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Research paper

Treatment of human T-cell acute lymphoblastic leukemia cells with CFTR inhibitor CFTRinh-172

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ABSTRACT

Our previous studies have demonstrated that a previously unrecognized role of CFTR in hematopoiesis and acute leukemia. Here, we show that CFTR inhibitor CFTR-inh172 possesses ability to inhibit human T-cell acute lymphoblastic leukemia cells. In detail, CFTR-inh172 inhibited cell proliferation, promoted apoptosis and arrested the cell cycle in human T-cell acute lymphoblastic leukemia cell CCRF-CEM, JURKAT and MOLT-4. Furthermore, transcriptome analysis reveals that CFTR-inh172 induces significant alteration of gene expression related to apoptosis and proliferation. These findings demonstrate the potential of CFTR inhibitor CFTR-inh172 in human T-cell acute lymphoblastic leukemia treatment.

1. Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) is well-known as an ATP-binding cassette (ABC) transporter superfamily cAMP-activated anion channel [1]. Over the past few years, we revealed a previously unrecognized role of CFTR, which is independent of its channel function, in the Wnt signaling pathway crucial for both primitive and definitive hematopoiesis by zebrafish model, providing a potential correlation between CFTR and blood disease [2]. Subsequently, we found that the mean level of CFTR expression in acute leukemia primary leukemia cells and cell lines, including T-cell acute lymphoblastic leukemia, was markedly higher than normal mononuclear cells (MNCs) and nonleukemia cell line [3]. CFTRinh-172, a classic CFTR inhibitor, exerted a significant anti-proliferative, apoptosis- inducing and cell cycle-arresting effect on CFTR-high Ph+ leukemia cells but had little effect on normal cells [3,4].

T-cell acute lymphoblastic leukemia (T-ALL) is noteworthy for its unique clinical and biological features, and accounts for 10–15% of newly diagnosed acute lymphoblastic leukemia cases in children, which is the most common pediatric malignancy, and 25% of cases in adults [5]. T-ALL arises in the thymus from an immature thymocyte as a consequence of a stepwise accumulation of genetic and epigenetic aberrations. Crucial biological processes such as differentiation, self-renewal capacity, proliferation, and apoptosis are targeted and deranged by several types of neoplasia-associated genetic alteration [5]. Although event-free survival (EFS) rates have been steadily improving with recent advances in therapy, recurrent disease is very difficult to salvage, and relatively few new drugs have been developed for children with resistant disease [6].

To elucidate the role of CFTR and its inhibitor CFTR-inh172 in leukemia cells further, we proposed that CFTR inhibitor CFTR-inh172 may have potential to treat T-cell acute lymphoblastic leukemia, since the mean level of CFTR expression protein in T-ALL primary leukemia cells was markedly higher than normal control [3]. In this present study, we show that CFTRinh-172 elicits significant anti-proliferative, apoptotic and cell cycle arrest phenotypes in T-cell acute lymphoblastic leukemia cell line CCRF-CEM, JURKAT and MOLT-4, accompanied by remarkable alteration of transcriptome.

2. Materials and methods

2.1. Cell culture

CCRF-CEM, JURKAT and MOLT-4 T-ALL cell line was provided from Cellbank of Chinese Academy of Science. Peripheral blood samples of T-cell acute lymphoma leukemia (T-ALL) patients were obtained from

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West China Hospital. The patients were diagnosed as primary T-ALL according to 2008 WHO classification of tumours of haematopoietic and FAB (French–American–British) classification criteria, and their information is given in Supplementary Table 1. The work on patients' samples was approved by the above medical institutions with patients' consent. Human lymphocytes were separated by kit with procedure provided by manufacture (LTS1077, TBDsciences, Tianjin, China). Human normal T cells, collected from authors, were separated using EasySep™ Direct Human T Cell Isolation Kit (STEMCELL Technologies Inc.). All Cells, including cell line and primary cell, were cultured in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (FBS) and grown at 37 °C in a 5% CO_2 atmosphere.

2.2. Cell proliferation and TUNEL assay

Cell proliferation was evaluated by the conversion of 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) to formazan using a cell counting kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan). Briefly, 96-well plates were seeded with 1000 cells/well. After 24 and 48 h, 10 µl of CCK-8 solution were added to each well. The samples were incubated at 37 °C for 4 h and the absorbance was measured at 450 nm using a microplate reader (Thermo Multiskan MK3, Waltham, USA). DeadEnd™ Fluorometric TUNEL System (Promega) was used for TUNEL assay with performance as manufacture described.

2.3. Analysis of the cell cycle and cell apoptosis

For cell cycle analysis, 1×10^6 cells were harvested, washed twice with ice-cold phosphate buffered saline (PBS), and fixed with ice-cold 70% ethanol overnight. Following two washes with PBS, fixed cells were incubated in RNase (25 µg/ml) at 37 °C for 30 min, followed by staining of the DNA with propidium iodide (PI) (50 µg/ml) (Sigma) in the dark at 37 °C for 30 min. For cell apoptosis analysis, cells were stained with FITC Annexin V and PI according to the manufacturer's instructions (FITC Annexin V Apoptosis Detection Kit I, BD Biosciences) to assess the level of apoptosis. The data were expressed as the percentages of early apoptotic cells (Annexin $V^+/PI-$) and late apoptotic cells (Annexin V+/PI+). Analysis was performed on a FACScan cytometer (BD) using CellQuest software.

