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■ RESEARCH ARTICLE

Investigation of Functional Role of a Novel Biomarker: CCNC Gene Deletion on Human Leukemia Cancer

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ABSTRACT: Leukemia is deadly cancer that increases the number of leucocytes in the blood or bone marrow. Even though the recent improvements in treatment protocols, the survival rate for five years after diagnosis is only 29.5%. Therefore, it is crucial to understand the biomarker and its molecular mechanism of how leukemia develops tumor progression. This study analyzed the genomic alteration and clinical profiles of 1978 leukemia patients using the cBioPortal database. We found that nine out of ten genes were deleted in chromosome 6q16.1-2. Also, we discovered that the CCNC gene deletion is enriched in deceased patients (9.09%) compared to living patients (1.33%). To further understand how CCNC gene deletion affects leukemia cancer progression, we silenced the CCNC gene using small interfering RNA (siRNA) to mimic CCNC gene deletion using the Jurkat leukemia cancer cell line. Our result showed that CCNC knockdown significantly increased cancer cell migration. We found that CCNC deletion or knockdown is a crucial novel biomarker that increases cancer cell migration and may cause poor prognosis in leukemia patients. This finding may support the current limitation of diagnosis and improve the accuracy of predicting the prognosis of leukemia patients.

KEYWORDS: Cellular and Molecular Biology, Cancer Biology, Genetics, Leukemia, CCNC, Patient Survival.

■ Introduction

Leukemia is cancer found in blood and blood-forming tissues, including the bone marrow and the lymphatic system.[1] Leukemia usually involves white blood cells, as the bone marrow of leukemia patients produces an excessive amount of abnormal white blood cells that cannot function properly. Leukemia is the most common cancer in childhood and teens, accounting for almost one out of three cancers.[2] Most childhood leukemias are acute lymphocytic leukemia (ALL). On the other hand, Acute Myeloid Leukemia (AML) is more common in older adults. Leukemia accounts for 10% of all diagnosed cancers in the United States. Currently, leukemia has a relative survival rate of 5 years, about 29.5% after diagnosis.[3]

A cancer biomarker refers to a substance or a process indicative of cancer in the body, including proteins or genes.[4] Biomarkers help detect cancer; about 40% of cancers can be cured if they are detected early enough through biomarkers. There are cancer biomarker types: Genetic, epigenetic, proteomic, and glycomic.[5] This study focused on genetic biomarkers, which refer to genes enriched in deceased cancer patients.

Genes are commonly deleted in leukemia patients. Gene deletions, which occur because of mutations, can eliminate tumor-suppressor genes and promote cancer.[6] Gene deletions refer to the loss of both copies of genes, thus leaving zero copies of the gene. Previous research showed that 68.2% (144/211) of adult B-ALL patients carried gene deletions, and the frequency is much higher in Ph+B-ALL patients.[7] IKZF1 gene deletion is the most common gene deletion in adult B-ALL, followed by CDKN2A/B deletion.[7]

Searching for a novel biomarker is essential to develop a novel leukemia diagnosis method and treatment. Therefore, we searched for a novel biomarker significantly affecting the leukemia patient's survival. Consequently, we analyzed 1978 leukemia patient genomics and found that the CCNC gene was deleted in deceased patients. Next, we analyzed the functional role of CCNC gene deletion in leukemia cells using siRNA transfection. Overall, this study found that CCNC gene deletion or knockdown is a crucial biomarker that increases cancer cell migration in leukemia cancer cells.

■ Methods and Materials

cBioPortal patient data analysis:

cBioPortal is an open-source platform that provides access to comprehensive cancer genomics data and allows users to visualize, analyze, and interpret cancer genomics data. Therefore, it can be used to analyze patient survival data in the context of cancer genomics. A total of 1,551 leukemia patients' information and genomic data were retrieved from cBioPortal.

Cell culture and maintenance:

The human leukemia cancer cell line Jurkat was purchased from the Korean Cell Line Bank (Seoul, Korea). Jurkat cells were cultured in RPMI-1640 medium (Gibco) supplemented with 10% HyClone™ fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin and streptomycin (Gibco) in a 5% CO_2 incubator at 37°C.

siRNA transfection in Jurkat cells:

A total of 100,000 cells were prepared in each well on a 6-well culture plate. Then the siRNA stock was prepared with 10 pmole/μL. In the separate tube, 1 nM of siRNA was prepared with different volumes of INTERFERin reagent (Polyplus), each with 8 μL, 4 μL, and 12 μL, as well as siRNA

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negative control with 8 µL. The mixture samples were then incubated for 10 minutes at room temperature. Finally, the transfection mix was added to the cells in serum containing RPMI-1640 medium. The cells were incubated for 48 hours. The following siRNA sequence targeting CCNC was used: 5'-CUGUAUCCUCCUUUGAUGA=tt-3'(anti-sense), 5'-UCAUGAAAGGAGGAUACAG=tt-3' (sense).

