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Original Article

Comparative Analysis of HULC, NEAT1, FENDRR, and EMG3 Long Non-coding RNA Expression Profiles in K562 Chronic Myeloid Leukemia Cells Treated with Hydroxycarbamide, Cyclophosphamide, and Cytarabine Chemotherapy Agents Versus Thiosemicarbazone Complexes

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Abstract

Background: Chronic myeloid leukemia (CML) is a complex hematological malignancy characterized by abnormal myeloid cell proliferation. Long non-coding ribonucleic acids (lncRNAs) have gained attention for their role in cancer, including CML. This study investigated the expression patterns of lncRNAs NEAT1, EMG3, FENDRR, and HULC in K562 CML cells under various drug treatments. More precisely, the study evaluated the impact of chemotherapy agents hydroxycarbamide (HU), cyclophosphamide (CP), cytarabine (Arac), and thiosemicarbazone (TSC) complexes on lncRNA expression profiles.

Methods: The K562 CML cell line was treated with chemotherapy drugs and TSC complexes at varying concentrations for different periods. Then, RNA extraction and complementary DNA synthesis were performed, and the expression levels of HULC, NEAT1, FENDRR, and EMG3 lncRNAs were examined by real-time polymerase chain reaction. The results were statistically analyzed using REST software.

Results: CP treatment resulted in the significant upregulation of NEAT1, EMG3, and HULC, while HULC exhibited downregulation at lower concentrations and longer durations. HU treatment led to the consistent upregulation of FENDRR and HULC, indicating concentration-dependent responses. Moreover, combined CP and Arac treatment revealed concentration-dependent effects on lncRNAs, with NEAT1 and EMG3 displaying optimal responses at specific concentrations and durations. Complex drug treatments yielded diverse outcomes. NEAT1 responded positively to Complex 1 but negatively to Complex 3. In addition, EMG3 showed marked upregulation under Complex 3. FENDRR and HULC demonstrated variable expression changes under different concentrations and durations, emphasizing the intricate regulatory dynamics. TSC nickel and copper treatments had concentration-dependent effects on lncRNA expression. Finally, NEAT1, EMG3, and HULC displayed sensitivity to specific concentrations and durations, highlighting their potential responsiveness to these treatments.

Conclusion: Overall, our findings provide insights into the dynamic expression of lncRNAs in response to drug treatments in CML. It is expected that understanding these regulatory mechanisms paves the way for targeted therapeutic interventions in CML, optimizing treatment strategies for improved patient outcomes.

Keywords: Chronic myeloid leukemia, Thiosemicarbazone, Cyclophosphamide, Cytarabine, Hydroxycarbamide

Introduction

Chronic myeloid leukemia (CML) is a hematological malignancy that is characterized by the abnormal proliferation of myeloid cells in the bone marrow.[1] It is a complex disease that has various genetic and molecular alterations that contribute to its pathogenesis.[2] In recent years, there has been growing interest in the role of long non-coding ribonucleic acids (lncRNAs) in cancer, including CML.[3] LncRNAs are a class of non-coding RNA molecules that are longer than 200 nucleotides and have been found to play an important role in various cellular processes, such as gene expression regulation, chromatin remodeling, and cell signaling.[4,5] According to epidemiological studies, the outcomes of CML have

considerably improved over the past two decades.[6] However, the survival levels of CML patients vary across regions, highlighting the need for comprehensive epidemiological research to evaluate the global burden of CML[7]. Data from the Global Burden of Disease study 2017 has been used to assess the distribution of CML burden based on various factors, such as age, gender, social-demographic index (SDI), and countries.[6] The study has analyzed incidence cases, death cases, and disability-adjusted life-years to understand the disease burden and its trends from 1990 to 2017. Over the past 28 years, there has been a noticeable reduction in the global disease burden associated with CML, particularly in regions with higher SDI scores. Conversely, lower SDI countries have witnessed an upward trend in the incidence and mortality rates of CML cases.[8] This observed pattern suggests a substantial disparity in CML healthcare outcomes among regions with varying levels of socio-economic development. An encouraging trend is the steady decline in the age-standardized rate of CML incidence on a global scale, underscoring an overall reduction in new CML cases over the past three decades.[9] However, it is noteworthy that CML primarily afflicts individuals aged 20 and older, with its incidence escalating as individuals advance in age, regardless of gender.[10] Furthermore, there exists a significant gender discrepancy, with males exhibiting a higher incidence of CML compared to females. This disparity is most pronounced in the age group of 75–80 years.[11]

Nuclear enriched abundant transcript 1 (NEAT1) is one of the most extensively studied lncRNAs in the context of cancer.[12] NEAT1 has been shown to be dysregulated in various types of cancer, including leukemia, and has been involved in tumor growth, metastasis, and drug resistance.[13] For example, in chronic lymphocytic leukemia, NEAT1 is highly expressed regardless of cytogenetic groups or clinical outcome.[14] In acute promyelocytic leukemia, the inhibition of NEAT1 has been found to impair myeloid differentiation.[15] Additionally, NEAT1 has been reported to modulate imatinib-induced apoptosis in CML cells.[16] These studies suggest that NEAT1 may be a potential therapeutic target in leukemia. Highly upregulated in liver cancer (HULC) is an lncRNA initially identified in hepatocellular carcinoma, but its dysregulation has also been observed in other cancers, including leukemia.[17] In a study on acute myeloid leukemia, the overexpression of the lncRNA PANDAR, which is located adjacent to HULC, was associated with adverse prognosis.[18] This indicates the potential role of HULC in leukemia pathogenesis and prognosis. Nonetheless, further investigation is needed to understand the specific functions and mechanisms of HULC in CML. EMG3 is another lncRNA that has been found to be dysregulated in cancer, including leukemia.[19] However, specific studies on EMG3 in the context of CML are rare. Further research is required to explore the expression patterns and functional roles of EMG3 in CML. FOXF1 adjacent non-coding developmental regulatory RNA (FENDRR) is a well-studied lncRNA that has been involved in a variety of biological processes, including development and disease.[20] Although there is limited research specifically focusing on FENDRR in CML, it has been shown to be dysregulated in other types of cancer and has been associated with tumor progression and metastasis. Accordingly, the potential involvement of FENDRR in CML warrants further investigation to elucidate its role in disease pathogenesis and therapy response.

Thus, this study aims to investigate the expression profiles of HULC, EMG3, and FENDRR lncRNAs in K562 CML cells. It focuses on analyzing their expression levels in response to different chemotherapy agents, including hydroxycarbamide (HU), cyclophosphamide (CP), cytarabine (Arac), and thiosemicarbazone (TSC) complexes. By comparing the expression patterns of these lncRNAs in treated cells with untreated control cells, the study seeks to identify potential associations between their expression levels and the response to therapy.

Materials and Methods

Study Design and Cell Culture

The research was performed at the Research Science Center of the Islamic Azad University of Zanjan from April to September 2019. The K562 CML cell line was employed as the experimental model. The cells were obtained from the Pasteur Institute of Iran at Passage 1 and were cultured with 80% density (2×10^5 cells/cm^2).

