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RNPS1 functions as an oncogenic splicing factor in cervical cancer cells

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Abstract

Numerous recent studies suggest that cancer-specific splicing alteration is a critical contributor to the pathogenesis of cancer. RNA-binding protein with serine-rich domain 1, RNPS1, is an essential regulator of the splicing process. However, the defined role of RNPS1 in tumorigenesis still remains elusive. We report here that the expression of RNPS1 is higher in cervical carcinoma samples from The Cancer Genome Atlas (TCGA-cervical squamous cell carcinoma and endocervical adenocarcinoma) compared to the normal tissues. Consistently, the expression of *RNPS1* was high in cervical cancer cells compared to a normal cell line. This study shows for the first time that RNPS1 promotes cell proliferation and colony-forming ability of cervical cancer cells. Importantly, RNPS1 positively regulates migration-invasion of cervical cancer cells. Intriguingly, depletion of RNPS1 increases the chemosensitivity against the chemotherapeutic drug doxorubicin in cervical cancer cells. Further, we characterized the genome-wide isoform switching stimulated by RNPS1 in cervical cancer cell. Mechanistically, RNA-sequencing analysis showed that RNPS1 regulates the generation of tumor-associated isoforms of key genes, particularly *Rac1b*, *RhoA*, *MDM4*, and *WDR1*, through alternative splicing. RNPS1 regulates the splicing of *Rac1* pre-mRNA via a specific alternative splicing switch and promotes the formation of its tumorigenic splice variant, *Rac1b*. While the transcriptional regulation of RhoA has been well studied, the role of alternative splicing in RhoA upregulation in cancer cells is largely unknown. Here, we have shown that the knockdown of RNPS1 in cervical cancer cells leads to the skipping of exons encoding the RAS domain of RhoA, consequently causing decreased expression of RhoA. Collectively, we conclude that the gain of RNPS1 expression may be associated with tumor progression in cervical carcinoma. RNPS1-mediated alternative splicing favors an active Rac1b/RhoA signaling axis that could contribute to cervical cancer cell invasion and

Abbreviations: APC/C, anaphase-promoting complex/cyclosome; AS, alternative splicing; CASP1, Caspase 1; CASP4, Caspase 4; CTSV, Cathepsin V; Dox, doxorubicin; ECM, extracellular matrix; HDF, human dermal fibroblast; HIV, human immunodeficiency virus; HPV, human papillomavirus; hrHPV, High-risk human papillomavirus; MMP9, matrix metalloproteinases; PAP, Papanicolaou; RNPS1, RNA-binding protein with serine-rich domain 1; TCGA, The Cancer Genome Atlas; TIMP3, tissue inhibitors of matrix metalloproteinases; UCEC, uterine corpus endometrial carcinoma; WDR1, WD-repeat domain 1.

metastasis. Thus, our work unveils a novel role of RNPS1 in the development of cervical cancer.

KEYWORDS
alternative splicing, cervical cancer, EJC, Rac1, RhoA, RNPS1

1 | INTRODUCTION

Cancer develops primarily as a result of sequential genetic changes and genomic instability, causing constitutive expression of oncogenes and repression of tumor-suppressor genes. As a result, cancer cells gain unique capabilities such as increased replicative potential, insensitivity to growth-inhibitory signals, delayed apoptosis, persistent angiogenesis, and tissue invasion.

Gynecological cancers are the second most diagnosed cancer in women, following breast cancer. Cervical cancer is the fourth most frequent cancer globally and the most common malignancy in developing countries.[1] Cervical cancer is linked to a number of risk factors, including sexually transmitted infections such as human papillomavirus (HPV) and human immunodeficiency virus, smoking, alcohol consumption, prolonged use of oral contraceptives, and a family history of cervical cancer.[2] Long-term infection with HPV is the major risk factor for cervical cancer.[3] HPV infections trigger a variety of cellular alterations, including alternative splicing, which leads to malignant transformation. Aberrant splicing events in cancer-related genes also result in chemo- and radioresistance.[4]

Interestingly, a recent systematic analysis by Kahles et al. revealed that alternative splicing events are more common in cancer tissues than normal tissues. Alterations in splicing factor expression levels appear to be the main drivers of abnormal splicing profiles.[5] The key transacting regulatory factors in splicing- SR proteins and the hnRNPs are altered in cervical cancer. As a representative example, the SR protein, SRSF10, controls the splicing of the *IL1RAP* gene and generates the oncogenic splice isoform, MIL1RAP, in cervical cancer. As a result, cancerous cells can avoid macrophage phagocytosis and evade the immune system.[6] The *FAS* receptor gene is another example that undergoes alternative splicing in cancer cells. The FAS ligand secreted by cytotoxic T cells activates the FAS receptor, which results in apoptosis via a death-signaling cascade. *FAS* receptor has three short mRNA variants that lack the transmembrane domain. As a result, the protein isoforms translated from these variants are probably released by the cancer cells as decoy receptors for the FAS ligand, thus aiding cancer cells in avoiding apoptosis.[7,8]

Alternative splicing also plays a critical role in promoting the invasion or migration capability of cancer cells. Cell migration is a highly orchestrated multistage process involving changes in the cytoskeleton, cell-substrate adhesions and remodeling of the extracellular matrix (ECM). The cancer cells acquire a pro-migratory phenotype to invade the stroma, migrate toward blood vessels and enter the bloodstream. Rho GTPases are one of the vital regulators of cytoskeletal dynamics, thereby playing pivotal roles in cell migration event. Most Rho GTPases remain inactive when bound to GDP and get activated by exchanging the bound GDP nucleotide for GTP. The most highly conserved Rho family members across eukaryotic species are Rho, Rac, and Cdc42. Briefly, Rac and Rho regulate the formation of lamellipodia, whereas Cdc42 promotes the development of filopodia and the coordinated action helps the cell in directional migration.[9] Intriguingly, *Rac1*, *RhoA*, and *Cdc42* undergo alternative splicing to alter the expression and/or functions of Rho GTPases. The *Rac1* pre-mRNA produces two splice variants, *Rac1* and *Rac1b*. The splice variant *Rac1b*, produced from the inclusion of an alternative exon 3b, encodes a constitutively active GTPase protein.[10,11] Rac1b is considered a pro-tumorigenic GTPase that promotes cellular transformation.[12] Likewise, alternative splicing of *Cdc42*, resulting in the switching of the *CDC42-v2* variant to the *CDC42-v1* variant, is associated with malignant transformation.[13]

Similar to SR protein, RNPS1 also functions in constitutive and alternative splicing.[14,15] Studies have also confirmed the role of RNPS1 in enhancing the formation of spliceosomal A complex and its presence in spliceosome B and C complexes.[16,17] Interestingly, the transcriptome-wide analysis revealed that the knockdown of RNPS1 in human cell lines leads to the mis-splicing of a large number of mRNAs due to the de-repression of cryptic splice sites. As a result, RNPS1 is a critical effector molecule in promoting splicing fidelity.[18,19] However, the role of RNPS1 in tumorigenesis remains elusive.

In this study, we have checked whether RNPS1 contributes to the development of cervical cancer. Our findings revealed for the first time that RNPS1 plays an important role in regulating key hallmarks of cancer, such as survival, migration and invasion. Interestingly, we found that RNPS1 modulates the alternative

splicing of cancer-specific genes to generate oncogenic isoforms.

2 | MATERIALS AND METHODS

2.1 | Cell culture

HeLa, SiHa, and human dermal fibroblast (HDF) cell lines were maintained in Dulbecco's Modified Eagle's Medium with high glucose (Himedia) supplemented with 10% (v/v) fetal bovine serum (Himedia) and 1% penicillin/streptomycin (Himedia) at 37°C in a humidified atmosphere containing 5% CO_2.

2.1.1 | siRNA transfection

For siRNA transfection, cells were seeded in six-well plate at 50% confluency and reverse-transfected using Lipofectamine RNAi MAX (Life technologies). In brief, 60 pmol siRNA and 3 μl RNAiMAX were each diluted in 100 μl OptiMEM (Life technologies). Both dilutions were mixed, vortexed briefly, incubated for 15 min at RT and added to the cells. Then, 24 hr after transfection, the medium was replaced. siRNA target sequence for RNPS1 are: RNPS1_1 5′-AAGGAAGACCAGTAGGAAA-3′ and RNPS1_2 5′-GGATCGCTCAAAAGATAAA-3′. The luciferase siRNA: 5′-AACGUACGCGGAAUACUUCGATT-3′ served as the negative control.

2.1.2 | RNA isolation and cDNA synthesis

Total cellular RNA was extracted with TRIzol reagent (Invitrogen) and chloroform separation. In order to remove genomic DNA contamination from isolated RNA, total RNA was incubated with DNase I (Promega). cDNA was prepared by reverse transcribing DNase-treated total RNA using the high capacity cDNA reverse transcription kit (Applied Biosystems).

2.1.3 | End-point PCR and quantitative real-time PCR

Then, 1 μl of diluted cDNA (1:3 dilution of the cDNA) was used as template in end-point PCR reactions with 0.15 μM gene-specific primers and EmeraldAmp MAX HS PCR Master Mix (Takara). The PCR products were analyzed on 2% agarose gel. qRT-PCR was performed using PowerUp Sybr green master mix (Invitrogen). DNase I treated cDNA was used as a template for the qPCR. For each reaction, 5 μl of 2X Sybr green master mix was mixed with 0.25 μM of each primer, 1 μl of diluted cDNA (1:3 dilution of the cDNA) and the rest were filled with nuclease-free water. Specific primers were used for quantifying gene expression (Supplementary file) and normalized with β-actin expression. Relative gene expression was calculated using the $\Delta\Delta C_T$ method.

