Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

Original article

Evaluation the Effect of Chemotherapy Drugs and Thiosemicarbazone Complexes on the Expression of URHC and CASC15 LncRNAs in Acute Lymphoblastic Leukemia Cell Line

Arezoo Hassani [1], Golnaz Asaadi Tehrani [1*], Neda Zahmatkesh [1], Sina Mirzaahmadi [1]

[1] Department of Genetics, Zanjan Branch, Islamic Azad University, Zanjan, Iran

ARTICLE INFO

Article History:
Received: 10/01/2023
Accepted: 12/03/2023

Keywords:
Acute lymphoblastic leukemia
Chemotherapy
Thiosemicarbazone
lncRNA

***Corresponding authors:**
Golnaz Asaadi Tehrani, Ph.D
Department of Genetics, Faculty of Medical and sciences, Zanjan Branch, Islamic Azad University, Etemadeyeh, Moalem St, Zanjan, Iran
Email: Golnaz_ asaadi@yahoo.com

Abstract

Background: Acute lymphoblastic leukemia (ALL) is a common type of leukemia in children, accounting for about 30% of pediatric malignancies. Researchers aimed to assess the impact of chemotherapy drugs and Thiosemicarbazone complexes on the expression of URHC and CASC15 LncRNAs in Jurkat E6.1 cell line, which represents Acute Lymphoblastic Leukemia. Thiosemicarbazone complexes are a group of chemical compounds known for their antitumor activities.

Material and methods: Optimal doses of chemotherapy drugs and thiosemicarbazone complexes were prepared and administered to the cell line at different time points (24, 48, and 72 hours). RNA extraction and cDNA synthesis were performed, and the expression of URHC, CASC15, and GAPDH genes was measured using Real-time PCR. Statistical analysis was conducted on the obtained results.

Results: The study found that complex 3, a combination of two chemotherapy drugs (Cytarabine and 6MP) at specific concentrations (1 mM and 5 mM, respectively), significantly reduced the expression of URHC after 72 hours. Similarly, treatment with Arac at 5 mM for 72 hours showed the most effective concentration and time for decreasing the expression of CASC15 LncRNAs.

Conclusion: In conclusion, the research demonstrated that the studied drugs had a positive impact on reducing the expression of CASC15 and URHC onco-lncRNAs. However, the optimal effects varied depending on the concentrations and treatment durations. These findings highlight the potential of the examined drugs in targeting specific LncRNAs associated with Acute Lymphoblastic Leukemia.

Please cite this article as: Hassani A, Asaadi Tehrani G, Zahmatkesh N, Mirzaahmadi S. Evaluation the Effect of Chemotherapy Drugs and Thiosemicarbazone Complexes on the Expression of URHC and CASC15 LncRNAs in Acute Lymphoblastic Leukemia Cell Line. Iranian Journal of Blood and Cancer. 2023; 15(1): 1-9.

1. Introduction

Acute lymphoblastic leukemia (ALL) is a hematological malignancy characterized by poor differentiation, growth, and aggregation of lymphoid progenitor cells in the bone marrow and/or extramedullary locations (1, 2). ALL exhibits a bimodal distribution: the first peak occurs around 5 years of age, and the second peak occurs around 50 years of age. It primarily affects pediatric patients, with 80% of cases occurring in children and 20% in adults (3-5). CASC15 has been found to be highly expressed in acute leukemia cells with RUNX1 translocations. Its

expression promotes apoptosis and reduces hematopoietic engraftment. CASC15 acts as a barrier to cell growth, and its expression is notably high in patients with RUNX1 translocations. Elevated CASC15 levels may hinder differentiation and reduce the proliferation rate. Furthermore, the expression of CASC15 in leukemic cells may indicate their specific differentiation stage. The expression profile of CASC15 shows a bimodal distribution, with a peak in common lymphoid progenitors (CLP) and a secondary peak in large pre-B cells during hematopoiesis. Constitutive over-expression of CASC15 prevents the decrease typically observed in the pre-B-cell stage, resulting in myeloid bias and inhibition of B-cell growth in transplanted animals. CASC15's cellular activity may also play a role in modulating cell survival during development (6).

Loss-of-function studies have demonstrated that URHC inhibits cancer cell growth and progression through the cell cycle, suggesting that targeting URHC could be a potential diagnostic and therapeutic strategy for cancer (7). However, the exact regulatory mechanisms governing URHC's role in cancer signaling remain largely unclear [8]. Additionally, it is uncertain whether the essential functions of URHC in cancer are linked to miR-5007-3p dysregulation (8). DNA homolog subfamily B member 9 (DNAJB9), also known as MDJ-1 and ERDJ4, belongs to the DNAJ protein family (9). DNAJB9 has been found to prevent apoptosis in SK-N-SH cells induced by endoplasmic reticulum (ER) stress (10).

