatin condensation (17). Moreover, we found that the active fragment of p17 binds PKC-δ and enhances its apoptotic kinase activity, triggering histone H2BS14 phosphorylation and chromatin condensation (18). Most recently, we show that zyxin binds acinus, which is regulated by Akt, and diminishes acinus proteolytic cleavage and chromatin condensation (19).

Cell cycle regulation plays a key role in proliferation, apoptosis, and differentiation of hematopoietic cells (20). There are two mammalian A-type cyclins, cyclin A1 and A2. Whereas cyclin A1 is

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

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doi:10.1158/0008-5472.CAN-08-0021

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limited to male germ cells, cyclin A2 is widely expressed. Cyclin A2 regulates both G_1-S and G_2-M transition, and cyclin A1 is critical for passage of spermatocytes into meiosis I (21). In addition to expression in male germ cells, cyclin A1 is also found in hematopoietic stem cells and primitive precursors (22, 23). Elevated levels of cyclin A1 have been detected in several leukemic cell lines and in patients with myeloid hematologic malignancies (23, 24). Transgenic mouse model shows that cyclin A1 overexpression results in abnormal myelopoiesis, supporting an important role of cyclin A1 in hematopoiesis and the etiology of myeloid leukemia (25). It has been shown before that c-myb can directly transactivate the promoter of cyclin A1 and might be involved in the high-level expression of cyclin A1 observed in acute myeloid leukemia (26). In this study, we show that acinus also regulates cyclin A1, but not cyclin A2, expression in human leukemia cells, and this process is regulated by SRPK2 phosphorylation. Manipulation of SRPK2 or acinus protein level significantly affects cell cycle profile and mediates cell proliferation. Interestingly, we found that both SRPK2 and acinus are strongly overexpressed and acinus is phosphorylated in human patients with myeloid hematologic malignancies.

Materials and Methods

Cells and reagents. A panel of human leukemic cell lines derived from myeloid lineage including HEL (erythroblasts), KG-1 (myeloblasts), K-562 (erythroblasts), HL-60 (late myeloblasts), U-937 (monoblasts), and NB-4 (promyelocytes) and two lymphoid cell lines B-JAD and DG75 were maintained in RPMI 1640 supplemented with 10% FCS and 100 units penicillin-streptomycin at 37°C with 5% CO_2 atmosphere in a humidified incubator. Anti–caspase-3 and α-tubulin antibodies were from Santa Cruz Biotechnology, Inc. Anti-Myc, acinus, phosphorylated Akt-473, and Akt antibodies were from Cell Signaling. Active Akt protein was from Upstate Biotechnology, Inc. Phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein/extracellular signal-regulated kinase kinase 1 (MEK1) inhibitors were from Calbiochem. All clinical samples were obtained with informed consent with approval by the Emory University Institutional Review Board. All the chemicals not included above were from Sigma.

Yeast two-hybrid screen. Two-hybrid screening was conducted using Y190 yeast strain containing the HIS3 and β-galactosidase reporter genes and the pAS2-1 and pACT2 expression vectors. The experiments were executed exactly as described (27).

Coimmunoprecipitation and *in vitro* binding assays. A 10-cm plate of HEK293 cells or PC12 cells was washed once in PBS, lysed in 1 mL lysis buffer [50 mmol/L Tris (pH 7.4), 40 mmol/L NaCl, 1 mmol/L EDTA, 0.5% Triton X-100, 1.5 mmol/L Na_3VO_4, 50 mmol/L NaF, 10 mmol/L sodium PPi, 10 mmol/L sodium β-glycerophosphate, 1 mmol/L phenylmethylsulfonyl fluoride, 5 mg/mL aprotinin, 1 mg/mL leupeptin, 1 mg/mL pepstatin A], and centrifuged for 10 min at 14,000 × g at 4°C. The supernatant was transferred to a fresh tube. Experimental procedures for coimmunoprecipitation and *in vitro* binding assays are as described (27). After SDS-PAGE, the samples were transferred to a nitrocellular membrane. Western blotting analysis was performed with a variety of antibody.

Immunofluorescent staining of SRPK2, acinus, and Akt. HEK293 cells were cotransfected with HA-Akt or Flag-SRPK2 and glutathione *S*-transferase (GST)–acinus. Cells were fixed with cold (−20°C) methanol for 5 min and then rehydrated by PBS for 1 min. Nonspecific sites were blocked by incubating with 200 μL of 1% bovine serum albumin (BSA) in PBS at 37°C for 15 min. A mouse monoclonal antibody against HA was diluted 1:200 in PBS containing 1% BSA and incubated with the coverslips at 37°C for 1 h. Cells were then washed with 1% BSA/PBS for 10 min at room temperature before incubating with a 1:200 dilution of Texas Red–labeled goat anti-mouse IgG antibody at room temperature for 45 min, and then the coverslips were rinsed with a 1% BSA/PBS solution for 10 min. Then the cells were stained with 4,6-diamidino-2-phenylindole for another 10 min at room temperature. The coverslips containing the cells were then mounted with AquaMount (Lerner Laboratories) containing 0.01% 1,4-diazobicyclo(2,2,2)octane. Cells were examined under a fluorescence microscope.

Cell synchronization. The cells were initially plated at a density of $\sim 1 \times 10^6$ cells/mL in a 10-cm dish. One day after seeding, the cells were incubated with 2 mmol/L thymidine–containing medium, and 24 h later, the medium was removed and the cells were washed twice with prewarmed PBS at 37°C and incubated in fresh thymidine-free medium for 10 h. The cells were then cultured in medium supplemented with 2 mmol/L thymidine for an additional 16 h. After aspirating the medium, the cells were washed thrice with PBS prewarmed at 37°C and then incubated in fresh medium. At various times after release from the second thymidine block, the cells were harvested and lysis.

Flow cytometric analysis of cell cycle status. The flow cytometric evaluation of the cell cycle status was performed by a modification of a published method (28). Briefly, 2×10^6 control or small interfering RNA (siRNA)–treated K562 cells were centrifuged, washed twice with ice-cold PBS, and fixed in 70% ethanol. Tubes containing the cell pellets were stored at −20°C for at least 24 h. After this, the cells were centrifuged at 1,000 × g for 10 min, and the supernatant was discarded. The pellets were resuspended in 30 μL of phosphate/citrate buffer [0.2 Na_2HPO_4/0.1 citric acid (pH 7.5)] at room temperature for 30 min. Cells were then washed with 5 mL of PBS and incubated with propidium iodide (20 μg/mL)/RNase A (20 μg/mL) in PBS for 30 min. The samples were analyzed on a Coulter Elite flow cytometer.

Immunoblotting and immunohistochemistry on bone marrows. Bone marrow samples were obtained from patients in heparinized tubes and bone marrow mononuclear cells (peripheral blood mononuclear cells) were isolated by centrifugation through Ficoll-Hypaque. The cell lysates were obtained and applied to SDS-PAGE, followed by immunoblotting analysis against anti-SRPK2 (1:2,000), anti–p-acinus S422 (1:1,000), anti-acinus (1:1,000), and anti–cyclin A1 (1:1,000) and cyclin A2 (1:2,000). The blocking/wash reagent was 5% milk in PBS with 0.5% Tween 20.

Bone marrow samples and sections. Bone marrow samples collected from patients at time of diagnosis were used in this study with approval from the ethics committee. Bone marrow samples from 5 healthy adults and 10 patients with acute myelogenous leukemia (AML) were obtained as archival specimens from the Department of Pathology, Lund University, University Hospital in Malmö. The patient samples were obtained at the time of diagnosis and contained 90% leukemic blasts. The patient samples were divided into the AML subtypes M1 and M2 according to the French American and British classification system. Paraffin-embedded tissue samples were deparaffinized and boiled in 0.01 mol/L citrate buffer (pH 6.0) for 10 min. The staining procedure was performed using a semiautomatic staining machine (Ventana ES, Ventana Inc.). The specimens were viewed with a Nikon 800 microscope. The staining intensities of antibodies in leukemic bone marrows were scored from 0 to 3. Negative cells were scored as 0, cells that had weak staining or had intensities similar to that of normal bone marrow were scored as 1, and cells with strong and very strong staining were scored as 2 or 3, respectively.

