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LETTER TO THE EDITOR

Novel endogenous endoplasmic reticulum transmembrane protein SURF4 suppresses cell death by negatively regulating the STING-STAT6 axis in myeloid leukemia

Dear Editor,

Myeloid differentiation was shown to be associated with reduced leukemic cell burden and leukemia-initiating cells and improved survival [1]. Reactive oxygen species (ROS) are associated with leukemia and can induce endoplasmic reticulum (ER) stress. ER stress induces several mechanisms, including cell death [2]. The stimulator of interferon genes (STING) is also an ER transmembrane protein and promotes anti-tumor immunity by linking innate and adaptive immunity [3]. Signal transducer and activator of transcription 6 (STAT6) plays a prominent role in adaptive immunity by transducing signals from extracellular cytokines and inducing apoptosis in cancer cells [4]. Surfeit 4 (SURF4) is a multi-pass ER transmembrane protein that participates in the ER-Golgi compartment, and *SURF4* was found to be amplified and highly expressed in leukemic cells (Supplementary Figure S1A) and several cancer types, including blood cancers [5]. STING interacts with STAT6 in the ER, and TANK-binding kinase 1 (TBK1) activates pSTAT6^{Y641}, leading to anti-tumor effects in cancer cells [6]. Interestingly, SURF4 binds to STING [7], but it remains unclear how the STING-TBK1-STAT6 axis yields anti-tumor effects in blood cancers. The study methods are detailed in the Supplementary Materials.

To determine the function of SURF4 in myeloid leukemic cells, we transduced multiple short hairpin RNA (shRNA) constructs targeting *SURF4* in myeloid malignancies. Three shRNAs targeting distinctive regions within the *SURF4* transcript provided effective knockdown in THP1, HL60 and K562 myeloid leukemic cells (Supplementary Figure S1B). *SURF4* shRNA-transfected THP1, HL60 and K562 myeloid leukemic cells showed significantly reduced proliferation compared to control cells (Figure 1A). Further, we found an increased number of apoptotic cells in *SURF4* shRNA-mediated THP1, HL60 and K562 myeloid leukemic cells (Figure 1B, Supplementary Figure S1C) and single-guide RNA (sgRNA) deletion of *Surf4* in normal hematopoietic progenitor cells (Supplementary Figure S1D). It is reported that AKT and ERK signaling pathways are implicated in myeloid leukemia, ROSs are associated with leukemia, and prolonged ROS elevation to activate the Jun N-terminal kinase (JNK)/c-JUN signaling pathway [8]. Increased phospho-JNK (pJNK) expression and decreased pERK and pAKT expression were detected in these cells (Supplementary Figures S1E-F). Interestingly, *SURF4* shRNA-mediated THP1 and HL60 cells and sgRNA deletion of *Surf4* in *Mll/Af9* cells exhibited significantly increased differentiation (Supplementary Figure S1G-H) and accumulation of ROS (Supplementary Figure S1I), respectively. In addition, the silencing of *SURF4* led to tumor growth arrest in vivo (Figure 1C). No differences in the cell cycle status were observed from the *SURF4* shRNA-mediated THP1 and K562 cells (Supplementary Figure S1J). Collectively, SURF4 suppressed myeloid differentiation and ROS production and inhibited cell death in myeloid leukemic cells.

Prolonged ER stress initiates the mechanisms of cell death [2]. Signaling apoptosis in response to ER stress is mainly associated with the apoptotic PKR-like ER kinase (PERK), which phosphorylates the eukaryotic initiation factor 2α (eIF2α) and C/EBP homologous protein (CHOP) axis. We then tested whether SURF4 contributed to the cell death effects of combinatorial treatments with paclitaxel (Figure 1D, Supplementary Figure S2A), cytarabine (Figure 1E, Supplementary Figure S2B), tunicamycin (Figure 1F, Supplementary Figure S3A), interleukin 4 (IL4) (Figure 1G, Supplementary Figure S3B), or STING

Abbreviations: ROS, Reactive oxygen species; ER, endoplasmic reticulum; STING, stimulator of interferon genes; STAT6, signal transducer and activator of transcription 6; SURF4, surfeit 4; TBK1, TANK-binding kinase 1; shRNA, short hairpin RNA; sgRNA, single-guide RNA; AML, acute myeloid leukemia; scRNA-seq, single-cell RNA sequencing; UMAP, uniform manifold approximation and projection; HSCs, hematopoietic stem cells; cDCs, conventional dendritic cells; GO, Gene Ontology.