Total RNA extraction:

AccuPrep Universal RNA Extraction Kit (Bioneer) was used to extract total RNA from the Jurkat cultured cells, followed by the manufactured protocol.

cDNA synthesis:

To synthesize the cDNA library, TOPscriptTM Reverse Transcriptase (Enzynomics) was used, and a total of 10 µL reaction condition was prepared with 10X reaction buffer, reverse transcriptase enzyme, dNTP, oligo dT primer, and 1 µg of extracted RNA were used for each sample to proceed cDNA synthesis. The samples were incubated in the following condition: 45 ˚C for 1 hour, 95 ˚C for 5 minutes, and infinite for storage at 15 ˚C.

Polymerase chain reaction:

AccuPower® PCR PreMix (Bioneer) was used to amplify CCNC and GAPDH cDNA. A total of 20 µL reactions were made. 10 pmol of forward primer and reverse primer were added with 100 ng of cDNA. The PCR reaction proceeded in the following condition: 95 ˚C for 5 minutes (step 1), 95 ˚C for 30 seconds (step 2), 58 ˚C for 30 seconds (step 3), 72 ˚C for 15 seconds (step 4), repeat from step 2 to 4 for 29 times, 72 ˚C for 5 minutes (step 5), and infinite for storage at 12 ˚C.

Cell migration:

The cell migration was analyzed using the transwell assay (Corning). First, Jurkat cells were added with cell media without fetal bovine serum cell media in the upper chamber. Then, the cell media with fetal bovine serum was added to the bottom chamber to stimulate cell migration. Finally, the cells were incubated for 96 h, and the cells migrated to the bottom chamber was imaged.

Results and Discussion

Table 1: Top 10 deleted genes that are significantly enriched in deceased leukemia patients.

Gene	Cytoband	Living group	Deceased group	p-Value	q-Value	Most enriched in
CCNC	6q16.2	7 (1.33%)	14 (9.09%)	5.86E-06	8.27E-04	DECEASED
EPHA7	6q16.1	7 (1.33%)	14 (9.09%)	5.86E-06	8.27E-04	DECEASED
PRDM13	6q16.2	7 (1.33%)	14 (9.09%)	5.86E-06	8.27E-04	DECEASED
USP45	6q16.2	7 (1.33%)	14 (9.09%)	5.86E-06	8.27E-04	DECEASED
COQ3	6q16.2	8 (1.52%)	14 (9.09%)	1.73E-05	2.27E-03	DECEASED
FAXC	6q16.2	8 (1.52%)	14 (9.09%)	1.73E-05	2.27E-03	DECEASED
MCHR2	6q16.2	8 (1.52%)	14 (9.09%)	1.73E-05	2.27E-03	DECEASED
PNISR	6q16.2	8 (1.52%)	14 (9.09%)	1.73E-05	2.27E-03	DECEASED
SIM1	6q16.3	8 (1.52%)	14 (9.09%)	1.73E-05	2.27E-03	DECEASED
ADD3	10q25.1-q25.2	16 (3.04%)	19 (12.34%)	2.45E-05	3.17E-03	DECEASED

We analyzed leukemia patients' genomics using the cBioPortal genomic database to find the novel leukemia biomarker. Through this, we further investigated the deletion of genes from the DNA of the leukemic patients. As a result, we found the top ten deleted genes at a higher level in deceased leukemia patients: CCNC, EPHA7, PRDM13, USP45, COQ3, FAXC, MCHR2, PNISR, SIM1, and ADD3. Nine of these genes are located in chromosome 6, with one exception, which is located in chromosome 10 (Table 1). This study focused on CCNC gene deletion, as CCNC has not been well studied in leukemia cancer cells. Also, CCNC was one of the top-ranked enriched deleted genes in the deceased group: comparing the data from living and deceased categories, only 1.33% were living, while 9.09% were deceased. Thus, we found ten novel genes were deleted in deceased leukemia patients, making them novel leukemia biomarkers.