2.4. Transcriptome analysis

We collected CCRF-CEM, JURKAT and MOLT-4 cells treated with DMSO/CFTRinh-172 and sent to Chengdu life baseline technology co., LTD for transcriptome analysis. Briefly, DNase I degraded double-stranded and single-stranded DNA contaminant in RNA samples; mRNA molecules were purified from total RNA using oligo(dT)-attached magnetic beads; mRNAs were fragmented into small pieces using fragmentation reagent; First-strand cDNA was generated using random hexamer-primed reverse transcription, then was followed by a second-strand cDNA synthesis; the synthesized cDNA was subjected to end-repair and then was 3' adenylated; adaptors were ligated to the ends of these 3' adenylated cDNA fragments to amplify the cDNA fragments with adaptors from previous step; PCR products were purified with the SPRI beads, and dissolved in EB solution; library was validated on the Agilent Technologies 2100 bioanalyzer and sequenced by IlluminaHiSeq™ 4000. The gene expression levels were calculated by the method of fragments per kilobase of exon per million mapped reads (FRKM). The most significant diff ;erential expression levels (log2 fold change ≥ 1; probability > 0.8) in the transcriptome were subjected to enrichment and functional analysis.

2.5. Quantitative real-time RT–PCR (qPCR) analysis and statistics

Total RNA of each sample was prepared with TRIzol (Invitrogen, 15596-018) and cDNA was synthesized from 1 µg of RNA with PrimeScript RT reagent Kit (Takara, DRR037A). qPCR with three independent biological replicates and three technical replicates was performed with the SYBR Green detection method with 7500 real-time PCR system (Applied Biosystems). The primers used to detect genes are from PrimerBank website (https://pga.mgh.harvard.edu/primerbank/). All detected genes expression was relative to β-actin. Quantitative data shows the mean + SD. The comparative CT (cycle threshold) method (also known as ΔΔCT method) was used to analyze the data. Statistical significance is defined as $P < 0.05$(*), $P < 0.01$(**), $P < 0.001$(***).

2.6. Statistical analysis

Data are presented as the mean ± SD. Statistical analysis was calculated by a one-way ANOVA with Dunnett as a post-test and the two-tailed unpaired *t*-test using GraphPad prism software. P-values of < 0.05 were considered statistically significant.

3. Results

3.1. CFTR inhibitor CFTR-inh172 inhibits cell proliferation in T-ALL cells

We first studied the effect of CFTR-inh172 on the growth and survival of T-cell acute lymphoblastic leukemia CCRF-CEM, JURKAT and MOLT-4 cells using CCK8 assay. The concentrations of CFTR-inh172 we used for this assay ranged from 5 to 40 µM. We found a concentration-dependent inhibition of the growth of all three T-ALL cell lines after 24 h and 48 h of treatment with CFTR-inh172. The IC50 s ranged from 7 to 15 µM in these cells. Exposure to CFTRinh-172 at 40 µM resulted in greater than 70% growth inhibition of these cells at 48 h (Fig. 1). These results show the more sensitivities of T-ALL cells to CFTRinh-172 than chronic myelogenous leukemia K562 and B-cell acute lymphoblastic leukemia SUP-B15 cells described before [3].

Furthermore, we also found that CFTRinh-172 exerted a significant anti-proliferative effect on the primary T-ALL cells (Supplementary Fig. 1 and Table 1). However, CFTRinh-172 had little effect on the human primary normal T cells (Supplementary Fig. 2).

3.2. CFTR inhibitor CFTR-inh172 induces apoptosis in T-ALL cells

We labeled leukemic cells with Annexin V and PI and found that early and late apoptosis was increased in CCRF-CEM, JURKAT and MOLT-4 cells treated with 20 µM CFTR-inh172 (Fig. 2). Furthermore, TUNEL (TdT-mediated dUTP Nick-End Labeling) assay also indicated the increased apoptosis in these cells with treatment with 20 µM CFTR-inh172 (Supplementary Fig. 3).

3.3. CFTR-inh172 treatment blocked the cell cycle of T-ALL cells

Cell cycle analysis indicated that the percentage of CCRF-CEM, JURKAT and MOLT-4 cells treated with 20 µM CFTR-inh172 in the SubG1, G1-phase was higher than in control cells, while the percentage of S-phase cells in these cells treated with 20 µM CFTR-inh172 was obviously decreased when compared to control cells (Fig. 3). These results indicated that CFTR-inh172 treatment arrested CCRF-CEM, MOLT-4 and JURKAT cells in the SubG1 and G1 phase.

3.4. Transcriptome analysis showed that significant changes of gene expression after CFTR-inh172 treatment in T-cell acute lymphoblastic leukemia cells

To further understand the molecular mechanism underlying CFTR-inh172 function in T-ALL cells, we treated CCRF-CEM, JURKAT and MOLT-4 cells with 20 µM CFTR-inh172 or DMSO for 24 h and performed transcriptome analysis (Fig. 4A). In comparison to control cells, 71 genes were up-regulated and 48 genes were down-regulated

