Regulating TKT activity inhibits proliferation of human acute lymphoblastic leukemia cells

Fang-Liang Huang
Children's Medical Center, Taichung Veterans General Hospital

Yao-Ming Chang
Academia Sinica

Cheng-Yung Lin
MacKay Medical College

Sheng-Jie Yu
Taichung Veterans General Hospital

Jing-Tong Fu
Taichung Veterans General Hospital

Ting-Yu Chou
Taichung Veterans General Hospital

Sih-Wen Yeh
Children's Medical Center, Taichung Veterans General Hospital

En-Chih Liao
MacKay Medical College

Chia-Ling Li
lingboxer@gmail.com
Children's Medical Center, Taichung Veterans General Hospital

Research Article

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Abstract

Background

Among pediatric blood cancers, acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy. Within ALL, the T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10 to 15% of all pediatric cases, and ~ 25% of adult cases. Their overall survival rate is 80%. The overall prevalence of ALL was 4/100,000 population. In Taiwan, it accounts for 25 to 30% of all childhood cancers and with ~ 200 newly diagnosed cases every year. Its recurrence and relapse after treatment remain problematic. It is therefore the need to develop new therapies for patients with T-ALL. Recent studies suggested regulating energy metabolism is a novel approach to inhibit tumor growth, likely a promising treatment of this cancer. Transketolase (TKT) is an important enzyme for modulating glucose metabolize in the pentose phosphate pathway.

Methods

Human T-ALL cell lines were treated with different doses of niclosamide and primary T-ALL PBMCs were analyzed by RNA sequencing. Cell viability were analyzed by CCK-8 assay. Human T-ALL cells treated with niclosamide were also conducted for Western blotting analysis and TKT activity assay. Metabolism evaluation of T-ALL cells were analyzed by ATP assay and seahorse analyses. Last, the effect of knockdown TKT on T-ALL tumor growth was examined in a T-ALL xenograft murine model. Tumor samples from T-ALL xenograft murine model were analyzed by hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) staining.

Results

In our study, we demonstrated that niclosamide reduced cell viability of T-ALL cells, and it also reduced expressions of TKT, TKTL1/2, transaldolase. In addition, niclosamide inhibited TKT enzyme activity, aerobic metabolism and glycolysis, finally leading to reduced ATP production. The tumor growth of xenograft T-ALL mice were inhibited by the knockdown of TKT.

Conclusions

Findings showed that niclosamide inhibits T-ALL cell growth by inhibiting TKT and energy metabolism.

1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is an immature lymphoid tumor characterized by malignant hematopoietic cells that diffusely infiltrate the bone marrow, and these cells express immature T cell markers[1, 2]. In Europe, the United States, and Japan, T-ALL accounts for 10 to 15% of pediatric ALL
cases, and 20 to 25% of adult ALL cases[3]. In children, T-ALL has an 85% overall complete remission rate, mainly due to advances in treatment. In contrast, the similar rate in adult T-ALL remains at about 40%[4]. In addition, a portion of T-ALL patients (20 to 25%) are completely refractory from the start of treatment[5]. Due to the low response to standard chemotherapy, T-ALL children often die from disease recurrence. When these cancer cells proliferate, the disease reappears. Therefore, there are still unmet medical needs. The medical problems that need to be solved include the high risk of recurrence after the first remission, and the treatment becomes a challenging problem after recurrence[6]. These issues still need to be resolved.

The main pathway of glucose consumption for cancer is the transformation to lactate, producing adenosine triphosphate (ATP). This is a much faster process than going through the tricarboxylic acid cycle (TCA cycle) in mitochondria. However, this pathway has low energy output resulting in consuming more glucose and producing other metabolites[7]. This cancer hallmark is an escape way for tumor cells to bypass immune attack, and thus can proliferate and maintain malignancy[8]. Altered metabolism of cancer cells can be the result of impaired function of mitochondria. Since the glycolytic pathway is an intermediate and signaling network, targeting this pathway is a promising therapeutic approach[9].