2.2 | Western blotting

Cell lysates were prepared by sonication in ice-cold RIPA buffer. Total protein was estimated using Bradford reagent (Pierce) and resolved on SDS-PAGE. The proteins were transferred to 0.22 μm nitrocellulose membrane (Himedia) and followed by blocking in 5% skimmed milk in TBST for 1 hr at RT. The membrane was incubated with the respective primary antibody (Supplementary file) at 4°C overnight. After washing with TBST, the membrane was incubated with HRP-conjugated secondary antibody anti-rabbit IgG (H + L) (CST, 7074 S) or anti-mouse IgG (H + L) (CST, 7076 S), 1:5,000 diluted in TBST containing 1% skimmed milk for 1 hr at room temperature.

2.3 | Wound healing assay

Control siRNA and RNPS1 knockdown cells were seeded in six-well plate and allowed to grow until confluency and then serum-starved for 12 hr. Confluent monolayers were scratched with a 200 μl pipette tip. Plates were washed with PBS to remove nonadherent cells, and fresh medium containing 2% FBS was added. The wound was photographed at regular intervals at ×5 magnification. The extent of wound closure was quantified using TScratch software. The percentage of wound closure was calculated using the formula $[(A_0 - A_t)/A_0] \times 100$, where A_0 is the wound area at 0 hr and A_t is the area of the wound at t hr.

2.4 | Transwell migration and invasion assay

For transwell migration, 24-well cell culture inserts (Nunc, Thermo Fischer Scientific) were used, and cells were seeded (0.8×10^5 cells per insert) on the top chamber of the inserts with the incomplete media. Cells were allowed to migrate through the insert's membrane to the lower side containing complete media. After 24 hr, inserts were removed, and cells migrated on the bottom

layer of the inserts were fixed with 100% methanol and stained using 0.1% crystal violet. The upper side of the membrane was wiped with cotton swabs to remove non-migrated cells. Images of the migrated cells were taken using a microscope. For quantification, 10% acetic acid solution was used to destain the insert membrane, and absorbance measurements were taken at 595 nm. Invasion assays were performed as described above, but the cells were allowed to migrate through a membrane insert pre-coated with matrigel (Corning). The cells were photographed at ×5 magnification with an inverted microscope (ZEISS Axio Vert A1).

2.5 | Colony formation assay

Control siRNA and RNPS1 knockdown cells were seeded in a six-well plate at a density of 1×10^3 cells/well. The cells were grown for 7 days, and then colonies were fixed with 100% methanol and stained with crystal violet. For quantification, 10% acetic acid solution was used and absorbance measurements were taken at 595 nm.

2.6 | Cell cycle analysis

Cells were washed with PBS and trypsinized. Fixation and permeabilization of the cells were done with ice-cold 70% ethanol at −20°C overnight. The next day, cells were washed with PBS, followed by centrifugation. Cell pellets were treated with RNase solution and then stained with propidium iodide to stain the DNA and analyzed by flow cytometry. The data were analyzed using FCS Express 5 software.

2.7 | Flow cytometric analysis of apoptotic cell death

Control siRNA and RNPS1 knockdown cells were treated with 5 μM of doxorubicin for 48 hr. Dead cells in the media were harvested and adherent cells were trypsinized. Cell pellets were then washed with PBS and resuspended in a binding buffer (Invitrogen). The cells were then stained with Annexin V/PI (Invitrogen) and analyzed by flow cytometry.

2.7.1 | RNA sequencing and computational analysis

Available RNA-Seq datasets of RNPS1 knockdown in HeLa cells were used for RNA-Seq data analysis (E-MTAB-6564). Adaptor contamination was removed from raw reads using cutadapt (version 3.3.0). The reads were aligned against the human genome (version 38, GENCODE release) and Salmon (version 1.9.0) was used to compute estimates for transcript abundance using the -validateMappings parameters. Differential transcript usage was calculated with IsoformSwitchAnalyzeR (version 1.18.0) and the DEXSeq method. Significance thresholds were delta isoform fraction $|dIF| > 0.1$ and adjusted p-value (isoform_switch_q_value) <.05.

2.7.2 | Statistical analysis

All the quantitative data are represented as mean ± *SD*. Agarose gel images and chemiluminescence images were quantified by ImageJ software. In all the statistical tests, $p < .05$ was considered significant. Statistical significances were calculated with the Student's t test using GraphPad PRISM.

3 | RESULTS

3.1 | Effect of RNPS1 knockdown on proliferation and clonogenic potential of cervical cancer cells

To determine whether RNPS1 has a role in cervical cancer tumorigenesis and progression, we first analyzed the expression of RNPS1 in TCGA samples using the OncoDB web resource. The data revealed that RNPS1 is overexpressed in cervical squamous cell carcinoma and endocervical adenocarcinoma (TCGA) samples compared to normal tissue (Figure 1a). We then investigated the expression of RNPS1 in HeLa, SiHa, and HDF cell lines. Consistent with the TCGA data, *RNPS1* was highly expressed in cervical cancer cells compared to the normal cell line, HDF (Figure 1b).

Since RNPS1 is significantly upregulated in cervical cancer, we hypothesized that RNPS1 might function as an oncogene. Therefore, to understand the role of RNPS1 in cervical cancer, the expression of RNPS1 was silenced in cervical cancer cell lines, HeLa and SiHa. RNPS1 was knocked down efficiently using two specific siRNAs targeting different regions of *RNPS1* mRNA (Figure 1c). Next, the effect of RNPS1 knockdown on the proliferation of cervical cancer cells was examined and observed a decrease in the cell numbers of HeLa and SiHa cells (Figure 1d). Together, these suggest that RNPS1 is involved in the positive regulation of the proliferation of cervical cancer cells.

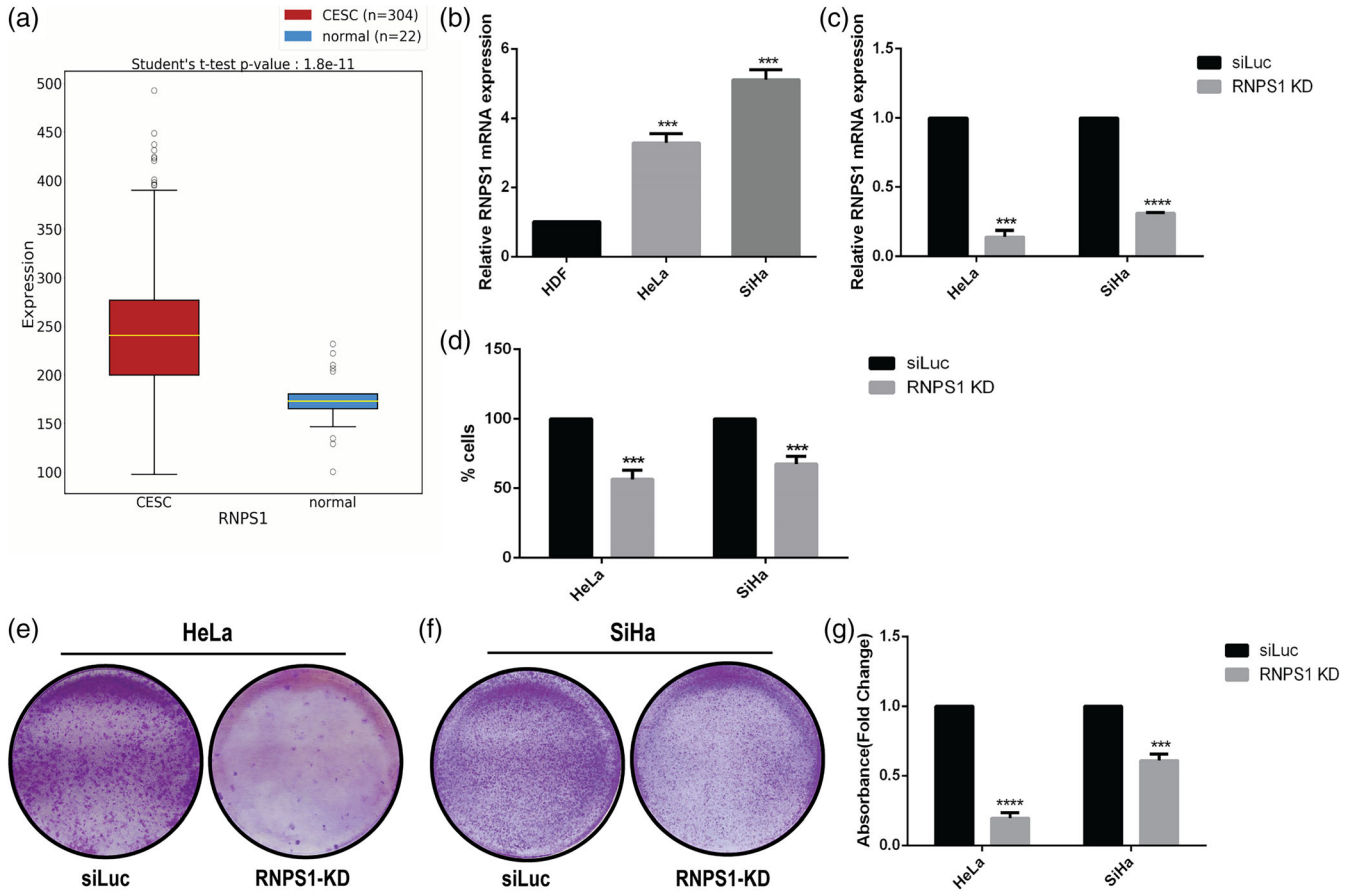


FIGURE 1 Expression levels of *RNPS1* in cervical cancer cell lines and tissues and the effect of its knockdown on cervical cancer cell proliferation and survival. (a) Expression of *RNPS1* in normal versus primary cervical cancer tissue from TCGA database using OncoDB web resource. (b) qRT-PCR shows the relative expression of *RNPS1* in human cervical cancer cell lines (HeLa and SiHa) and normal cell line, human dermal fibroblast (HDF). β-actin was used as a normalization control. (c) The expression level of *RNPS1* mRNA upon knockdown of RNPS1 by siRNA in HeLa and SiHa by qRT-PCR. (d) Estimation of cell proliferation by Trypan blue staining in control and RNPS1 knockdown cervical cancer cells. (e, f) Effect of RNPS1 knockdown on the clonogenic potential of cervical cancer cells using colony formation assay. (g) The graphical representation of estimation of colony forming ability of RNPS1 knockdown cervical cancer cells. The average absorbance was represented as fold change. Absorbance from control cells was normalized to 1, whereas clonogenic potential in RNPS1-depleted cells was measured as a fold change. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $^{***}p \leq .001$, $^{****}p \leq .0001$