Cell Culture and Drug Preparation

The K562 CML cells were cultured in Dulbecco's modified Eagle's medium (90%) supplemented with 10% fetal bovine serum. They were maintained at 37°C with 5% CO_2 for a span of six days. The regular passage of cells was conducted every two days, involving their transfer to fresh growth media. After the fourth passage, the cell density per cm^2 was calculated to be 104×10^3 cells/cm^2. Then, the cells were divided into control and sample groups for subsequent procedures.

Cytotoxicity Assay and Half-Maximal Inhibitory Concentration Determination

The cytotoxicity and IC_{50} values of TSC nickel/copper (Ni/Cu) complexes were determined using the 4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The K562 cells were exposed to CuHL1 and NiHL1 for 24 hours, 48 hours, and 72 hours. CuHL1 exhibited cytotoxicity values of 20 ± 2.5, 17 ± 1.5, and 16 ± 1.0 after 24 hours, 48 hours, and 72 hours, respectively. The IC_{50} values for NiHL1 were 104 ± 3.5, 61 ± 4.0, and 48 ± 2.0 after 24 hours, 48 hours, and 72 hours, respectively.

Evaluation of Half-Maximal Inhibitory Concentration Values for Chemotherapeutic Agents

The 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide assay was similarly utilized to determine IC_{50} values for various chemotherapeutic agents. These agents included CP, Arac, HU, CP + Arac, HU + CP, HU + Arac, and the TSC Ni/Cu complexes. The drugs were dissolved in H_2O and tested at 24-hour, 48-hour, and 72-hour intervals, while drug-free cells served as controls.

Ribonucleic Acid Extraction, Complementary Deoxyribonucleic Acid Synthesis, and Real-Time Polymerase Chain Reaction

After the specified treatment periods, RNA was extracted from K562 cells using a total RNA extraction kit. The extracted RNA was quantitatively assessed and purified by a spectrophotometer , ensuring a 260/280 nm ratio of 1.8–2.2. The amount of extracted pure RNA ranged from 0.5 µg to 1 µg. Subsequently, cDNA synthesis was performed using the Easy cDNA Reverse Transcription kit according to the manufacturer's guidelines. RT-PCR was conducted by a Rotor-Gene Q RT-PCR cycler using the Ampliqon SYBR Green Master Mix High ROX kit. The expression levels of HULC, NEAT1, FENDRR, EMG3 lncRNAs, along with *GAPDH* as the reference gene, underwent evaluation.

Confirmation of Gene Expression and Statistical Analysis

Gene presence was confirmed through electrophoresis on a 2% agarose gel, displaying PCR product lengths of 300 bp. These products were sequenced and verified by Fanavaran Gene Company. In addition, Δct values were computed for both case and control samples, and a 2-ΔΔct (fold change) was determined for each sample. Eventually, the expression levels of lncRNAs (HULC, NEAT1, FENDRR, and EMG3) were assessed using the Livak method and REST software (version), with statistical significance defined at $P < 0.05$.

Results

Drug Concentrations and Complex Combinations

Employing a case-control study design, this research focused on the K562 CML cell line as its primary statistical population. Different concentrations of HU (1200 µM, 1400 µM, and 1600 µM), CP (50 µM, 100 µM, and 200 µM), and Arac (4 µM, 7 µM, and 10 µM) drugs at 24-hour, 48-hour, and 72-hour intervals were administered to investigate the impact of various pharmacological agents. Additionally, the effect of the Ni-TSC complex at various concentrations was explored over 24 hours (100.5 µM, 104 µM, and 107.5 µM), 48 hours (57 µM, 61 µM, and 65 µM), and 72 hours (46 µM, 48 µM, and 50 µM). Furthermore, the Cu-TSC complex at different concentrations was assessed over 24 hours (17.5 µM, 20 µM, and 22.5 µM), 48 hours (15.5 µM, 17 µM, and 18.5 µM), and 72 hours (15 µM, 16 µM, and 17 µM). The combinations of the Arac + CP complex (4 µM Arac + 50 µM CP, 7 µM Arac + 100 µM CP, 10 µM Arac + 200 µM CP) were administered over 24 hours, 48 hours, and 72 hours, as well as the HU + CP complex (1200 µM HU + 50 µM CP, 1400 µM HU + 200 µM CP, 1600 µM HU + 100 µM CP) and HU + Arac complex (1200 µM HU + 4 µM Arac, 1400 µM HU + 7 µM Arac, 1600 µM HU + 10 µM Arac), both administered over the same time intervals. Additionally, the CP + Arac + HU complex (50 µM CP + 4 µM Arac + 1200 µM HU, 100 µM CP + 7 µM Arac + 1400 µM HU, 200 µM CP + 10 µM Arac + 1600 µM HU) and the CP + Arac + HU complex (50 µM CP + 7 µM Arac + 1600 µM HU, 100 µM CP + 10 µM Arac + 1200 µM HU, 200 µM CP + 4 µM Arac + 1400 µM HU) were studied over 24 hours, 48 hours, and 72 hours of preparation. Subsequently, these cells were subjected to drug treatments, followed by RNA extraction, cDNA synthesis, and RT-PCR analysis. Our primary objective was to scrutinize the alterations in LncRNA *FENDRR* gene expression within human K562 cancer cells. An attempt was made to establish a nexus between changes in LncRNA FENDRR expression and the administration of chemotherapy drugs and TSC complexes, employing quantitative RT-PCR methodologies.

Effects of Cyclophosphamide Treatment on the Expression of Long Non-Coding Ribonucleic Acids