Transketolase (TKT) is encoded by the TKT gene. It is an enzyme used both in the pentose phosphate pathway in all organisms, and in the Calvin cycle of photosynthesis in plants. In mammals, TKT connects the pentose phosphate pathway to glycolysis, feeding excess sugar phosphates into the main carbohydrate metabolic pathways. Cancer cells, even with abundant oxygen supply, tend to generate ATP using glycolysis, despite lower ATP per glucose molecule, rather than using the oxidative phosphorylation as in normal cells. This phenomenon of aerobic glycolysis is known as the “Warburg effect.” In glycolysis, the glucose uptake by cancer cells is higher than normal cells, with lactic acid produced from pyruvate. Thus the pH of tumor cells is lower[10]. In rapidly growing tumors, the insufficient blood supply and high energy consumption lead to hypoxia. Consequently, oxidative phosphorylation is inactivated, mitochondrial respiration is then switched to glycolysis, accompanied by mitochondrial dysfunction[11, 12].

TKT overexpression has been recently demonstrated to correlate with tumorigenesis, such as (a) development of hepatocellular carcinoma in a non-metabolic manner via its nuclear localization and EGFR pathway[13], (b) in peritoneal metastases of ovarian cancer[14] and (c) in esophageal cancer by promoting cell invasion via mediating the EMT process. Recent advances in medical technology have led to new treatment strategies based on deeper knowledge on the molecular and cellular mechanisms of tumorigenesis. A promising strategy is key enzyme inhibitions in glycolysis resulting in less energy production. For example, in esophageal cancer, anti-TKT inhibited cell migration and invasion[15] or in human colon cancer cells, inhibiting transketolase-like-1 gene expression significantly reduced TKT levels and markedly inhibited cell proliferation[16]. These results all suggested that TKT may serve as a target for novel anticancer therapies. However, there is no report regarding TKT inhibition on treating T-ALL. Here, we hypothesized that knockdown TKT has anticancer effects on TALL. In this study, we investigated the anticancer effect by targeting TKT in the T-ALL model.
2. Materials and Methods

Cells and Drug Treatments

We kept at 37 °C, two human TALL cell lines: Jurkat and CCRFCEM (obtained from the American Type Culture Collection; ATCC), in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with the following: 10% heat inactivated fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences), 1 mM sodium pyruvate (HyClone; GE Healthcare Life Sciences), 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were maintained in a humidified atmosphere containing 95% air and 5% CO₂. Cells were then treated with niclosamide (ACROS Organics™) at different doses and time courses.

Primary T-ALL Cells

Primary T-ALL cells were harvested from PBMCs of either patients with T-ALL or healthy controls in Taichung Veterans General Hospital. For PBMC culture, 8 mL of blood were collected in sodium citrate tubes (Vacutainer® CPT™, BD, USA) after being taken from patients or healthy controls. PBMCs were purified through centrifugation down a density gradient. Cells were cultured in RPMI-1640, supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 25 mM HEPES, and 2 mM L-glutamine. The study protocol was approved by the Institutional Review Board of Taichung Veterans General Hospital, Taiwan (NO. CG19384A).

Isolation of RNA

Cells were collected from in vitro treatments, and harvested after drug treatments. Total RNA was extracted using the Amersham RNASpin Mini Kit (Cytiva) according to manufacturer's instructions.