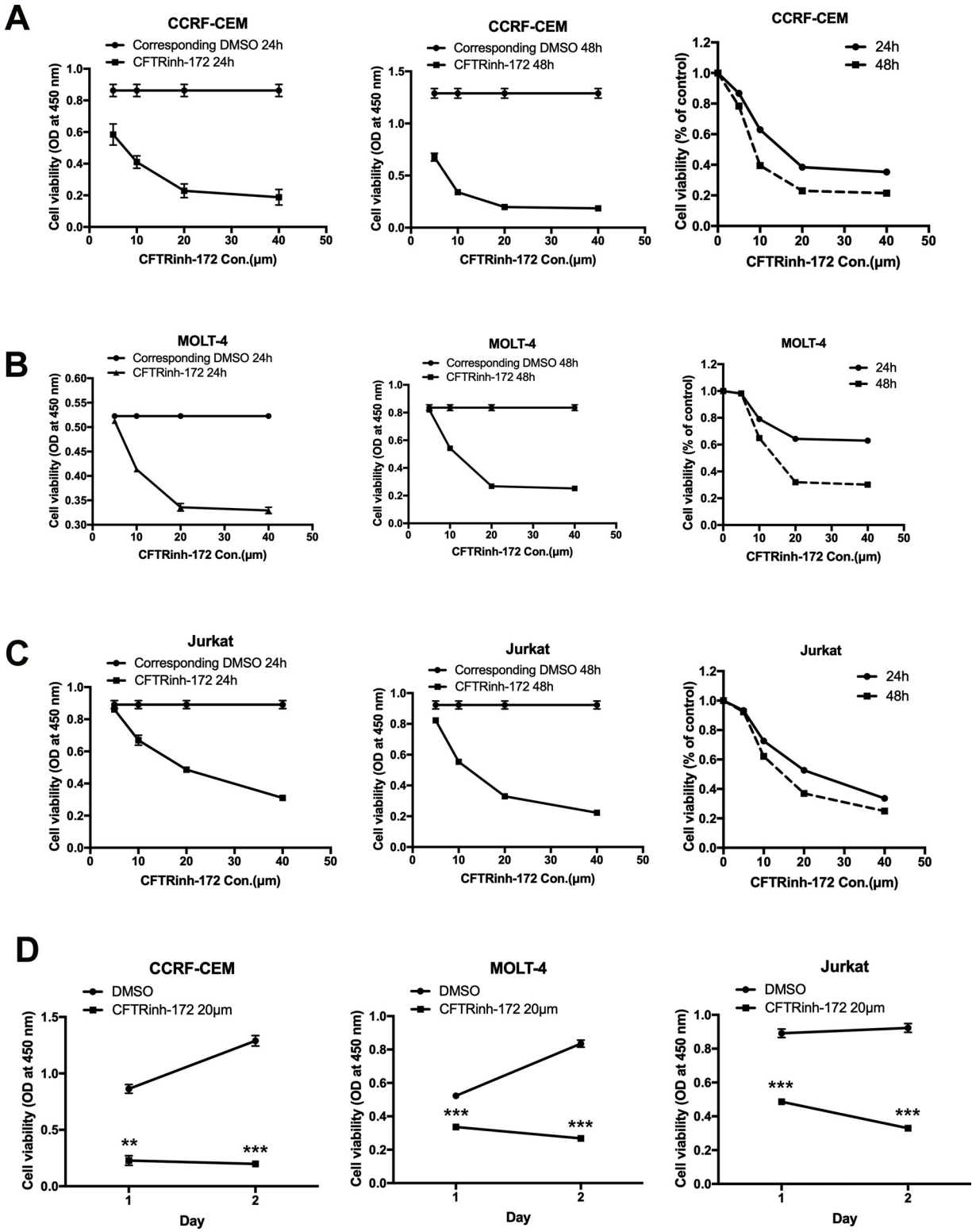

Fig. 1. Effects of CFTRinh-172 treatment on proliferation in T-ALL cells.
CCK-8 assays revealed that treatment of CCRF-CEM, JURKAT and MOLT-4 cells with CFTRinh-172 reduced cell proliferation in a concentration-dependent and time-dependent manner. Data are shown as means ± SD of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 vs DMSO.

significantly in CCRF-CEM cells treated with CFTR-inh172 (log2 fold change ≥ 1; probability > 0.8) (Supplementary Table 2, SRA accession: PRJNA530806, release date: 2019-05-01); 54 genes were up-regulated and 44 genes were down-regulated significantly in JURKAT cells treated with CFTR-inh172 (Supplementary Table 3, SRA accession: PRJNA559264, release date: 2019-08-25); 61 genes were up-regulated and 51 genes were down-regulated significantly in MOLT-4 cells treated with CFTR-inh172 (Supplementary Table 4, SRA accession: PRJNA559241, release date: 2019-08-25). GO and KEGG pathway enrichment analysis demonstrated that the genes up-regulated or down-regulated by CFTR-inh172 were involved in various signaling pathways, including the Wnt/TGF-beta signaling pathway, the cell cycle,