The effect of RNPS1 knockdown on the survival of cervical cancer cells was determined using a colony formation assay. This assay basically assesses the ability of a cell to undergo "unlimited" division. The knockdown of RNPS1 decreases the number of colonies compared to the control (Figure 1e–g). This indicates that RNPS1 promotes the clonogenic ability of HeLa and SiHa cells. Thus, RNPS1 provides a survival advantage to cervical cancer cells.

3.2 | Downregulation of RNPS1 alters the cell cycle progression of cervical cancer cells

Next, to gain deeper insight into the observed altered proliferation of RNPS1-KD (knockdown) cells, we evaluated the effect of loss of RNPS1 on cell cycle progression by flow cytometry. The cell cycle analysis showed that HeLa cells were distributed more in G2/M-phase and less in the S phase after the depletion of RNPS1, which suggests that RNPS1-KD HeLa cells were arrested at the G2/M phase (Figure 2a). Similarly, SiHa cells were distributed less in the S-phase after the knockdown of RNPS1 (Figure 2b). Moreover, qPCR analyses of M phase-related genes showed a significant reduction in the mRNA expression of *ANAPC5* and *ANAPC7* (Figure 2c,d). In addition, RNPS1 knockdown altered the expression of essential cell cycle proteins, Survivin, and CDKN2B (Figure 7c–e). Taken together, these results suggest that RNPS1 is a regulator of the cell cycle.

In cancer, the apoptotic pathway is typically inhibited and faulty regulation of apoptosis is an important event

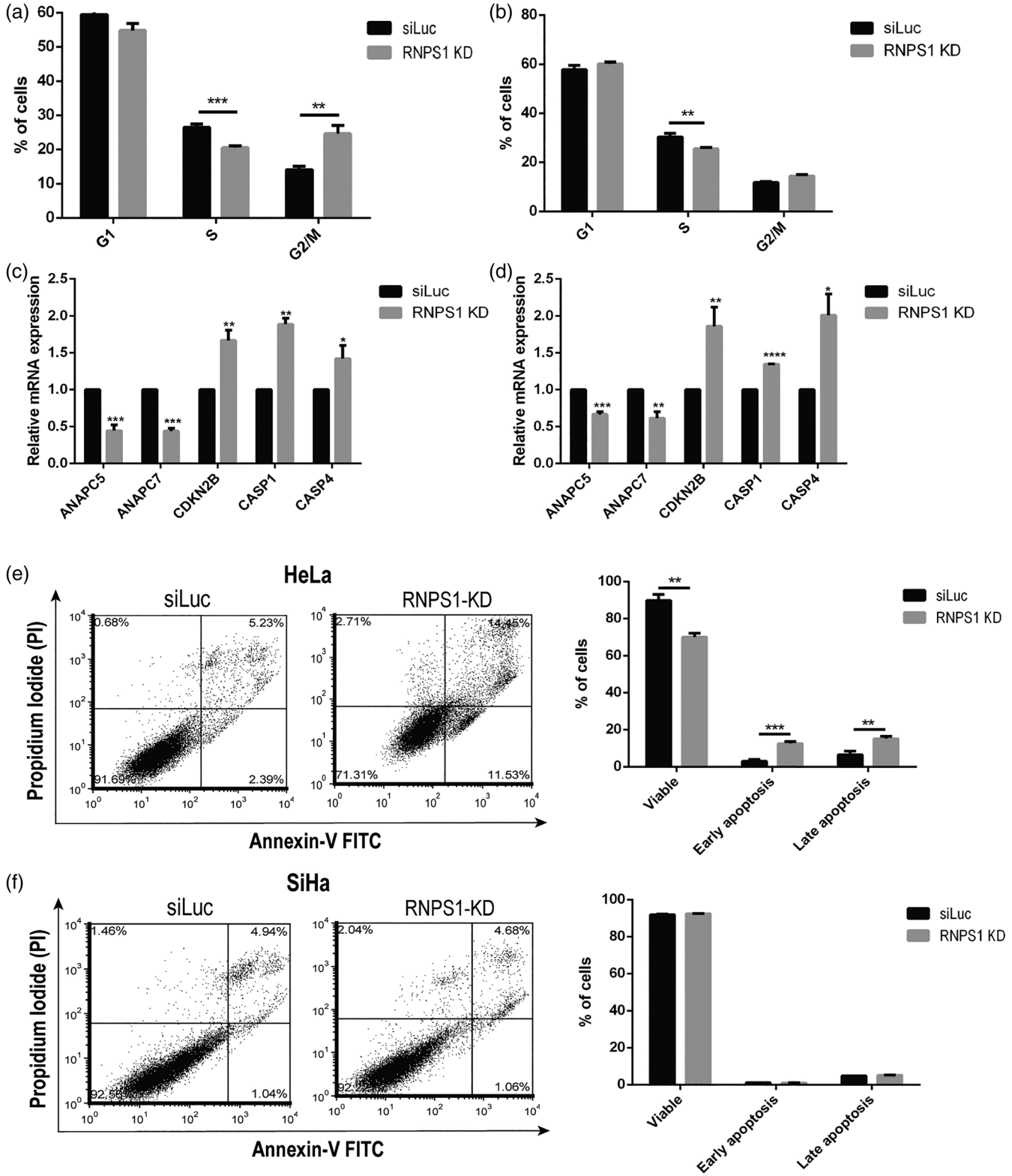


FIGURE 2 Effect of RNPS1 knockdown on cell cycle progression and apoptosis. (a, b) Cell cycle analysis was performed using flow cytometry in (a) HeLa and (b) SiHa cells transfected with siRNA-RNPS1 and its graphical representation in terms of the % distribution of cells. (c, d) qRT-PCR of genes related to cell cycle and caspase family in RNPS1 knockdown (c) HeLa and (d) SiHa cells normalized to β-actin. (e) Apoptosis rate was quantified using flow cytometry in HeLa cells transfected with the indicated siRNA and its graphical representation in terms of % distribution of cells (right panel). (f) Apoptosis rate was quantified using flow cytometry in RNPS1 knockdown SiHa cells. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $*p \leq .05$, $**p \leq .01$, $***p \leq .001$, $****p \leq .0001$

in the development of cancer. Therefore, the effect of RNPS1 depletion on the apoptosis of HeLa and SiHa cells was assessed. Flow cytometry analysis with Annexin V and PI staining revealed that the apoptotic rates of knockdown HeLa cells were higher than those of the control group cells (Figure 2e). In line with previous reports, the data suggest that RNPS1 regulates apoptosis in HeLa cells.[20,21] On the contrary, the depletion of RNPS1 in SiHa cells did not trigger apoptosis (Figure 2f). Hence, RNPS1 probably regulates apoptosis in a cancer cell-specific manner. Nevertheless, the levels of Caspase family members, particularly *CASP1* (Caspase 1) and *CASP4* (Caspase 4) (Figure 2c,d), were increased upon knockdown of RNPS1 in both HeLa and SiHa cells.

3.3 | Silencing of RNPS1 decreases migration and invasion of cervical cancer cells

Migration is a critical event during the malignant transformation of cancer cells. To assess the functional effect of RNPS1 knockdown on the migration of cervical cancer cells, HeLa and SiHa, wound healing and transwell migration assays were performed. In the wound healing assay, we found that migration was inhibited in cervical cancer cells upon RNPS1 knockdown (Figure 3a,b). The migration potential of RNPS1-KD cervical cancer cells was further assessed using serum as a chemoattractant by transwell migration assay. Crystal violet staining of the membrane showed that the number of migrated cells in RNPS1-KD cells was less compared to the control cells (Figure 3c,d). This suggests that RNPS1-KD cells have lower migration potential compared to the control cells.

Besides migration, invasion of neighboring tissues is another essential hallmark of metastatic cancer cells. In this regard, RNPS1 knockdown cells exhibited markedly decreased invasive ability than the negative control cells. The number of cells that invaded the lower part of the matrigel-coated transwell insert was substantially reduced in RNPS1 KD cells compared to the control cells (Figure 3d,e). Hence, our data indicate that RNPS1 facilitates the invasion of cervical cancer cells.

