SHQ1 regulation of RNA splicing is required for T-lymphoblastic leukemia cell survival

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T-acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy with complicated heterogeneity. Although expression profiling reveals common elevated genes in distinct T-ALL subtypes, little is known about their functional role(s) and regulatory mechanism(s). We here show that SHQ1, an H/ACA snoRNP assembly factor involved in snRNA pseudouridylation, is highly expressed in T-ALL. Mechanistically, oncogenic NOTCH1 directly binds to the SHQ1 promoter and activates its transcription. SHQ1 depletion induces T-ALL cell death in vitro and prolongs animal survival in murine T-ALL models. RNA-Seq reveals that SHQ1 depletion impairs widespread RNA splicing, and MYC is one of the most prominently downregulated genes due to inefficient splicing. MYC overexpression significantly rescues T-ALL cell death resulted from SHQ1 inactivation. We herein report a mechanism of NOTCH1–SHQ1–MYC axis in T-cell leukemogenesis. These findings not only shed light on the role of SHQ1 in RNA splicing and tumorigenesis, but also provide additional insight into MYC regulation.
-cell acute lymphoblastic leukemia (T-ALL) is a lethal and aggressive hematological malignancy that frequently affects children and adolescents, and accounts for approximately 10–15% of newly diagnosed pediatric ALL. Although clinical complete remission is approaching 90% due to the implementation of intensive chemotherapy protocols, the outcomes of patients with relapsed or refractory T-ALL remain poor, with cure rates of less than 40%. This clinical challenge has fueled considerable research into the molecular understanding of T-ALL pathogenesis which has yielded immense progress in the past decade. Gene expression profiling of T-ALL cases has led to the identification of subgroups of T-ALL, each characterized by aberrant expression of one particular transcription factor such as TAL1, TLX1, and LM01/2. Genome-wide sequencing has identified numerous somatic gene mutations in T-ALL, in which NOTCH1 gain-of-function mutations are found in >50% of T-ALL cases and FBW7, the gene encoding the NOTCH1 E3 ligase, is mutated with impaired activity at the rate of 12%. These findings have vaulted the dysregulated NOTCH1 signaling to the center of T-ALL pathogenesis.

Activation of NOTCH1 signaling initiates with association to ligands of the Delta/Serrate/Lag-2 (DSL) family on neighboring cells. This interaction elicits a series of proteolytic cleavage terminated by γ-secretase. As a result, intracellular NOTCH1 (ICN1) is released from the cell membrane and translocates into the nucleus, activating downstream responder genes by forming a transcriptional activation complex. Persistent activation of NOTCH1 signaling, due to NOTCH1 gain-of-function and/or FBW7 loss-of-function mutations, triggers overexpression of multiple oncogenes in T-ALL. MYC has been demonstrated as a major downstream target of NOTCH1 which plays an essential role in T-cell leukemogenesis.

Pseudouridine (Ψ), a C5-glycoside isomer of uridine, is the most abundant posttranscriptional modification in cellular RNAs. Pseudouridines in ribosomal RNA (rRNA) and small nuclear (snRNA) are essential for the correct function of the ribosome and spliceosome. In higher eukaryotes, pseudouridylation is mainly governed by a family of box H/ACA snoRNPs (small nucleolar ribonucleoproteins), consisting of a unique box H/ACA snoRNA and four common core proteins (Cbf5/NAP57/Dyskerin, Nhp2/L7Ae, Nop10, and Gar1). The RNA component serves as a guide that base pairs with the substrate RNA and directs the enzyme Cbf5 to carry out the pseudouridylation reaction at a specific site. During the assembly process of H/ACA snoRNPs, SHQ1 functions as an assembly chaperone that protects the Cbf5 protein complexes from non-specific RNA binding and aggregation before its assembly with H/ACA snoRNA. As such, abrogation of SHQ1 activity results in assembly failure and loss of H/ACA snoRNP function. Despite well-documented mechanism of SHQ1 in H/ACA snoRNP biogenesis, little is known about its precise functional role, especially in human diseases such as cancer.

We herein define a vital role of SHQ1 in supporting T-cell leukemogenesis. Sustained SHQ1 expression, induced by oncogenic NOTCH1, is essential for T-ALL cell growth in vitro and leukemogenesis in vivo. The profound role of SHQ1 in leukemogenesis relies on successful H/ACA snoRNP assembly, enabling efficient global pre-mRNA splicing. We also identify MYC, whose splicing and expression are highly dependent on SHQ1, as an important downstream effector mediating the tumor-supporting role of SHQ1. These findings provide important insights into how SHQ1-mediated RNA modification and pre-mRNA splicing affect tumorigenesis, and also deepen our understanding of posttranscriptional regulation of oncogene MYC.

**Results**

**SHQ1 is highly expressed in T-ALL.** To screen common elevated genes with tumorigenic potential, we compared gene expression profiles of 117 diagnostic pediatric T-ALLs with 7 normal bone marrow (BM) controls. A total of 97 genes were enriched with more than 1.5-fold upregulation in T-ALL (p < 0.01, unpaired t-test). One notable finding in this gene cluster was SHQ1, encoding an essential factor involved in H/ACA snoRNP biogenesis and RNA splicing (Fig. 1a and Supplementary Data 1). Analysis of the Cancer Cell Line Encyclopedia (CCLE) demonstrated SHQ1 is most highly expressed in T-ALL among 1036 human cancer cell lines (Fig. 1b). Assessment of multiple human leukemia databases confirmed significant increase in SHQ1 expression in T-ALL as compared to normal BM (Fig. 1c) or other hematological malignancies (Fig. 1d). In addition, previously published genome-wide expression profiling data from normal and malignant T cells confirmed significant higher SHQ1 expression in primary human T-ALL (n = 64) as compared to CD4+CD8+ normal human thymocytes (Fig. 1e).