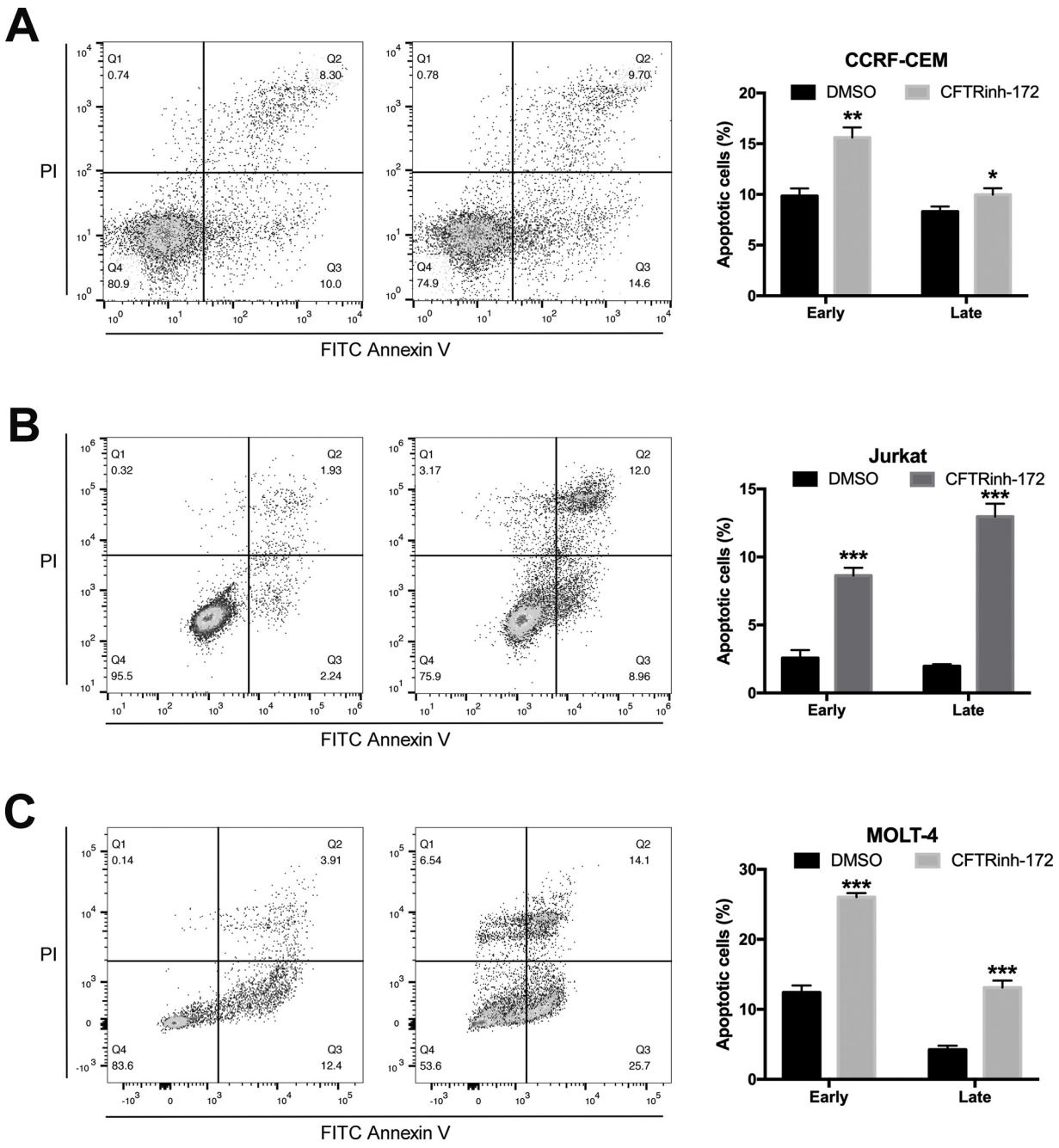

Fig. 2. Effects of CFTRinh-172 treatment on apoptosis in leukemic cells.
CCRF-CEM, JURKAT and MOLT-4 cells treated with CFTRinh-172 were analyzed by flow cytometry for Annexin V + /PI− and Annexin V + /PI + cells. Data are shown as means ± SD of three independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 vs DMSO.

apoptotic process, cell proliferation, programmed cell death, etc (Supplementary Figs. 4–8).

Then, we selected 12 representative genes with significant expression change for further identification in CCRF-CEM, MOLT-4 and JURKAT cells. Protein phosphatase 1, regulatory subunit 15A (PPP1R15A), also known as growth arrest and DNA damage-inducible protein GADD34, plays a vital role in promoting cell death and the unfolded protein response (UPR) [7]; jun, an important component of activating protein-1 (AP-1) transcription factor, is closely related to cell proliferation, apoptosis and malignant transformation [8]; CEBPB play roles in a wide range of cellular processes, such as cellular apoptosis, proliferation, adipocyte differentiation, carbohydrate metabolism and inflammation, and is associated with ER stress and contributes to tumor cell death and migration [9]; NGFR, also known as p75 neurotrophin receptor (p75NTR) and CD271, is a cell surface receptor that belongs to the tumor necrosis factor receptor superfamily, which contributes to the tumor-initiating capacity of a number of malignancies [10]; Thioredoxin-interacting protein (TXNIP) is an endogenous inhibitor of thioredoxin (TRX) and regulates the oxidative stress in cells facing various stress together with glutathione, playing an essential role in diverse biological processes, including regulation of oxidative stress, inflammation, glucose and lipid metabolism, and cell apoptosis [11]; The activating transcription factor 3 (ATF3) is an ATF/CREB family member whose expression is rapidly induced by a wide range of cellular stresses including DNA damage, cellular injury and oxidative stress, and strongly associated with tumor cell apoptosis [12]; C1orf167, Chromosome 1 open reading frame 167, has been explored associated with cancer development by high-throughput sequencing [13]; Midkine