Since RNPS1 influences the metastatic potential of cervical cancer cells, we next investigated the effect of RNPS1 knockdown on the expression of genes related to migration and invasion. Interestingly, we observed a significant decrease in the expression of genes such as *CTSV* (Cathepsin V) mRNA (Figure 3f,g) and N-Cadherin protein (Figure 7c–e) upon knockdown of RNPS1. The knockdown of RNPS1 also decreased the active form of MMP9 (matrix metalloproteinases) in HeLa (Figure 7c–e). MMPs and cathepsins are proteolytic enzymes that degrade the ECM and help the cancer cell migrate and invade secondary sites.[22] However, no change in the mRNA level of *TIMP3* (tissue inhibitors of matrix metalloproteinases) (Figure 3f,g), an inhibitor of MMPs, was detected. These data indicate that RNPS1 facilitates cell migration and invasion in cervical cancer cells. In summary, these results demonstrate that RNPS1 promotes cell proliferation, colony-forming ability, migration and invasion potential, thereby supporting the notion that RNPS1 exerts an oncogenic role in human cervical cancer cells.

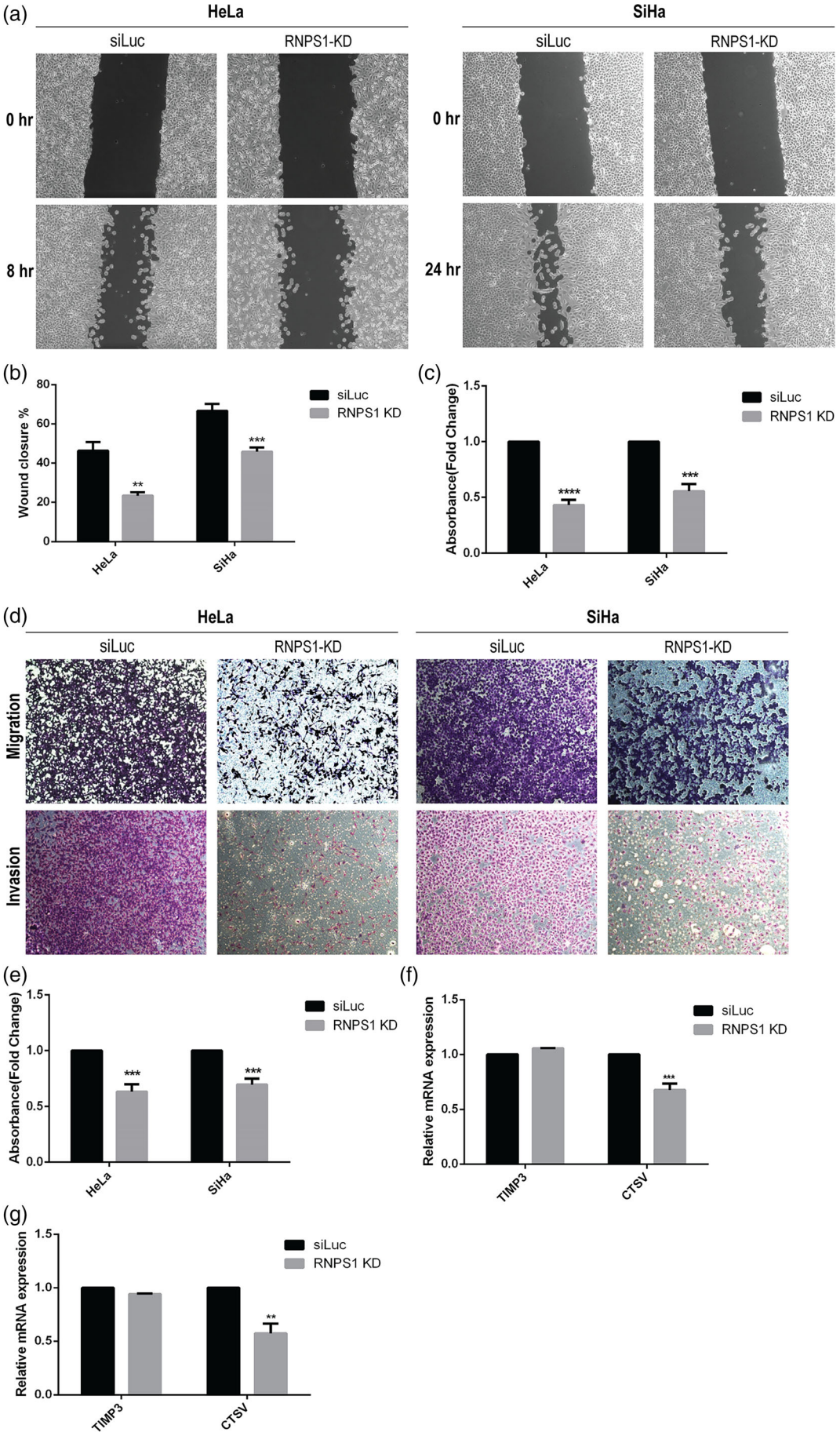
3.4 | Silencing of RNPS1 enhances chemosensitivity against the drug doxorubicin

Growing evidence suggests that drug resistance is one of the main obstacles to the successful treatment of cancer. In this regard, we determined the role of RNPS1 in the chemoresistance of cervical cancer cells. RNPS1 knockdown HeLa and SiHa cells were treated with doxorubicin (Dox). Dox is an anthracycline and a widely used anticancer drug in chemotherapy to treat various types of cancer. Notably, Dox treatment dramatically reduced the viability and concomitantly increased the apoptosis of RNPS1-KD cervical cancer cells compared to the control cells (Figure 4a,b). Taken together, the data suggest that depletion of RNPS1 enhances the chemosensitivity against the drug doxorubicin in cervical cancer cells.

3.4.1 | RNPS1 regulates the generation of cancer-specific splice isoforms

RNPS1 is known to regulate alternative splicing in *D. melanogaster* and human cells. In this regard, overexpression of RNPS1 in cervical cancer cells might deregulate splicing and probably leads to differential usage of transcript isoforms (isoform switching) of the same gene. To investigate the role of RNPS1 in modulating the splicing events of cancer-specific splice isoforms, the existing RNA-Seq dataset of RNPS1-KD in HeLa cells was analyzed. Differential transcript usage analysis was performed using the IsoformSwitchAnalyzeR package. The result revealed 219 isoform switching after RNPS1 knockdown (Supplementary Table S1).

Intriguingly, RNA-Seq analysis revealed that the knockdown of RNPS1 leads to the downregulation of the tumor-associated isoform of *Rac1*, *Rac1b* (Figure 5b). Rac1b is a hyperactive variant of the small GTPase Rac1.[23] Rac1 is a member of the Rho family of GTPases that acts as a molecular switch during signal



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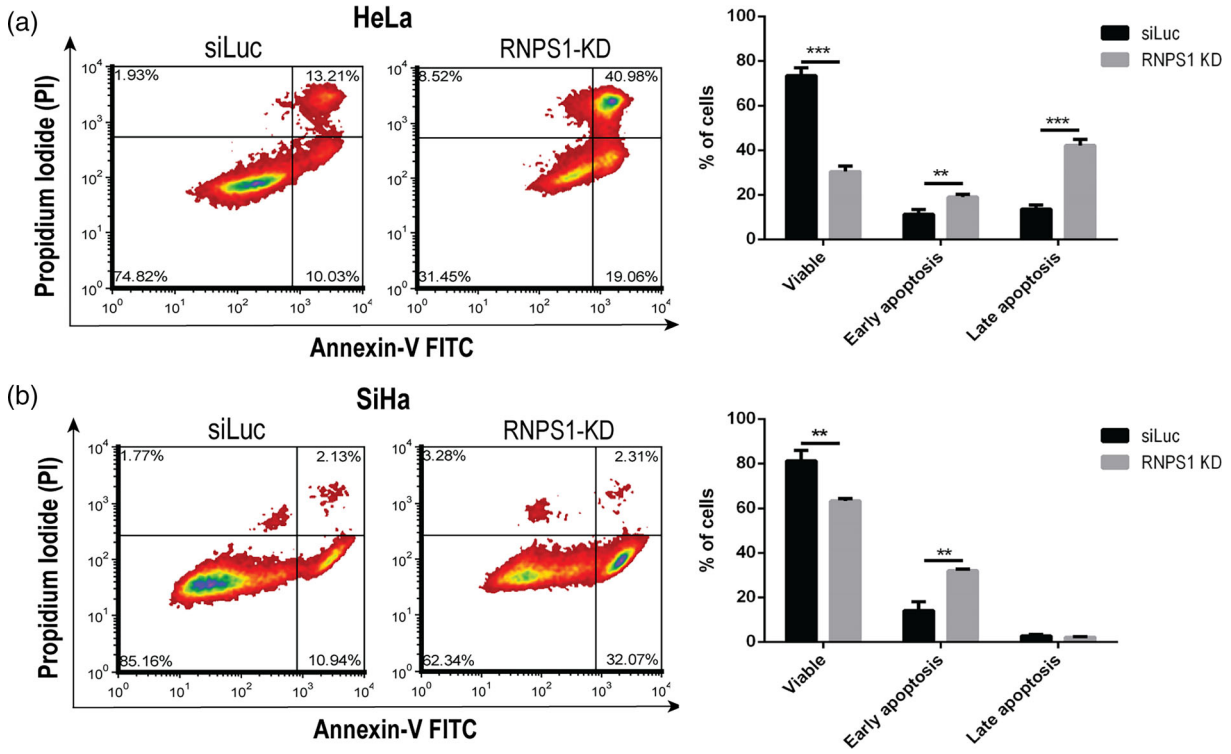