Furthermore, primary T-ALL cells harboring NOTCH1 activating mutations showed higher SHQ1 protein expression than a T-ALL case with wild-type NOTCH1 or normal thymocytes (Fig. 1f). In line with the observations in human T-ALL, murine T-ALL cells with NOTCH1 activating mutations/truncations had greater SHQ1 expression than normal thymocytes (Fig. 1g). Taken together, we identify a global upregulation of SHQ1 in T-ALL.

**NOTCH1 directly activates SHQ1 expression in T-ALL.** To understand the molecular mechanism underlying SHQ1 upregulation in T-ALL, we performed in silico analysis to identify potential transcription factor binding cis-elements in the SHQ1 locus. NOTCH1, GATA3, TAL1 and MYC were predicted to activate the SHQ1 promoter (Supplementary Fig. 1a). We individually knocked down each of these transcription factors in human T-ALL JURKAT cells and found that only depletion of NOTCH1 induced SHQ1 downregulation (Supplementary Fig. 1b-1f). We then blocked NOTCH signaling using γ-secretase inhibitor (GSI) Compound E in T-ALL cells. As shown in Fig. 2a, NOTCH inactivation decreased SHQ1 mRNA and protein levels in seven T-ALL cell lines and four primary patient-derived T-ALL cells. Again, genetic inactivation of NOTCH1 by expression of dominant negative MAML (DNMAML) in HPB-ALL cells diminished SHQ1 mRNA (Supplementary Fig. 2a). Conversely, overexpression of intracellular NOTCH1 in either human HPB-ALL or murine T6E cells remarkably bolstered SHQ1 expression in the presence of NOTCH inhibitor (Supplementary Fig. 2b). Moreover, gene expression profiling in 174 primary T-ALLs revealed a strong and significant correlation between SHQ1 and NOTCH1-regulated signature genes including NOTCH1 itself (Fig. 2b) as well as NOTCH3 or LZTFL1 (Supplementary Fig. 2c).

Using three individual normal thymus specimens as control, we further verified these findings in 11 Chinese T-ALL patient samples, and found that SHQ1 was generally more abundant in leukemia cells with enhanced NOTCH1 activation, as judged by ICN1 production (Fig. 2c). These data support the presence of NOTCH1–SHQ1 axis in human T-ALL.

Importantly, removal of Compound E immediately reversed GSI-mediated inhibition of SHQ1 expression, even in the presence of protein synthesis inhibitor cycloheximide (Supplementary Fig. 2d), suggesting a direct NOTCH1 transcriptional activation of SHQ1. In support of this prediction, chromatin immunoprecipitation-sequencing (ChIP-Seq) analysis revealed a NOTCH1 binding signal proximal to the SHQ1 transcription
start site. The association of NOTCH1 was completely abolished in the NOTCH off state. Similarly, NOTCH1 inhibition compromised the binding of RBPJ, an essential component of the NOTCH1 transcriptional complex, to the SHQ1 promoter (Fig. 2d). Conventional ChIP validated NOTCH1 association with the SHQ1 promoter, with the HES1 promoter analyzed as a positive control. Consistently, blockade of ICN1 generation by Compound E disrupted this interaction (Fig. 2e). Tentative NOTCH1 binding sites of SHQ1 were constructed into a luciferase reporter vector; wild-type, but not mutant, DNA sequences are sufficient to activate luciferase expression when co-transfected with ICN1-expressing constructs (Fig. 2f). Our data provide compelling evidence that nuclear NOTCH1 specifically binds to the SHQ1 promoter for direct transcriptional activation which may be the primary molecular mechanism underlying elevated SHQ1 expression in T-ALL.
SHQ1 is required for T-ALL cell survival. To clarify the role of SHQ1 in T-ALL cell viability, we knocked it down in human T-ALL cells with two short hairpin RNAs (shRNAs) (Fig. 3a and Supplementary Fig. 3a). These shRNAs were cloned into modified pLKO.1 vector, in which the puromycin-resistant gene was substituted by the green fluorescent protein (GFP) gene. As shown in Fig. 3b and Supplementary Fig. 3b, not only SHQ1 deficiency in HPB-ALL and KOPTK1 cells but also in primary T-ALL resulted in marked cell growth inhibition. Consistently, SHQ1-depleted cells lost growth advantage as percentages of GFP+ populations dramatically declined over time, while cells expressing control shRNA remained unaffected (Fig. 3c and Supplementary Fig. 3c). Notably, SHQ1 ablation induced noticeable apoptotic cell death in HPB-ALL, KOPTK1, and primary T-ALL cells, yet minimally affected normal bone marrow cells (Fig. 3d, e and Supplementary Fig. 4a-4b). In addition, we also analyzed SHQ1 function in normal murine thymus and murine T-ALL cells, and consistently found heightened cell death in leukemia cells upon SHQ1 inactivation as compared to normal thymocytes (Supplementary Fig. 4c). Time course cell viability assay showed that SHQ1 ablation resulted in poor cell survival in T-ALL but not in normal bone marrow, confirming an essential role of SHQ1 in the survival of transformed T cells (Fig. 3f). Cell cycle was not affected as a result of SHQ1 deficiency (Supplementary Fig. 4d-4e), suggesting that SHQ1 inactivation imposes a cytotoxic, rather than cytostatic, effect on T-ALL cells.