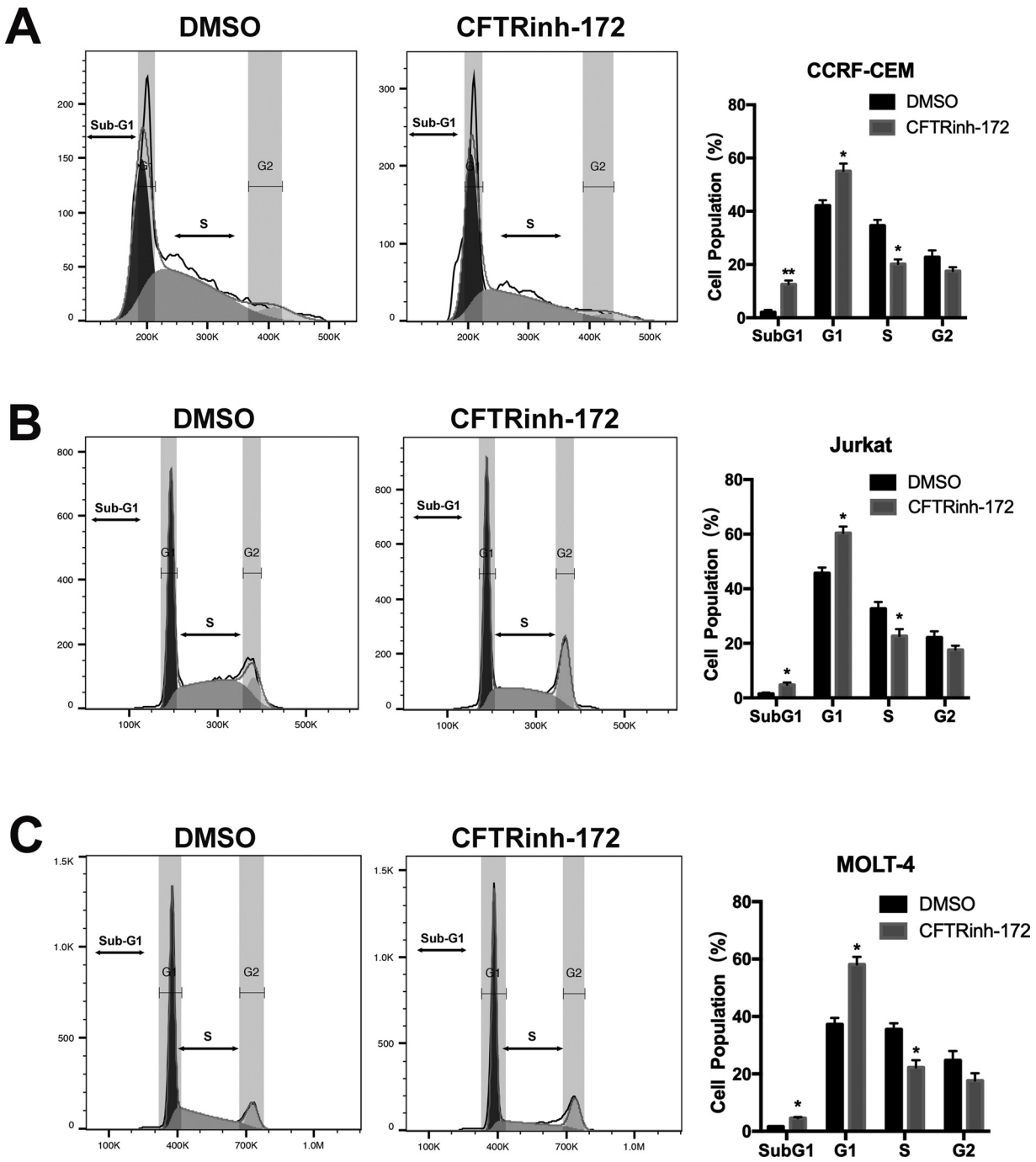

Fig. 3. Effects of CFTRinh-172 treatment on cell cycle progression in leukemic cells. Flow cytometric analysis of the cell cycle following PI staining; representative histograms of CCRF-CEM, JURKAT and MOLT-4 cells treated with CFTRinh-172 are shown. The data are expressed as the percentage of cells in the SubG1, G1 phase, S phase and G2 phase of the cell cycle. Data are shown as means ± SD of three independent experiments. **P < 0.01, ***P < 0.001 vs DMSO.

(MDK) is a heparin-binding growth factor expressed at abnormally high levels in several human cancers, which has been found to exacerbate disease by promoting many tumor specific functions, including cell growth, tumor cell survival, cell migrations, and carcinogenesis [14]; Rock1p1, key factors in the Rho/Rho-associated coiled-coil-forming protein kinase (Rock) pathway, is important in TGF-β inhibition of cell proliferation and links signaling components with regulation of cell-cycle progression [15]; IGHV3-33-2 and IGHV3-35 are belong to immunoglobulin heavy chain protein, which effect on growth, viability and capacity for proliferation of cancer cells [16]; DNA damage-inducible transcript 3 (Ddit3), a member of the CCAAT/enhancer-binding protein family of transcription factors, is known to be important apoptotic signaling molecules [17]. Quantitative PCR results showed that all detected genes demonstrated similar expression pattern in tested cell lines, suggesting that CFTR regulated T-ALL apoptosis, cell cycle, and cell proliferation via various signaling pathway (Fig. 5).