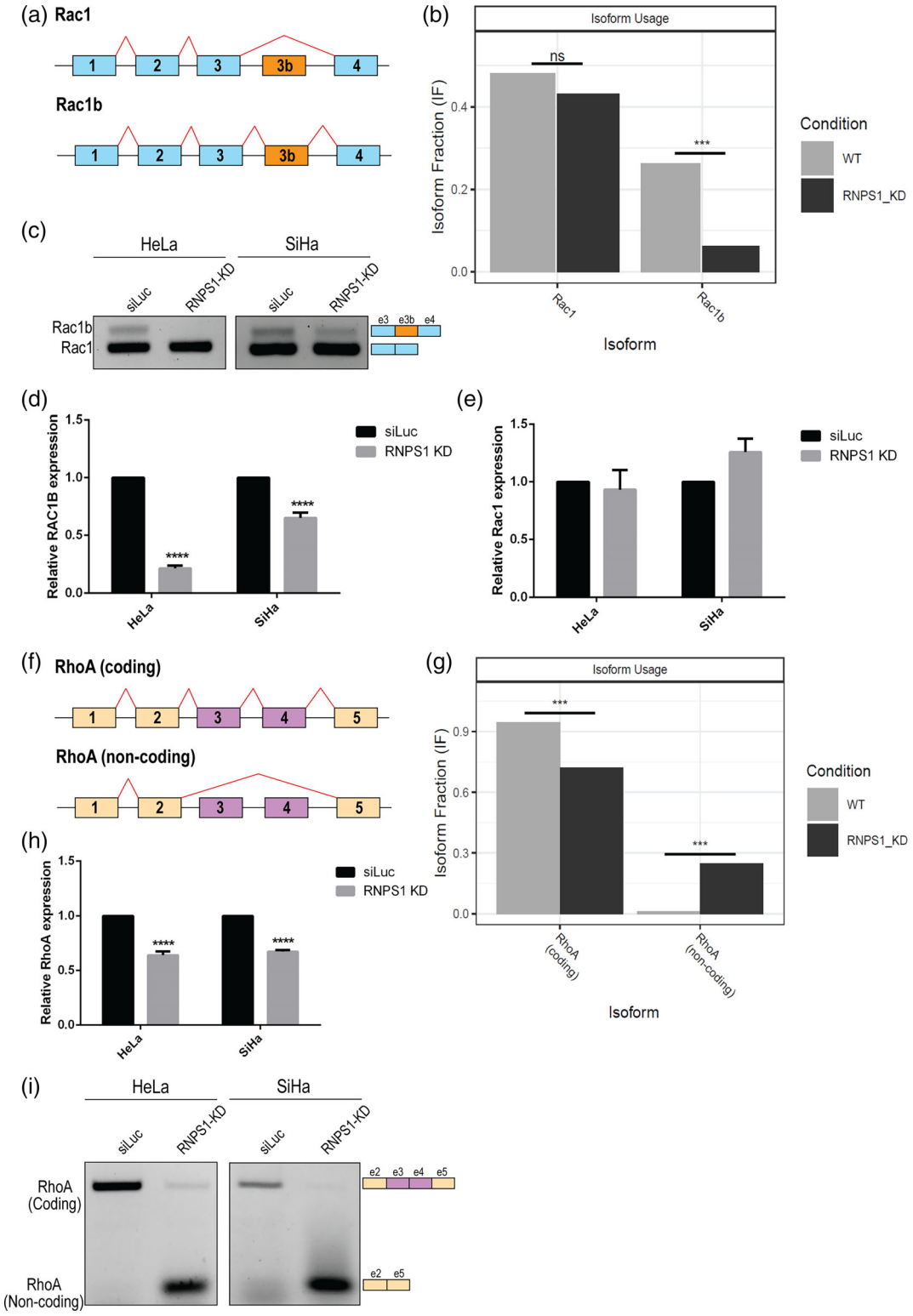
FIGURE 4 Silencing of RNPS1 enhances chemosensitivity against doxorubicin. RNPS1 knockdown cells were treated with 5 μM of doxorubicin for 48 hr. The apoptosis rate was quantified using flow cytometry (left panel) and its graphical representation in terms of % distribution of cells (right panel). (a) HeLa cells and (b) SiHa cells. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $^{**}p \leq .01$, $^{***}p \leq .001$

transduction. Rho GTPases play a central role in regulating cell protrusions, adhesion, and polarization and are, therefore, involved in cell migration processes.[24] Rac1b differs from Rac1 by the inclusion of an additional exon (exon 3b) that results in an impaired GTP-hydrolysis and increased GDP to GTP exchange rates (Figure 5a). In line with RNA-Seq analysis, qPCR and RT-PCR analysis demonstrated that RNPS1 knockdown results in the skipping of exon 3b in *Rac1* mRNA and consequently repressed the expression of *Rac1b* in cervical cancer cells (Figure 5c,d). Consistently, Western blot analysis showed downregulation of Rac1b protein level in RNPS1 depleted cervical cancer cells, HeLa and SiHa (Figure 7c–e). Importantly, RNPS1 knockdown did not alter the expression of *Rac1* mRNA in cervical cancer cells (Figure 5e). Taken together, these suggest that RNPS1 modulates the alternative splicing of *Rac1b*, a cancer-specific splice variant of *Rac1*.

Another essential member of the Rho family of GTPases is RhoA. Remarkably, Isoform-Switch analysis showed that RhoA is also a target of RNPS1 (Figure 5g). RhoA controls the cytoskeletal organization, cell migration, cytokinesis, and cell cycle through interaction with downstream effectors.[24] RhoA controls the contractility of actomyosin, which is necessary to produce the traction forces that pull the cell body in the direction of migration. Depletion of RNPS1 leads to a switch of the *RhoA* splice variant from a coding variant to a noncoding

FIGURE 3 Effect of RNPS1 knockdown on migration and invasion potential of cervical cancer cells. (a) Wound healing assay to determine the migration of HeLa and SiHa cells upon knockdown of RNPS1. (b) Graphical representation of the migration potential of RNPS1 knockdown cervical cancer cells in comparison to control cells. (c) Graphical representation of the migration potential of RNPS1 knockdown cervical cancer cells in comparison to control cells using Boyden chamber assay. The average absorbance was represented as fold change. Absorbance from control cells was normalized to 1, whereas migration in RNPS1-depleted cells was measured as a fold change. (d) Effect of RNPS1 knockdown on migration and invasion of cervical cancer cells using Boyden chamber assay. (e) Graphical representation of the invasive potential of RNPS1 knockdown cervical cancer cells in comparison to control cells. (f, g) qRT-PCR of genes related to migration and invasion in RNPS1 knockdown (f) HeLa and (g) SiHa cells normalized to β-actin. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $^{**}p \leq .01$, $^{***}p \leq .001$, $^{****}p \leq .0001$

variant lacking exons 3 and 4 (Figure 5f). The skipping of exons 3 and 4 leads to a loss of the Ras domain in the noncoding variant of *RhoA*. In accordance with the RNA-Seq analysis, the qPCR and RT-PCR analysis showed that the knockdown of RNPS1 results in the downregulation of the *RhoA* coding isoform (Figure 5h,i). Further, Western blot analysis showed downregulation of RhoA protein level in RNPS1 depleted cervical cancer cells, HeLa



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and SiHa (Figure 7c–e). Together, these findings confirmed that RNPS1 controls the activation of the Rho-GTPase signaling cascade in cervical cancer cells by regulating the alternative splicing of *Rac1b* and *RhoA*.

Furthermore, we explored the isoform switching on MDM4, a negative regulator of p53. We found that RNPS1 knockdown leads to a switch of the *MDM4* splicing isoform from stable full-length *MDM4-FL* (coding) to unstable *MDM4-S* (noncoding) lacking exon 6 (Figure 6a,-b). Next, RT-PCR and qPCR analysis were performed to validate the splicing event of MDM4 mRNA in RNPS1-KD cervical cancer cell lines. Consistent with the RNA-Seq analysis, RT-PCR analysis showed that depletion of RNPS1 leads to the skipping of exon 6 in the *MDM4* mRNA (Figure 6c). Similarly, the qPCR analysis revealed that the knockdown of RNPS1 caused a marked reduction of the stable *MDM4-FL* isoform and upregulation of the unstable *MDM4-S* isoform in HeLa and SiHa cells (Figure 6d). Interestingly, we observed that total *MDM4* mRNA levels remained unaffected in RNPS1-KD cervical cancer cells (Figure 6e), indicating that RNPS1-mediated posttranscriptional event probably regulates MDM4 abundance in cervical cancer cells.

Another essential target of RNPS1 is WDR1 (WD-repeat domain 1), which is required for actin dynamics in processes such as cell migration and cytokinesis.[25] RNA-Seq data showed that depletion of RNPS1 in cervical cancer cells results in the skipping of exons three, four and five in *WDR1* mRNA, yielding a truncated isoform of *WDR1* named *WDRΔ35* (Figure 6f,g). This finding was further validated by qPCR analyses (Figure 6h).

Additionally, RNPS1 mediates isoform switching of *CDKN2C*, *KIFC1*, and *CEP72* transcripts, as evidenced by differential usage of isoforms of the target genes upon RNPS1 knockdown (Supplementary Figure S1). Conclusively, these results imply that RNPS1 regulates the splicing events of cancer-associated genes and enables cervical cancer cells to generate protein isoforms favoring tumorigenesis.

3.4.2 | Knockdown of RNPS1 modulates Notch1 and JNK signaling molecules

Several signaling pathways contribute to the development of cancer. Therefore, we further explored the downstream targets of RNPS1 by analyzing the expression of genes involved in diverse signaling pathways in cancer cells. We found that the knockdown of RNPS1 upregulates the expression of *Notch1* (Figure 7a,b). In line with this, it has been previously reported that downregulation of Notch1 expression is a crucial event in HPV-induced carcinogenesis of aggressive cervical cancers as well as cervical carcinoma cells such as HeLa and SiHa.[26] Thus, RNPS1 negatively modulates the expression of *Notch1* and likely aids in the tumorigenesis of cervical carcinoma.