Cyclophosphamide is commonly administered simultaneously or sequentially with other anti-tumor drugs (11). Cytarabine is an inhibitor of ribonucleotide reductase and is specifically designed for the S-phase of the cell cycle. Mercaptopurine, a purine analog, is an anti-metabolite used in the treatment of acute lymphocytic leukemia. It functions similarly to the hypoxanthine compound (12). Methotrexate (MTX) is a well-known anti-cancer medication (13). MTX reduces the quantity of folate carriers by inhibiting dihydrofolate reductase (DHFR), subsequently suppressing de novo synthesis of purine and pyrimidine precursors (14-16). Thiosemicarbazones (TSC) are a family of ligands that have received attention due to their flexible coordination towards metal ions, structural topology, and potential applications (17). They exhibit a wide range of bioactivities, including antiviral and antibacterial functions (18). This is mainly due to its anti-viral (19), anti-bacterial functions (20). DNA interaction, antitumor, and antioxidant properties have all been linked to thiosemicarbazones (21). Various thiosemicarbazone compounds have been tested in vitro and in vivo for their anti-proliferative effects against different types of malignancies (22, 23). For example, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone has been extensively studied in the treatment of cervical, colon, and metastatic renal cancer. Additionally, several thiosemicarbazones and related compounds have demonstrated toxic effects on different leukemia cells (24, 25). Other researchers have reported that thiosemicarbazones derived from 2-benzoylpyridine exhibit anti-leukemia activity.

Different investigations have demonstrated the oncogenic roles of URHC and CASC15 lncRNAs in various types of leukemia. In this study, our aim was to investigate whether downregulation of these lncRNAs through thiosemicarbazone complexes could exhibit anti-tumoric effects on T-ALL cells. To the best of our knowledge, this is the first study to evaluate the expression alterations of these aforementioned onco-lncRNAs in T cells with acute lymphoblastic leukemia when treated with thiosemicarbazone complexes of Ni and Cu. Our primary objectives were to examine the differential expression of the lncRNAs URHC and CASC15 in T cells with acute lymphoblastic leukemia and to assess how thiosemicarbazone complexes of Ni and Cu could impact the expression of these lncRNAs. Furthermore, we aimed to compare the effects of thiosemicarbazone complexes of Ni and Cu with the efficacy of cyclophosphamide, methotrexate, mercaptopurine, cytarabine, a combination of methotrexate and cyclophosphamide (MTX+CP), a drug complex of cyclophosphamide and cytarabine (CP+Arac), and a complex of cytarabine and mercaptopurine (Arac+6-mp) on the expression alterations of two lncRNAs, URHC and CASC15, in T cells with acute lymphoblastic leukemia using the jurkat E6.1 cell line.

2. Method and material

The current case-control study was conducted from April to September 2019 at the Research Science Center of the Islamic Azad University of Zanjan. For this purpose, the acute Jurkat E6.1 human lymphoblastic leukemia cell line was purchased from the Pasteur Institute of Iran in Passage 1 with a density of 80% (2×10^5 cells/cm^2).

2.1 Cell culture and preparation of drug at different concentrations

First, the Jurkat E6.1 cell line was cultured in Dulbecco's Modified Eagle's Medium (RPMI 90%) medium containing 10% FBS and incubated at 37 °C with 5% carbon dioxide for a duration of six days. Afterward, three passages were applied to the cells every two days, and in each cell passage, the cells were inserted into a flask with new culture medium. Then, the cells of the fourth passage were selected for the next steps, and the cells were counted and stained with Trypan blue. The number of cells was determined to be 3×10^4 cells/cm^2. Cells were divided into control and sample groups
Cytotoxicity and IC50 values of Ni and Cu thiosemicarbazone complexes were calculated by MTT assay, and the related results of the combination of CuHL1 were as follows:

- 24 hours: 20 ± 2.5
- 48 hours: 17 ± 1.5
- 72 hours: 16 ± 1.0

The IC50 value for NiHL1 was as follows:

- 24 hours: 104 ± 3.5
- 48 hours: 61 ± 4.0
- 72 hours: 48 ± 2.0

After preparing the desired concentrations of drug compounds for cyclophosphamide, methotrexate, mercaptopurine, and cytarabine, the following drug combinations were prepared:

- Methotrexate and cyclophosphamide (MTX+CP) drug complex
- Cyclophosphamide and cytarabine (CP+Arac)
- Cytarabine and mercaptopurine complex (Arac+6mp)
- Thiosemicarbazone complexes of Ni and Cu

IC50 values of the prepared drugs were analyzed by MTT assay, and the drug treatment groups were prepared in H2O solvent with specific concentrations detailed in **Table 1**. The prepared concentrations were used in three-time groups for durations of 24, 48, and 72 hours, and the drug-free cells were used as the control groups. The next steps were performed in the following order.