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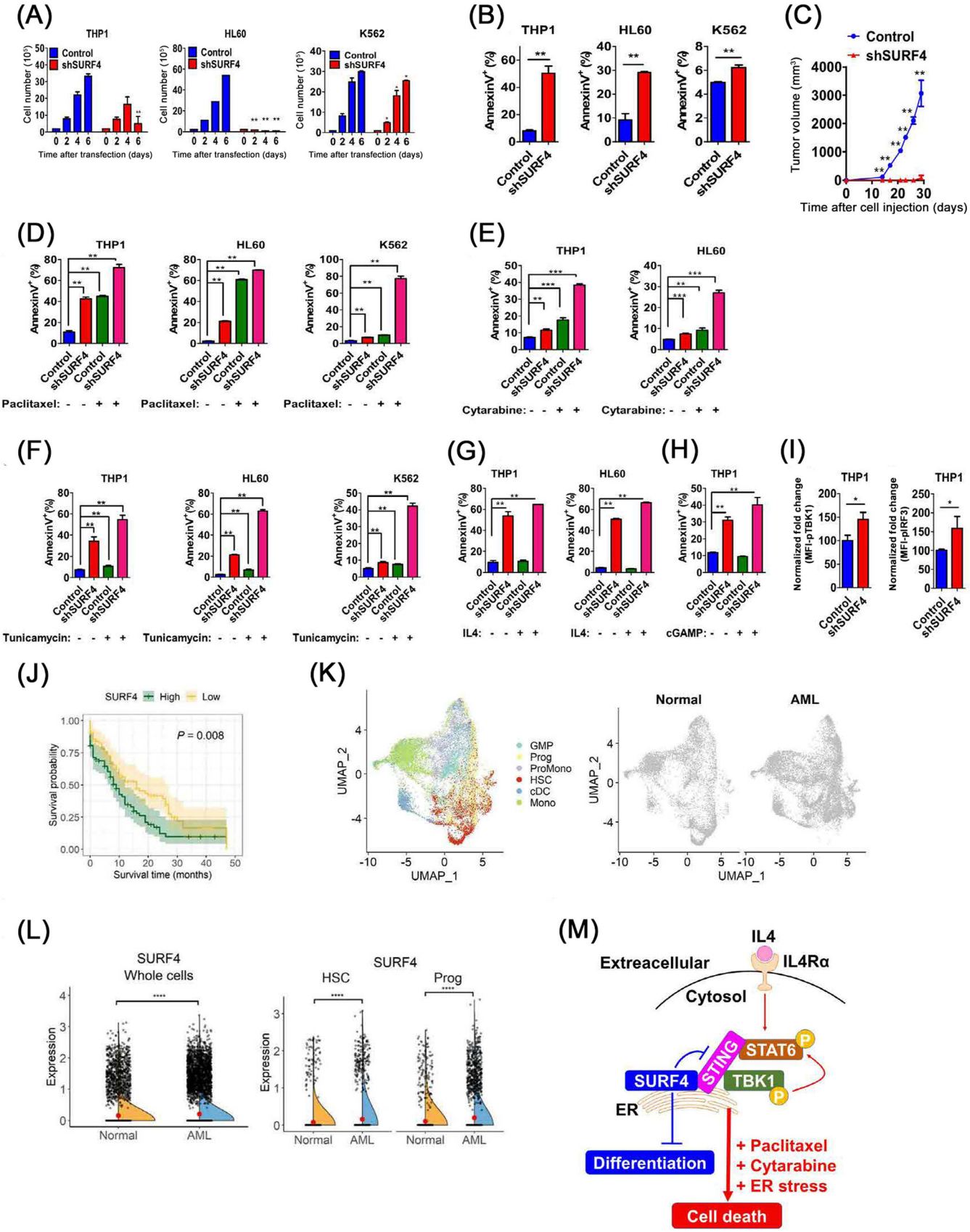
(A)
THP1
HL60
K562
Control
shSURF4
Cell number (10^5)
Time after transfection (days)
(B)
AnnexinV$^+$ (%)
(C)
Tumor volume (mm^3)
Time after cell injection (days)
(D)
Paclitaxel: - - + +
(E)
Cytarabine: - - + +
(F)
Tunicamycin: - - + +
(G)
IL4: - - + +
(H)
cGAMP: - - + +
(I)
Normalized fold change (MFI-pTBK1)
Normalized fold change (MFI-pIRF3)
(J)
SURF4 High Low
P = 0.008
Survival probability
Survival time (months)
(K)
GMP
Prog
ProMono
HSC
cDC
Mono
UMAP_1
UMAP_2
Normal
AML
(L)
SURF4
Whole cells
HSC
Prog
Expression
(M)
IL4
Extreacellular
IL4Rα
Cytosol
STING
STAT6
P
SURF4
TBK1
ER
Differentiation
+ Paclitaxel
+ Cytarabine
+ ER stress
Cell death