Results

Acinus binds SRPK2. Acinus contains a few RS domains in the COOH terminus and regulates pre-mRNA splicing (14). To look for the binding targets, we conducted a yeast two-hybrid analysis using the COOH terminal domain (228–583 amino acids) of acinus S as bait. One of 10 independent positive clones encodes the NH_2 terminal fragment of SRPK2 protein (amino acids 73–443). We observed interactions between the COOH terminal portion of acinus and SRPK2 NH_2 terminal domain (73–443 amino acids) regardless of which protein was used as bait or prey. By contrast, the NH_2 terminal portion of acinus failed to interact with SRPK2 (Fig. 1*A*). To verify the interaction between these two proteins,

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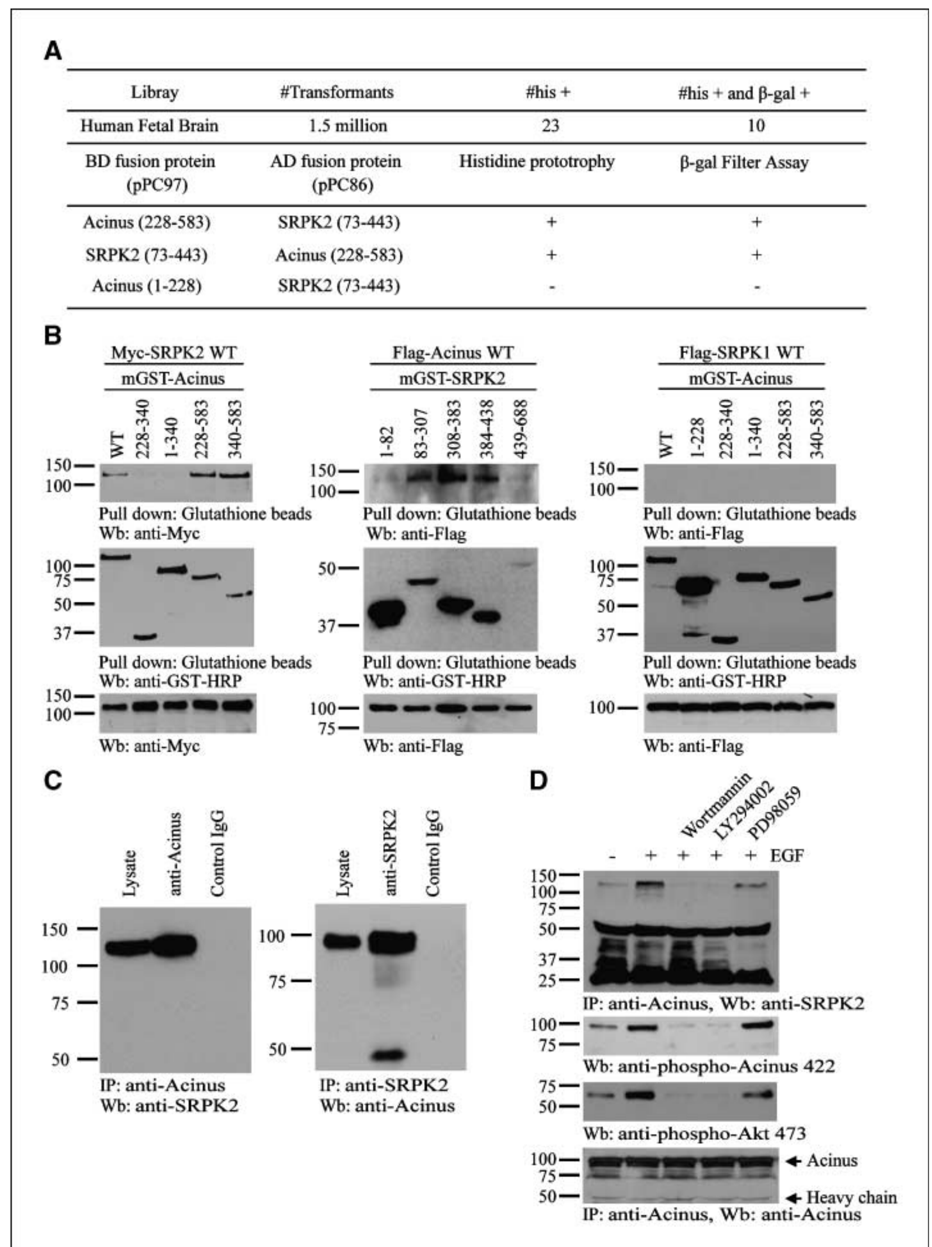

Figure 1. Acinus binds SRPK2. *A,* yeast two-hybrid screen searching for the binding targets of the CTD of acinus. *B,* the CTD of acinus associates with the middle spacer in SRPK2. Various GST-tagged acinus constructs were cotransfected with SRPK2 into HEK293 cells. Transfected acinus proteins were pulled down with glutathione beads. The COOH terminal end but not the NH_2 terminal domain of acinus associates with SRPK2 (*top left*). GST-tagged SRPK2 fragments were cotransfected into HEK293 cells with Flag-acinus. SRPK2 proteins were pulled down with glutathione beads. The middle region of SRPK2 from 308 to 383 interacted with acinus (*top middle*). The expression of the transfected constructs was confirmed (*middle* and *bottom middle*). SRPK1 does not bind to acinus (*right*). *C,* endogenous acinus binds to SRPK2 in mouse brain. Acinus coimmunoprecipitated with SRPK2 regardless of acinus or SRPK2 antibody used. *D,* PI3K signaling mediates the association between SRPK2 and acinus. K562 cells were pretreated with various pharmacologic inhibitors (20 nmol/L Wortmannin, 10 μmol/L LY294002, 10 μmol/L PD98059) for 30 min, followed by 50 ng/mL EGF for 10 min. Endogenous acinus was immunoprecipitated with anti-acinus antibody. PI3K inhibitors but not MEK1 pretreatment abolished SRPK2 binding to acinus (*top*). Acinus S422 and Akt S473 phosphorylation were verified (*second* and *third panels*). Equal amount of acinus was immunoprecipitated (*bottom*).

we conducted a binding assay. In HEK293 cells, transfected flag-SRPK2 strongly bound to both acinus CTD fragments (amino acids 228–583 and 340–583), and the full-length acinus also associated with SRPK2; by contrast, SRPK2 did not interact with the middle RRM (amino acids 228–340) or acinus-NTD (amino acids 1–340), consistent with our yeast two-hybrid findings (Fig. 1*B, left*). Mapping assay using a variety of SRPK2 fragments reveals that the middle region (amino acids 308–383), but not the extreme NH_2 or COOH terminus of SRPK2, is essential for interacting with acinus (Fig. 1*B, middle*). SRPK1 and SRPK2 share very high homology. mSRPK1 has two stretches of basic amino acids (11–21 and 265–277 amino acids), which may function as nuclear localization signals, whereas mSRPK2 has one of these basic amino acid regions (264–276 amino acids); instead, it contains a proline-rich domain (21–43 amino acids) in the NH_2 terminus with unknown function. Moreover, mSRPK2 has an acidic domain (287–405 amino acids), which is unique to this kinase (29). To assess whether SRPK1 also binds acinus, we conducted coimmunoprecipitation study and found that SRPK1 did not interact with acinus (Fig. 1*B, right*), indicating the association between acinus and SRPK2 is specific.

To explore whether endogenous acinus and SRPK2 could associate with each other in mouse brain, we performed immunoprecipitation study. Acinus and SRPK2 robustly bound to each other no matter whether acinus or SRPK2 antibody was used. In contrast, control IgG failed to precipitate either protein, underscoring that the interaction between acinus and SRPK2 is specific (Fig. 1*C*). Our previous study reveals acinus is a physiologic substrate of Akt. To examine whether the interaction between these two proteins are regulated by PI3K signaling, we pretreated K562 cells with various pharmacologic inhibitors, followed by epidermal growth factor (EGF) stimulation. EGF elicited robust association

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between acinus and SRPK2, which was completely disrupted by PI3K inhibitors Wortmannin and LY294002; in contrast, MEK1 inhibitor PD98059 failed to block the interaction (Fig. 1*D*), suggesting that PI3K/Akt signaling regulates the interaction between these two proteins. We made similar observation in PC12 cells in response to nerve growth factor stimulation (data not shown). Hence, acinus strongly binds to SRPK2 in mammalian cells.