2.2 RNA extraction, cDNA synthesis, and Real-Time PCR

After cell maintenance in the aforementioned time periods (24, 48, 72), RNA extraction was done using the Total RNA Extraction kit manufactured by PARS Co. Mashhad, IR Iran (Cat #: A101231). Quantitative purification of purified RNA was performed by a spectrophotometer, and the concentration of purified RNA was determined to be 0.5-1 µg. For cDNA synthesis, the cDNA Reverse Transcription kit (50 tests) of Pars tous company (Cat No. A101161) was used, and cDNA was made according to the kit's instructions. Primer sequences (5-3) used for CASC15, URHC, and GAPDH are listed in **Table 2.** The reactions were adjusted at a 20 µl volume, and the amplification was performed using the Rotor-Gene Q real-time PCR cycler (Qiagen, Hilden, Germany) with Ampliqon SYBR green Master Mix High ROX (Cat No. A325402) according to the kit's instructions.

2.3 Investigating Gene Expression Confirmation and Statistical Analysis:

The PCR product was electrophoresed on a 2% agarose gel to confirm the presence of the LncRNAs, and the length of the fractions was equal to 300 bp. The fractions were then sequenced in a direct direction and approved by Fanavaran Gene Company. After calculating the Δct for all cases and control samples, $2^{-\Delta\Delta ct}$ (fold change) was obtained for each sample. To evaluate the expression of URHC and CASC15 lncRNAs by Real-time PCR, the Livak method and Rest program (2002) were used, respectively. A significance level of $P < 0.05$ was considered.

3. Results

Comparing the results of the reference gene expression in drug-treated groups and control groups, and also calculating the correlation coefficient and standard deviation, showed the same numbers of $2^{-\Delta\Delta ct}$ and Ratio. These results confirm the ineffectiveness of the reference gene in response to the implemented drugs.

3.1 Expression alteration of URHC and CASC15 treated with MTX, CP, AraC, 6-MP

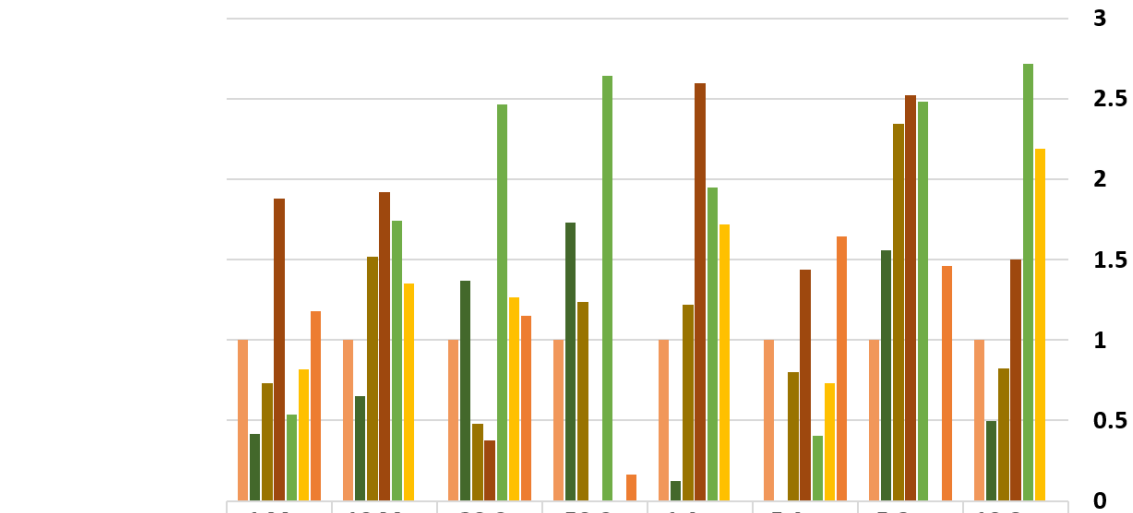
- Expression alteration of URHC LncRNA treated with MTX decreased down to 1µM (0.82) after 48 h of treatment and down to 1µM (0.537) after 72 h of treatment, which were statistically significant ($P < 0.001$).
- Changes in the expression of URHC LncRNA treated with CP only decreased to 50µM (0.164) after 24 h of treatment, which was statistically significant.
- In the expression of URHC LncRNA treated with AraC, a significant reduction in expression was observed after 48 h of treatment with the concentration of 5µM (0.731) and after 72 h of treatment with 5µM (0.406).

Table 1. Cyclophosphamide, methotrexate, mercaptopurine, cytarabine, complex of (MTX + CP) complex of (CP + Arac), complex of (Arac + 6mp) concentrations.