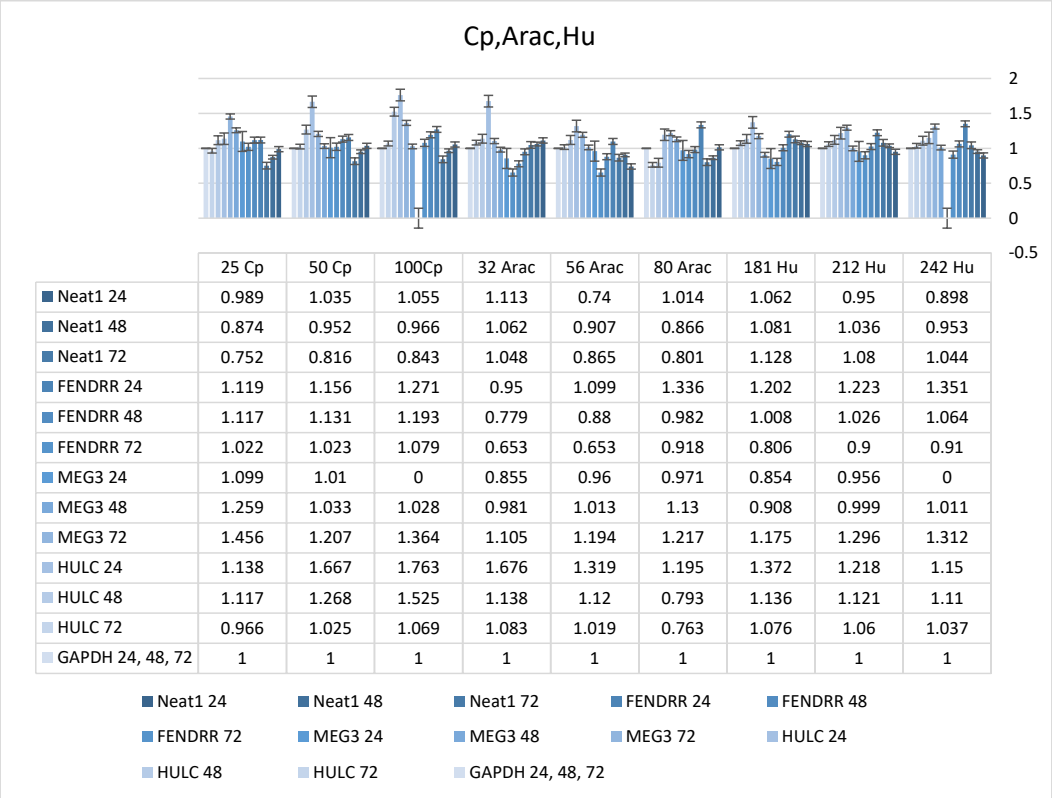
In our investigation of lncRNA FENDRR expression changes under CP treatment, significant alterations were observed across various drug concentrations and durations. Notably, CP treatment led to the significant upregulation of lncRNA FENDRR expression ($P < 0.001$) at all concentrations (25 µM, 50 µM, and 100 µM) and time points (24 hours, 48 hours, and 72 hours).

Similarly, the expression changes of lncRNA EMG3 under CP treatment were examined, and the findings indicated the significant upregulation of lncRNA EMG3 at concentrations of 25 µM and 50 µM at 24 hours, 48 hours, and 72 hours ($P < 0.001$). However, there was no significant expression change at the concentration of 100 µM and a 48-hour duration ($P = 0.489$).

The results related to lncRNA HULC expression changes under CP treatment revealed a dose-dependent response. Specifically, lncRNA HULC exhibited a significant increase in expression at higher CP concentrations (50 µM and 100 µM) and the 24-hour duration ($P < 0.001$). Conversely, a significant downregulation was detected at a concentration of 25 µM and the 72-hour duration ($P < 0.001$).

Moreover, the expression changes of lncRNA NEAT1 under CP treatment demonstrated distinct patterns. At a concentration of 25 µM and 24-hour duration, lncRNA NEAT1 displayed a significant downregulation ($P < 0.001$). However, no significant changes were found at concentrations of 50 µM and 100 µM at the same duration. At 48-hour and 72-hour durations, a consistent downregulation was observed across all CP concentrations (25 µM, 50 µM, and 100 µM; $P < 0.001$).

Overall, our results highlight the dynamic and concentration-dependent effects of CP treatment on the expression of these lncRNAs in our experimental K562 cell line model, underscoring their intricate regulatory role in response to chemotherapy (Figure 1).



	25 Cp	50 Cp	100Cp	32 Arac	56 Arac	80 Arac	181 Hu	212 Hu	242 Hu
Neat1 24	0.989	1.035	1.055	1.113	0.74	1.014	1.062	0.95	0.898
Neat1 48	0.874	0.952	0.966	1.062	0.907	0.866	1.081	1.036	0.953
Neat1 72	0.752	0.816	0.843	1.048	0.865	0.801	1.128	1.08	1.044
FENDRR 24	1.119	1.156	1.271	0.95	1.099	1.336	1.202	1.223	1.351
FENDRR 48	1.117	1.131	1.193	0.779	0.88	0.982	1.008	1.026	1.064
FENDRR 72	1.022	1.023	1.079	0.653	0.653	0.918	0.806	0.9	0.91
MEG3 24	1.099	1.01	0	0.855	0.96	0.971	0.854	0.956	0
MEG3 48	1.259	1.033	1.028	0.981	1.013	1.13	0.908	0.999	1.011
MEG3 72	1.456	1.207	1.364	1.105	1.194	1.217	1.175	1.296	1.312
HULC 24	1.138	1.667	1.763	1.676	1.319	1.195	1.372	1.218	1.15
HULC 48	1.117	1.268	1.525	1.138	1.12	0.793	1.136	1.121	1.11
HULC 72	0.966	1.025	1.069	1.083	1.019	0.763	1.076	1.06	1.037
GAPDH 24, 48, 72	1	1	1	1	1	1	1	1	1

Figure 1. Effects of CP, Arac, and HU Treatments on the Expression of LncRNAs. *Note*. CP: Cyclophosphamide; Arac: Cytarabine; HU: Hydroxycarbamide; LncRNA: Long non-coding ribonucleic acid

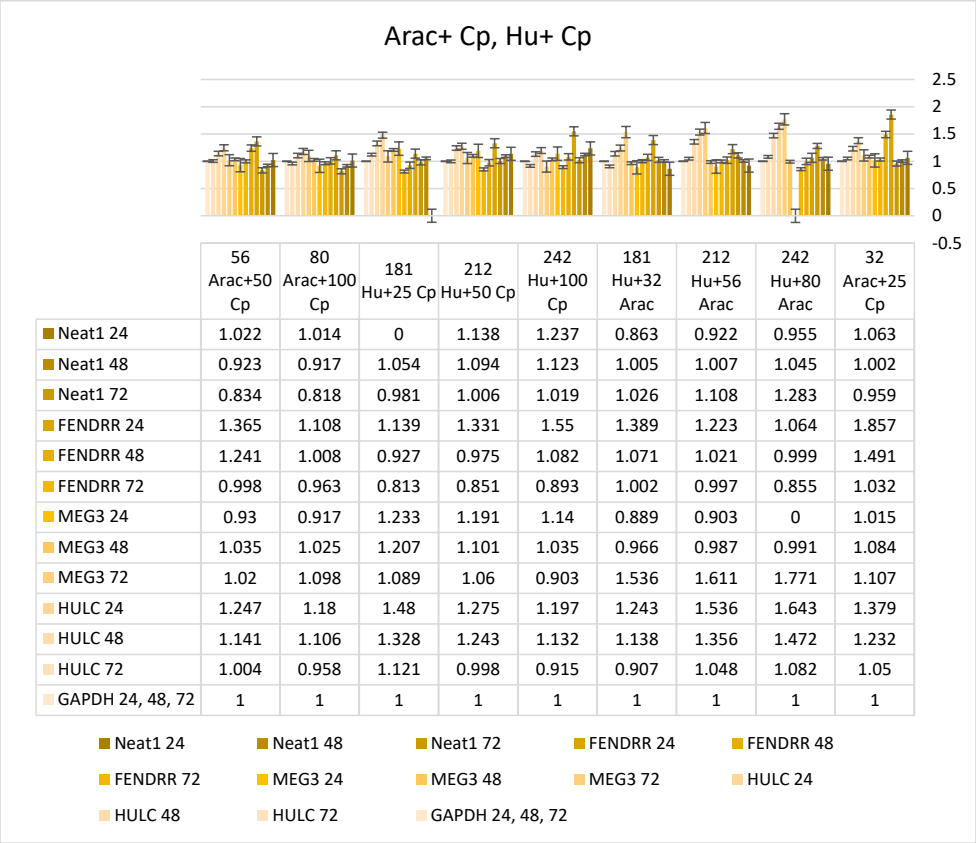
Effects of Cyclophosphamide-Cytarabine Treatment on the Expression of Long Non-Coding Ribonucleic Acids