RNA Sequencing

The quality of the raw reads were checked using FastQC (v0.11.9). Adapters, low quality bases and reads were trimmed with Trimmomatic (v0.36), using these parameters ILLUMINAACLIP: Adapter.fa:2:30:10 LEADING: 3 TRAILING: 3 SLIDINGWINDOW: 4:15 MINLEN: 36. After trimming, clean reads were aligned to the reference genome using HISAT2 (v2.2.1). StringTie (2.2.1) was used to normalize read counts. FeatureCounts (v1.6.2) were used to summarize reads. Differential expression analyses were performed using either DEGseq (v2.2.1) without biological replicates, or DESeq2 (1.34.0) with biological replicates. Differential expressions of gene sets were filtered based on the absolute value of log2fold change ≥ 2 and adjusted p-value < 0.005 according to results of DEGseq. Differentially expressed gene sets at statistical significance were filtered based on the absolute value of log2fold change ≥ 2 and adjusted p-value < 0.05 according to the results of DESeq2. To identify biological processes and pathways that are significantly enriched by the differentially expressed genes, the gene set enrichment analyses (or GSEA) were performed with the ClusterProfiler (4.2.2). The database of the Kyoto encyclopedia of genes and genomes (or KEGG) was used for annotation. Pathway visualization was performed using Pathview (1.34.0).
**CCK-8 assay**

Jurkat and CCRFCEM cells were seeded at a density of $1 \times 10^5$ cells/well in a 96-well cell culture plate. Jurkat cells were treated at 37°C for 24 h, with either vehicle control (DMSO) or with different doses of niclosamide. Afterward, cell viability was determined with the CCK-8 kit (cat. no.96992, Sigma-Aldrich) according to the manufacturer's instructions. In addition, optical density was measured at 450 nm with a microplate reader (BioTek Synergy HT).

**Western blot analysis**

Jurkat and CCRFCEM cells were seeded at a density of $1.5 \times 10^6$ cells/well in a 12well cell culture plate. Jurkat cells were treated with either vehicle control (DMSO) or niclosamide (1.0, 2.0 and 4.0 µM). CCRFCEM cells were treated with either vehicle control (DMSO) or niclosamide (1.0, 2.0 and 4.0 µM). Both cell cultures were kept similarly at 37 °C for 24 h. After niclosamide treatment, cells were harvested and levels of protein expressions were evaluated with western blotting using appropriate antibodies. In brief, cells were first washed with PBS before collection. Cells were lysed in RIPA buffer (Biomed, Taiwan), and centrifuged at 13,000 g for 10 min at 4 °C. The supernatant was used for protein quantification with a Coomassie protein assay reagent (Thermo Fisher Scientific, Inc.). Fixed amounts of protein (15 µg/lane) were loaded into each well and separated by SDS-PAGE. Samples were finally transferred to the PVDF membrane. Immunoblotting was conducted using the following antibodies: Transketolase (TKT) (cat. no. sc-390179; Santa Cruz; 1:1,000 dilution), Transketolase Like 1/2 (TKTL1/2) (cat. no. sc-514513; Santa Cruz; 1:500 dilution), Transaldolase (cat. no. sc-166230; Santa Cruz; 1:1,000 dilution) and βactin (cat. no. 4970S; Cell Signaling; 1:5,000 dilution), followed by incubation with secondary antimouse IgG, HRPlinked antibody (cat. no. 7076P2; Cell Signaling Technology; 1:7,000 dilution) or goat antirabbit IgG antibody (cat. no. 7074P2; Cell Signaling Technology; 1:7,000 dilution). Labeled proteins were detected using the ECL Detection Kit (Millipore) from the Alliance Q9 (UVITEC, UK). Normalization was performed with the βactin antibody.

**Measuring ATP levels**

ATP measurements were carried out with the ATP Assay Kit Colorimetric/Fluorometric kit (ab83355, Abcam, USA) according to the manufacturer’s instructions. In brief, $1 \times 10^6$ cells were washed in cold PBS and then homogenized in 100 µl of ATP assay buffer. Cells were centrifuged at 4 °C at 13,000 g and then the supernatant was collected. Standard samples were prepared according to manufacturer instructions. Test samples and background controls were prepared in a 96-well plate according to manufacturer’s instructions for colorimetric assay. Finally, plates were read with a plate reader (Enspire 2300-0000/PerkinElmer) at OD 570 nm.