4. Discussion

Our previous work showed that the regulatory role of CFTR-dependent Wnt signaling in hematopoiesis is evolutionally conserved in human [2]. This is highlighted by concomitant upregulation of CFTR and Wnt signaling in human blood system [2]. Aberrantly activated Wnt signaling has been implicated in human leukemia pathogenesis

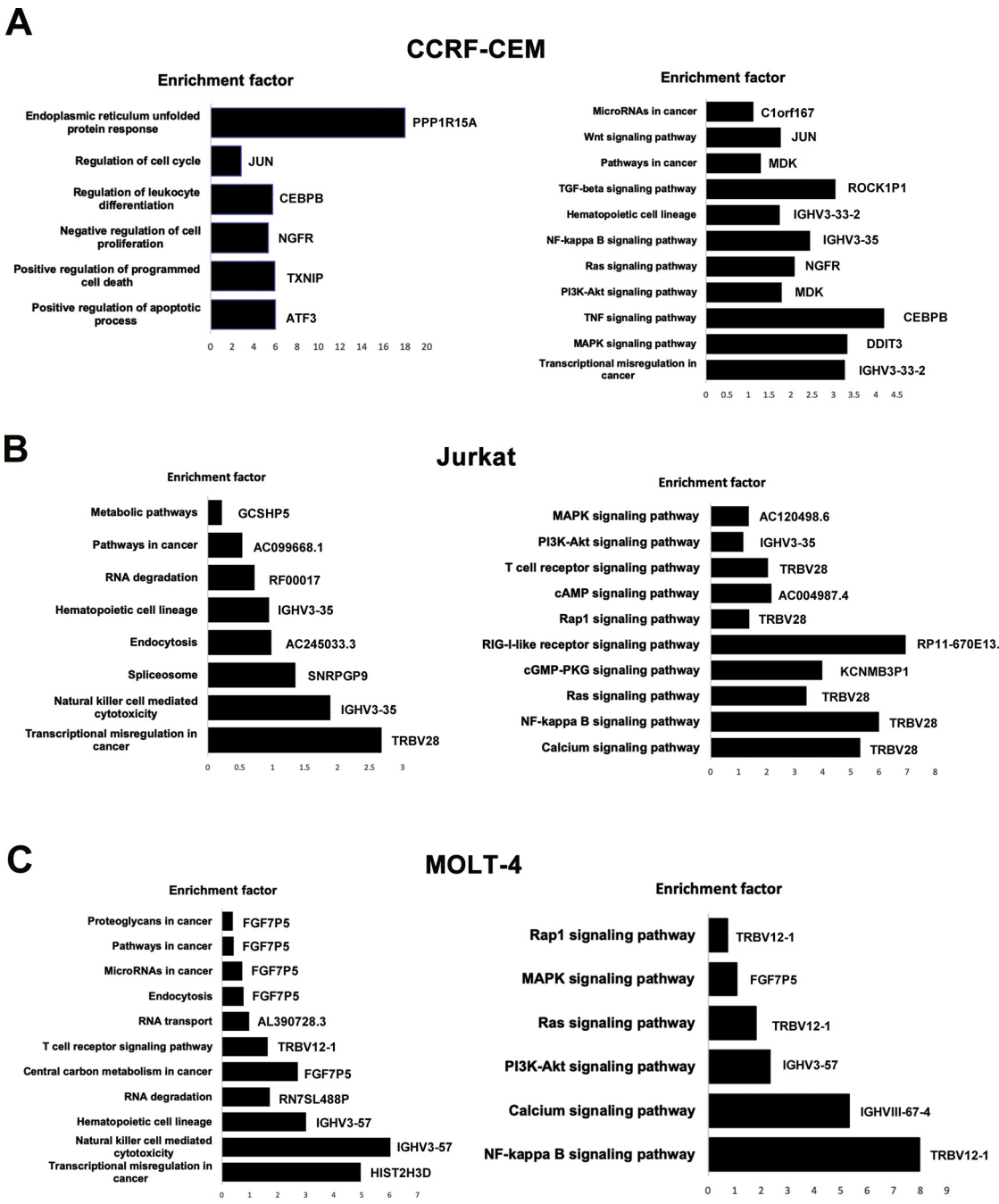

Fig. 4. CFTRinh-172 treatment in T-ALL cells affects the expression of components of apoptotic and proliferative pathway. GO (left pannel) and pathway (right pannel) analysis of gene expression in CCRF-CEM (A), Jurkat (B) and MOLT-4 (C) cells treated with 20 μm CFTRinh-172. Key gene of each progress is shown at the right side of bar.

[18,19]. The demonstrated CFTR involvement in Wnt-dependent hematopoiesis prompted us to propose whether the expression levels of CFTR are associated with leukemia. On the other side, it is important to note that arsenic, an ancient Chinese remedy that has attracted worldwide interest because of its substantial anti-leukemia activity [20], has also been shown to promote ubiquitinylation and lysosomal degradation of CFTR in human airway epithelial cells [21]. Our previous study not only offered novel and important mechanistic insights that explain the observed therapeutic effect of arsenic in acute leukemia, but also suggested that CFTR may be have potential to be a new target to treat leukemia.