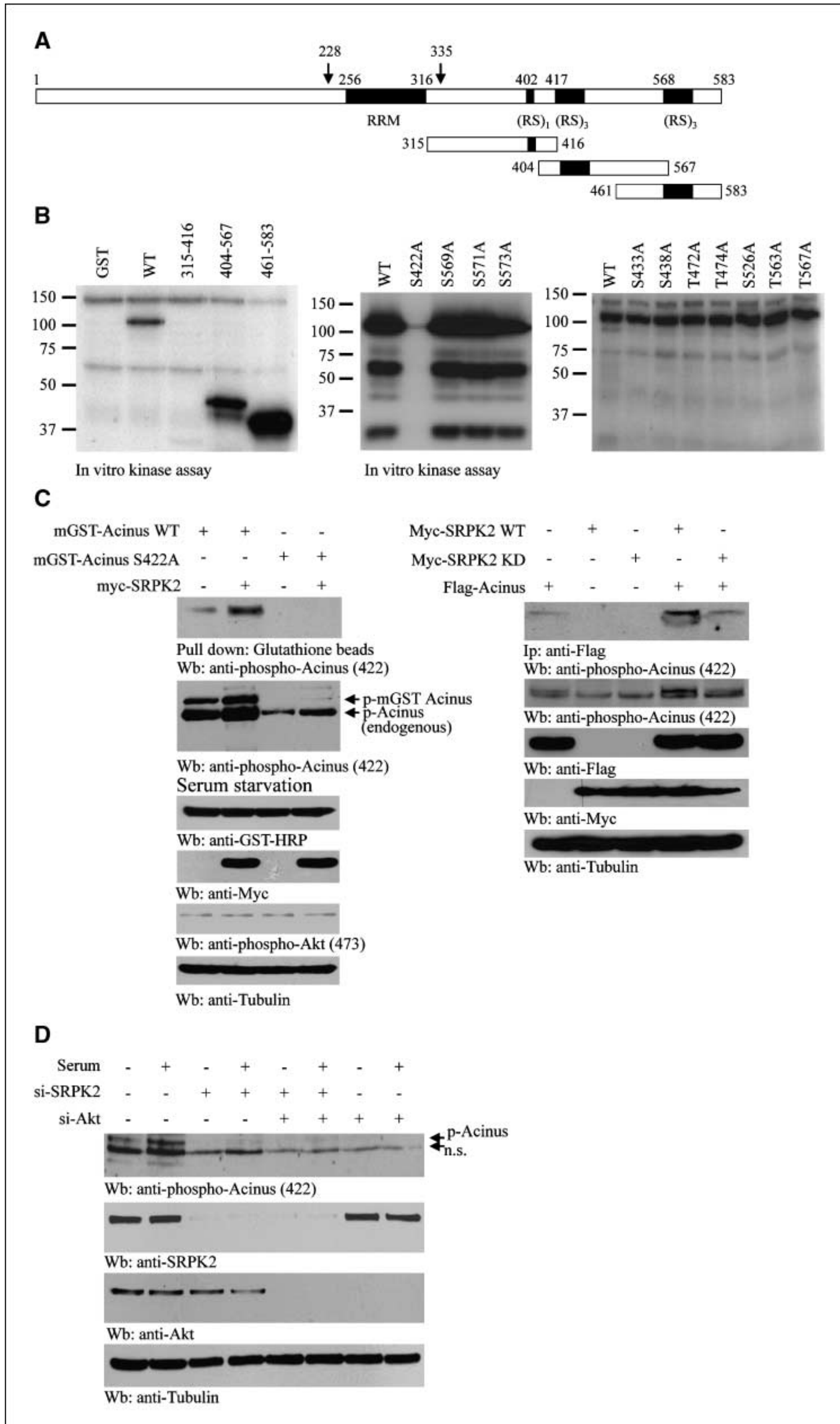

Figure 2. SRPK2 phosphorylates acinus on serine 422. *A*, diagram of acinus S. Acinus S possesses three RS motifs as indicated. The three fragments, with each containing the RS dipeptide repeat motif, are indicated with residue numbers. *B, in vitro* SRPK2 kinase assay. Purified recombinant GST fusion proteins were incubated with purified His-SRPK2 at 30°C for 30 min. Both fragments *B* and *C* were robustly phosphorylated, whereas fragment *A* was not (*left*). S422 residue in acinus S was phosphorylated by SRPK2. Purified GST-acinus proteins were incubated with purified SRPK2 in the presence of [γ-^{32}P]ATP. S422A mutant substantially decreased acinus phosphorylation (*middle* and *right*). *C,* wild-type but not KD SRPK2 phosphorylates acinus. GST-acinus wild type and S422A were transfected into HEK293 cells in the presence or absence of SRPK2. Transfected acinus was pulled down with glutathione beads. Whereas S422 site was markedly phosphorylated in wild-type acinus, no phosphorylation was detected in S422A mutant (*top left*). The expression of transfected constructs was verified (*second* to *bottom left panels*). Flag-acinus and Myc-SRPK2 wild type or KD were transfected into HEK293 cells. Acinus was immunoprecipitated with anti-Flag antibody and probed with anti–phosphorylated S422 antibody. Wild-type SRPK2 potently phosphorylated acinus, whereas SRPK2-KD failed (*top right*). The expression of transfected constructs was verified (*second* to *bottom right panels*). *D,* acinus S can be phosphorylated in intact cells. HEK293 cells were transfected with siRNA for SRPK2 or Akt and followed by serum starvation overnight. In control samples, serum triggered potent S422 phosphorylation. Knocking down of SRPK2 or Akt blocked acinus S422 phosphorylation (*top*). The depletion of SRPK2 and Akt was confirmed (*second* and *third panels*).

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SRPK2 phosphorylates acinus on serine 422. Acinus contains a few RS motifs in the COOH terminus. To explore whether it can be phosphorylated by SRPK2, we prepared GST-recombinant proteins from three fragments of acinus, with each containing a putative phosphorylation domain. We examined their ability to be phosphorylated by SRPK2 through *in vitro* kinase assay in the presence of [γ-^{32}P]ATP. Fragments (amino acids 404–567) and (amino acids 461–583) and full-length acinus were robustly phosphorylated by SRPK2. By contrast, fragment (amino acids 315–416) or GST alone was not phosphorylated (Fig. 2*A* and *B, left*). Mutation with S422A but not S569A, S571A, S573A or other residues in acinus abolished the phosphorylation of full-length acinus, suggesting that S422 residue is the major phosphorylation site by SRPK2 *in vitro* (Fig. 2*B, middle* and *right*). Interestingly, we have previously shown that S422 in acinus can also be phosphorylated by Akt (17). To explore whether acinus can be phosphorylated by SRPK2 in intact cells, we transfected GST-tagged acinus wild-type or S422A into HEK293 cells alone or in combination with Myc-SRPK2 wild-type construct. The transfected cells were serum starved overnight, and the transfected proteins were pulled down and monitored by immunoblotting with anti–phosphorylated acinus S422 antibody. Compared with control, SRPK2 robustly provoked acinus phosphorylation, and acinus S422A was not phosphorylated regardless of single transfection or in a combination with SRPK2 (Fig. 2*C, top left*). Furthermore, the endogenous acinus phosphorylation was also regulated by transfected SRPK2 (Fig. 2*C, second left*). Akt was not activated in the serum-starved cells (*bottom*), indicating that SRPK2 is responsible for acinus S422 phosphorylation. Transfection with a kinase-dead (KD; K110A) SRPK2-KD markedly diminished kinase activity of SRPK2 on acinus (Fig. 2*C, top right*), demonstrating that SRPK2 contributes to acinus S422 phosphorylation in mammalian cells. To explore whether acinus is a physiologic substrate of SRPK2, we depleted SRPK2 or Akt in HEK293 cells, respectively. Ten percent fetal bovine serum (FBS) strongly increased acinus phosphorylation in serum-starved cells. Knocking down of Akt or SRPK2 by the si-RNA abolished S422 phosphorylation in acinus. The band below acinus might be a nonspecific band (Fig. 2D, top panel). Ablation of either Akt or SRPK2 diminishes acinus S422 phosphorylation suggests that both kinases are necessary for acinus S422 phosphorylation. Collectively, these data support that acinus acts as a physiologic substrate of SRPK2.

SRPK2 but not Akt redistributes acinus in the nucleus. Our previous study shows that acinus resides in the nuclear speckles, colocalizing with SC35, a nuclear speckle marker (17). Overexpression of SRPK2 causes disassembly of cotransfected SF2/ASF and SC35 (29). To explore the effect of SRPK2 phosphorylation on acinus subcellular localization, we conducted immunofluorescent staining on HEK293 cells transfected with various constructs. Like wild-type acinus, both acinus (S422A) and acinus (S422, 573A) also distributed in the nuclear speckles. However, acinus (S422D) uniformly localized in the whole nucleoplasm (Fig. 3*A, top*), indicating acinus phosphorylation by either SRPK2 or Akt is sufficient to redistribute its subcellular localization. Wild-type SRPK2 mainly localized in the cytoplasm and a fraction of the kinase was visible in the nucleus, whereas SRPK2-KD exclusively occurred to the cytoplasm, confirming the previous reports (10, 29). To distinguish which kinase accounts for the redistribution of acinus (S422D) in the nucleus, we cotransfected SRPK2 wild type or KD into HEK293 cells with wild-type or unphosphorylatable acinus constructs. We found that acinus (S422D) homogenously resided in the whole nucleoplasm regardless of SRPK2 wild type or KD; by contrast, wild-type acinus remained in the nuclear speckle in the presence of SRPK2-KD, and it occurred in the nucleoplasm when cotransfected with wild-type SRPK2. Nonetheless, acinus (S422A) constantly localized in the nuclear speckles irrespective of SRPK2 wild type or KD (Fig. 3*A, bottom*), suggesting that SRPK2 kinase activity is responsible for acinus nuclear redistribution. We conducted the similar experiments with wild-type Akt, constitutively active Akt-CA or Akt-KD. HA-Akt wild-type, Akt-CA, and Akt-KD alone predominantly occurred in the cytoplasm, but it also distributed in the nucleus when cotransfected with acinus, fitting with previous finding that acinus binds to Akt (17). Both acinus wild-type and S422A remained in the nuclear speckles no matter which version of Akt was cotransfected (Fig. 3*B*). Thus, these results support that SRPK2 but not Akt phosphorylation of acinus on S422 translocates acinus from the nuclear speckles to the nucleoplasm.