To evaluate the role of SHQ1 in different tumor types, we inactivated SHQ1 in B-ALL (RS4;11), acute myeloid leukemia (AML) (HL-60), chronic myeloid leukemia (CML) (K562), and lung cancer (A549) cell lines. B-ALL, AML, and CML cells were all sensitive to SHQ1 inactivation, whereas lung cancer cells were not (Supplementary Fig. 5a). In line with this observation, high SHQ1 expression was associated with poor prognosis of T-cell lymphoma and AML, while it predicted favorable outcome in lung cancer (Supplementary Fig. 5b). Based on these data from various tumor types, we reason that SHQ1 may exert variable or even opposing roles in different tumor contexts.

SHQ1 depletion impedes T-cell leukemogenesis in vivo. To assess the role of SHQ1 in leukemogenesis, we established a doxycycline-inducible SHQ1 knockout JURKAT cell line using CRISPR/Cas9 system and transplanted these cells into immunocompromised NPG mice, using non-specific single-guide RNA (sgRNA) targeting GFP as a control. Prior to transplantation, inducible SHQ1 deletion was confirmed to suppress JURKAT cell growth in vitro (Supplementary Fig. 6). At 10 days post engraftment, animals were randomly divided into two groups. Doxycycline (200 mg kg⁻¹) was administrated for consecutive 7 days to induce Cas9 expression and sgRNA-mediated cleavage (Fig. 4a). Mice bearing sgGFP-expressing JURKAT cells succumbed to T-ALL within 35 days, regardless of doxycycline treatment or not. In contrast, doxycycline treatment significantly improved animal survival rates and prolonged life span of mice transplanted with sgSHQ1-expressing JURKAT cells (Fig. 4b), associated with efficient SHQ1 depletion (Fig. 4c). Consistently, mice bearing SHQ1-deficient cells manifested ameliorated spleenomegaly and more reddish bones (Fig. 4d), as well as suppressed CD45+ leukemia cell dissemination in the BM and spleen (Fig. 4e, f). Immunohistochemical (HIC) staining confirmed that SHQ1 deficiency significantly decreased human CD45+ leukemia burden and cell proliferation (reflected by proliferating cell nuclear antigen (PCNA) staining) in the spleen (Fig. 4f). Similar results were obtained when we examined another HPB-ALL xenografts in which shRNA-mediated SHQ1 silencing also decreased T-ALL burden in vivo (Supplementary Fig. 7).

We next evaluated the role of SHQ1 in NOTCH1-induced T-ALL using retroviral ICN1 transduction and fetal liver cell transplant model. The retroviral vector MSCV-ires-GFP allows co-expression of ICN1 and shRNA-of-interest in one construct. Retroviruses expressing empty vector, ICN1/control shRNA or ICN1/murine SHQ1 shRNA, with GFP as an expression marker, were transduced into hematopoietic stem/progenitor cells (HSPCs) from fetal livers of donor mice. We then transplanted these HSPCs into irradiated recipients and assessed the onset of frank leukemia among these mice (Fig. 5a). Mice expressing ICN1/control shRNA succumbed to T-ALL in about 2 months, whereas most mice expressing ICN1/murine SHQ1 shRNA, with GFP as an expression marker, were transduced into hematopoietic stem/progenitor cells (HSPCs) from fetal livers of donor mice. When we examined another HPB-ALL xenograft model in which shRNA-mediated SHQ1 silencing also decreased T-ALL burden in vivo (Supplementary Fig. 7).