Next, we demonstrated that the mean expression level of CFTR in primary acute leukemia cells and cell line was markedly higher than that in normal human mononuclear cells (MNCs) and nonleukemia cell line. Furthermore, we revealed that CFTR interacts with PP2AA in the cytosol, resulting in PP2A complex inactivation and increased degradation of PP2A substrates via the lysosomal/proteasome pathway, which protected and maintained the continuous activation of BCR-ABL and the canonical Wnt/β-catenin signaling pathway in Philadelphia chromosome–positive (Ph+) acute leukemia [3]. Also, high-expression CFTR plays an important role in the development of Ph+ leukemia through the HDAC2-mediated PTEN pathway [4]. Taken together with our present work, the robust suppression of cell proliferation in acute lymphoblastic leukemia cell lines by the interference with CFTR or CFTR inhibitor CFTR-inh172 [3,4] suggests CFTR as a new target in the development of target gene therapy.

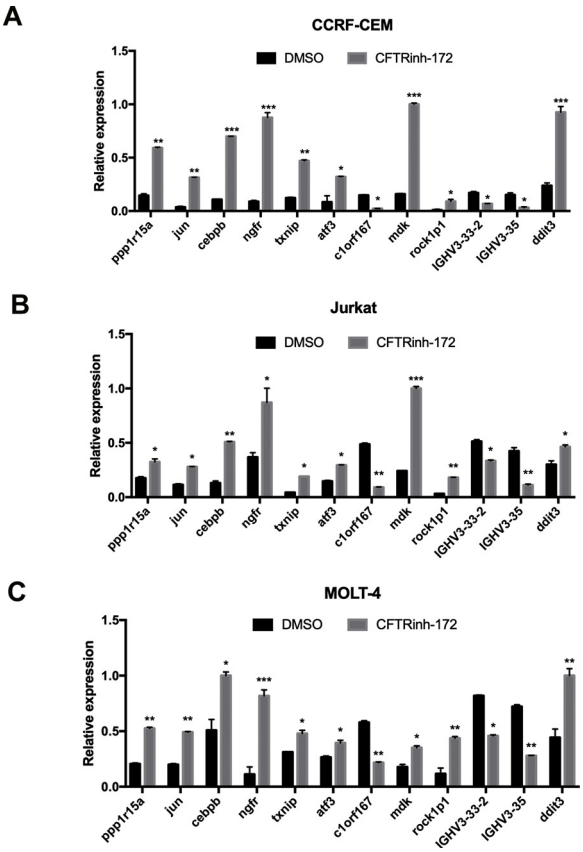

Fig. 5. Key molecules affected by CFTRinh-172 treatment were verified by real-time PCR in all detected T-ALL cells. (A) CCRF-CEM, (B) Jurkat and (C) MOLT-4 cells. The data represent the mean ± SD from three experiments. *P < 0.05, **P < 0.01 vs DMSO.

Author contributions statement

M. L., and H. S. conceived and designed the experiments; M. L., H. L., Y. C. and Z. L. performed the experiments; M. L., H. S. and X. Z. analyzed the data; Y. L. and H. C. C coordinated the project; H.S. and M. L. wrote the paper.

Declaration of Competing Interest

All the authors listed declare no competing financial interests and have approved the manuscript that is enclosed.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.106225.

References

[1] D.C. Gadsby, P. Vergani, L. Csanady, The ABC protein turned chloride channel whose failure causes cystic fibrosis, Nature 440 (7083) (2006) 477–483, https://doi.org/10.1038/nature04712.

[2] H. Sun, Y. Wang, J. Zhang, Y. Chen, Y. Liu, Z. Lin, M. Liu, K. Sheng, H. Liao, K.S. Tsang, X. Zhang, X. Jiang, W. Xu, M. Mao, H.C. Chan, CFTR mutation enhances Dishevelled degradation and results in impairment of Wnt-dependent hematopoiesis, Cell Death Dis. 9 (3) (2018) 275, https://doi.org/10.1038/s41419-018-0311-9.

[3] X. Yang, T. Yan, Y. Gong, X. Liu, H. Sun, W. Xu, C. Wang, D. Naren, Y. Zheng, High CFTR expression in Philadelphia chromosome-positive acute leukemia protects and maintains continuous activation of BCR-ABL and related signaling pathways in combination with PP2A, Oncotarget 8 (15) (2017) 24437–24448, https://doi.org/10.18632/oncotarget.15510.

[4] T. Yan, Y. Leng, X. Yang, Y. Gong, H. Sun, K. Wang, W. Xu, Y. Zheng, D. Naren, R. Shi, High-expressing cystic fibrosis transmembrane conductance regulator interacts with histone deacetylase 2 to promote the development of Ph+ leukemia through the HDAC2-mediated PTEN pathway, Leuk. Res. 57 (2017) 9–19, https://doi.org/10.1016/j.leukres.2017.02.004.

[5] K. Karrman, B. Johansson, Pediatric T-cell acute lymphoblastic leukemia, Genes Chromosomes Cancer 56 (2) (2017) 89–116, https://doi.org/10.1002/gcc.22416.

[6] E.A. Raetz, D.T. Teachey, T-cell acute lymphoblastic leukemia, Hematol. Am. Soc. Hematol. Educ. Program 2016 (1) (2016) 580–588, https://doi.org/10.1182/asheducation-2016.1.580.

[7] Y. Song, S. Liu, Z. Zhao, Y. Zhang, Y. Yang, B. Luo, Relationship between PPP1R15A gene polymorphism (rs611251) and Epstein-Barr virus-associated tumors, Acta Virol. 61 (4) (2017) 445–452, https://doi.org/10.4149/av_2017_407.