PI3K signaling mediates the association between Akt and acinus. Acinus S422 phosphorylation is essential for this interaction (17). To investigate whether SRPK2 phosphorylating acinus plays any role in their association, we cotransfected SRPK2 into HEK293 cells with various acinus constructs. GST pull-down assay shows that both acinus (S422A) and acinus (S422, 573A) displayed lower affinity to SRPK2 than wild-type acinus. Notably, acinus (S422D) and acinus (S422, 573D) revealed a slightly enhanced binding activity than wild-type counterpart (Fig. 3*C*). Acinus binds both Akt and SRPK2. To assess whether Akt plays any role in mediating the association between acinus and SRPK2, we cotransfected acinus wild-type and S422A into HEK293 cells with SRPK2 wild type or KD. Compared with wild-type SRPK2, the binding activity to both wild-type acinus and S422A by SRPK2-KD slightly decreased. Depletion of Akt did not affect the association between wild-type acinus and wild-type SRPK2. However, it evidently decreased the interaction between S422A and SRPK2. Knocking down Akt strongly diminished the interaction between wild-type acinus and SRPK2-KD and completely abolished the binding by S422A to SRPK2-KD (Fig. 3*D, top*). These data suggest that S422 phosphorylation in acinus by SRPK2 is important for its binding to SRPK2, and Akt is dispensable for the acinus/SRPK2 complex formation. However, when SRPK2 kinase activity is low, Akt is critical for acinus binding to SRPK2.

SRPK2 is required for cyclin A1 expression. The transcripts of most genes that encode apoptotic regulators are subjected to alternative splicing, which can result in the production of antiapoptotic or proapoptotic protein isoforms (30). SRPKs are cleaved *in vivo* upon apoptotic stimuli, which can be prevented by bcl-2 or caspase inhibitors (31). Moreover, SRPKs are cell cycle–regulated protein kinases. Probably, some of the apoptosis or cell cycle–related proteins are mediated by SRPK2. To test this notion, we transfected SRPK2 into HeLa cells and K562 cells and monitored the expression of various CDKs, cyclins, caspases, and DNA fragmentation factor (DFF). Disappointingly, none of the examined CDKs, caspases, or DFFs was altered. Strikingly, cyclin A1, but not cyclin A2 or cyclin B1, was evidently up-regulated (Fig. 4*A*). Notably, cyclin D1 was slightly enhanced upon SRPK2 overexpression as well. Cyclin A1 up-regulation by SRPK2 was dependent on its kinase activity, as SRPK2-KD failed to trigger cyclin A1 expression. These results suggest that SRPK2 selectively affects cyclin A1 expression. To determine whether SRPK2 regulates cyclin A1 transcription, we conducted luciferase assay with cyclin A1 promoter containing construct. Coexpression of

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SRPK2 with the cyclin A1 promoter construct significantly increased the reporter activity in HEK293 cells in a dose-dependent manner. By contrast, SRPK2-KD failed. As a positive control, MyB also potently activated cyclin A1 promoter (Fig. 4*B, top*). To investigate whether SRPK2 is required for cyclin A1 promoter activation, we depleted SRPK2 with siRNA. Luciferase activity was gradually decreased, as SRPK2 was progressively knocked down (Fig. 4*B, bottom*). To examine whether acinus is involved in SRPK2-regulated cyclin A1 expression, we cotransfected various acinus constructs and siRNA of acinus with SRPK2. Depletion of acinus in SRPK2 overexpressed cells completely abolished the stimulatory effect (Fig. 4*C, lane 3*), suggesting that acinus acts downstream of

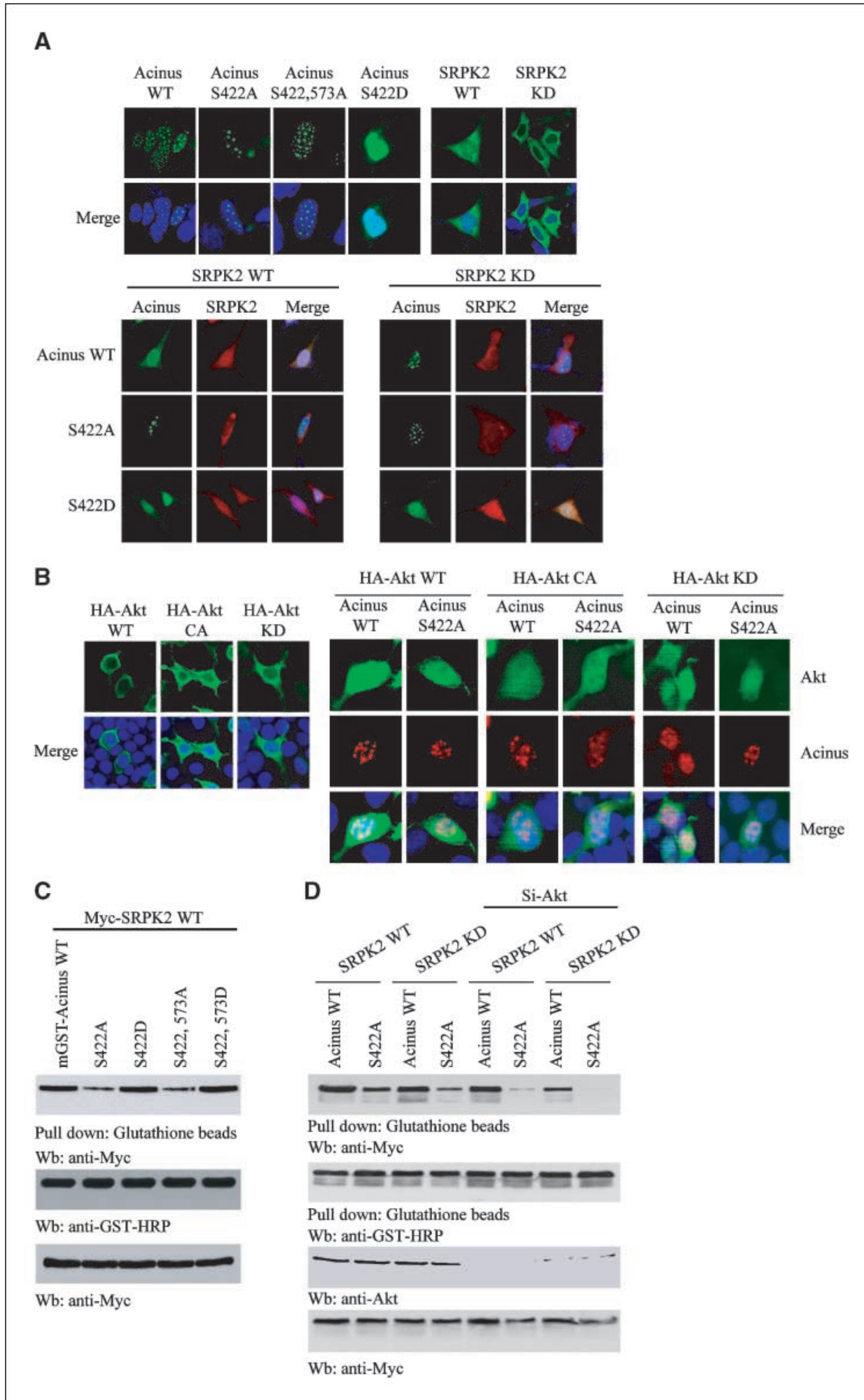

Figure 3. SRPK2 but not Akt redistributes acinus in the nucleus. *A,* acinus phosphorylation mimetic mutant S422D redistributes in the nucleus. Wild-type acinus and S422A mutants resided in the nuclear speckles, whereas S422D occurred in the whole nucleoplasm. Wild-type SRPK2 was mainly localized in the cytoplasm, and a portion of it was also distributed in the nucleus. SRPK2-KD exclusively localized in the cytoplasm. SRPK2 phosphorylation triggers acinus relocation from the nuclear speckle to the nucleoplasm. Wild-type acinus redistributed in the nucleoplasm when cotransfected with wild-type SRPK2, and it localized in the nuclear speckles when cotransfected with SRPK2-KD. S422D resided in the nucleoplasm regardless of SRPK2 wild type or KD. *B,* Akt cannot relocate acinus from the nuclear speckles. All Akt proteins (wild type, CA, and KD) and acinus-S colocalized in the nuclear speckles of transfected cells. *C,* S422A exhibited lower affinity to SRPK2. Myc-SRPK2 was cotransfected into HEK293 cells with various GST-tagged acinus constructs. Transfected acinus proteins were pulled down with glutathione beads and probed with anti-myc antibody. S422A mutants exhibited decreased binding activity to SRPK2 (*top*). The expression of transfected constructs was confirmed (*middle* and *bottom*). *D,* Akt enhances the interaction between SRPK2 and acinus, when SRPK2 kinase activity is low. Acinus and SRPK2 were cotransfected into HEK293 cells, followed by knocking down of Akt with siRNA. Wild type and SRPK2-KD displayed the similar affinity to wild-type acinus and lower binding activity to S422A. Depletion of Akt slightly decreased the affinity of wild-type SRPK2 to acinus, whereas SRPK2-KD binding to acinus wild-type was evidently reduced and completely abolished to acinus S422A (*top*). The expression of transfected constructs and Akt protein level were confirmed (*second* to *bottom panels*).