The investigation into the expression changes of lncRNAs under Arac + CP treatment yielded insightful findings, shedding light on the most efficient concentrations and durations within each group. At 24 hours, the most efficient concentration for NEAT1 was detected at the concentration of 56 μM Arac + 50 μM CP, resulting in a significant increase in expression ($P < 0.001$). Simultaneously, EMG3 showed optimal downregulation at this concentration ($P < 0.001$). FENDRR, on the other hand, exhibited the highest decrease in expression at the concentration of 80 μM Arac + 100 μM CP ($P < 0.001$). Within the same timeframe, HULC displayed upregulation at concentrations of 32 μM Arac + 25 μM CP, 56 μM Arac + 50 μM CP, and 80 μM Arac + 100 μM CP ($P < 0.001$). At 48 hours, the efficacy of 56 μM Arac + 50 μM CP in downregulating NEAT1 and EMG3 persisted ($P < 0.001$), while FENDRR maintained its reduced expression at the concentration of 80 μM Arac + 100 μM CP ($P < 0.001$). Meanwhile, HULC continued to show upregulation at the same concentrations ($P < 0.001$). At the 72-hour interval, 56 μM Arac + 50 μM CP remained the optimal concentration for NEAT1 and EMG3 downregulation ($P < 0.001$). FENDRR also demonstrated continued downregulation at the concentration of 80 μM Arac + 100 μM CP ($P < 0.001$). HULC, however, represented divergent results, with the 56 μM Arac + 50 μM CP concentration stimulating upregulation ($P < 0.001$), while the concentrations of 32 μM Arac + 25 μM CP and 80 μM Arac + 100 μM CP revealed no significant alteration ($P = 0.504$). These findings pinpoint the specific concentrations and durations of Arac + CP treatment that induce significant alterations in lncRNA expression, offering valuable insights into targeted therapeutic approaches in the context of CML (Figure 2).

Effects of Cytarabine Treatment on the Expression of Long Non-Coding Ribonucleic Acids

The results related to lncRNA expression changes under Arac treatment in K562 CML cells confirmed considerable alterations in the expression profiles of NEAT1, EMG3, FENDRR, and HULC lncRNAs at various concentrations and durations. The most effective concentrations and timepoints were identified within each group.

For NEAT1, the optimal concentration and timepoint for inducing expression changes were 56 μM Arac over 24 hours, resulting in a substantial increase in expression (1.099-fold, $P < 0.001$). Regarding EMG3, a concentration of 80 μM Arac over 72 hours was most effective, leading to a noticeable upregulation (1.217-fold, $P < 0.001$). Similarly, FENDRR exhibited its highest response at a concentration of 56 μM Arac over 72 hours, showing a significant increase in expression (1.217-fold, $P < 0.001$). Eventually, as regards HULC, the most efficient concentration and duration were 32 μM Arac over 24 hours, causing a significant upregulation (1.676-fold, $P < 0.001$).



	56 Arac+50 Cp	80 Arac+100 Cp	181 Hu+25 Cp	212 Hu+50 Cp	242 Hu+100 Cp	181 Hu+32 Arac	212 Hu+56 Arac	242 Hu+80 Arac	32 Arac+25 Cp
Neat1 24	1.022	1.014	0	1.138	1.237	0.863	0.922	0.955	1.063
Neat1 48	0.923	0.917	1.054	1.094	1.123	1.005	1.007	1.045	1.002
Neat1 72	0.834	0.818	0.981	1.006	1.019	1.026	1.108	1.283	0.959
FENDRR 24	1.365	1.108	1.139	1.331	1.55	1.389	1.223	1.064	1.857
FENDRR 48	1.241	1.008	0.927	0.975	1.082	1.071	1.021	0.999	1.491
FENDRR 72	0.998	0.963	0.813	0.851	0.893	1.002	0.997	0.855	1.032
MEG3 24	0.93	0.917	1.233	1.191	1.14	0.889	0.903	0	1.015
MEG3 48	1.035	1.025	1.207	1.101	1.035	0.966	0.987	0.991	1.084
MEG3 72	1.02	1.098	1.089	1.06	0.903	1.536	1.611	1.771	1.107
HULC 24	1.247	1.18	1.48	1.275	1.197	1.243	1.536	1.643	1.379
HULC 48	1.141	1.106	1.328	1.243	1.132	1.138	1.356	1.472	1.232
HULC 72	1.004	0.958	1.121	0.998	0.915	0.907	1.048	1.082	1.05
GAPDH 24, 48, 72	1	1	1	1	1	1	1	1	1

Figure 2. Effects of Arac + CP and HU + CP Treatments on the Expression of LncRNAs. *Note*. CP: Cyclophosphamide; Arac: Cytarabine; HU: Hydroxycarbamide; LncRNA: Long non-coding ribonucleic acid

These findings demonstrate the sensitivity of these lncRNAs to Arac treatment, with distinct optimal conditions for modulating their expression. These data provide valuable insights into the regulatory role of these lncRNAs in response to Arac therapy in K562 leukemia cells, potentially paving the way for targeted therapeutic interventions in CML (Figure 1).

Effects of Hydroxycarbamide-Cyclophosphamide Treatment on the Expression of Long Non-Coding Ribonucleic Acids

The investigation into the expression changes of various lncRNAs under the HU + CP treatment yielded significant findings. Notably, with respect to lncRNA NEAT1, the most effective concentrations and durations were 212 μM HU + 50 μM CP for 24 hours and 212 μM HU + 50 μM CP for 48 hours, with expression levels of 1.138 ($P < 0.001$) and 1.094 ($P < 0.001$), respectively. Similarly, concerning lncRNA EMG3, the highest impact was noted at concentrations of 212 μM HU + 50 μM CP for 24 hours and 212 μM HU + 50 μM CP for 48 hours, recording expression levels of 1.191 ($P < 0.001$) and 1.101 ($P < 0.001$), respectively. As regards lncRNA FENDRR, optimal outcomes were evident at 212 μM HU + 50 μM CP for 24 hours, with an expression level of 1.331 ($P < 0.001$), and at 212 μM HU + 50 μM CP for 48 hours, with an expression level of 0.975 ($P < 0.001$). Conversely, the expression changes of lncRNA HULC represented the highest effect at concentrations of 181 μM HU + 25 μM CP for 24 hours and 181 μM HU + 25 μM CP for 48 hours, indicating expression levels of 1.48 ($P < 0.001$) and 1.328 ($P < 0.001$), respectively. These results underscore the significance of specific drug concentrations and durations in modulating the expression of these lncRNAs under HU + CP treatment, offering valuable insights into potential therapeutic strategies (Figure 2).