FIGURE 1 Impact of SURF4 on myeloid leukemic cells. (A) *SURF4* shRNA-mediated THP1, HL60, and K562 cells were counted every two days. (B) Quantification of apoptotic cells from *SURF4* shRNA-mediated THP1, HL60, and K562 cells. (C) Tumorigenicity was analyzed with a xenograft model of NOD-SCID mice. Subcutaneous injection of *SURF4* shRNA-mediated HL60 cells was performed and tumor growth was quantified ($n = 5$). (D-F) Quantification of the apoptotic cells from *SURF4* shRNA-mediated THP1, HL60, and K562 cells after paclitaxel (D), cytarabine (E), or tunicamycin (F) treatment. (G) Quantification of the apoptotic cells from *SURF4* shRNA-mediated THP1 and HL60 cells after IL4 treatment. (H) Quantification of the apoptotic cells from *SURF4* shRNA-mediated THP1 cells after cGAMP treatment. (I) Quantification of the normalized fold change in MFI for the indicated phospho-protein in *SURF4* shRNA-mediated THP1 cells. (J) Survival analyses for *SURF4*. Overall survival for 4 years with Kaplan-Meier curve stratified by *SURF4* expression. (K) UMAP visualization of cells from normal and AML patients. In the UMAP plot, each point represents a cell, and the space of the point reflects the location of the cell in low-dimensional space based on transcriptional similarity. The left panel is colored by the cell type of bone marrow, and the right panel shows UMAPs separated by donor origin. (L) Gene expression levels of *SURF4* in whole cells, progenitors, and HSCs. The Y-axis of the violin plot is log-normalized counts of *SURF4*, and the described p-value was derived using the Wilcox rank-sum test comparing the gene expression levels in the normal and AML cells. (M) SURF4 negatively regulates STAT6 and STING functions and suppresses differentiation and cell death in myeloid leukemic cells. Error bars indicated the S.E.M. (**** $P \leq 0.001$, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$). ($n = 2$ independent experiments and *3* total measurements per group and treatment).

Abbreviations: ROS, Reactive oxygen species; ER, endoplasmic reticulum; STING, stimulator of interferon genes; STAT6, signal transducer and activator of transcription 6; SURF4, surfeit 4; sgRNA, single-guide RNA; AML, acute myeloid leukemia; scRNA-seq, single-cell RNA sequencing; UMAP, uniform manifold approximation and projection; cDCs, conventional dendritic cells; GO, Gene Ontology.