Drug	*Concentration in 24h*	*Concentration in 48h*	*Concentration in 72h*
Control	-	-	-
MTX	1,10 µm	1,10 µm	1,10 µm
CP	20,50 µm	20,50 µm	20,50 µm
Arac	1,5 µm	1,5 µm	1,5 µm
6mp	5,10 µm	5,10 µm	5,10 µm
Cu	17.5,20 µm	15.5 ,17µm	15,16 µm
Ni	100.5,104 µm	51,61 µm	42,4 µm
MTX+ CP	1 MTX +20 CP µm	1 MTX +20 CP µm	1 MTX +20 CP µm
CP+ Arac	20 CP +1 Arac µm	20 CP +1 Arac µm	20 CP +1 Arac µm
Arac+6mp	1 Arac +56mp µm	1 Arac +56mp µm	1 Arac +56mp µm

Table 2. Primer sequences of studied LncRNAs

Gene and LncRNA name	*Forward sequence*	*Reverse sequence*
CASC15	CACACGCATGGAAAACCCAG	GAGGACCTGAGCTGTAAGCC
URHC	TGTTTATGTGAGAGGAGAAAG	CACTAGAGGTCTGCAAATAAAGTGA
GAPDH	ACCACAGTCCATGCCATC	TCCACCACCCTGTTGCTGTA



	1 Mtx	10 Mtx	20 Cp	50 Cp	1 Arac	5 Arac	5 6mp	10 6mp
URHC24	1.181	0	1.153	0.164	0	1.643	1.459	0
URHC48	0.82	1.351	1.266	0	1.72	0.731	0	2.188
URHC72	0.537	1.741	2.466	2.642	1.946	0.406	2.48	2.714
CASC15 24	1.88	1.919	0.377	0	2.598	1.438	2.523	1.5
CASC15 48	0.733	1.52	0.478	1.237	1.221	0.798	2.342	0.821
CASC15 72	0.417	0.652	1.366	1.728	0.123	0.003	1.56	0.497
GAPDH 24,48,72	1	1	1	1	1	1	1	1

Figure 1. Comparison of URHC, CASC15 and GAPDH genes expression alternation treated with MTX, CP, Arac,6MP chemotherapy drugs. Various concentrations of MTX (1 and 10 µM), CP (20 and 50 µM), AraC (1, 5 µM) and 6MP (5, 10 µM) in different treatment times (24, 48,72 compared with each other).

• Changes in the expression of URHC LncRNA treated with 6-mp showed that none of the implemented concentrations and times were effective in reducing the expression of the mentioned LncRNA.
• Statistical analysis for the expression of CASC15 LncRNA, as an onco LncRNA, treated with MTX compared to the control group after 48 h was 1μM (0.733) and decreased to 1μM (0.417) and 10μM (0.652) after 72 h, with a significant rate of ($P < 0.001$).
• In cells treated with CP, changes in CASC15 expression were significantly reduced after 24 h of treatment with the concentration of 20μM (0.377) and after 48 h with 20μM (0.478). This decrease was statistically significant ($P < 0.001$).
• The results of changes in the expression of CASC15 LncRNA treated with AraC showed a significant reduction after 48 h with 5μM (0.798). Furthermore, a meaningful decrease in expression was observed after 72 h for all concentrations (1μM (0.123) and 5μM (0.003).
• Changes in the expression of CASC15 LncRNA treated with 6-mp drug after 48 h with the concentration of 10μM (0.821) and after 72 h with 10μM (0.497) showed a significant decrease in expression. (**Figure. 1**).

3.2 Expression alteration of URHC and CASC15 treated with Cu, Ni, thiosemicarbazone complexes

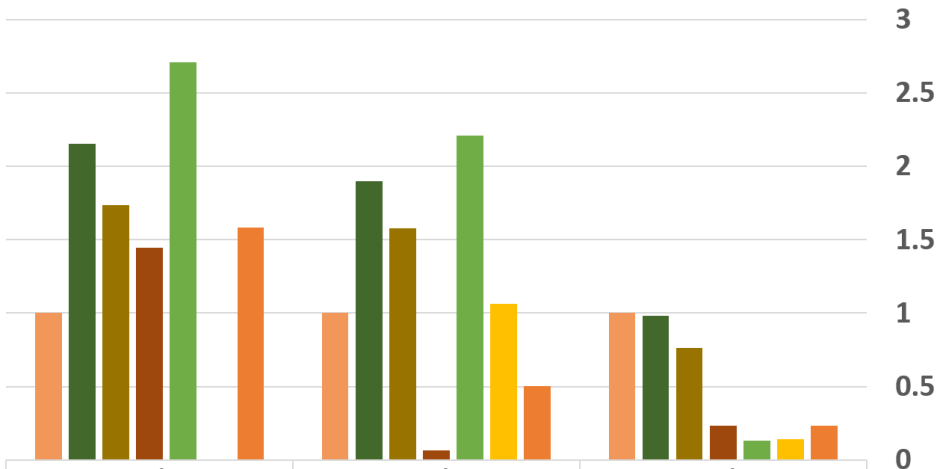
• The results of changes in gene expression showed a significant decrease at the first 24 h after treatment with Cu with the concentration of 17.5μM (0.357), and after 48 h of treatment, the result was elevated up to 0.935.
• Changes in the expression of URHC gene treated with Ni showed a significant decrease in expression after 24 h of treatment, 104μM (0.647) after 48 h of treatment, and 61μM (0.879).
• Similarly, the results of changes in CASC15 expression after the first 24 h of treatment with Cu at 17.5μM (0.123), and after 48 h with 15.5μM (0.74) showed a significant decrease in expression.
• Changes in the expression of CASC15 LncRNA treated with Ni showed a significant decrease after 48 h of treatment with 51μM (0.714) and 61μM (0.992) and after 72 h of treatment with the concentrations of 46μM (0.027) and 48μM (0.597). (**Figure. 2**).