**TKT activity assay**

TKT activity was measured with the Transketolase Activity Assay Kit (Fluorometric) (ab273310, Abcam, USA) according to the manufacturer’s instructions. In brief, cells ($4 \times 10^5$) were homogenized in a 100 µl
TKT Assay buffer for lysis, and then centrifuged at 4 °C at 10,000 g. Supernatants were then collected. Standard samples were prepared according to manufacturer instructions. Test samples and background control were prepared in a 96-well plate according to manufacturer instructions for fluorometric assay. Fluorescence was measured immediately at 30 sec intervals for 30 to 45 min at 37°C.

**Seahorse analyses**

Cells were plated in Seahorse XF 6-well plates at respective optimal densities one day prior to measurements. These cells were subjected to the experimental conditions as described above, and incubated in Seahorse XF Assay Media at 37°C for 1 hr without CO₂ just before assay. Substrate concentrations were as follows: 1 µM for Oligo and FCCP, 1 µM /0.5 µM for Rot/AA, and 5 mM for succinate. All reagents were from Seahorse Bioscience. Basic conditions of glycolysis, maximal glycolytic capacity, and non-glycolytic activity were measured with the Seahorse XF-6 Extracellular Flux Analyzer.

**T-ALL xenograft murine model**

Female NOD/SCID mice aged 8 weeks with body weight 18 to 22 g were obtained from the National Laboratory Animal Center (Taipei, Taiwan). Mice were housed under specific pathogen-free conditions with 12:12-h dark/light cycle, with food and water ad libitum. All experiments were approved by the Animal Care and Use Committee of Kaohsiung Veterans General Hospital (IACUC NO. 2021-2022-A003-MOST). CCRFCEM cells (1x10⁶ cells/100 µl/mouse), suspended in a 1:1 mixture of BD Matrigel™ (basement membrane matrix, growth factor reduced, phenol redfree; BD Biosciences, cat. no. 356231) and RPMI-1640 medium were injected subcutaneously into the ank region of NOD/SCID mice. Tumors were periodically measured with a digital caliper 3 times/week until sacrifice. Mice were randomly divided into different experiment groups (6 mice/group) that included: (a) vehicle control group, (b) niclosamide (20 mg/kg) treatment group, (c) α-KG (10 mg/kg) treatment group, and (d) α-KG (10 mg/kg) plus niclosamide (20 mg/kg) treatment group. Niclosamide and α-KG treatments were delivered through intraperitoneal injections, 3 times/week, beginning on day 7 after induction. Mice were sacrificed on day 28, and tumor samples collected for H&E and IHC stainings.

**Hematoxylin and eosin (H&E) staining**

Tumor tissues were removed from the xenograft mice and fixed in 10% formalin for 24 h at room temperature. Tissues were embedded in paraffin, and then sectioned for staining with hematoxylin and eosin (H&E). Microscopic images were captured under light microscopy (AXIOVERT, ZEISS).

**Immunohistochemistry (IHC) staining**

After tumor tissues had been removed from the xenograft mice, specimens were fixed in 10% phosphatebuffered formalin, dissected and then embedded in paraffin. Paraffin sections (3 µm in thickness) were incubated with 0. % hydrogen peroxide for 15 min, blocked for 1 h at room temperature,
and then incubated with Transketolase (TKT) (cat. no. sc-390179; Santa Cruz) and Ki-67 (cat. no. 9449S; Cell Signaling) overnight at 4 °C. After washing with TBST, sections were processed with the Epredia™ UltraVision™ Quanto Detection System HRP DAB kit (Epredia™ TL-060-QHD), and when required, immediately stained with DAB (Epredia™).