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Fig. 2 Intracellular NOTCH1 directly binds to and activates SHQ1. a Seven T-ALL cell lines and four primary T-ALL cells were subjected to GSI (Compound E, 1 μM) or DMSO treatment for 24 h. SHQ1 mRNA and protein levels were subsequently determined by quantitative polymerase chain reaction (qPCR) and immunoblots. b Correlation of SHQ1 expression with NOTCH1 in 174 primary T-ALL samples (GSE13159) with mRNA levels presented as log2 median-entered intensity. Pearson’s correlation coefficient (R) = 0.481, p < 0.001, paired t-test. c Immunoblots of SHQ1 and ICN1 in additional 11 primary T-ALL and 3 normal thymus samples. d Chromatin landscapes around the SHQ1 locus in HPB-ALL (GSE58406) and CUTLL1 (GSE51800) cells. The associations of nuclear NOTCH1 and RBPJ with the SHQ1 transcriptional start region are shown with respect to NOTCH1 active or inactive state. e Binding of ICN1 to the SHQ1 or HES1 promoter was analyzed by ChIP in SIL-ALL cells with or without GSI treatment (Compound E, 1 μM) for 24 h. Averages of fold enrichment between ICN1 and isotype IgG are shown. α-N1 denotes antibodies against ICN1. f Schematic presentation of two potential RBPJ-binding sites proximal to the SHQ1 transcription initiation site. Luciferase assays were carried out with SHQ1 DNA sequence (−441 to −194) or HES1 promoter cloned into pGL3-basic vector; reporter activities relative to empty pGL3 vector without ICN1 are presented as average fold induction. Data shown represent the means (± SEM) of three biological replicates; *p < 0.05, **p < 0.01, ***p < 0.001, unpaired t-test (a, e, f).
**Fig. 3** SHQ1 depletion impairs T-ALL cell survival while it spares normal bone marrow cells. **a** HPB-ALL and patient-derived T-ALL (#1) cells were infected with lentiviruses expressing control (Ctrl, blue) or SHQ1 shRNA (sh1-red or sh2-purple) with GFP as an expression marker. SHQ1 mRNA levels were analyzed in GFP+ cells. **b** Live GFP+ cells were counted at the indicated time points and cell growth was plotted as shown. Cell counts of those expressing control shRNA, SHQ1 shRNA-1, or -2 are marked as blue square, red circle, or purple diamond. **c** Percentages of GFP+ cell populations were analyzed by flow cytometry at the indicated time points post infection. **d**, **e** Apoptotic cell death was analyzed by Annexin V-PI staining 6 days post infection and quantified in e (blue: control shRNA, purple: SHQ1 shRNA-2), along with immunoblots of SHQ1. Long exposure of SHQ1 bands in bone marrow cells is also shown (Long exp). **f** Cell viability was assessed by MTT assay in two T-ALL cell lines (HPB-ALL, blue and KOPTK1, green), two primary T-ALL cells (#1, red and #3, purple), and two normal human BM cells (#1, black and #2, brown) expressing either control or SHQ1 shRNA. Absorbance readings of SHQ1-deficient cells relative to control cells were plotted as shown. Data shown represent the means (±SEM) of three biological replicates; *p < 0.05, **p < 0.01, ***p < 0.001, unpaired t-test (a, b, e).
Fig. 4 SHQ1 knockout significantly decreases leukemia burden in the human T-ALL xenograft. a Schematic representation of human T-ALL xenograft. Two million JURKAT cells, expressing inducible CRISPR-Cas9 and sgRNA targeting GFP or SHQ1, were injected into irradiated NPG mice (1 gray). At 10 days post engraftment, doxycycline or normal saline was introduced into each allocated group for consecutive 7 days. b Kaplan-Meier survival curves of JURKAT xenografts in each group as indicated (n = 5 for each group). Survival of doxycycline-treated or untreated sgSHQ1 mice (purple and red lines) are compared with p value provided as shown (log-rank test). Between 28 and 35 days post engraftment, treated (blue line) or untreated (black line) sgGFP mice became moribund and 5 mice were taken out in each group and killed for assessment of leukemia burden. c Cas9 and SHQ1 were analyzed in human CD45+ bone marrow cells obtained from representative mice in each group. d Representative spleen and bone images of mice carrying JURKAT cells with or without SHQ1 expression. e Human CD45+ cells from spleen and bone marrow (as shown in d) were analyzed by flow cytometry. Data from five individual mice were plotted and shown on the right. f Representative immunohistological images of SHQ1, human CD45, and PCNA in spleen sections from indicated mice. Scale bar, 50 μm. Histological stain was quantified using ImageJ; *p < 0.05, **p < 0.01, ***p < 0.001, unpaired t-test (e, f)
**SHQ1 depletion affects pre-mRNA splicing.** As a crucial factor for the assembly of H/ACA snoRNPs, SHQ1 is often found to be associated with the degradation of the associated snoRNAs. We confirmed the diminished expression of scaU93, an H/ACA snoRNA responsible for U2 snRNA pseudouridylation at the 5′ site, in SHQ1-depleted HPB-ALL cells (Fig. 6a). As expected, an in vitro N-cyclohexyl-N-(2-morpholinoethyl)-carboximid-methoxytoluene-sulfonate (CMC)-primer extension pseudouridylation assay manifested less U2 snRNA pseudouridylation in SHQ1-deficient cells (Supplementary Fig. 9). We reasoned that inefficient U2 snRNA modification would impair mRNA splicing. RNA-Seq analysis was performed in HPB-ALL and KOPTK1 cells expressing SHQ1 or control shRNA to assess pre-mRNA splicing. Splicing analysis was restricted to reads directly spanning exon—intron or exon–exon junction sequences (HPB-ALL, 61,456 junctions in 293T cells; KOPTK1, 78,976 junctions in 7905 genes). Reads spanning exon–intron junctions are considered as unspliced form (a) and reads across exon–exon junctions as spliced form (b). Intron retention (IR) ratio (a/b) reflects the magnitude of pre-mRNA accumulation (Fig. 6b). As predicted, SHQ1 depletion caused widespread intron retention reflected by increased IR ratios (Fig. 6c). Indeed, 81% of genes in HPB-ALL and 73% of genes in KOPTK1 cells exhibited differential intron retention upon SHQ1 inactivation (Fig. 6d). Intron-retaining pre-mRNAs often fail to complete mRNA maturation and are commonly degraded via quality control mechanisms. We then focused on the most downregulated genes with splicing alteration in SHQ1 loss and selected five candidates, RNA polymerase II-associated protein 2 (RPAP2), ATP citrate lyase (ACLY), checkpoint kinase 1 (CHEK1), MYC, and cyclin-dependent kinase 6 (CDK6) (Fig. 6d). Their increased intron retentions were validated in SHQ1-depleted HPB-ALL (Fig. 6e) and primary T-ALL cells (Supplementary Fig. 10), consequently leading to decreased mature mRNA and protein levels (Fig. 6e–f). As a major proto-oncogene in T-ALL, MYC gained our attention. To test whether SHQ1 globally affects T-ALL-associated oncogenes or specifically regulates MYC, we examined MYC, AKT1, NOTCH1, TALI, and LMO2 expression in response to SHQ1 loss. Only MYC expression was significantly decreased (Fig. 6g), which is consistent with the findings from RNA-Seq (Fig. 6d and data not shown). We thus identify MYC as one of the most prominent genes downstream of the NOTCH1–SHQ1 axis.