3.3 Expression alteration of URHC and CASC15 treated with Complexes (MTX + CP) and (CP+ AraC)

• Changes in URHC LncRNA expression under treatment with the studied complexes decreased significantly after 24 h of treatment in complex3, complex2 with a rate of (0.502) and (0.233) respectively. A significant decrease in expression was observed at 48 h for complex3 (0.143) and at 72 h for complex3 (0.133) with a significant rate of ($P < 0.05$).
• Also, changes in the expression of CASC15 LncRNA treated with the studied complexes decreased significantly after 24 h with complex2 and complex3 treatment by the rate of (0.067) and (0.234), at 48 h with Complex3 (0.761), and after 72 h with Complex3 (0.981) ($P < 0.05$). (**Figure. 3**).

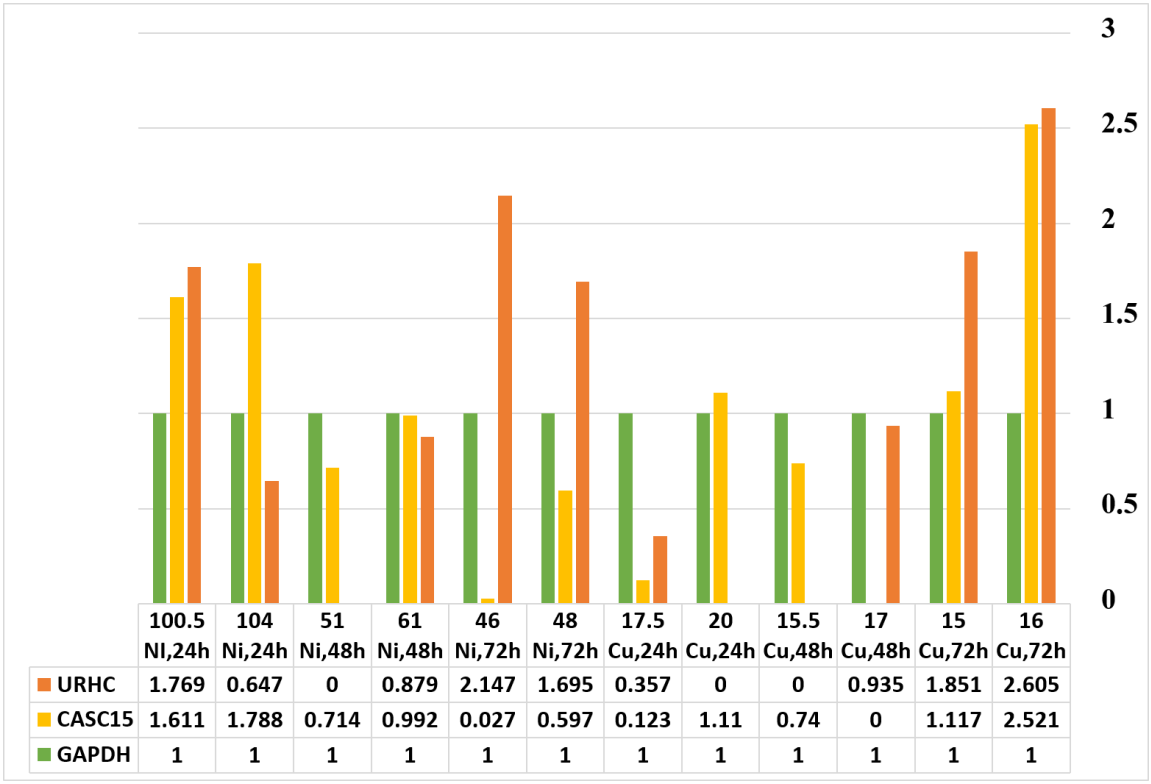
4. Discussion

Acute lymphoblastic leukemia (ALL) is still a fatal hematologic malignancy. T-cell acute lymphoblastic leukemia (T-ALL) is a highly diverse malignant hematological disease resulting from T-cell progenitor. Therefore, innovative treatment techniques are desperately needed. The aim of the present study was to determine the optimal dosage of thiosemicarbazone complexes and their effect on the expression alteration of URHC and CASC15 onco-LncRNAs compared to other routine chemotherapeutic agents used in clinics. The comparison of results between the studied groups showed the highest significant decrease in URHC expression, indicating the highest effect of the drug complex 3 (1μM of cytarabine and 5μM of 6MP) (0.133) after 72 hours of treatment. The highest significant decrease in expression of the lncRNA CASC15 was observed when treated with Arac at 5μM (0.003) after 72 hours. These results indicate the positive effect of the studied drugs at specific times and different concentrations on the expression of the aforementioned onco-LncRNAs. The comparison of the results of these reduction alterations in most groups showed that the effects of drugs mostly depend on their time and concentrations. In 2018, Wu SX et al. described CASC15's participation in GC carcinogenesis, showing that down- or up-regulation of CASC15 prevented or promoted cell proliferation, induced cell cycle arrest and cell death when CASC15 interacted with EZH2 and WDR5, and was involved in CDKN1A modulation in the nucleus (26). In 2019, Shenga and colleagues revealed that CASC15 silencing reduced melanoma cell motility and invasion by suppressing the EMT pathway. Activation of the Wnt/β-catenin signaling pathway increased the multiplication, invasion, and metastasis of melanoma cells (27). In 2017, Thilini et al. revealed that CASC15 is a preserved lncRNA associated with increased diagnoses of B-acute lymphoblastic leukemia (B-ALL) and pediatric acute myeloid leukemia (AML) in



	Complex1	Complex 2	Complex 3
URHC24	1.585	0.502	0.233
URHC48	0	1.061	0.143
URHC72	2.708	2.208	0.133
CASC15 24	1.445	0.067	0.234
CASC15 48	1.736	1.579	0.761
CASC15 72	2.154	1.901	0.981
GAPDH 24,48,72	1	1	1

Figure 2. URHC, CASC15 and GAPDH genes expression alternation treated with Complex 1, 2, 3.



	100.5 Nl,24h	104 Ni,24h	51 Ni,48h	61 Ni,48h	46 Ni,72h	48 Ni,72h	17.5 Cu,24h	20 Cu,24h	15.5 Cu,48h	17 Cu,48h	15 Cu,72h	16 Cu,72h
URHC	1.769	0.647	0	0.879	2.147	1.695	0.357	0	0	0.935	1.851	2.605
CASC15	1.611	1.788	0.714	0.992	0.027	0.597	0.123	1.11	0.74	0	1.117	2.521
GAPDH	1	1	1	1	1	1	1	1	1	1	1	1

Figure 3. URHC, CASC15 and GAPDH treated with Ni,Cu Thiosemicarbazone complexes.