Statistical analyses

Data were expressed as mean ± standard error of mean. Statistical analyses were performed using oneway ANOVA followed by Tukey’s test for posthoc comparisons. Results were analyzed using the software GraphPad Prism (version 6; GraphPad Software, Inc.). Statistical significance was set at p < 0.05.

3. Results

3.1 Down-regulation of autophagy in T-ALL

We have previously reported that niclosamide suppresses Tcell acute lymphoblastic leukemia growth through activation of apoptosis and autophagy[17]. Here, we further compared expressions of autophagy-related genes between clinical PBMC samples obtained from T-ALL patients and healthy controls. KEGG autophagy pathway graph rendered by Pathview revealed down-regulated genes (in green), such as LC3 and p62 (Fig. 1A) that were involved in the autophagic pathway in T-ALL patients’ PBMC samples when compared with healthy controls. On the other hand, the same genes showed significant up-regulations in niclosamide-treated CCRF-CEM cells when compared with WT CCRF-CEM cells (Fig. 1B).

3.2 Niclosamide inhibited cell viability of T-ALL

To investigate the cell viability of T-ALL cells after niclosamide treatment, CCRFCEM and Jurkat cells were treated with different doses of niclosamide for 24 h. Results showed that niclosamide had inhibited T-ALL cell viability in a dose-dependent manner (Fig. 2).

3.3 Niclosamide inhibited ATP production in T-ALL cells

As shown in Fig. 2, cancer cell death was likely related to reduced sources of energy metabolism. Therefore we further determined possible changes in the final ATP production of T-ALL after niclosamide treatments. CCRFCEM and Jurkat cells were initially treated with different doses of niclosamide or vehicle control for 24 h. Results showed that the relative contribution of ATP production was lower in niclosamide treated CCRFCEM and Jurkat cells compared with vehicle controls (Fig. 3). Niclosamide also significantly inhibited ATP production at a dose-dependent manner. Taken together, our findings suggested that niclosamide inhibits ATP production in T-ALL in a dose-dependent manner.
3.4 Niclosamide inhibited pentose phosphate pathway related protein expressions in T-ALL cells

Metabolic reprogramming is considered a hallmark of cancer development. Cancer cells prefer glycolysis over oxidative phosphorylation to generate energy. Most leukemias are highly dependent on glycolysis and can be targeted using glycolytic inhibitors. TKT, TKTL1/2 and transaldolase (TALDO1) played important roles in connecting the pentose phosphate pathway (PPP) to glycolysis. Therefore, to investigate the effects of niclosamide on PPP pathway-related protein expressions, CCRFCEM and Jurkat human T leukemia cells were initially treated with different doses of niclosamide or vehicle control for 24 h. Results showed that niclosamide had inhibited expressions of PPP pathway-related proteins, such as TKT, TKTL1/2 and transaldolase, in CCRFCEM and Jurkat cells at a dose-dependent manner (Fig. 4A, B). Taken together, niclosamide was found to inhibit PPP related protein expressions of T-ALL cells in a dose-dependent manner.

3.5 Niclosamide inhibited TKT activity in T-ALL cells

TKT is the key enzyme of the non-oxidative phase of PPP and TKT provides a reversible link between glycolysis and PPP. As shown in Fig. 4, since niclosamide treatment reduces TKT protein levels, it is natural to further explore whether the activity of TKT enzyme is altered after the niclosamide treatment. To this end, CCRFCEM and Jurkat cells were first treated with niclosamide or vehicle control for 24 h. We found that niclosamide (at 4 µM) had inhibited TKT activity during 0 to 45 min in both CCRFCEM and Jurkat cells (Fig. 5). Findings suggested that in T-ALL cells, TKT activity was significantly inhibited after niclosamide treatment.