Effects of Hydroxycarbamide Treatment on the Expression of Long Non-Coding Ribonucleic Acids

In our investigation of the expression changes of lncRNAs NEAT1, EMG3, FENDRR, and HULC under HU treatment, various drug concentrations and drug-consuming durations were examined to identify the most efficient conditions.

Regarding lncRNA NEAT1, significant changes were observed in expression at different drug concentrations and durations. Notably, the concentration of 212 μM HU for 24 hours exhibited the highest effect on lncRNA NEAT1 expression, resulting in a significant decrease (0.950, $P < 0.001$).

In the case of lncRNA EMG3, the impact of HU treatment was notable. The concentration of 242 μM HU for 24 hours led to no expression, indicating a substantial inhibitory effect. Additionally, 212 μM HU for 72 hours demonstrated the highest upregulation (1.296, $P < 0.001$).

Based on our investigation of lncRNA FENDRR,

consistent and significant expression changes were found across different concentrations and durations. The concentration of 212 μM HU for 24 hours induced the highest upregulation level (1.202, $P < 0.001$).

Finally, concerning lncRNA HULC, there were consistent and noticeable expression changes under HU treatment. The concentration of 181 μM HU for 24 hours could induce the most considerable upregulation (1.372, $P < 0.001$).

These findings highlight the sensitivity of lncRNAs NEAT1, EMG3, FENDRR, and HULC to HU treatment, with specific concentrations and durations exerting distinct effects on their expression profiles. The 212 μM HU concentration for 24 hours was most effective in modulating the expression of lncRNA NEAT1, while for lncRNA EMG3, the 242 μM HU concentration for 24 hours led to significant suppression. Additionally, lncRNA FENDRR exhibited notable upregulation with 212 μM HU for 24 hours. Ultimately, lncRNA HULC showed significant upregulation under the 181 μM HU concentration for 24 hours. These findings provide valuable insights into the potential therapeutic applications of HU in modulating the expression of these lncRNAs in the context of cancer treatment (Figure 1).

Effects of Hydroxycarbamide-Cytarabine Treatment on the Expression of Long Non-Coding Ribonucleic Acids

Significant results were observed in the investigation of lncRNA NEAT1 expression changes under HU + Arac treatment. At 24 hours, the highest concentration was found to be 242 μM HU + 80 μM Arac, with a significantly increased expression level of 0.955 ($P < 0.001$). Over 48 hours, the optimal concentration remained consistent at 242 μM HU + 80 μM Arac, displaying a considerable elevation in expression (1.045, $P < 0.001$). Similarly, at 72 hours, the most noticeable concentration continued to be 242 μM HU + 80 μM Arac, revealing a significant increase in expression (1.283, $P < 0.001$).

The investigation of the expression changes of lncRNA EMG3 under HU + Arac treatment indicated significant findings. At 24 hours, 212 μM HU + 56 μM Arac was the highest concentration, resulting in a notably elevated expression level (0.903, $P < 0.001$). Over 48 hours, the concentration of 181 μM HU + 32 μM Arac demonstrated the highest efficiency, with an increased expression level of 0.966 ($P < 0.001$). The concentration of 212 μM HU + 56 μM Arac had the highest effect at 72 hours, yielding an expression level of 1.611 ($P < 0.001$).

Significant outcomes were obtained regarding FENDRR lncRNA expression changes under HU + Arac drug treatment. At 24 hours, the most effective concentration was 242 μM HU + 80 μM Arac, showcasing a considerable elevation in expression (1.064, $P < 0.001$). Over 48 hours, the concentration of 181 μM HU + 32 μM Arac had the highest effect, with an increased expression level of 1.071 ($P < 0.001$). However, at 72 hours, the expression level remained consistent across various concentrations, representing the lack of significant changes ($P = 0.505$).

Eventually, the examination of lncRNA HULC expression changes under HU + Arac treatment demonstrated significant results. At 24 hours, the concentration of 242 μM HU + 80 μM Arac was most effective, yielding a significantly increased level of expression (1.643, $P < 0.001$). Over 48 hours, the optimal concentration remained consistent at 242 μM HU + 80 μM Arac, displaying a noticeable elevation in expression (1.472, $P < 0.001$). Conversely, at 72 hours, the expression level was most effective at the concentration of 212 μM HU + 56 μM Arac, with a notable increase (1.048, $P < 0.001$).

These findings confirm that the expression changes of lncRNAs NEAT1, EMG3, FENDRR, and HULC under HU + Arac treatment depend on concentration and duration, shedding light on potential avenues for targeted therapeutic interventions in the context of acute lymphoblastic leukemia (Figure 2).

Effects of Different Complex Drug Treatments on the Expression of Long Non-Coding Ribonucleic Acids

The expression changes of lncRNAs NEAT1, EMG3, FENDRR, and HULC were rigorously investigated under the influence of complex drug treatments, and the results revealed distinct patterns of expression alterations across different concentrations and durations.

For lncRNA NEAT1, the most significant changes were observed at 24 hours, with Complex 1 showing a remarkable increase (1.141-fold) and Complex 3 displaying a significant decrease (1.094-fold) in expression. At 48 hours, Complex 2 exhibited the most considerable upregulation (1.062-fold), whereas Complex 3 illustrated a significant reduction (1.048-fold). At 72 hours, Complex 3 showed the highest level of downregulation (0.945-fold), while Complex 2 represented a noticeable decrease (0.967-fold).

Regarding lncRNA EMG3, the most significant impacts were recorded at 48 hours, with Complex 3 demonstrating the highest upregulation (1.166-fold). Complex 2 also displayed notable upregulation at this timepoint (1.061-fold). Similarly, Complex 2 exhibited the highest level of increase (1.193-fold) at 72 hours, closely followed by Complex 3 (1.211-fold).

Examining lncRNA FENDRR, the most noticeable alterations were identified at 24 hours, with Complex 3 illustrating the highest upregulation (1.181-fold), while Complex 1 represented a moderate increase (1.028-fold). At 48 hours, Complex 3 continued to show the highest upregulation (1.092-fold). Contrarily, Complex 2 displayed considerable downregulation (0.975-fold). At 72 hours, Complex 3 demonstrated consistent upregulation (0.958-fold), while Complex 2 depicted significant downregulation (0.920-fold).