children. The anticipated transcriptional targets of the YY1 transcription factor were enriched in differentially regulated genes after CASC15 knockdown. CASC15 increases YY1-mediated control of the SOX4 promoter, providing insights into its activity (6). In silico analysis by Grasedieck et al. in AML cells revealed that the survival-associated lncRNA CASC15 serves as a proto-oncogene, affecting proliferation and differentiation. Its expression is connected to DNA methylation-affecting mutations and splicing, possibly involving an intragenic lineage-specific CTCF site (28). According to earlier research on the CASC15 gene in various malignancies, the findings of our investigations revealed that this gene, which plays a role in onco-LncRNAs, had reduced expression in acute lymphoblastic leukemia treated with the suggested medicines. The quantities and timing of these changes in expression were varied. Nevertheless, the diminishing alterations in CASC15 gene expression in most groups showed that the pharmacological compounds' efficacy was time and intake dependent. Treatment with Arac at 5μM (0.003) for 72 hours was the most effective concentration and time in lowering CASC15 gene expression among the groups treated with pharmacological compounds. Gu and coworkers showed in 2018 that down-regulation of lncRNA-URHC might increase E-cadherin expression while decreasing N-cadherin, vimentin, and snail expression. As a last thought Colorectal cancer development can be slowed by inhibiting lncRNA-URHC expression (29). High URHC expression can increase cell proliferation and prevent apoptosis by suppressing ZAK expression through inactivation of the ERK/MAPK pathway, according to a 2014 study by Wei-Hua and colleagues. These discoveries might lead to the discovery of a new mechanism and therapeutic targets for HCC therapy (30). In 2002, Kunwei and colleagues found that silencing the URHC gene inhibited the growth of HCC cells. URHC also positively controlled the amount of DNAJB9 by sponging miR-5007-3p. These findings shed light on the role of URHC as a miRNA sponge in HCC (31). The results of URHC expression changes in treatment with the mentioned drugs, as well as a comparison of the results with previous studies, revealed that URHC onco-LncRNAs are associated with increased expression in acute lymphoblastic leukemia, and we observed a decrease in the expression of this LncRNA when treated with various drugs. Most of the reduced expressions were detected among treatment groups with time and with increasing concentration, confirming drug dependency on concentration and period of usage. Complex3, a combination of 1μM cytarabine and 5μM of 6MP drug, had the most effective dosage and time of drug impact in decreasing URHC expression at a rate of (0.133) after 72h. This decrease in expression shows that combining two chemotherapeutic medicines as the complex was effective in decreasing URHC expression. In conclusion, the CASC15 and URHC LncRNAs were chosen by conducted literature review. For the first time we detected that URHC is downregulated through treatment with thiosemicarbazone complexes loaded with different chemotherapeutic agents. both the carcinogenic role of CASC15 and URHC LncRNAs and other main genes have been identified in acute lymphoblastic leukemia, treating with various doses the complex (methotrexate + cyclophosphamide) and complex (Cyclophosphamide + Cytarabine) in the thesis, as well as the different doses of CASC15 LncRNA URHC in acute lymphoblastic leukemia. Considering the optimum effects of medicines in decreasing the expression of the investigated genes, it is possible to infer that these effects might vary depending on concentration and duration. The results revealed that the medicines tested had a strong potential to decrease expression of CASC15 and URHC onco-LncRNAs in the control, therapy and reduction of the growth of this kind of leukemia as a result of mutations in CASC15 URHC in acute lymphoblastic leukemia. Furthermore, to consolidate the found out results of the present investigation, further bioinformatic discoveries including RNA-seq or microarray data analysis should be conducted. In addition, further in vitro analysis is required to disclose precise mechanism of action after being suppressed or downregulated with different anti-cancer agents. Ultimately, in vivo studies can be implemented to validate our results.