agonist cyclic GMP-AMPs (cGAMP) (Figure 1H, Supplementary Figure S3C) on *SURF4* shRNA-mediated myeloid leukemic cells, respectively. These molecules were shown to activate ER stress, STAT6 and STING signaling pathways, and led to cell death in cancer cells [2–4, 6]. Increased cell death was detected in *SURF4* shRNA-mediated myeloid leukemic cells after combinatorial treatment with paclitaxel, cytarabine, tunicamycin, IL4, or cGAMP. Western blotting after paclitaxel, tunicamycin, IL4, or cGAMP treatment of *SURF4* shRNA-mediated THP1 cells showed activation of cleaved caspase 9, caspase 3, cleavage of poly(ADP-ribose) polymerase 1 (PARP1) (Supplementary Figure S4A), phospho-eukaryotic initiation factor 2α (peIF2α), activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP) (Supplementary Figure S4B), pSTAT6 (Supplementary Figure S4C-D), pTBK1 and pIRF3 (Supplementary Figure S4E), respectively. We quantified the intensity of these western blots. Silencing of *SURF4* increased pSTAT6 levels in association with depleted *SURF4* levels with and/or without IL4 treatment (Supplementary Figure S4D, F). pTBK1 and pIRF3 are downstream targets of the STING signaling pathway, and increased pTBK1 and pIRF3 expression were detected in *SURF4* shRNA-mediated THP1 cells (Figure 1I, Supplementary Figure S4E). Collectively, depletion of *SURF4* synergistically induces apoptosis in myeloid leukemic cells via anti-cancer drugs, such as paclitaxel, cytarabine, and ER stress inducers, such as tunicamycin. IL4-dependent pSTAT6 and/or STING activation-induced apoptosis was also increased in *SURF4*-silenced leukemic cells.

Next, we explored the overall survival of acute myeloid leukemia (AML) patients based on relative levels of *SURF4* expression from the cancer genome atlas (TCGA). Individuals with high *SURF4* expression had significantly shorter survival than those with low *SURF4* expression (Figure 1J). Further, to characterize the expression of *SURF4* in AML, we downloaded and explored the AML single-cell RNA sequencing (scRNA-seq) datasets [9] (Figure 1K). The scRNA-seq data from bone marrow (BM) cells of normal healthy donors and AML patients were subjected to uniform manifold approximation and projection (UMAP) analysis. *SURF4* was highly expressed in the total population and displayed remarkably high expression in hematopoietic stem cells (HSCs) and progenitor cells from AML patients (Figure 1L). However, there are no differences in *SURF4* expression in the AML cells from conventional dendritic cells (cDCs), granulocyte-macrophage progenitor (GMP) cells, monocytes (mono), and promonocytes (promono) compared with normal healthy donors (Supplementary Figure S5A). Gene ontology (GO) analysis revealed that *SURF4* might be associated with differentially expressed immune responses [3, 4, 6, 7] (Supplementary Figure S5B). Collectively, the expression of *SURF4* was significantly increased in AML patients, suggesting that SURF4 is relevant to the pathogenesis of hematological malignancies (Figure 1M).

In this study, we demonstrated that SURF4 suppressed myeloid differentiation and inhibited cell death in myeloid leukemic cells via negatively regulating the STING-TBK1-STAT6 axis. Thus, we propose that the inhibition of SURF4, such as using monoclonal antibodies and/or aptamer, may be used as a potential therapeutic strategy for the treatment of hematological malignancies.

DECLARATIONS

AUTHOR CONTRIBUTIONS

J.K. and H.L. designed the research, analyzed the data, and wrote the manuscript. J.K. carried out most of the experimental work with the help of H.L., C.M.H., J.H.N., H.J.Y., W.H.C., H.S.K., and C.H. Y.H.K. and D.L. directed the research.

CONFLICT OF INTEREST

No conflicts of interest to disclose.

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DATA AVAILABILITY STATEMENT

The scRNA-seq bone marrow cell data from normal and AML patients were downloaded from the GSE116256 database. The data used and/or analyzed for this study will be available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal experiments were approved by the Animal Ethics Committee of the Pusan National University School of Medicine (PNU-2022-0141).

CONSENT FOR PUBLICATION

Not applicable.

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SUPPORTING INFORMATION

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