Finally, in the case of lncRNA HULC, the highest alterations occurred at 24 hours, with Complex 2 showing the highest level of upregulation (1.198-fold), while

Complex 3 exhibited a meaningful increase (1.066-fold). At 48 hours, Complex 1 illustrated the most notable upregulation (1.142-fold), whereas Complex 3 displayed significant downregulation (0.847-fold). At 72 hours, Complex 1 demonstrated the most considerable increase (1.007-fold), while Complex 3 represented noticeable downregulation (0.715-fold).

In general, the expression changes of these lncRNAs under complex drug treatments were found to be concentration-dependent and time-dependent, highlighting the intricate regulatory dynamics within the investigated cellular systems (Figure 3).

Effects of Different Complex Drug Treatments on the Expression of Long Non-Coding Ribonucleic Acids

The investigation into the expression changes of various lncRNAs under complex drug treatments yielded significant findings. For lncRNA NEAT1, the most effective outcomes were observed at concentrations of 1.065 μM after 24 hours, 1.021 μM after 48 hours, and 0.648 μM after 72 hours, all displaying statistically significant alterations ($P < 0.001$). In the case of lncRNA EMG3, the highest expression changes occurred at concentrations of 1.238 μM after 48 hours and 1.411 μM after 72 hours ($P < 0.001$). Conversely, lncRNA FENDRR exhibited less significant alterations, with significant changes detected at concentrations of 0.868 μM after 24 hours, 0.894 μM after 48 hours, and 1.044 μM after 72 hours ($P < 0.001$). Eventually, lncRNA HULC depicted notable changes in expression at concentrations of 0.957 μM after 24 hours and 0.608 μM after 72 hours ($P < 0.001$). These results underline the concentration-dependent and duration-dependent effects of complex drug treatments on the expression of these lncRNAs, shedding light on potential therapeutic avenues for the treatment of CML (Figure 3).

Effects of Nickel-Thiosemicarbazone Treatments on the Expression of Long Non-Coding Ribonucleic Acids

The results of our investigation regarding the expression changes of various lncRNAs under Ni-TSC treatment indicated distinct patterns of gene expression alterations. Notably, when examining the expression of NEAT1 lncRNA, it was observed that treatment with 25 μM Ni for 24 hours yielded a significant increase in expression (1.074, $P < 0.001$), suggesting its potential responsiveness to this concentration and duration. Similarly, at 48 hours, 11.5 μM Ni treatment induced a notable increase in NEAT1 expression (1.011, $P < 0.001$).

In contrast, our study of EMG3 lncRNA expression revealed contrasting trends. Treatment with 12 μM Ni for 48 hours resulted in a considerable increase in expression (1.086, $P < 0.001$), while 25 μM Ni for 24 hours exhibited a similar effect (1.301, $P < 0.001$). These findings imply that EMG3 lncRNA expression might be particularly influenced by specific concentrations and durations.

As regards FENDRR lncRNA, our analysis showed less significant changes in response to Ni-TSC treatment. Remarkably, the expression of FENDRR lncRNA remained relatively stable across various concentrations and durations, with minimal deviations from baseline levels.

Ultimately, a concentration-dependent response was observed when assessing the expression of HULC lncRNA. Specifically, 26 μM Ni treatment for 24 hours

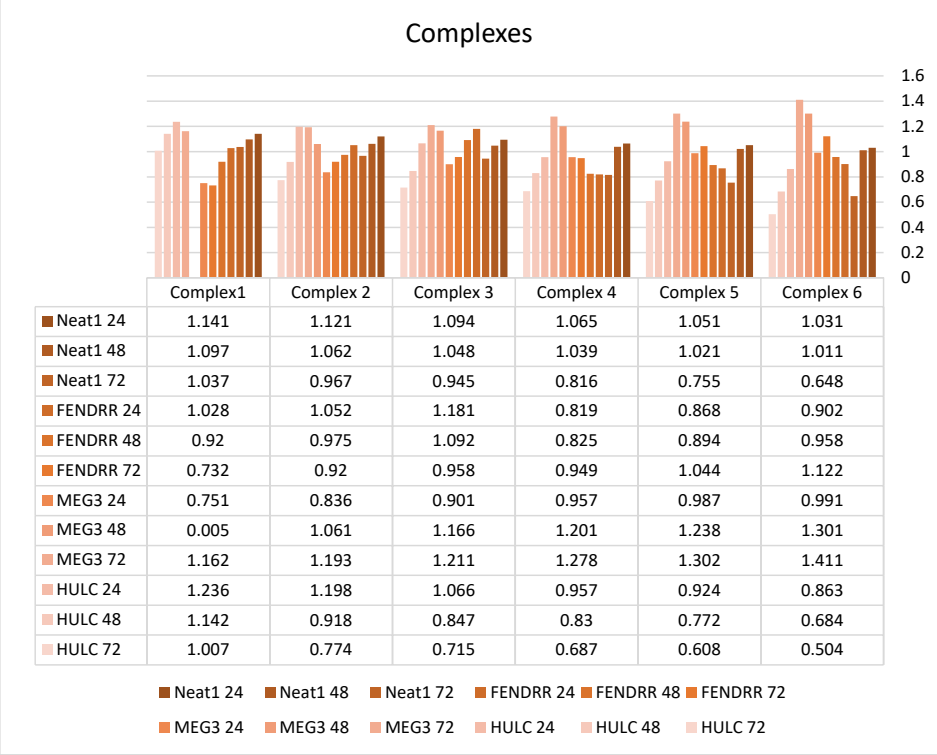


Figure 3. Effects of Different Complex Drug Treatments on the Expression of LncRNAs. *Note*. LncRNA: Long non-coding ribonucleic acid

resulted in a noticeable increase in HULC expression (1.092, $P<0.001$), suggesting that this concentration and duration may be most effective in modulating HULC expression. Contrarily, 11.5 µM Ni treatment for 48 hours led to a decrease in HULC expression (0.911, $P<0.001$), indicating a distinct response pattern.

Overall, our results confirmed that the expression changes of these lncRNAs under Ni-TSC treatment vary in a concentration-dependent and time-dependent manner. NEAT1 and EMG3 lncRNAs displayed notable responsiveness to specific concentrations and durations, while FENDRR lncRNA exhibited relative stability. HULC lncRNA, on the other hand, demonstrated concentration-dependent effects. These findings shed light on the intricate regulatory mechanisms governing lncRNA expression under the influence of Ni-TSC treatment and provide valuable insights for further investigations in this area (Figure 4).

Effects of Copper-Thiosemicarbazone Treatments on the Expression of Long Non-Coding Ribonucleic Acids

In the investigation of lncRNA NEAT1 expression changes under Cu-TSC treatment, the most effective concentration and time combination was found to be 11.25 µM Cu over 24 hours, where expression showed a significant decrease ($P<0.001$). Similarly, the most effective concentration and duration in the evaluation of the expression changes of EMG3 lncRNA under Cu-TSC treatment were determined to be 9.25 µM Cu for 48 hours, resulting in a remarkable increase in expression ($P<0.001$). Conversely, in the analysis of FENDRR lncRNA expression changes under Cu-TSC treatment, the most optimal condition was found to be 8 µM Cu over 48 hours, leading to a significant upregulation in expression ($P<0.001$). In the examination of lncRNA HULC expression changes under Cu-TSC treatment, the most noticeable concentration and duration were identified as 8.75 µM Cu for 24 hours, showcasing a marked increase in expression ($P<0.001$).