Declaration of interest

None.

Acknowledgements

This research is based on a master's thesis titled " Evaluation of the effect of chemotherapy drugs and thiosemicarbazone complexes on expression of URHC, CASC 15 LncRNAs Acute Lymphoblastic Leukemia cell line" which was accomplished in 2019 with the support of the Islamic Azad University of Zanjan's Research Science Center, the Pasteur Institute of Iran, and the Faculty of Science of the Islamic Azad University of Zanjan.

References

1. Pui CH CD PD, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. Med. 2009;360(26):2730-41.
2. Kantarjian HM OBS ST, et al. Results of treatment with hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. J Clin Oncol. 2000;18(3):547-61.
3. abbour E OBS KM, Kantarjian H. New insights into the pathophysiology and therapy of adult acute lymphoblastic leukemia. Cancer. 2015;121(15):2517-28.
4. Jemal A TR MT, et al. Cancer Statistics. Cancer J Clin. 2004;54(1):8-29.
5. Siegel RL MK JA. Cancer statistics. Clin. 2015;65(1):5-29.
6. Thilini R. Fernando JRC, Matteo Zampini, Norma I. Rodriguez-Malave, Michael O. Alberti, Jaime Anguiano, Tiffany M. Tran, Jayanth K. Palanichamy, Jasmine Gajeton, Nolan M. Ung, Cody J. Aros, Ella V. Waters, David Casero, Giuseppe Basso, Martina Pigazzi & Dinesh S. Rao. The lncRNA CASC15 regulates SOX4 expression in RUNX1-rearranged acute leukemia
Molecular Cancer. 2017;16(1):126.
7. Xu WH ZJ DZea. Long non-coding RNA URHC regulates cell proliferation and apoptosis via ZAK through the ERK/MAPK signaling pathway in hepatocellular carcinoma. Biol.2014;10(7):664-76.
8. Yang B JC, Wang J, Guo X, Chen Y, Xu R, Peng L, Liu J, Li L Identification of microRNAs associated with lymphangiogenesis in human gastric cancer. Clin Transl Oncol. 2014;16:374-9.
9. Lee HJ KJ KK, et al. Genotoxic stress/p53-induced DNAJB9 inhibits the pro-apoptotic function of p53. Cell Death Differ. 2015;22(1):86-95.
10. Kurisu J HA MH, et al. MDG1/ERdj4, an ER-resident DnaJ family member, suppresses cell death induced by ER stress. Genes Cells. 2003;8(2):189-202.
11. Schreffler CSaS. Cyclophosphamide. Encyclopedia of Toxicology. 2014;1:709–11.
12. Chabner BA R, D.P., Paz-Ares, L., Carbonero, R.G., Calabresi, P. Antineoplastic Agents. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 2001:1389–459.
13. Prodanowich FM JRT, C. Pezon, T. Fasihi, and R. S. Kirsner. Methotrexate reduces incidence of vascular diseases in veterans with psoriasis or rheumatoid arthritis. J Am Acad Dermatol. 2005;52: 262-7.
14. J. Neradil GP aRV. New mechanisms for an old drug; DHFR- and nonDHFR-mediated effects of methotrexate in cancer cells. Europepmc. 2012;25 2S87-92.
15. J. Cudmore MS JS, and R. Zarychanski. Methotrexate and trimethoprimsulfamethoxazole: toxicity from this combination continues to occur. Can Fam Physician. 2014;60: 53-6.
16. Hiroyuki Imanishi NO, Mariko Yagi , Yukari Noro , Yuka Moriya , Tsutomu Nakamura , Akira Hayakawa , Yasuhiro Takeshima , Toshiyuki Sakaeda , Masafumi Matsuo , Katsuhiko Okumura Genetic polymorphisms associated with adverse events and elimination of methotrexate in childhood acute lymphoblastic leukemia and malignant lymphoma. Hum Genet. 2007;52(2):166-71.
17. Lobana TS, Sharma, R., Bawa, G. and Khanna, S. Bonding and Structure Trends of Thiosemicarbazone Derivatives of Metals—An Overview. Coordination Chemistry. 2009;253:977-1055.
18. Richardson DSKaDR. The Evolution of Iron Chelators for the Treatment of Iron Overload Disease and Cancer. Pharmacological Reviews. 2005;57 (4):547-83.