3.6 Niclosamide inhibited mitochondrial aerobic metabolism and glycolysis in T-ALL cells.

Furthermore, we used the Seahorse machine to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in T-ALL cells. We evaluated glycolysis by analyzing the ECAR, and we also measured mitochondrial aerobic metabolism based on OCR. We found that both OCR and ECAR were inhibited after niclosamide treatment (Fig. 6, 7). In response to niclosamide, we observed significant inhibitions in ATP-production coupled respiration (Fig. 6) and glycolysis (Fig. 7). Results indicated that niclosamide treatment inhibited mitochondrial aerobic metabolism and glycolysis in T-ALL cells.

3.7 Downregulation of TKT improved phenotype of xenograft T-ALL mouse model

To verify results found in in vitro experiments, that inhibiting TKT retarded T-ALL cell growth,, we further used a known TKT inhibitor, α-KG, to treat the xenograft T-ALL mice. We aimed to validate if tumor growth
is also inhibited. In brief, to confirm that the metabolic changes caused by TKT-knockdown can affect cancer growth in vivo, we used the xenograft T-ALL murine model for our test. Results showed that knockdown of TKT by addition of α-KG markedly reduced the tumor volume (Fig. 8). Niclosamide treatment or niclosamide plus α-KG treatment also significantly reduced tumor volume compared with vehicle controls.

3.8 Niclosamide improved pathology of xenograft T-ALL mice

Since downregulation of TKT inhibited tumor growth in xenograft T-ALL mice, we further investigated whether downregulation of TKT improves the pathology of xenograft T-ALL mice. Hence tumor samples were examined by H&E staining and IHC staining. H&E staining showed that tumor masses contained leukemia cells, with a discohesive pattern of medium to large atypical lymphoid cells displaying signs of brisk apoptosis and mitotic activity. Compared with the vehicle controls, the experimental groups showed more degenerative tumor cells with smudge nuclei and more apoptosis associated with niclosamide treatment, α-KG treatment and α-KG plus niclosamide treatments (Fig. 9A). IHC staining showed that TKT and Ki-67 expressions were lowered with niclosamide, α-KG and α-KG plus niclosamide treatments compared with the vehicle controls (Fig. 9B, C).

3.9 Niclosamide down-regulated PPP genes

Further bioinformatics analysis of CCRF-CEM cells treated with niclosamide revealed downregulation of key enzymes related to PPP, such as TKT, transaldolase and G6PD (Fig. 10A). The enrichment plot and heat map of differentially expressed genes indicated marked suppressions of PPP in niclosamide-treated CCRF-CEM cells. Gene Set Enrichment Analysis (GSEA) revealed that the PPP genes were highly enriched in control CCRF-CEM cells, with a concomitant drop in expressions of TKT, transaldolase and G6PD in niclosamide-treated CCRF-CEM cells (Fig. 10B). Taken together, niclosamide was found to inhibit key enzymes of PPP in T-ALL cells.

4. Discussion

Our principal finding is that niclosamide inhibited T-ALL cell viability. In addition, niclosamide also inhibited glycolysis pathway-related protein expressions, TKT activity and ATP production, including mitochondrial aerobic metabolism and glycolysis. Furthermore, we found that downregulation of TKT inhibited tumor growth in the xenograft T-ALL mouse model. In summary, niclosamide improved T-ALL by regulating energy metabolism.

Metabolic reprogramming is an important hallmark of cancer. During the development of malignant tumors, cancer cells consume more glucose than normal cells to support abnormal cell growth, enhancing their ability to metastasize, and adapting to the stress of survival in the harsh tumor microenvironment[18]. TKT is the major reversible enzyme in the nonoxidative branch of PPP[19]. Previous studies showed that TKT is upregulated in various tumors like colorectal cancer[20], ovarian...
cancer[14] and lung adenocarcinoma[21]. However, the expression of TKT in T-ALL remains unknown. Our present results indicated that PPP related proteins, such as TKT, TKTL1/2, transaldolase were suppressed. Inhibition of TKT reduced tumor growth in the xenograft T-ALL mouse model. These findings confirm TKT as a prognostic biomarker and therapeutic target in T-ALL.