**SHQ1 modulates MYC gene splicing and expression.** To further analyze MYC pre-mRNA splicing, we expressed a minigene carrying MYC intron 2 and flanking exons in 293T cells
(Supplementary Fig. 11). SHQ1 knockdown led to noticeable accumulation of unspliced RNA, resulting in less mature RNA. We observed a more profound defect on minigene splicing upon pharmacological inhibition of NOTCH which was significantly reversed by exogenous expression of SHQ1 (Fig. 7a). Endogenous MYC in HPB-ALL cells was also assessed with primers designed to amplify pre-mRNA (E1–I1 and E2–I2) and mature mRNA (E1–E2 and E2–E3). As shown in Fig. 7b, mature MYC mRNA, but not pre-mRNA, was significantly reduced in SHQ1 knockdown cells. Again, deregulation of endogenous MYC splicing upon NOTCH inhibition was efficiently rescued by SHQ1 overexpression (Supplementary Fig. 12). More importantly, diminished MYC expression upon SHQ1 ablation in vivo was also confirmed in JURKAT cell xenograft (Fig. 7c) and fetal liver cell transplant (Fig. 7d).

Fig. 6 SHQ1 depletion affects pre-mRNA splicing efficiency. HPB-ALL or KOPTK1 cells were infected with lentiviruses expressing control shRNA (Ctrl) or SHQ1 shRNA (sh1 or sh2). a H/ACA snoRNA scaU93, base paired with U2 snRNA and guiding its pseudouridylation, in HPB-ALL cells expressing control shRNA (blue), SHQ1 shRNA-1 (red), or shRNA-2 (purple) was analyzed by qPCR. b Schematic of intron retention (IR) analysis. Exon–intron reads (a) relative to exon–exon reads (b) are defined as IR ratio which reflects the magnitude of pre-mRNA accumulation. c Empirical cumulative distribution of IR coefficients for 6809 genes in HPB-ALL cells. Curves represent IR ratio in control (black) and SHQ1 shRNA-2-mediated SHQ1 depletion (red). A rightward shift in the curve representing SHQ1-depleted cells indicates increased IR, namely increased pre-mRNA accumulation upon SHQ1 knockdown. d Genome-wide splicing and gene expression were analyzed in HPB-ALL and KOPTK1 cells. Numbers of genes with altered IR ratio were plotted in red at the pie chart, and those with no intron retention (NIR) were marked in black. Genes with IR ratios (SHQ1 knockdown vs control) more than 1.15 are considered aberrant intron retention. Heatmaps of mature mRNA expression from intron-retained genes are presented at the bottom (red indicates increased and blue decreased). e, f Validation of candidate genes shown in the box from d by assessing IR ratio, mature mRNA (e), and protein expression (f) in HPB-ALL cells. g Analysis of T-ALL associated oncogene expression in HPB-ALL cells by qPCR. In e and g, blue, red, or purple bars represent indicated RNA quantifications in control shRNA, SHQ1 shRNA-1, or -2-expressing cells. Data shown represent the means (±SEM) of three biological replicates; *p < 0.05, **p < 0.01, ***p < 0.001, unpaired t-test (a, e, g) or Kolmogorov-Smirnov test (c).
We next analyzed MYC-regulated aerobic glycolysis upon SHQ1 deficiency and found significantly impaired glucose uptake and lactate secretion in SHQ1-depleted HPB-ALL and KOPTK1 cells (Fig. 7e and Supplementary Fig. 13a), associated with downregulation of MYC target genes implicated in glycolysis (Fig. 7f and Supplementary Fig. 13b). Notably, these glycolysis defects were efficiently rescued by enforced MYC expression (Supplementary Fig. 13c). To evaluate the role of SHQ1-regulated MYC in cell viability, we overexpressed MYC in JURKAT cells, in which doxycycline treatment induced SHQ1 knockout (Supplementary Fig. 6). MYC-overexpressed cells significantly ameliorated cell apoptosis resulting from SHQ1 depletion (Fig. 7g). Enforced MYC expression also rescued cell death caused by SHQ1 inactivation in HPB-ALL and KOPTK1 cells (Supplementary Fig. 13d).
**Fig. 7** SHQ1 regulation of MYC gene splicing and expression is important for T-ALL cell survival. a MYC minigene splicing was analyzed in 293T cells expressing control (Ctrl) or SHQ1 shRNA (sh1 or sh2) (top), and also in 293T cells expressing SHQ1 or empty vector in the presence of DMSO or GSI (Compound E, 1 μM) (bottom). Semi-quantitative RT-PCR was performed to analyze unspliced and spliced RNA. Splicing efficiency was calculated and shown as the ratio of spliced verse total RNA. b Schematic of MYC gene with three exons and two introns. Specific primer sets were designed to amplify pre-mRNA (E1–I1 and E2–I2) and mature mRNA (E1–E2 and E2–E3) (left). Endogenous MYC pre-mRNA and mature mRNA in HPB-ALL were analyzed by qPCR (right). Blue, red, or purple bars represent indicated RNA quantities in control shRNA, SHQ1 shRNA-1, or -2-expressing cells. c Representative immunohistological images of MYC in spleen sections from mice bearing sgSHQ1-JURKAT cells injected with or without doxycycline (Fig. 4d). Scale bar, 50 μm. Histological stain was quantified using ImageJ and plotted on the right. d Immunoblots of SHQ1 and MYC in GFP+ cells from 3 fetal liver HSPC transplant samples in Fig. 5d. e HPB-ALL cells were infected with lentiviruses expressing control (Ctrl, blue) or SHQ1 shRNA (sh1 or sh2) (top), and also in 293T cells expressing SHQ1 or empty vector in the presence of DMSO or GSI (Compound E, 1 μM) (bottom). Semi-quantitative RT-PCR was performed to analyze unspliced and spliced RNA. Splicing efficiency was calculated and shown as the ratio of spliced verse total RNA. b Schematic of MYC gene with three exons and two introns. Specific primer sets were designed to amplify pre-mRNA (E1–I1 and E2–I2) and mature mRNA (E1–E2 and E2–E3) (left). Endogenous MYC pre-mRNA and mature mRNA in HPB-ALL were analyzed by qPCR (right). Blue, red, or purple bars represent indicated RNA quantities in control shRNA, SHQ1 shRNA-1, or -2-expressing cells. c Representative immunohistological images of MYC in spleen sections from mice bearing sgSHQ1-JURKAT cells injected with or without doxycycline (Fig. 4d). Scale bar, 50 μm. Histological stain was quantified using ImageJ and plotted on the right. d Immunoblots of SHQ1 and MYC in GFP+ cells from 3 fetal liver HSPC transplant samples in Fig. 5d. e HPB-ALL cells were infected with lentiviruses expressing control (Ctrl, blue) or SHQ1 shRNA (sh1 or sh2, purple) as denoted. Sorted GFP+ cells were used for cell growth analysis (left). In all, 8946 cells were infected with MSCV-shControl (Ctrl) or MSCV-shSHQ1 (shmSHQ1, purple) with PBS treatment as a control (Ctrl, black; shmSHQ1, blue), followed by cell growth analysis (right). Data shown represent the means (±SEM) of three biological replicates; *p < 0.05, **p < 0.01, ***p < 0.001; n.s. non-significant, unpaired t-test (b, c, e, f, g, h).