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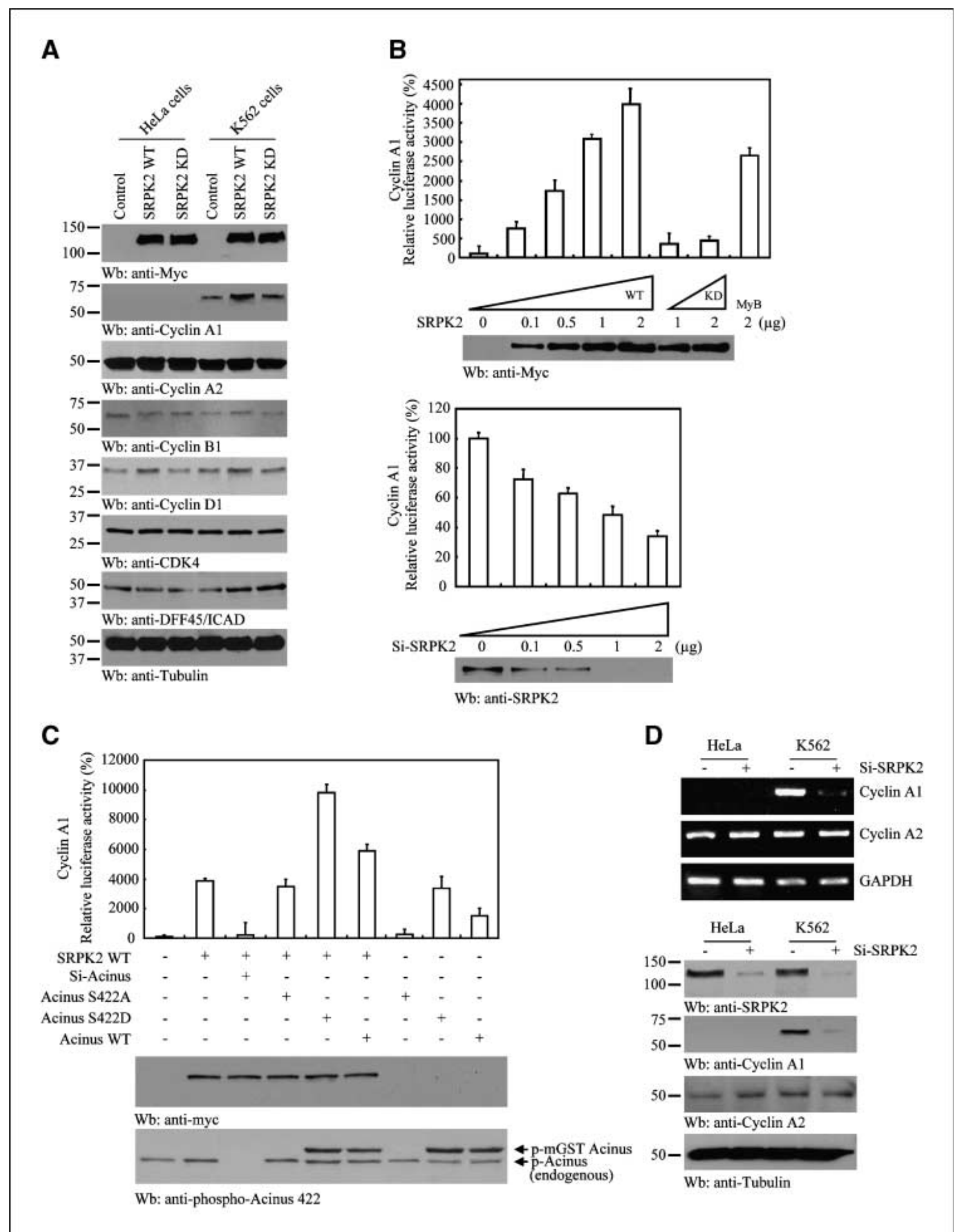

Figure 4. SRPK2 is required for cyclin A1 expression. *A,* SRPK2 overexpression up-regulates cyclin A1 expression. HeLa cells and K562 cells were transfected with SRPK2 wild type and KD. The expression of various cell cycle–related and apoptosis-related proteins was monitored by immunoblotting. Cyclin A1 but not cyclin A2 or cyclin B1 was selectively increased in SRPK2 wild-type cells, and the stimulatory effect was lost in KD sample (*second, third,* and *fourth panels*). Interestingly, cyclin D1 was also weakly enhanced in SRPK2 overexpressed cells (*fifth panel*). CDK4 and DFF/ICAD expression levels were not affected by SRPK2 (*sixth* and *seventh panels*). *B,* SRPK2 regulates cyclin A1 promoter activity. Different amounts of SRPK2 wild type and KD were coexpressed with a cyclin A1 promoter construct (335-bp fragment). Empty vector was used to match the same total amount of DNA in all experiments. *Columns,* mean for three independent experiments; *bars,* SE. SRPK2-mediated cyclin A1 promoter activation in a dose-dependent manner, and KD lost its activity. SRPK2 is required for cyclin A1 promoter activation. Endogenous SRPK2 was depleted from HEK293 cells, transfected with cyclin A1 promoter construct. Ablation of SRPK2 decreased cyclin A1promoter luciferase activity. *Columns,* means of three independent experiments; *bars,* SD. *C,* acinus mediates SRPK2 activity on cyclin A1 expression. Various acinus constructs and siRNA of acinus were cotransfected with SRPK2 wild type into HEK293 cells. Depletion of acinus blocked SRPK2 activity. Unphosphorylatable mutant S422A decreased SRPK2 effect, whereas S422D substantially increased SRPK2 activity. *Columns,* means of three independent experiments; *bars,* SD. *D,* SRPK2 controls cyclin A1 expression in human leukemia cells. SRPK2 siRNA and control RNAi were transfected into HeLa, HL-60, and K562 cells. The total RNA was extracted, and RT-PCR was conducted. Ablation of SRPK2 abolished cyclin A1 but not cyclin A2 expression (*top* and *second panels*). The cell lysates were analyzed with immunoblotting with anti-SRPK2, cyclin A1, and cyclinA2 antibodies, respectively. Depletion of SRPK2 substantially attenuated cyclin A1 but not cyclin A2 expression.

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SRPK2. Compared with wild-type acinus, transfection of phosphorylation mimetic acinus, S422D, up-regulated cyclin A1 promoter activity, whereas unphosphorylatable mutant S422A decreased the activity (*lanes 7–9*). Coexpression of wild-type acinus and SRPK2 further enhanced luciferase activity. The maximal activity occurred to phosphorylation mimetic, acinus S422D. By contrast, unphosphorylatable mutant S422A attenuated the activity (*lanes 4–6*). These results suggest that acinus might be necessary but not sufficient to mediate all of SRPK2 effects. To test whether SRPK2 actually affects cyclin A1 expression, we transfected human K562 leukemia cells and HeLa cells with siRNA to knock down SRPK2. Reverse transcription–PCR (RT-PCR) analysis shows that depletion of SRPK2 substantially abrogated cyclin A1 expression without influencing cyclin A2 transcription in K562 cells, and HeLa cells did not express cyclin A1 (Fig. 4*D, top*), underscoring that SRPK2 influences cyclin A1 transcription. Cyclin A1 protein levels were substantially blocked when SRPK2 was knocked down. Cyclin A2 remained stable in both cells regardless of SRPK2 expression level (Fig. 4*D, bottom*). Therefore, SRPK2 regulates cyclin A1 transcription and protein expression, for which its kinase activity is indispensable.