Maintaining stable glucose metabolism is an important requirement for the survival and progression of cancer cells. Cancer cells rely on aerobic glycolysis to meet metabolic demands. The growth of cancer cells is dominated by metabolism switching from mitochondrial respiration to glycolysis during hypoxia[11, 12]. In our study, we found that niclosamide inhibited ATP production of T-ALL, and through inhibitions of aerobic metabolism and glycolysis, especially by suppressing expressions and activities of those enzymes in PPP, to reduce the energy source of cancer cells, leading to cell death. These results are consistent with previous reports. Many compounds have anticancer effects in preclinical experiments. These compounds include Phloretin, WZB117, Fasentin are glucose transporter blockers[22, 23]. Another glucose analog, 2-deoxyglucose (2-DG), showed promising anticancer effects in preclinical models. 2-DG inhibits glycolysis and depletes ATP, has been applied to treat lymphoma[24] and breast cancer[25]. Therefore, based on our previous findings and results from the literature, tumor glycolysis appears to be an ideal target for therapeutic intervention.

A number of studies reported that the anticancer effect of niclosamide is mediated through apoptosis, inhibiting tumor cell migration, invasion, and proliferation[26]. Our results demonstrated that niclosamide is a potent TKT inhibitor, which can inhibit TKT activity, leading to suppression of T-ALL cell proliferation and tumor growth. Niclosamide treatment in T-ALL not only inhibited the expression of TKT protein, but also reduced TKT activity, more effectively inhibited aerobic metabolism and glycolysis of cancer cells, and it thus reduced their energy sources. Our further experiments showed that, in addition to niclosamide treatment, adding α-KG effectively inhibited tumor growth of T-ALL. We validated that niclosamide treatment significantly inhibited viability of T-ALL. However, α-KG plus niclosamide treatment did not yield a better effect in vivo. Such a discrepancy in results needs to be carefully studied in future.

Some limitations in our study are as follows. First, our clinical sample size was too small. In the future, we plan to increase the number of clinical cases for further study. Second, establishment of TKT knockout mice model may be a better strategy for studying TKT in the future.

In conclusion, we discovered that reducing the ATP production, including OCR and ECAR levels via knockdown of TKT is an important factor in suppressing cell proliferation of T-ALL. This is a first study showing that niclosamide has an anti-leukemia effect through downregulating TKT and energy metabolism.

Declarations

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

The present clinical study protocol was approved by the Institutional Review Board of Taichung Veterans General Hospital, Taiwan (NO. CG19384A). The present animal experimental protocol were approved by the Animal Care and Use Committee of Kaohsiung Veterans General Hospital (IACUC NO. 2021-2022-A003-MOST).

Authors' contributions


Availability of data and materials

Data Availability Statement: Data sharing is not applicable to this article.

References


**Figures**
KEGG pathway graph rendered by Pathview. KEGG Pathview showing autophagy pathway genes up or down regulated in (A) T-ALL patients vs. healthy control and in (B) niclosamide (2 mM; 24 h) treated CCRF-CEM cells compared to WT CCRF-CEM cells.

**Figure 2**

A

![Graph A](image)

B

![Graph B](image)

**Figure 2**

Niclosamide effectively inhibits the viability of T-ALL cells in a dose-dependent manner. Human T-ALL cells were treated with niclosamide for 24 h. Cell viability and proliferation was measured by CCK-8 assays. CCK-8 assays showed that niclosamide inhibited the proliferation of (A) CCRF-CEM and (B) Jurkat cells dose-dependently. The results are expressed as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, vs. the control group; #P<0.05, ##P<0.01, ###P<0.001, ####P<0.0001, vs. the DMSO group.