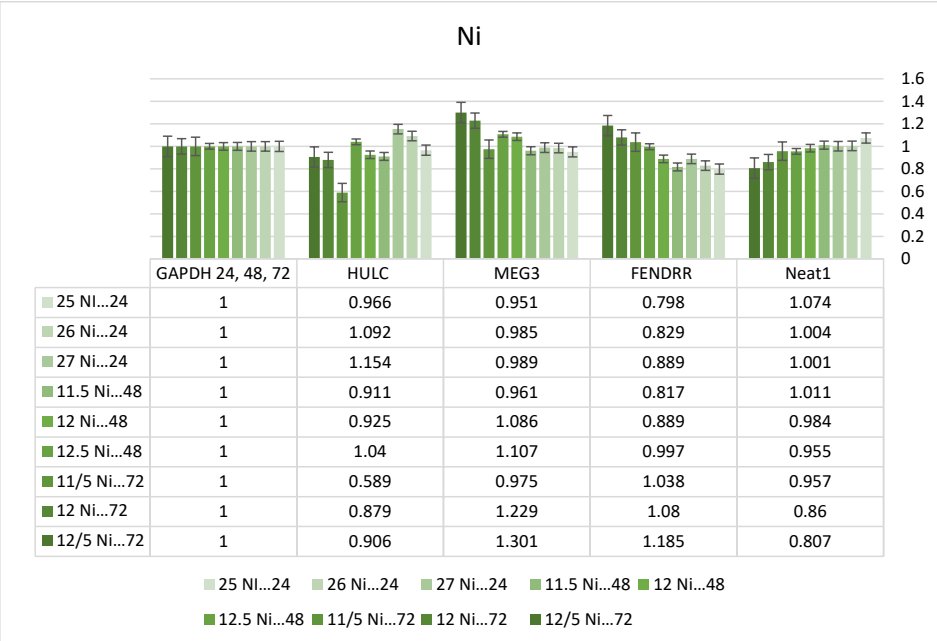
These findings underscore the distinct responses of various lncRNAs to Cu-TSC treatment, highlighting the importance of both concentration and duration in modulating their expression levels. These insights into lncRNA expression changes contribute to a deeper understanding of the molecular mechanisms underlying therapeutic interventions in the context of cancer treatment (Figure 5).

Discussion

Previous studies have also highlighted the effects of TSC complexes and chemotherapy drugs on the expression of key lncRNAs, such as NEAT1 and GAS5, in leukemic cell lines.[21-26]

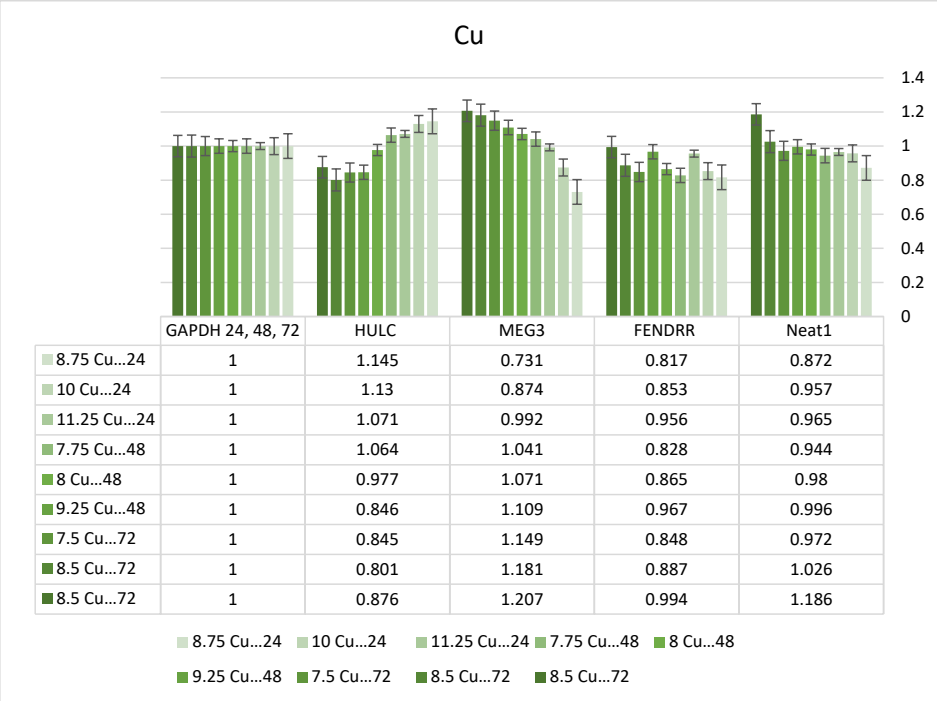
Our study delved into the intricate world of lncRNAs and their dynamic responses to various drug treatments, shedding light on the potential therapeutic implications for CML. CML is a complex hematological malignancy, and finding effective treatment for this malignancy is a challenge.[24,25] Nonetheless, understanding the modulation of lncRNA expression in response to different drugs provides a novel avenue for targeted therapeutic approaches.[26] This can potentially lead to more tailored and effective treatments for CML patients.

These findings provide critical insights into the modulation of lncRNA expression in the context of chemotherapy, offering a basis for the development of targeted treatment strategies. Significant alterations in the expression of lncRNA FENDRR, EMG3, HULC, and NEAT1 were observed under CP treatment. A notable upregulation of FENDRR was evident across



	GAPDH 24, 48, 72	HULC	MEG3	FENDRR	Neat1
25 Nİ...24	1	0.966	0.951	0.798	1.074
26 Ni...24	1	1.092	0.985	0.829	1.004
27 Ni...24	1	1.154	0.989	0.889	1.001
11.5 Ni...48	1	0.911	0.961	0.817	1.011
12 Ni...48	1	0.925	1.086	0.889	0.984
12.5 Ni...48	1	1.04	1.107	0.997	0.955
11/5 Ni...72	1	0.589	0.975	1.038	0.957
12 Ni...72	1	0.879	1.229	1.08	0.86
12/5 Ni...72	1	0.906	1.301	1.185	0.807

Figure 4. Effects of Nickel-Thiosemicarbazone Treatments on the Expression of LncRNAs. *Note*. LncRNA: Long non-coding ribonucleic acid