19. A Garoufis SH, N Hadjiliadis. Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents. Coordination Chemistry Reviews. 2009;253:1384-97.
20. Mehmet Aslantaş EK, Necmettin Demir, Ali E Şabik, Mehmet Tümer, Metin Kertmen. Synthesis, spectroscopic, structural characterization, electrochemical and antimicrobial activity studies of the Schiff base ligand and its transition metal complexes. Molecular and Biomolecular Spectroscopy. 2009;74:617-24.
21. Seyed Abolfazl Hosseini-Yazdi AMa AAKa, Václav Eigner b,Michal Dušek b, Majid Mahdavi c, Sina Soltani c, Farzaneh Lotfipour d, Jonathan White. Reactions of copper(II), nickel(II), and zinc(II) acetates with a new water-soluble 4-phenylthiosemicarbazone Schiff base ligand: Synthesis, characterization, unexpected cyclization, antimicrobial, antioxidant, and anticancer activities. Polyhedron. 2017;124:156–65.
22. Guo Z-L, Richardson DR, Kalinowski DS, Kovacevic Z, Tan-Un KC, Chan GC-F. The novel thiosemicarbazone, di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC), inhibits neuroblastoma growth in vitro and in vivo via multiple mechanisms. Journal of hematology & oncology. 2016;9(1):1-16.
23. Sardroud SJ, Hosseini-Yazdi SA, Mahdavi M, Poupon M, Skorepova E. Synthesis, characterization and in vitro evaluation of anticancer activity of a new water-soluble thiosemicarbazone ligand and its complexes. Polyhedron. 2020;175:114218.
24. Karp JE, Giles FJ, Gojo I, Morris L, Greer J, Johnson B, Thein M, Sznol M, Low J. A Phase I study of the novel ribonucleotide reductase inhibitor 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine®) in combination with the nucleoside analog fludarabine for patients with refractory acute leukemias and aggressive myeloproliferative disorders. Leukemia research. 2008;32(1):71-7.
25. Li M-X, Chen C-L, Ling C-S, Zhou J, Ji B-S, Wu Y-J, Niu J-Y. Cytotoxicity and structure–activity relationships of four α-N-heterocyclic thiosemicarbazone derivatives crystal structure of 2-acetylpyrazine thiosemicarbazone. Bioorganic & medicinal chemistry letters. 2009;19(10):2704-6.
26. Qiong Wu SX JM, Pingping Hui, Ting Wang, Wenying Meng, Min Shi, and Yugang Wang Long non-coding RNA CASC15 regulates gastric cancer cell proliferation, migration and epithelial mesenchymal transition by targeting CDKN1A and ZEB1. Mol Oncol. 2018 12(6):799–813.
27. Liang Sheng RW. Long Non-Coding RNA-CASC15 Promotes Cell Proliferation, Migration, and Invasion by Activating Wnt/β-Catenin Signaling Pathway in Melanoma. Pathobiology. 2020;87(1):20-9.
28. Grasedieck S, Ruess C, Krowiorz K, Lux S, Pochert N, Schwarzer A, Klusmann J-H, Jongen-Lavrencic M, Herold T, Bullinger L. The long non-coding RNA Cancer Susceptibility 15 (CASC15) is induced by isocitrate dehydrogenase (IDH) mutations and maintains an immature phenotype in adult acute myeloid leukemia. haematologica. 2020;105(9).
29. Z-G Gu G-HS J-HL, W-X Huang, Z-H Qian, J Qiu. Effects of long non-coding RNA URHC on proliferation, apoptosis and invasion of colorectal cancer cells. Eur Rev Med Pharmacol Sci. 2018;22:1658-64.
30. Wei-Hua Xu J-BZ ZD, Xiao Li , Ti Zhou , Jie Liu , De-Sheng Wang , Wen-Jie Song , Ke-Feng Dou . Int J . 10.(7). Long non-coding RNA URHC regulates cell proliferation and apoptosis via ZAK through the ERK/MAPK signaling pathway in hepatocellular carcinoma. Biol Sci. 2014 10(7):664–76.

31. kunwei niu SQ, Xuan Zhang, Jimin Dai, Jianlin Wang, Ye Nie, Hong Zhang, Kaishan Tao, Wenjie Song. LncRNA URHC promotes cell proliferation by regulating a miR-5007-3p/DNAJB9 axis in hepatocellular carcinoma. 2020.