**Fig. 8** Model depicting the NOTCH1-SHQ1-MYC axis in promoting T-cell leukemogenesis. NOTCH1 directly activates SHQ1 transcription. The resulting accumulation of SHQ1 protein optimizes the assembly of H/ACA snoRNPs and snRNA pseudouridylation, potentiating MYC pre-mRNA splicing. In addition to NOTCH1 transcriptional regulation of MYC, NOTCH1 induces SHQ1 expression and enhances MYC RNA processing, ultimately maximizing MYC expression in T-ALL. See discussion for more details.

Discussions

Based on gene expression profiling analysis, we identify that SHQ1, whose expression is aberrantly upregulated in T-ALL, contributes to leukemogenesis. We demonstrate SHQ1, directly driven by NOTCH1, is required for T-ALL cell survival in vitro and expansion in vivo. This important role of SHQ1 attributes to the assembly of H/ACA snoRNPs that mediates snRNA pseudouridylation and mRNA splicing. We here provide mechanistic evidence demonstrating regulation of MYC splicing by SHQ1 as an important event involved in T-cell leukemogenesis (Fig. 8), thus revealing the tumor-supporting role...
of SHQ1 in human cancers that links SHQ1 to RNA splicing and leukemogenesis.

Regulatory mechanism and functional role of SHQ1 in human cancers are largely unknown. Using T-ALL as a model system, we reveal a molecular mechanism underlying SHQ1 expression in human cancers. NOTCH1 transcriptional complex directly binds to the promoter region of SHQ1 and activates its transcription. Given aberrant, sustaining NOTCH1 activation in T-ALL, this regulatory mechanism may explain common elevation of SHQ1 expression in various T-ALL subtypes. Consistently, previous publications suggest that SHQ1 expression is dependent on NOTCH1 activity in T-ALL. Being an important effector downstream of NOTCH1 signaling, enhanced SHQ1 expression is essential for T-ALL cell survival. Similarly, SHQ1 plays an essential role in B-ALL, AML, and CML cells, and high SHQ1 expression correlates with poor prognosis in T-cell lymphoma and AML. However, SHQ1 deficiency in lung cancer cell line A549 induces minimal cell death and higher expression of SHQ1 is associated with better overall survival in lung cancer patients. These findings are in agreement with prior reports that low-small cell lung cancers and prostate cancers are highly context dependent probably through regulating splicing of distinct downstream genes.