Aberrant activation of JNK signaling contributes to cervical cancer progression and malignancy. Intriguingly, RNPS1 substantially controls the expression of key genes of the JNK signaling pathway, such as *MAPK8*, *JAK3*, and *FGF2* (Figure 7a,b). Of note, *JAK3* mRNA in SiHa cells fell below the detection limit of our qPCR assay. However, further investigation showed that depletion of RNPS1 has no effect on the expression of downstream transcription factor STAT3 and its phosphorylated form phospho STAT3 (p-STAT3) (Figure 7c–e). Collectively, RNPS1 probably regulates the expression of various signaling molecules and contributes to cervical cancer development.

4 | DISCUSSION

The primary screening methods for patients with cervical cancer are Papanicolaou smear and colposcopy, which assess the morphology of cervical epithelial cells.[27,28] However, these tests occasionally suffer interviewer and intraviewer variability. Similarly, high-risk HPV testing has high sensitivity but low specificity, thereby leading to

FIGURE 5 Silencing of RNPS1 modulates alternative splicing of cancer-specific genes, *Rac1b* and *RhoA*. (a) The schematic representation of *Rac1* and *Rac1b* isoforms showing the alternate exon 3b. (b) Quantification of isoform fraction of *Rac1b* in wild type (Luc siRNA) and RNPS1 knockdown HeLa cells using IsoformSwitchAnalyzeR. Knockdown of RNPS1 leads to downregulation of tumor-associated isoform of *Rac1*, *Rac1b* (ENST00000356142.4). (c) RT-PCR analysis of *Rac1* exon 3b skipping with RNA from cervical cancer cells transfected with the indicated siRNA. (d) The expression level of *Rac1b* mRNA upon knockdown of RNPS1 in HeLa and SiHa was measured by qRT-PCR. (e) The expression level of *Rac1* mRNA upon knockdown of RNPS1 in cervical cancer cells by qRT-PCR. (f) Schematic representation of isoform switching in *RhoA* mRNA showing the alternate exons 3 and 4. (g) Quantification of isoform fraction of *RhoA* using IsoformSwitchAnalyzeR. Knockdown of RNPS1 in HeLa cells leads to downregulation of the coding isoform of *RhoA* (ENST00000418115.6) and upregulation of the noncoding isoform (ENST00000676712.2). (h) The expression level of *RhoA* mRNA upon knockdown of RNPS1 in cervical cancer cells was measured by qRT-PCR. (i) RT-PCR analysis of *RhoA* exons 3 and 4 skipping with RNA from cervical cancer cells transfected with the indicated siRNA. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $^{***}p \leq .001$, $^{****}p \leq .0001$

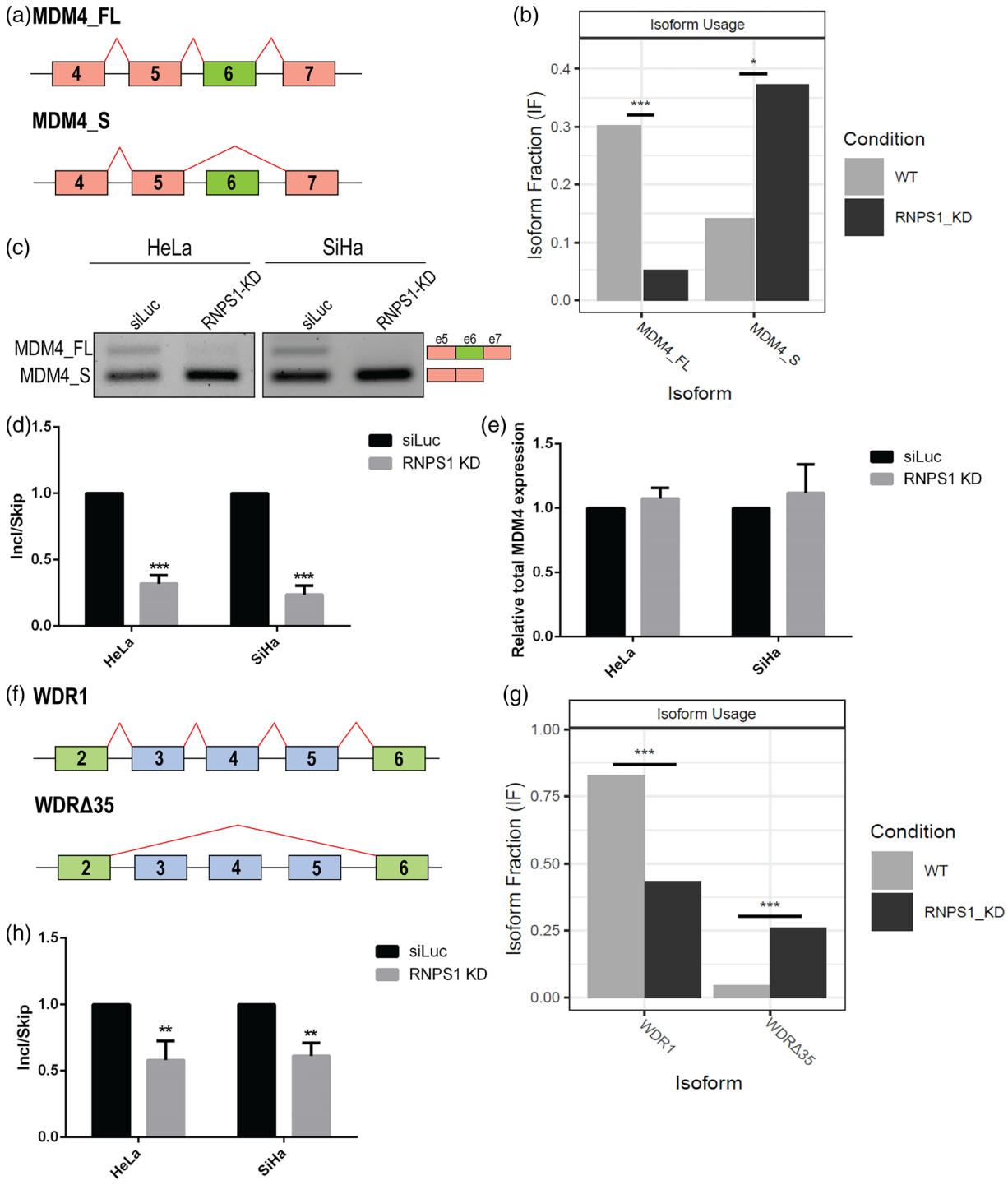

FIGURE 6 Silencing of RNPS1 modulates alternative splicing of cancer-specific genes, *MDM4* and *WDR1*. (a) Schematic representation of *MDM4_FL* and *MDM4_S* isoforms showing the alternate exon 6. (b) Quantification of isoform fraction of *MDM4* in wild type (Luc siRNA) and RNPS1 knockdown HeLa cells using IsoformSwitchAnalyzeR. Knockdown of RNPS1 leads to downregulation of *MDM4_FL* (ENST00000367182.8) and upregulation of *MDM4_S* (ENST00000391947.6). (c) RT-PCR analysis of *MDM4* exon 6 skipping with RNA from HeLa and SiHa cells transfected with the indicated siRNA. (d) A relative inclusion/skipping ratio (Incl/Skip) is plotted indicating that Incl/Skip ratio of *MDM4* decreases when RNPS1 is depleted. (e) The expression level of total *MDM4* mRNA upon knockdown of RNPS1 in cervical cancer cells was measured by qRT-PCR. (f) Schematic representation of *WDR1* isoform switching showing the alternate exons 3, 4, and 5. (g) Quantification of isoform fraction of *WDR1* using IsoformSwitchAnalyzeR. Knockdown of RNPS1 in HeLa cells leads to downregulation of *WDR1* (ENST00000499869.7) and upregulation of *WDRΔ35* (ENST00000502702.5). (h) A relative inclusion/skipping ratio (Incl/Skip) is plotted indicating that Incl/Skip ratio of *WDR1* decreases when RNPS1 is depleted. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $*p \leq .05$, $**p \leq .01$, $***p \leq .001$