Acinus phosphorylation by SRPK2 regulates its effect on cyclin A1 expression. Acinus is a component of the ASAP complex, which is composed of the proteins SAP18, RNPS1, and distinct isoforms of acinus. ASAP complex and acinus by itself affect RNA processing (14, 16). To explore the physiologic role of the SR splicing factor acinus in cyclin A1 expression, we cotransfected acinus with the cyclin A1 promoter construct into HEK293 cells. Wild-type acinus increased cyclin A1 promoter activity in a dose-dependent manner. Interestingly, acinus S422D strongly increased the reporter activity, whereas S422A evidently blocked the activation of cyclin A1 promoter (Fig. 5*A*). The

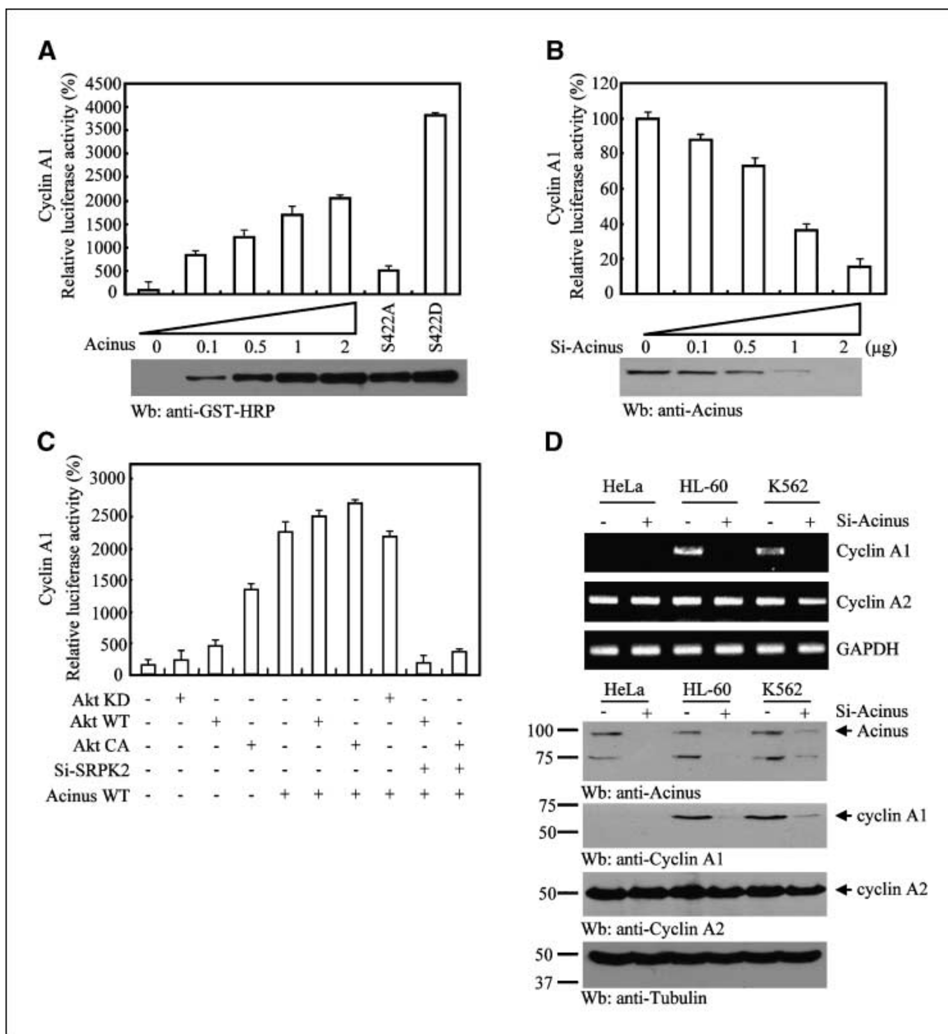

Figure 5. Acinus phosphorylation by SRPK2 regulates its effect on cyclin A1 expression. *A,* acinus mediates cyclin A1 promoter activity, which is regulated by S422 phosphorylation. Different amounts of acinus wild-type and phosphorylation mutants were coexpressed with a cyclin A1 promoter construct (335-bp fragment). Empty vector was used to match the same total amount of DNA in all experiments. *Columns,* means of three independent experiments; *bars,* SD. Acinus mediated cyclin A1 promoter activation in a dose-dependent manner. Acinus S422A lost its activity, whereas S422D strongly elevated cyclin A1 promoter activity. *B,* acinus is required for cyclin A1 expression. Endogenous acinus was knocked down from HEK293 cells, transfected with cyclin A1 promoter construct. Depletion of acinus reduced cyclin A1 promoter activity. *Columns,* means of three independent experiments; *bars,* SD. *C,* SRPK2 plays a more important role in activating acinus stimulatory activity. Constitutively active Akt-CA overexpression evidently increased cyclin A1 promoter activity (*lane 4*), but the effect was not as much as acinus overexpression (*lane 5*). Coexpression of Akt with acinus slightly increased acinus activity (*lanes 6* and *7*), which was almost completely abrogated in SRPK2-depleted samples (*lanes 9* and *10*). Akt-KD almost had no effect on acinus activity (*lane 8*). *Columns,* means of three independent experiments; *bars,* SD. *D,* acinus controls cyclin A1 expression in human leukemia cells. Acinus siRNA and control RNAi were transfected into HeLa, HL-60, and K562 cells. RT-PCR was conducted. Knocking down of acinus abolished cyclin A1 but not cyclin A2 expression (*top* and *second panels*). The cell lysates were analyzed with immunoblotting with anti-acinus, cyclin A1, and cyclin A2 antibodies, respectively. Depletion of SRPK2 prominently decreased cyclin A1 but not cyclin A2 expression.

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luciferase activity was steadily reduced, as endogenous acinus was increasingly depleted (Fig. 5*B*), supporting that acinus is essential for cyclin A1 expression. Because both Akt and SRPK2 can bind acinus and phosphorylate S422, we monitored cyclin A1 luciferase activity in HEK293 cells transfected with Akt or acinus alone or in a combination. Compared with control and Akt-KD, Akt wild-type slightly increased luciferase activity and Akt-CA significantly augmented the activity (Fig. 5*C, lanes 1–4*). In contrast, acinus overexpression elicited much more potent effect than Akt-CA, indicating acinus itself is much more important than Akt in this event. Cotransfection of acinus with Akt-wild-type weakly elevated the activity, which was further enhanced when cotransfected with Akt-CA. Notably, Akt-KD did not obviously affected acinus stimulatory effect, indicating that Akt phosphorylation is not essential for this action. However, depletion of SRPK2 by its siRNA almost completely eliminated acinus activity (Fig. 5*C, lanes 8* and *9*), underscoring that SRPK2 plays a much more important role in regulating acinus catalytic activity than Akt. To further explore whether acinus is required for cyclin A1 expression, we transfected HeLa, HL-60, and K562 cells with acinus siRNA. RT-PCR analysis shows that elimination of acinus completely blocked cyclin A1 expression without affecting cyclin A2 in both HL-60 and K562 cells (Fig. 5*D, top*). Consequently, cyclin A1 protein expression was diminished when acinus was depleted. Cyclin A2 was not affected irrespective of acinus expression level (Fig. 5*D, bottom*).

SRPK1 kinase activity is regulated during cell cycle (2). To assess whether endogenous acinus and SRPK2 regulate cyclin A1 expression in a cell cycle–dependent way, we synchronized HL60 cells in S phase via double thymidine incorporation and monitored cyclin A1 and cyclin A2 expression. SRPK2 expression was relatively stable in all cell phases. However, acinus was evidently augmented in G_1 and M phases compared with early S phase, later S phase, and G_2-M phases. Interestingly, we observed a cleaved band at ~75 kDa in G_1 and M phases, reminiscent of a proteolytic cleaved fragment (Supplementary Fig. S1, *top* and *second panels*). Strikingly, acinus was selectively phosphorylated in early S phase, gradually increased in late S phase, and peaked in G_2-M phase. Cyclin A1 expression pattern correlated with phosphorylated acinus S422 levels (*third* and *fourth left panels*). Akt phosphorylation also occurred in S and G_2-M phases. Cyclin A2 expression level remained relatively stable during the cell cycle (Supplementary Fig. S1, *fifth* and *bottom panel*). Therefore, acinus phosphorylation by SRPK2 mediates cyclin A1 expression in human leukemia cells. Collectively, these data show that SRPK2 regulates cyclin A1 expression by phosphorylating acinus. Although Akt also phosphorylates acinus in the same residue, SRPK2 plays a more critical role than Akt in the transcriptional regulation of cyclin A1 by acinus.