Our in-depth mechanistic analysis reveals that SHQ1 modulates MYC splicing and expression. In line with prior reports that NOTCH1 directly activates MYC transcription through miR-34 downstream of the SHQ1 promoter, SHQ1 enhances the expression of MYC in human lung cancers. In addition, SHQ1 may modulate MYC expression in a MYC-dependent manner. Moreover, our analysis reveals that SHQ1 expression is dependent on NOTCH1 activation in T-ALL, which may explain common elevation of SHQ1 expression in various T-ALL subtypes. Consistently, previous publications suggest that SHQ1 expression is dependent on NOTCH1 activity in T-ALL. In this case, SHQ1 expression is dependent on NOTCH1 activity in T-ALL. Being an important effector downstream of NOTCH1 signaling, enhanced SHQ1 expression is essential for T-ALL cell survival. Similarly, SHQ1 plays an essential role in B-ALL, AML, and CML cells, and high SHQ1 expression correlates with poor prognosis in T-cell lymphoma and AML. However, SHQ1 deficiency in lung cancer cell line A549 induces minimal cell death and higher expression of SHQ1 is associated with better overall survival in lung cancer patients. These findings are in agreement with prior reports that low-small cell lung cancers and prostate cancers are highly context dependent probably through regulating splicing of distinct downstream genes.

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Taken together, our data suggest that T-ALL may have an increased dependency on SHQ1-mediated snRNA pseudouridylation and functionally intact spliceosome. Oncogene activation has been shown to increase total RNA and protein production in various tumor contexts. While such increases in RNA and protein production may endow cancer cells with pro-tumorigenic hallmarks, this increase in synthesis may also generate heightened burden on cancer cells to process these macromolecules properly. Our findings provide an example that, in order to adapt the oncogenic stress, NOTCH1 activates the expression of SHQ1 which optimizes snRNA modification and maximizes spliceosomal potential to accommodate greater volumes of total RNA. As such, partial modulation or inhibition of spliceosome function may be detrimental to T-ALL cells. Hence, our findings highlight the indispensable role of SHQ1 for optimal spliceosome function in T-ALL which may offer therapeutic opportunity for T-ALL patients by targeting of SHQ1 or spliceosome.

Methods

Cell culture. The 293T, JURKAT, and CCRF-CEM cells were purchased from American Type Culture Collection (ATCC). Human T-ALL cell lines S11-ALL, HPB-ALL, KOPTK1, CUTL1, and DND41, and murine T-ALL cell lines T6E, GA2, and 8946 were kindly provided by Dr Warren Pear (University of Pennsylvania). T-ALL cell lines were grown in complete RPMI-1640 (Hyclone) supplemented with 10% fetal bovine serum (FBS, Hyclone), 1% penicillin/streptomycin (Hyclone), 1% non-essential amino acids (Gibco), 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), and 100 μM β-mercaptoethanol (Sigma). Primary T-ALL cells were co-cultured with M5-S-DI leukemia feeder cells in Witt-L medium supplemented with stem cell factor (SCF) (30 ng ml⁻¹), interleukin (IL)-2 (10 ng ml⁻¹), IL-7 (10 ng ml⁻¹), and insulin-like growth factor-1 (10 ng ml⁻¹). Human normal bone marrow cells were maintained in minimum essential medium α supplemented with 20% FBS and granulocyte colony-stimulating factor (30 ng ml⁻¹). The 293T cell was maintained in Dulbecco’s modified Eagle’s medium (Hyclone) containing 10% FBS and 1% penicillin/streptomycin (Hyclone). All cell lines were authenticated using the variable number of tandem repeats PCR assay, cultured for fewer than 6 months after resuscitation, and tested for mycoplasma contamination every 3 months using MycoAlert (Lonza). Human primary specimens were obtained with informed consents from Center for Center for Medical Genetics, Ghent University, Ghent, Belgium; Center for Cancer Research, Boston University School of Medicine, Boston, USA; Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China; Union Hospital and Tongji Hospital, Wuhan, China.

RNA extraction and quantitative real-time PCR. Total cellular RNA was extracted using TRIzol (Invitrogen) and random primed RNAs (1 μg) were reverse transcribed with RevertAid first-strand complementary DNA synthesis kit according to the manufacturer’s instructions (Thermo Scientific). Quantitative PCR was conducted using FAST SYBR Green Master Mix on CFX Connect Real-Time PCR System (Bio-Rad). Relative expression of the mRNA was calculated by 2^−ΔΔCt method and normalized to ACTIN. Specific PCR primer sequences are listed in Supplementary Table 1.