	GAPDH 24, 48, 72	HULC	MEG3	FENDRR	Neat1
8.75 Cu...24	1	1.145	0.731	0.817	0.872
10 Cu...24	1	1.13	0.874	0.853	0.957
11.25 Cu...24	1	1.071	0.992	0.956	0.965
7.75 Cu...48	1	1.064	1.041	0.828	0.944
8 Cu...48	1	0.977	1.071	0.865	0.98
9.25 Cu...48	1	0.846	1.109	0.967	0.996
7.5 Cu...72	1	0.845	1.149	0.848	0.972
8.5 Cu...72	1	0.801	1.181	0.887	1.026
8.5 Cu...72	1	0.876	1.207	0.994	1.186

Figure 5. Effects of Copper-Thiosemicarbazone Treatments on the Expression of LncRNAs. *Note*. LncRNA: Long non-coding ribonucleic acid

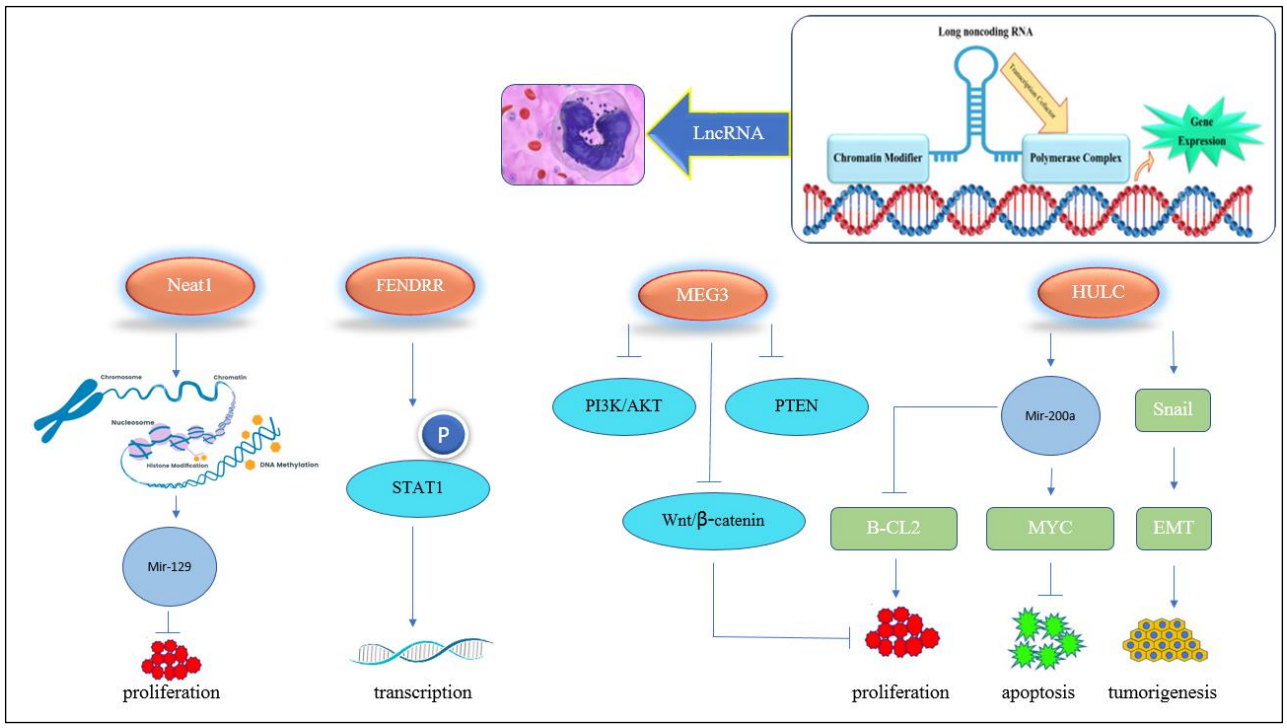


Figure 6. Association of HULC, NEAT1, FENDRR, and EMG3 Long Non-coding Ribonucleic Acid Expression Profiles in K562 Chronic Myeloid Leukemia Cells

all concentrations and durations, emphasizing its sensitivity to CP therapy. Conversely, EMG3 exhibited a concentration-dependent response, with increased expression at lower CP concentrations. Intriguingly, HULC displayed both upregulation and downregulation, contingent on concentration and duration. In addition, NEAT1 primarily responded with downregulation, except at specific concentrations and timepoints. These results underscore the intricate, context-specific effects of CP treatment on lncRNA expression. Based on the results, concentration and time-dependent responses were found when combining CP and Arac. Notably, NEAT1 and EMG3 responded optimally to 56 μM Arac + 50 μM CP over 24 hours, showcasing opposing expression changes. FENDRR favored the concentration of 80 μM Arac + 100 μM CP, maintaining downregulation over time. HULC

represented divergent responses, implying the complex interplay of these lncRNAs in the face of combination therapy. These findings offer potential avenues for targeted therapeutic approaches in CML. Our exploration of HU treatment revealed intriguing responses among lncRNAs. NEAT1 and EMG3 illustrated decreased expression, while FENDRR and HULC displayed significant upregulation. The concentration-dependent and duration-dependent modulation of lncRNAs highlights the multifaceted impacts of HU on CML. The combination of HU and Arac resulted in nuanced responses in lncRNA expression. NEAT1 and EMG3 responded optimally to 242 µM HU + 80 µM Arac, showcasing the concentration-dependent and time-dependent effects of this regimen. FENDRR depicted consistent upregulation, while HULC showed diverse responses, underscoring the complexity of combination therapies. In the context of complex drug treatments, distinct patterns emerged. NEAT1 displayed increased expression under Complex 1 and decreased expression under Complex 3. Additionally, EMG3 exhibited significant upregulation in Complex 3, while FENDRR and HULC illustrated varied expression changes across different concentrations and durations. Our investigation into Ni-TSC and Cu-TSC treatments highlighted unique responses among lncRNAs. NEAT1 represented sensitivity to Cu treatment, with downregulation at 11.25 µM Cu over 24 hours. EMG3 responded optimally to 9.25 µM Cu for 48 hours. FENDRR depicted a marked upregulation at 8 µM Cu for 48 hours. Likewise, HULC demonstrated distinct concentration-dependent and duration-dependent responses.

While previous studies have explored lncRNA expression in CML, our research provides a more comprehensive view by examining multiple drugs and their combinations (Figure 1). This expanded scope enhances our understanding of the nuanced responses of lncRNAs to treatment.

Conclusion

In general, our study underlines the complexity of lncRNA responses to various drug treatments in the context of CML. The concentration-dependent and duration-dependent nature of these responses emphasizes the importance of precise treatment regimens tailored to individual lncRNA profiles. These findings open avenues for targeted therapeutic interventions, moving us closer to more effective treatments for CML. Nonetheless, further research is warranted to elucidate the underlying mechanisms governing these lncRNA responses and to translate these discoveries into clinical applications.

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Software: Nastaran Yegani.
Supervision: Golnaz Asaadi Tehrani.
Validation: Haleh Yaghoubi.
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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

This study was approved by the Ethics Committee of Islamic Azad University, Zanjan Branch, Zanjan, Iran. Due to the absence of human subjects or laboratory animals, the study required no ethical code.

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Supplementary File

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