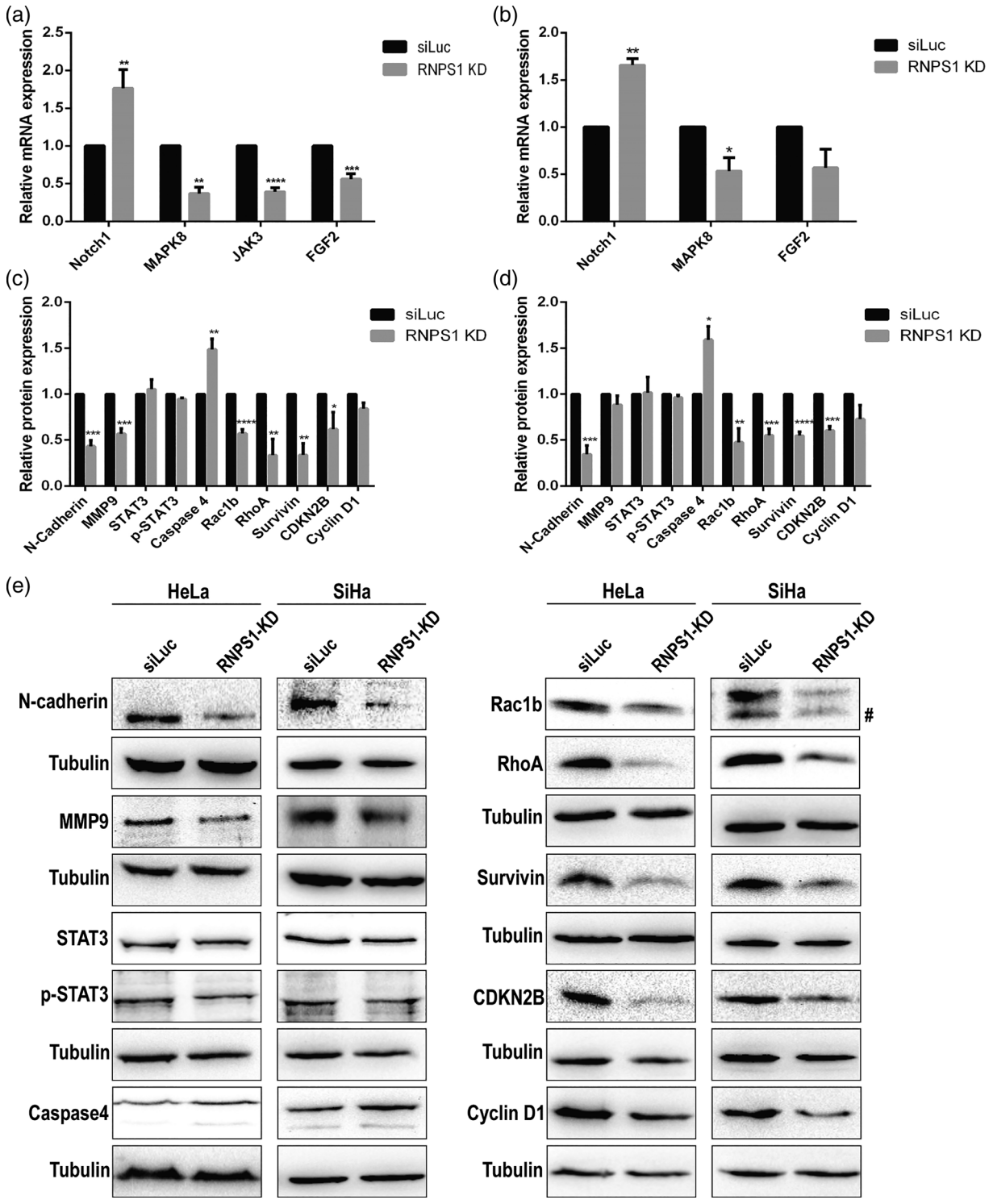

FIGURE 7 Effect of RNPS1 knockdown on various signaling molecules and markers of epithelial–mesenchymal transition (EPT). (a, b) qRT-PCR of genes related to Notch1 and JNK signaling in RNPS1 knockdown (a) HeLa and (b) SiHa cells normalized to β-actin. (c, d) Graph showing fold changes in protein levels of EMT markers and signaling molecules by western blotting with cell lysates from (c) HeLa and (d) SiHa cells transfected with the indicated siRNA. (e) Effect of RNPS1 knockdown on EMT markers and signaling molecules by Western blotting in HeLa and SiHa cells. Tubulin was used as a normalization control. Values are depicted as mean ± *SD* ($n = 3$) and *p*-values are depicted as $^{*}p \leq .05$, $^{**}p \leq .01$, $^{***}p \leq .001$, $^{****}p \leq .0001$. A band corresponding to non-specific protein is labeled with #

many unnecessary medical interventions. Hence, more assays are required to improve the routine screening approach and protein biomarker-based alternative screening is currently under investigation in cervical cancer research.[29] Emerging reports revealed that the expressions of splicing factors are frequently deregulated in

multiple types of cancer. The aberrant expression and/or function of splicing regulators in tumorigenesis have become an important scientific discovery. Therefore, a study on the expression and function of RNPS1 in the development of cervical cancer would comprehend whether RNPS1 can be used as a screening biomarker for cervical cancer.

In the current study, we found that RNPS1 is significantly upregulated in cervical cancer tissues compared to the normal tissue in the TCGA dataset. The goal of this study was to comprehensively characterize the functions of RNPS1 in cervical carcinoma and dissect the mechanisms involved. Functionally, we found that the knockdown of RNPS1 could significantly repress the proliferation and survival of cervical cancer cells. Similar to our findings, RNPS1 was recently reported to promote the proliferation of UCEC (uterine corpus endometrial carcinoma) tumor cells.[30] In addition, our results suggest RNPS1 promotes the clonogenic potential of cervical cancer cells. Further, we found that the downregulation of RNPS1 resulted in G2/M phase cell cycle arrest in HeLa cells. The anaphase-promoting complex/cyclosome (APC/C) is one of the key regulators of cell cycle progression. The APC/C mediates ubiquitin-dependent degradation of cell cycle regulatory proteins to control sister chromatid segregation and cytokinesis, hence crucial for the transition from prophase to telophase in mitosis.[31] Our data suggest that RNPS1 knockdown inhibits the expression of APC/C subunits *ANAPC5* and *ANAPC7*, presumably leading to cell cycle alteration upon depletion of RNPS1.

Furthermore, RNPS1 knockdown suppressed the migration and invasive potential of cervical cancer cells, as evinced by the decrease in the number of cells that migrated or invaded the lower part of the transwell insert. These findings were further supported by the decreased expression of *CTSV* mRNA and N-Cadherin protein upon RNPS1 knockdown. These proteins play a critical role in the proteolytic degradation of ECM, alteration of cell–ECM, and cell–cell interactions, resulting in ECM remodeling. This remodeling is essential during tumor invasion, metastasis, and modulation of the tumor microenvironment.[32] The knockdown of RNPS1 also decreased the expression of *MAPK8*, *JAK3*, and *FGF2*. The JNK pathway is involved in essential cellular processes, including proliferation and survival. This pathway is constitutively expressed in many cancers and results in enhanced proliferation, malignant transformation and drug resistance.[33,34] Accordingly, the knockdown of RNPS1 plausibly modulates JNK signaling pathway, resulting in enhanced chemosensitivity of RNPS1 KD cervical cancer cells.

Importantly, this study has shown for the first time that RNPS1 participates in the regulation of several oncogenic AS (alternative splicing) events. We found that RNPS1 is involved in the regulation of *Rac1* alternative splicing. RNPS1 knockdown caused the skipping of exon 3b in the *Rac1* mRNA, resulting in the downregulation of its active isoform, *Rac1b*. Rac1b exists primarily in the active GTP-bound state, rendering it constitutively active. Rac1b is overexpressed in multiple types of cancer as compared to normal tissues. Several studies documented that overexpression of Rac1b plays a pivotal role in tumor cell survival and malignant transformation.[12,23,35] Our findings indicate that RNPS1 is a critical regulator of the abundance of Rac1b in cervical cancer cells. In line with our study, a previous report has shown that the expression of Rac1b in colorectal cancer cells is based on an alternative splicing event by the SR proteins SRSF1 and SRSF3. Notably, Rac1b also confers chemoresistance in colorectal cancer cells against chemotherapeutic drugs 5-FU and OXA.[36] Hence, it is tempting to speculate that the chemosensitivity of cervical cancer cells in the absence of RNPS1 is also partly due to the depletion of the Rac1b protein.

Additionally, we demonstrate for the first time the role of a splicing factor in driving RhoA expression through an AS-based mechanism. We show that RNPS1 mediates the inclusion of exons 3 and 4 in the *RhoA* transcript giving rise to the coding splice isoform. In contrast, the absence of RNPS1 leads to the skipping of exons 3 and 4, resulting in a noncoding variant and a decrease in RhoA expression. Importantly, RhoA is one of the master regulators of cytoskeletal dynamics.[24] Cytoskeletal dynamic is required for invasive cancer metastasis and migration of cancer cells. Therefore, it is perceivable that RNPS1 promotes migration and invasion through modulation of the Rac1b/RhoA signaling axis.

Evidence suggests that the MDM4 protein is frequently overexpressed in melanoma cells. However, surprisingly, no correlation exists between MDM4 protein levels and total *MDM4* mRNA levels.[37] The *MDM4* gene produces two alternative transcripts, *MDM4-FL* and *MDM4-S*. *MDM4-FL* harbors exon 6 and encodes the full-length MDM4 protein, whereas the skipping of exon 6 causes the insertion of a premature stop codon and the generation of an unstable MDM4-S protein. A major consequence of skipping exon 6 in *MDM4* mRNA is a reduction in full-length MDM4 protein. This implies that the synthesis of MDM4-FL is majorly regulated at the post-transcriptional stage.[38] As a result, it has been suggested that MDM4 overexpression in many cancer cells is mediated by an alternative splicing switch that promotes exon 6 inclusion.[39] Consistent with previous reports, we

demonstrate that RNPS1 influences the inclusion of *MDM4* exon 6, generating the *MDM4-FL* transcript in cervical cancer cells. Of note, depletion of RNPS1 does not change the level of total *MDM4* mRNA. Taken together, these indicate that the alternative splicing of *MDM4* is regulated by multiple splicing factors, including RNPS1.

Furthermore, WDR1 is a highly conserved protein across all eukaryotes and is known to promote actin dynamics in cellular processes, including cytokinesis and cell migration.[25] Interestingly, WDR1 was found to be upregulated in the high metastatic cell line in gallbladder carcinoma compared to the low metastatic cell line.[40] Accordingly, the expression of WDR1 increased in invasive ductal carcinoma and high WDR1 levels correlated with poor survival in breast cancer and lung cancer patients.[41,42] Here, we show that the knockdown of RNPS1 decreased the expression of *WDR1* in cervical cancer cells and triggered an isoform switch from *WDR1* to *WDRΔ35*. We found out that *WDR1* and *WDRΔ35* are products of alternative splicing events mediated by RNPS1. *WDRΔ35* is a recently discovered truncated isoform of human *WDR1*. Nevertheless, the functional role of WDRΔ35 remains poorly characterized, which warrants future investigation.