**Figure 3**

A

![Graph C](image)

B

![Graph D](image)

**Figure 3**
Niclosamide effectively inhibits ATP production of T-ALL cells dose-dependently. The cells were treated with different doses of niclosamide for 24 h. ATP production were measured by ATP assays. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, vs. the control group; #P<0.05, ##P<0.01, ###P<0.001, ####P<0.0001, vs. the DMSO group. DMSO group: vehicle control group.

Figure 4

Niclosamide inhibits TKT, TKTL1/2 and transaldolase expressions of T-ALL cells in a dose-dependent manner. The cells were treated with different doses of niclosamide for 24 h, and the expression of TKT, TKTL1/2 and transaldolase were measured by western blot analysis in (A) CCRF-CEM and (B) Jurkat cells. β-actin protein was used in these experiments as the loading control. The results are expressed as mean ± SEM. *P<0.05, **P<0.01, ****P<0.0001, vs. the control group; #P<0.05, ##P<0.01, ###P<0.001, vs. the DMSO group. DMSO group: vehicle control group.
Niclosamide treatment inhibits TKT activity in T-ALL cells. The absolute specific TKT activities were determined and then compared. The results are expressed as mean ± SEM. ***P<0.001, vs. the DMSO group.
Niclosamide treatment affects mitochondrial oxygen consumption in T-ALL cells. Niclosamide treatment decreased oxygen consumption rate (OCR) in (A) CCRF-CEM and (B) Jurkat cells. Following a sequential addition of inhibitors of mitochondrial function: oligomycin, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP), and a combination of rotenone and antimycin A. Maximal respiration was measured following the addition of FCCP. The results are expressed as mean ± SEM. *p<0.05, ****p<0.0001 vs. control group.
Figure 7

Niclosamide treatment affects extracellular acidification rate (ECAR) in T-ALL cells. Niclosamide treatment decreased extracellular acidification rate (ECAR), non-glycolytic acidification and glycolytic capacity in (A) CCRF-CEM and (B) Jurkat cells. The results are expressed as mean ± SEM. **p<0.01, vs. control group.
Figure 8

Niclosamide and α-KG inhibits T-ALL xenograft growth in vivo. (A) NOD/SCID mice bearing CCRF-CEM tumors were randomly separated into four groups: vehicle control, 20 mg/kg niclosamide treatment, 10 mg/kg α-KG treatment, and 10 mg/kg α-KG combined with 20 mg/kg niclosamide treatment (n=6/group). Tumor volumes were recorded three times a week during the 27 days period. (B) The mice were subjected to euthanasia and the tumors from each group were collected and imaged at the end of the experiment. (C) Tumor volume for each group were measured on the last day of the experiment (day 28). The results are expressed as mean ± SEM. **P<0.01, ****P<0.0001, vs. the vehicle control group.
**Figure 9**

Niclosamide suppresses xenograft tumor proliferation and inhibits the production of TKT and Ki-67 in NOD/SCID mice. Tumor samples were subjected to (A) H&E staining and IHC analysis for (B) TKT and (C) Ki-67 at the end of the experiment. Slides were scanned on magnifications of x400 and x200. H&E, hematoxylin and eosin; IHC, immunohistochemistry. *P<0.05, vs. the vehicle control group.

**Figure 10**
KEGG pathway graph rendered by Pathview and GSEA enrichment plots. KEGG Pathview showing pentose phosphate pathway genes up or down regulated in (A) niclosamide (2 mM; 24 h) treated CCRF-CEM cells compared to WT CCRF-CEM cells. In figure (A), enzyme 2.2.1.1: transketolase; enzyme 2.2.1.2: transaldolase; enzyme: 1.1.1.49: G6PD. (B) GSEA enrichment plots of the HALLMARK PENTOSE PHOSPHATE PATHWAY and the corresponding heat map for control versus niclosamide (2 mM) treatment groups in CCRF-CEM cells. Transaldolase: TALDO1; Transketolase: TKT. G6PD: Glucose 6 phosphate dehydrogenase.

Supplementary Files

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- rawdataofwesternblotting.jpg