SRPK2 and acinus regulates cell cycle profile and leukemia cell proliferation. Cyclin A1 is essential for meiosis: targeted deletion of the *Ccna1* gene resulted in male sterility (32). Acinus regulates cyclin A1 expression (Figs. 4 and 5); thus, it is possible that acinus also plays some role in cell cycle and cell proliferation. To test this notion, we knocked down acinus and SRPK2 in K562 cells and monitored cell proliferation, respectively. As shown in Fig. 6*A*, the levels of endogenous acinus and SRPK2 were severely reduced after siRNA transfection (*top* and *second panels*). As expected, the steady-state levels of cyclin A1 was decreased more in acinus eliminated cells than SRPK2 ablated cells. As expected, cyclin A2 remained stable. Strikingly, however, acinus and SRPK2-RNA interference (RNAi) treatment significantly reduced the growth rate of these cells. Nevertheless, the extent to which Akt ablation led to cell proliferation suppression is less than those by acinus or SRPK2 elimination (Fig. 6*B*), suggesting that SRPK2 or acinus inactivation induces cell growth repression stronger than Akt ablation in K562 cells. BrdUrd incorporation confirmed this observation. SRPK2 and acinus depletion decreased BrdUrd-positive cells from 48% to 13% and 19%, respectively. Akt inactivation diminished it to 27% (Fig. 6*C*). To further explore the effect of SRPK2 and acinus on cell cycle, we monitored K562 cell cycle profile using fluorescence-activated cell sorting (FACS) analysis. Compared with control, SRPK2 or acinus ablation evidently triggered G_1 phase accumulation; Akt knockdown exhibited the similar pattern. Quantitative analysis reveals that G_1 phase percentage was substantially increased from 51.1% to 86.2%, 85.1%, and 76.3% in SRPK2, acinus, and Akt ablated cells, respectively. S-phase content was remarkably reduced, fitting with BrdUrd incorporation results (Fig. 6*D*). On the other hand, overexpression of acinus or wild-type SRPK2 provoked prominent G_2-M phase accumulation and G_1 phase decrease. By contrast, the effect by SRPK2-KD was substantially less than its wild-type counterpart. Quantitative analysis shows that acinus or SRPK2 transfection led to a quadrupling of G_2-M phase. The kinase activity of SRPK2 was critical, as we observed a little over doubling of cell number in SRPK2-KD transfected cells (Supplementary Fig. S2). To explore whether cyclin A1 is required for the dramatic cell cycle effects by acinus and SRPK2, we depleted cyclin A1 using its siRNA. Expression of acinus or SRPK2 or both triggered G_2-M phase arrest, ablation of cyclin A1 substantially abolished this cell cycle arrest effect (Supplementary Fig. S2). Thus, these data show that both

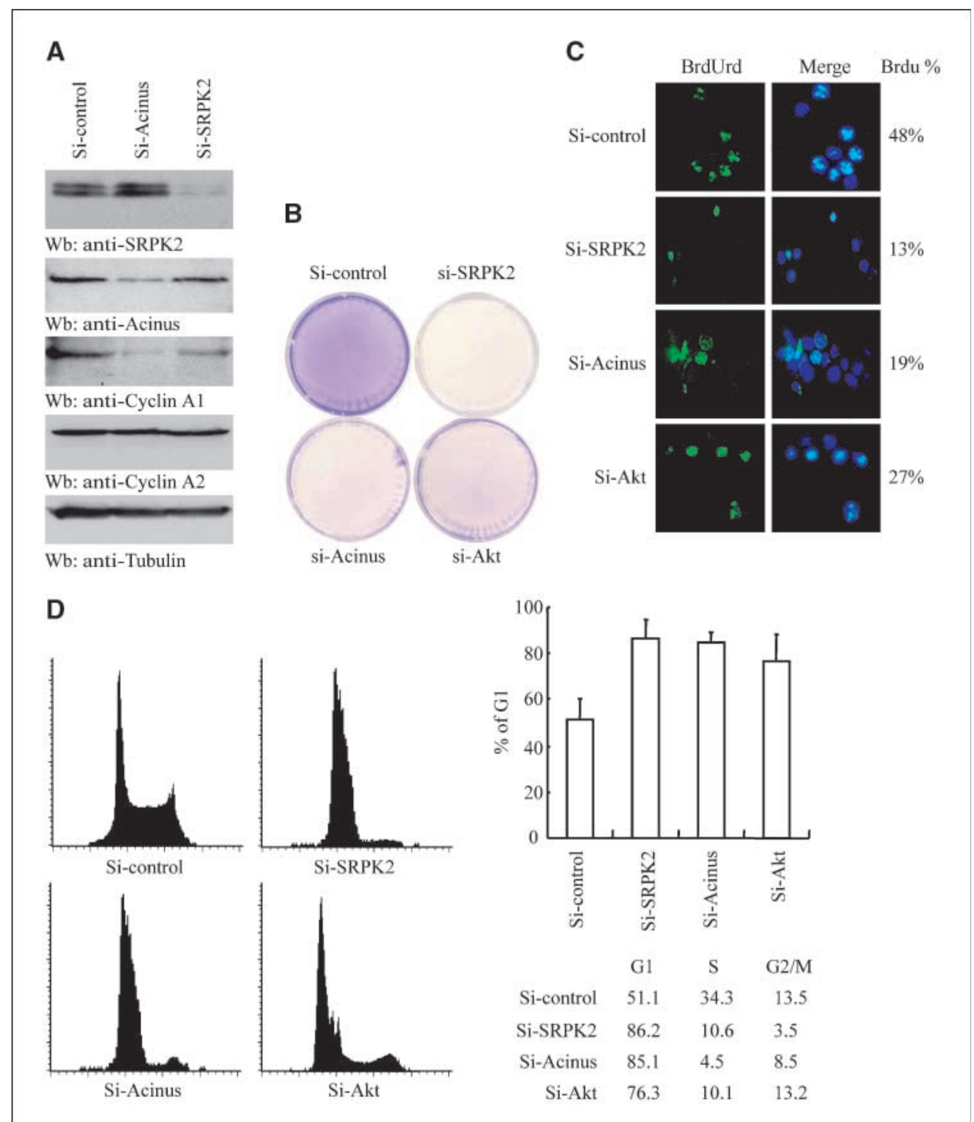

Figure 6. SRPK2 and acinus regulates cell cycle profile and leukemia cell proliferation. *A,* ablation of acinus or SRPK2 attenuates cyclin A1 expression. Immunoblotting analysis of K562 cells transfected with siRNA of acinus or SRPK2. *B,* depletion of SRPK2 or acinus strongly decreases K562 cell proliferation. K562 cells were treated with a control RNAi, acinus RNAi, SRPK2 RNAi, or Akt1 RNAi. The cells were stained with crystal violet 3 d after siRNA treatment. *C,* depletion of SRPK2 or acinus decreased BrdUrd incorporation. K562 cells treated with a control RNAi, acinus RNAi, SRPK2 RNAi, or Akt1 RNAi. The cells were labeled with BrdUrd and stained 1 d after RNAi treatment. *D,* inactivation of acinus or SRPK2 induces G_1 arrest in K562 cells. K562 cells were treated with various siRNA, and the cell cycle profiles were analyzed with FACS.

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acinus and SRPK2 are key players in the cell cycle and are essential for leukemia cell proliferation.

Expression of acinus and SRPK2 in leukemic cell lines and in leukemic bone marrows from patients with acute myeloid leukemia. To assess whether SRPK2 and acinus play any pathophysiologic role in primary patient leukemia, we monitored their expression levels in leukemia cell lines and leukemic bone marrows by immunoblotting analysis. Expression of acinus and SRPK2 was examined in a panel of human leukemic cell lines, the majority of which were derived from myeloid lineages. The overall level of acinus expression was relatively high and was comparable in all four myeloid cell lines, but was lower in DG-75 and BJAD lymphoid cell lines. Furthermore, subcellular localization of acinus seemed to be predominantly nuclear in leukemic cells of myeloid and lymphoid lineages. In contrast, the highest level of SRPK2 expression was detected in DG-75 and BJAD lymphoid cells. In myeloid leukemic cells, expression of SRPK2 varied with the highest level has been detected in NB4 and U-937 cells, the moderate level in K562 and HL-60 cells and the lowest level in HEL and KG_1 cells. Interestingly, the subcellular localization of SRPK2 was predominantly cytoplasmic in myeloid leukemic cell lines, but seemed to be both cytoplasmic and nuclear in lymphoid leukemic cells (Supplementary Fig. S3*A*). Next, we examined expression of acinus and SRPK2 in bone marrow samples from patients with acute myeloid leukemia by immunohistochemical analysis. Bone marrow specimens from five healthy donors were used as normal controls. Expression of acinus was detected in all five normal bone marrow samples. A majority of patients (n = 10) displayed elevated level of acinus expression. Similar to what was observed in leukemic cell lines, acinus was predominantly localized to the nucleus of leukemic blasts in leukemic bone marrows. Expression of SRPK2 was undetectable in normal bone marrows. However, in leukemic bone marrows (n = 5), high level of SRPK2 expression was observed. The subcellular localization of SRPK2 was predominantly cytoplasmic (Supplementary Fig. S3*B*). Immunoblotting analysis reveals that both SRPK2 and acinus were strongly overexpressed in some of the primary AML patients. Acinus S422 phosphorylation status tightly couples to SRPK2 expression pattern, further supporting that SRPK2 is the physiologic kinase for acinus. As predicted, cyclin A1 was selectively overexpressed in AML samples when acinus was highly phosphorylated; underscoring that acinus is essential for cyclin A1 expression (Supplementary Fig. S3*C*). Taken together, our findings show that SRPK2 phosphorylates acinus and regulates its stimulatory effects on cyclin A1 expression, contributing to leukemia cell proliferation.