In conclusion, our study uncovers an unknown functional role of RNPS1 in cervical cancer progression. The results suggest that RNPS1 promotes cell survival, invasion and migration of cervical cancer cells plausibly by activating the Rac1b/RhoA signaling axis. Our findings indicate that RNPS1 may function as an oncogene in cervical carcinoma and shed new light on the RNPS1-mediated RNA splicing mechanism that is harnessed by the cervical cancer cells to promote its progression. It would be interesting in future to evaluate the interplay between splicing factors during malignant transformation. Taken together, this study showed for the first time that RNPS1 might play a crucial role in the development of cervical cancer and therefore, targeting splicing factors could provide a new promising approach in therapeutic intervention for cervical cancer. However, to fully establish the clinical significance of these findings, further in-depth in vivo and clinical research are required.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: A worldwide analysis. Lancet Glob Health. 2020;8(2):e191–e203.
2. Johnson CA, James D, Marzan A, Armaos M. Cervical cancer: An overview of pathophysiology and management. Semin Oncol Nurs. 2019;35(2):166–174.
3. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. The Lancet. 2019;393(10167):169–182.
4. Francies FZ, Bassa S, Chatziioannou A, Kaufmann AM, Dlamini Z. Splicing genomics events in cervical cancer: Insights for phenotypic stratification and biomarker potency. Genes. 2021;12(2):130.
5. Kahles A, Lehmann K-V, Toussaint NC, et al. Comprehensive analysis of alternative splicing across tumors from 8,705 patients. Cancer Cell. 2018;34(2):211–224.e216.
6. Liu F, Dai M, Xu Q, et al. SRSF10-mediated IL1RAP alternative splicing regulates cervical cancer oncogenesis via mIL1RAP-NF-κB-CD47 axis. Oncogene. 2018;37(18):2394–2409.
7. Cascino I, Fiucci G, Papoff G, Ruberti G. Three functional soluble forms of the human apoptosis-inducing Fas molecule are produced by alternative splicing. J Immunol. 1995;154(6):2706.
8. Cheng J, Zhou T, Liu C, et al. Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science. 1994;263(5154):1759–1762.
9. Lawson CD, Ridley AJ. Rho GTPase signaling complexes in cell migration and invasion. J Cell Biol. 2017;217(2):447–457.
10. Schnelzer A, Prechtel D, Knaus U, et al. Rac1 in human breast cancer: Overexpression, mutation analysis, and characterization of a new isoform, Rac1b. Oncogene. 2000;19(26):3013–3020.
11. Fiegen D, Haeusler L-C, Blumenstein L, et al. Alternative splicing of Rac1 generates Rac1b, a self-activating GTPase. J Biol Chem. 2004;279(6):4743–4749.
12. Singh A, Karnoub AE, Palmby TR, Lengyel E, Sondek J, Der CJ. Rac1b, a tumor associated, constitutively active Rac1 splice variant, promotes cellular transformation. Oncogene. 2004;23(58):9369–9380.
13. He X, Yuan C, Yang J. Regulation and functional significance of CDC42 alternative splicing in ovarian cancer. Oncotarget. 2015;6(30):29651–29663. https://doi.org/10.18632/oncotarget.4865.
14. Mayeda A, Badolato J, Kobayashi R, Zhang MQ, Gardiner EM, Krainer AR. Purification and characterization of human RNPS1: A general activator of pre-mRNA splicing. EMBO J. 1999;18(16):4560–4570.
15. Fukumura K, Wakabayashi S, Kataoka N, et al. The exon junction complex controls the efficient and faithful splicing of a

subset of transcripts involved in mitotic cell-cycle progression. Int J Mol Sci. 2016;17(8):1153.
16. Bessonov S, Anokhina M, Will CL, Urlaub H, Luhrmann R. Isolation of an active step I spliceosome and composition of its RNP core. Nature. 2008;452(7189):846–850.
17. Trembley JH, Tatsumi S, Sakashita E, et al. Activation of pre-mRNA splicing by human RNPS1 is regulated by CK2 phosphorylation. Mol Cell Biol. 2005;25(4):1446–1457.
18. Boehm V, Britto-Borges T, Steckelberg A-L, et al. Exon junction complexes suppress spurious splice sites to safeguard transcriptome integrity. Mol Cell. 2018;72(3):482–495.e487.
19. Fukumura K, Inoue K, Mayeda A. Splicing activator RNPS1 suppresses errors in pre-mRNA splicing: A key factor for mRNA quality control. Biochem Biophys Res Commun. 2018; 496(3):921–926.
20. Michelle L, Cloutier A, Toutant J, et al. Proteins associated with the exon junction complex also control the alternative splicing of apoptotic regulators. Mol Cell Biol. 2012;32(5): 954–967.
21. Schwerk C, Prasad J, Degenhardt K, et al. ASAP, a novel protein complex involved in RNA processing and apoptosis. Mol Cell Biol. 2003;23(8):2981–2990.
22. Piperigkou Z, Kyriakopoulou K, Koutsakis C, Mastronikolis S, Karamanos NK. Key matrix remodeling enzymes: Functions and targeting in cancer. Cancer. 2021;13(6):1441.
23. Melzer C, Hass R, Lehnert H, Ungefroren H. RAC1B: A rho GTPase with versatile functions in malignant transformation and tumor progression. Cell. 2019;8(1):21.
24. Ridley AJ. Rho GTPases and cell migration. J Cell Sci. 2001; 114(15):2713–2722.
25. Ono S. Regulation of actin filament dynamics by actin depolymerizing factor/cofilin and actin-interacting protein 1: New blades for twisted filaments. Biochemistry. 2003;42(46):13363–13370.
26. Talora C, Sgroi DC, Crum CP, Dotto GP. Specific down-modulation of Notch1 signaling in cervical cancer cells is required for sustained HPV-E6/E7 expression and late steps of malignant transformation. Genes Dev. 2002;16(17):2252–2263.
27. Rokade A, Kshirsagar NS, Laddad MM. PAP smear versus colposcopy in symptomatic women and women with suspicious-looking cervix. J Nat Sc Biol Med. 2021;12:145–148.
28. Sharif YH. Clinical correlation of cervical cancer screening using Pap smear test. J Popul Ther Clin Pharmacol. 2022;29(1): e1–e8.
29. Güzel C, van Sten-Van't Hoff J, de Kok IMCM, et al. Molecular markers for cervical cancer screening. Expert Rev Proteomics. 2021;18(8):675–691.
30. Liu X, Ma H, Ma L, Li K, Kang Y. RNA-binding protein with serine-rich domain 1 regulates microsatellite instability of uterine corpus endometrial adenocarcinoma. Clinics. 2021;76:e3318.
31. Alfieri C, Zhang S, Barford D. Visualizing the complex functions and mechanisms of the anaphase promoting complex/cyclosome (APC/C). Open Biol. 2017;7(11):170204.
32. Quintero-Fabian S, Arreola R, Becerril-Villanueva E, et al. Role of matrix metalloproteinases in angiogenesis and cancer. Front Oncol. 2019;9:1370.
33. Morgan EL, Scarth JA, Patterson MR, et al. E6-mediated activation of JNK drives EGFR signalling to promote proliferation and viral oncoprotein expression in cervical cancer. Cell Death Differ. 2021;28(5):1669–1687.
34. Hu L, Zou F, Grandis JR, Johnson DE. The JNK pathway in drug resistance. Targeting cell survival pathways to enhance response to chemotherapy. Volume 3. Cambridge, Massachusetts: Academic Press, 2019; p. 87–100.
35. Stallings-Mann ML, Waldmann J, Zhang Y, et al. Matrix metalloproteinase induction of Rac1b, a key effector of lung cancer progression. Sci Transl Med. 2012;4(142):142ra195.
36. Goka ET, Lippman ME, Cho CH, Hu T. Rac1b: An emerging therapeutic target for chemoresistance in colorectal cancer. Drug resistance in colorectal cancer: Molecular mechanisms and therapeutic strategies. Volume 8. Cambridge, Massachusetts: Academic Press, 2020; p. 153–171.
37. Gembarska A, Luciani F, Fedele C, et al. MDM4 is a key therapeutic target in cutaneous melanoma. Nat Med. 2012;18(8): 1239–1247.
38. Bardot B, Toledo F. Targeting MDM4 splicing in cancers. Genes. 2017;8(2):82.
39. Dewaele M, Tabaglio T, Willekens K, et al. Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth. J Clin Invest. 2016;126(1):68–84.
40. Wang J-W, Peng S-Y, Li J-T, et al. Identification of metastasis-associated proteins involved in gallbladder carcinoma metastasis by proteomic analysis and functional exploration of chloride intracellular channel 1. Cancer Lett. 2009;281(1):71–81.
41. Lee JH, Kim JE, Kim BG, Han HH, Kang S, Cho NH. STAT3-induced WDR1 overexpression promotes breast cancer cell migration. Cell Signal. 2016;28(11):1753–1760.
42. Yuan B, Zhang R, Hu J, et al. WDR1 promotes cell growth and migration and contributes to malignant phenotypes of non-small cell lung cancer through ADF/cofilin-mediated actin dynamics. Int J Biol Sci. 2018;14(9):1067–1080.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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