Immunoblotting. Cells were lysed with RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 2 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM EDTA, 1 mM NaN₃, 0.5 mM leupeptin, and 50 μM 4 μM pepstatin) and protein concentrations determined using Bradford reagent (Bio-Rad). 30–50 μg total cellular proteins were then subjected to SDS-polyacrylamide gel and transferred to polyvinylidene difluoride membrane (Bio-Rad). After being blocked with 5% fat free milk, blots were generally incubated with primary antibodies at 4 °C overnight. Appropriate horseradish peroxidase-conjugated secondary antibodies were applied for 1–2 h at room temperature before detection with SuperSignal Chemiluminescent Substrate (Bio-Rad). Densitometric analyses of protein abundance were determined by ImageJ software. All uncropped blotting images are presented in Supplementary Fig. 15. 18S and 28S Antibodies used in the experiments include β-ACTIN (1:2000, A5441, Sigma-Aldrich), SHQ1 (1:300, ab110692, Abcam), MYC (1:1000, sc-764, Santa Cruz), TALI (1:1000, sc-393287, Santa Cruz), CHEKI (1:1000, sc-8408, Santa Cruz), CDK6 (1:1000, A1545, Abclonal), GATA3 (1:1000, A1638, Abclonal), ACY2 (1:1000, sc-332, Cell Signaling Technology), cleaved NOTCH1 (1:1000, #4147, Cell Signaling Technology), H2A (1:1000, #2016, Cell Signaling Technology), L3HHA (1:1000, #2012, Cell Signaling Technology), GLUT1 (1:1000, ab652, Abcam) and PKM2 (1:1000, ab38327, Abcam).
**ChIP assay.** ChIP was performed using human T-ALL SIL-ALL cells.¹⁰⁴ These cells were treated with dimethyl sulfoxide (DMSO) or Compound E (1 μM, Merck) for 10 min to fixate the nuclear matrix of the cell. Cells were subjected to a Bioruptor Pico Sonifier to shear chromatin DNA to a size range of 500–1000 bp. Precleared chromatin was immunoprecipitated with anti-serum against intracellular NOTCH1 or rabbit IgG (sc-3888, Santa Cruz Biotechnology) for 16 h at 4 °C. Antibody–chromatin complexes were pulled down with protein G agarose/salmon sperm DNA beads (Roche) (1 h, 4 °C). The eluted chromatin was reverse-cross-linked and treated with proteinase K (40 μg ml⁻¹). Immunoprecipitated DNA was purified by phenol/chloroform extraction, eluted by distilled H₂O, and quantified by QFX Connect Real-Time PCR System (Bio-Rad) using specific primers listed in Supplementary Table 1.

**Luciferase reporter assay.** The wild-type or mutant SHQ1 promoter sequences were amplified using specific primers listed in Supplementary Table 1. The resulting DNA fragments were constructed into pG3-basic luciferase reporter vector (Promega). To detect luciferase reporter activity, 0.8 μg pG3L expressing the SIPO1 promoter (or empty vector) and 0.2 μg pcDNA3-ICN1 plasmid, along with 50 ng pcRL-TK Renilla luciferase reporter construct, were co-transfected into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific). Luciferase activities were measured 24 h later using Dual Luciferase Reporter Assay System (Promega)⁴⁹. Firefly luciferase activities were normalized with Renilla luciferase control values, and relative to values from the empty vector lysate.

**Lentiviral or retroviral transduction.** For viral production, lentiviral vectors pLKO.1 or pCDH were used for plasmid construction and transfected into 293T cells simultaneously with helper plasmids (pMD2.G and psPAX2); retrovirus vectors MSCV-IRES-GFP or MigR1 were used for plasmid construction and transfected into 293T cells simultaneously with packaging plasmids (pCpg and pHIT). Viral supernatants were generally collected 6–24 h post transfection. Transduction of T-ALL cells was carried out as follows⁴⁸,⁴⁹,⁵⁰. 1 × 10⁶ JURKAT cells infected with inducible Cas9 and SHQ1 sgRNA (or GFP vectors MSCV-IRES-GFP or MigR1) were used for plasmid construction and transfected into 293T cells prior to additional 24 h of cell culture. Cells were then harvested and RNA was isolated, reverse transcribed, and subjected to PCR analysis (30 s at 94 °C, 1 min at 60 °C, 1 min at 72 °C) for 1× treatment, and the PCR products were separated by agarose gel electrophoresis and imaged using GelDoc (Bio-Rad). The ratio of intron reads to exon reads was calculated and normalized to the same live cell numbers. The heatmap was generated using Cluster 3.0 and Treeview software.

**Meningine assay.** Human MYC intron 2 and about 500 bp of flanking exonic sequences were amplified by PCR from genomic DNA and cloned into a hybrid minigene plasmid vector KS construct (a generous gift from Dr. Hai-Ning Du, Wuhan University). pcDNA3.1-SHQ1 shRNA or pcDNA3.1 GFP plasmid (1 μg) were individually transfected into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific) in a final volume of 1 ml. After 24 h, RNA expression was evaluated by further pLX-sgGFP or pLX-sgSHQ1 lentiviral transduction. Positive colonies were selected by PCR. Inducible Cas9 JURKAT cells were generated as described⁵⁰. day 13.5. Day 13.5 human genome reference assembly (hg19) using Bowtie 2. The ratio of exon–intron reads to exon–exon reads was calculated as junction IR. Mature mRNA expression was determined by normalized RPKM values (Reads Per Kilobases Per Million reads) of aligned exonic–exonic transcripts. We restricted the above-mentioned analyses to high confidence genes with an average of at least 10 total junction reads and RPKM ≥ 1 in the control samples. The heatmap was generated using Cluster 3.0 and Treeview software.

**Glucose uptake and lactate secretion assays.** Glucose uptake and lactate secretion analysis were carried out using respective colorimetric assay kits according to the manufacturer’s instruction (BioVision). Briefly, 4 × 10⁵ cells were inoculated in each well of 6-well plates. After 48 h of incubation, cell culture media were collected to quantify glucose and lactate. Consumed glucose and released lactate were calculated and normalized to the same live cell numbers.

**Statistical analysis.** Log-rank analysis was used to evaluate differences in Kaplan–Meier survival curves. Kolmogorov–Smirnov test was used to analyze normality of cumulative distribution of IR scores in splicing analysis. Other statistical analysis was calculated using two-tailed Student’s t-test, with p < 0.05 considered significant.

**Data availability.** RNA-Seq data that support the findings of this study have been deposited in Gene Expression Omnibus with the accession code GSE117264. All other relevant data are available from the corresponding authors.

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**Additional information**

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