Discussion

In the present study, we have uncovered a novel molecular mechanism by which the SR splicing factor acinus mediates cyclin A1 expression in human leukemia cells. This event is regulated by SRPK2, which directly binds and phosphorylates acinus on S422. Phosphorylation of acinus by SRPK2 up-regulates its stimulatory effect on cyclin A1. Moreover, ablation of SRPK2 or acinus arrest cell cycle at G_1 phase, resulting in cell proliferation decrease, whereas overexpression of acinus or SRPK2 substantially increases G_2-M phase. Furthermore, we show that acinus is highly expressed and phosphorylated in human patients with myeloid hematologic malignancies. Thus, this finding provides a molecular mechanism of how cyclin A1 is regulated in leukemia cells. SRPK was initially identified as a cell cycle–regulated protein kinase. SR proteins are phosphorylated strongest in M phase, followed by G_2 phase, and the activity fades away in S and G_1 phases (2). Here, we present compelling evidence demonstrating that SRPK2 specifically regulates cyclin A1 but not other cyclins or CDKs expression in human leukemia cell lines. Our data support that SRPK2 through phosphorylating acinus plays an essential role in cell cycle progression. Surprisingly, acinus is not uniformly expressed during the cell cycle, and acinus is actively cleaved and distinctive fragmentation activity occurs in different cell phases (Fig. 5). Cyclin A1 expression pattern tightly correlates with acinus S422 phosphorylation status; by contrast, cyclin A2 is relatively stable, supporting that SRPK2 phosphorylating acinus plays a key role in selectively mediating cyclin A1 expression.

RXRXXS/T is a consensus Akt phosphorylation element present in numerous Akt substrates. Our previous study shows that Akt phosphorylates acinus on both S422 and S573 residues, which reside in 417-423 RSRSRSR and 568-574 RSRSRST motifs. Interestingly, both residues fall into the RS dipeptide repeat domain, which is found in numerous SR splicing factors. Here, we show that SRPK2 selectively phosphorylates S422 but not S573 in acinus. SR proteins are all subjected to extensive phosphorylation on serine residues within their RS domain, and the phosphorylation status affects protein-protein interactions (33, 34) and regulates protein activity. Akt is a potent SR protein kinase, as there are several consensus motifs for Akt phosphorylation in the RS domain of SR proteins. For instance, both SF2/ASF and 9G8 are phosphorylated by Akt in a PI3K-dependent way and regulate fibronectin splicing, providing evidence of how SR protein activity is modified in response to extracellular stimulation (35). Moreover, Akt can also phosphorylate the SR protein Srp40 and modify alternative splicing of PKCβII (36).

A subset of SR proteins shuttles continuously between the nucleus and the cytoplasm, indicating the existence of cytoplasmic activities for shuttling SR proteins (37). However, acinus predominantly resides in the nucleus. Akt translocates into the nucleus, where it phosphorylates acinus (17). SRPK2 mainly occurs in the cytoplasm, but a fraction of it also distributes in the nucleus. Presumably, nuclear SRPK2 is involved with phosphorylating nuclear acinus. Overexpression of SRPKs disassemble nuclear speckles and redistribute SR proteins (2, 29). Here, we show that overexpression of SRPK2 but not Akt triggers redistribution of acinus in the nucleus. In addition, SRPK2 kinase activity is required for this action (Fig. 3), suggesting that acinus phosphorylation by SRPK2 is essential for this process. Although both SRPK2 and Akt can phosphorylate S422 on acinus, they elicit different outcomes in acinus localization, indicating that these two kinases have distinctive effects in SR protein redistribution. In agreement with this finding, Akt and SR protein kinases Clk and SRPK2 reveal opposite effects on alternative splicing (35). Although both Akt and SRPK2 can boost acinus activity on cyclin A1, SRPK2 displays a much more potent effect than Akt. Furthermore, ablation of SRPK2 substantially blocks acinus activity even in the presence of active Akt, underscoring the observation that SRPK2 is absolutely required for acinus stimulatory effect on cyclin A1 expression (Figs. 4 and 5). Acinus binds both SRPK2 and Akt through its RS domain containing COOH terminus (Fig. 1). Presumably, growth factors trigger Akt nuclear translocation and elicit the tertiary complex formation in the nucleus. S422 phosphorylation is required for acinus to bind Akt (17). It also affects acinus binding affinity to SRPK2 (Fig. 3*D*). Interestingly, we found that depletion of Akt weaken the association between acinus and SRPK2, indicating

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that Akt might somehow mediate SRPK2 binding to acinus. Moreover, depletion of either SRPK2 or Akt abolishes acinus phosphorylation in HEK293 cells (Fig. 2*D*). It is tempting to speculate that Akt phosphorylates SRPK2 and provokes its interaction with S422 phosphorylated acinus. Clearly, further work is necessary to explore this hypothesis.

Human SRPK1 is highly enriched in pancreas, whereas SRPK2 is abundantly expressed in brain, although both are coexpressed in other human tissues (3). Interestingly, both SRPK1 and SRPK2 are highly expressed in testis (29). SRPK1 selectively phosphorylates human protamine 1 in testis, which might implicate in sperm chromatin condensation and repress transcriptional activity (38). On the other hand, human cyclin A1 is also highly expressed in testis and faintly expressed in brain among all of the normal tissues (22). Cyclin A1 deficiency results in spermatocyte arrest before first meiotic division. Thus, cyclin A1 is essential for passage of spermatocytes into meiosis I (32). Here, we provide compelling evidence supporting that SRPK2 regulates cyclin A1 expression in human leukemia cells by phosphorylating acinus. Ablation of acinus or SRPK2 reduces cyclin A1 but not A2 expression level and attenuates cell proliferation (Figs. 4 and 5). This finding is consistent with the previous report that the cyclin A1–deficient spermatocyte meiotic arrest is accompanied by a block in the activation of MPF kinase, a Cdk1/cyclin B complex critical for G_2-M transition in the meiotic cell cycle (39). Conceivably, SRPK2 might also play some role in spermatogenesis. Presumably, SRPK2/acinus/cyclin A1 signaling cascade might apply in testis as well.

Interestingly, cyclin A1 is greatly expressed in a subset of primary leukemia samples. The highest frequency of cyclin A1 overexpression occurs in acute myelocytic leukemias. Cyclin A1 expression was also detected in normal CD34(+) progenitor cells (23, 24). We show that acinus phosphorylation is sufficient and necessary for cyclin A1 expression (Figs. 4 and 5). Moreover, depletion of acinus attenuates human leukemia cell proliferation, whereas overexpression of acinus enhances cell division (Fig. 6). Furthermore, we show that acinus is overexpressed and robustly phosphorylated in a panel of human patients with hematologic malignancies (Supplementary Fig. S3). These data further support that acinus plays a critical role in leukemia progression. It remains unknown exactly how acinus regulates cyclin A1 promoter activity. We cannot rule out the possibility that it binds other transcription factors, including c-MycB, and coordinately regulates cyclin A1 transcription. SF2/ASF has been shown to control the cytoplasmic mRNA stability of a specific mRNA (40). Acinus contains an RRM motif, implicated in binding RNA. It is plausible that acinus might also stabilize cyclin A1 mRNA and enhance its translation as well. Collectively, our finding that SRPK2 phosphorylating acinus and elevating its activity on cyclin A1 expression provides insight in the novel function of pre-mRNA machinery in cell cycle and tumorigenesis.

Disclosure of Potential Conflicts of Interest

K. Ye: Emory University School of Medicine employee. The other authors disclosed no potential conflicts of interest.

Acknowledgments

Received 1/3/2008; revised 3/6/2008; accepted 3/19/2008.

Grant support: NIH grant RO1 NS045627 (K. Ye).

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We thank Dr. C. Müller-Tidow (Department of Medicine, Hematology, and Oncology, University of Münster) for cyclin A1–luciferase plasmid and Dr. X. Fu (University of California-San Diego) for SRPK2 constructs.

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