[8] S.Y. Guo, X.D. Zhu, L.Y. Ge, S. Qu, L. Li, F. Su, Y. Guo, RNAi-mediated knockdown of the c-jun gene sensitizes radioresistant human nasopharyngeal carcinoma cell line CNE-2R to radiation, Oncol. Rep. 33 (3) (2015) 1155–1160, https://doi.org/10.3892/or.2014.3692.

[9] Y. Bai, Y. Wei, L. Wu, J. Wei, X. Wang, Y. Bai, C/EBP beta mediates endoplasmic reticulum stress regulated inflammatory response and extracellular matrix degradation in LPS-stimulated human periodontal ligament cells, Int. J. Mol. Sci. 17 (3) (2016) 385, https://doi.org/10.3390/ijms17030385.

[10] C. Chen, J.H. Shin, J.T. Eggold, M.K. Chung, L.H. Zhang, J. Lee, J.B. Sunwoo, ESM1 mediates NGFR-induced invasion and metastasis in murine oral squamous cell carcinoma, Oncotarget 7 (43) (2016) 70738–70749, https://doi.org/10.18632/oncotarget.12210.

[11] L. Xu, X. Lin, M. Guan, Y. Zeng, Y. Liu, Verapamil attenuated prediabetic neuropathy in high-fat diet-fed mice through inhibiting TXNIP-mediated apoptosis and inflammation, Oxid. Med. Cell. Longev. 2019 (2019) 1896041, , https://doi.org/10.1155/2019/1896041.

[12] Z. You, J. Xu, B. Li, H. Ye, L. Chen, Y. Liu, X. Xiong, The mechanism of ATF3 repression of epithelial-mesenchymal transition and suppression of cell viability in cholangiocarcinoma via p53 signal pathway, J. Cell. Mol. Med. 23 (3) (2019) 2184–2193, https://doi.org/10.1111/jcmm.14132.

[13] K. Wang, S.T. Yuen, J. Xu, S.P. Lee, H.H. Yan, S.T. Shi, H.C. Siu, S. Deng, K.M. Chu, S. Law, K.H. Chan, A.S. Chan, W.Y. Tsui, S.L. Ho, A.K. Chan, J.L. Man, V. Foglizzo, M.K. Ng, A.S. Chan, Y.P. Ching, G.H. Cheng, T. Xie, J. Fernandez, V.S. Li, H. Clevers, P.A. Rejto, M. Mao, S.Y. Leung, Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer, Nat. Genet. 46 (6) (2014) 573–582, https://doi.org/10.1038/ng.2983.

[14] M.C. Ma, Y.J. Chen, T.J. Chiu, J. Lan, C.T. Liu, Y.C. Chen, H.H. Tien, Y.Y. Chen, Positive expression of Midkine predicts early recurrence and poor prognosis of initially resectable combined hepatocellular cholangiocarcinoma, BMC Cancer 18 (1) (2018) 227, https://doi.org/10.1186/s12885-018-4146-7.

[15] N.A. Bhowmick, M. Ghiassi, M. Aakre, K. Brown, V. Singh, H.L. Moses, TGF-beta-induced RhoA and p160ROCK activation is involved in the inhibition of Cdc25A with resultant cell-cycle arrest, Proc. Natl. Acad. Sci. U. S. A. 100 (26) (2003) 15548–15553, https://doi.org/10.1073/pnas.2536483100.

[16] Z. Chen, X. Qiu, J. Gu, Immunoglobulin expression in non-lymphoid lineage and neoplastic cells, Am. J. Pathol. 174 (4) (2009) 1139–1148, https://doi.org/10.2353/ajpath.2009.080879.

[17] S.B. Syc-Mazurek, K.A. Fernandes, M.P. Wilson, P. Shrager, R.T. Libby, Together JUN and DDIT3 (CHOP) control retinal ganglion cell death after axonal injury, Mol. Neurodegener. 12 (1) (2017) 71, https://doi.org/10.1186/s13024-017-0214-8.

[18] N.I. Khan, K.F. Bradstock, L.J. Bendall, Activation of Wnt/beta-catenin pathway mediates growth and survival in B-cell progenitor acute lymphoblastic leukaemia, Br. J. Haematol. 138 (3) (2007) 338–348, https://doi.org/10.1111/j.1365-2141.2007.06667.x.

[19] T.C. Luis, M. Ichii, M.H. Brugman, P. Kincade, F.J. Staal, Wnt signaling strength regulates normal hematopoiesis and its deregulation is involved in leukemia development, Leukemia 26 (3) (2012) 414–421, https://doi.org/10.1038/leu.2011.387.

[20] B.C. Bornhauser, L. Bonapace, D. Lindholm, R. Martinez, G. Cario, M. Schrappe, F.K. Niggli, B.W. Schafer, J.P. Bourquin, Low-dose arsenic trioxide sensitizes glucocorticoid-resistant acute lymphoblastic leukemia cells to dexamethasone via an Akt-dependent pathway, Blood 110 (6) (2007) 2084–2091, https://doi.org/10.1182/blood-2006-12-060970.

[21] J.M. Bomberger, B.A. Coutermarsh, R.L. Barnaby, B.A. Stanton, Arsenic promotes ubiquitinylation and lysosomal degradation of cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels in human airway epithelial cells, J. Biol. Chem. 287 (21) (2012) 17130–17139, https://doi.org/10.1074/jbc.M111.338855.