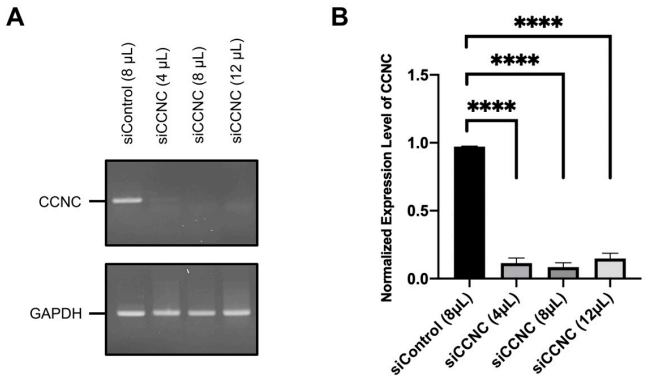

Figure 1: siRNA targeting CCNC decreased CCNC expression level in human leukemia Jurkat cells (A) Agarose gel representing the amplified CCNC and GAPDH cDNA. (B) Bar graph illustrating mean and standard deviation of the normalized expression level of CCNC. One-way ANOVA with Tukey's post hoc test was performed to calculate the p-value. $p < 0.0001$ (****).

We found that CCNC gene deletion was enriched in deceased leukemia patients. To further investigate the function of CCNC deletion on leukemia cancer cell development, we silenced the CCNC gene using siRNA transfection. Four siRNA transfection conditions were tested for this experiment to optimize the siRNA transfection: siControl, siCCNC (4 µL, 8 µL, 12 µL). Then, the amplification of two genes (CCNC and GAPDH) was analyzed in agarose gel electrophoresis. GAPDH was used to normalize the CCNC expression level. Finally, the band intensity of CCNC was quantified, and the normalized expression level of CCNC was calculated. The results show that all three CCNC siRNA transfection conditions significantly decreased the expression level of CCNC in human leukemia Jurkat cells (Figure 1). Thus, our designed siRNA targeting CCNC successfully silenced the CCNC gene expression.

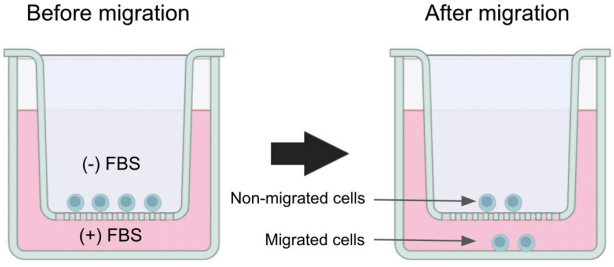

Figure 2: The representation of the concept of migration assay using transwell. Jurkat cells were placed on the upper chamber of the transwell, which has small holes that cancer cells can pass through to the bottom chamber. The migration of cells towards FBS-containing media was measured as an indicator of cell migration ability.

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Cell migration is essential in many biological processes, such as embryological development, tissue formation, immune defense or inflammation, and cancer progression. Especially cell migration is one of the initial processes of cancer metastasis. Since CCNC deletion was enriched in deceased leukemia patients, we hypothesize that CCNC silencing may increase cell migration. Cancer cells are known to localize around the human blood vessels, which contain abundant nutrients such as glucose and oxygen. Cancer cells can sense the nutrients and move towards high-nutrition conditions. Therefore, in this experiment, we designed a transwell experiment that provides two different conditions: with and without FBS, which contains high nutrition for cancer cells. First, we placed the Jurkat cells on the upper chamber of the transwell (Figure 2). The upper chamber transwell has small holes that cancer cells can pass through to the bottom chamber. This transwell assay can measure the cell migration ability that cancer cells move towards the nutrition full environment.

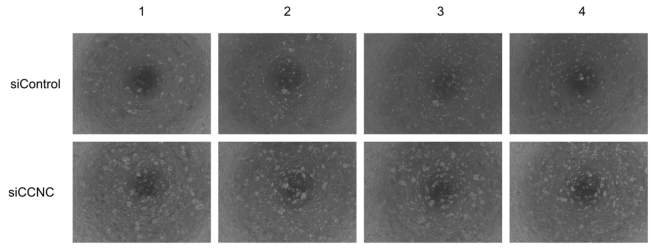

Figure 3: CCNC gene silencing increased Jurkat leukemia cell migration. The migrated cells were then photographed, and the number of migrated cells was quantified. Four replicate samples were analyzed after transfecting siControl and siCCNC.

We underwent CCNC silencing to find the novel function of the CCNC gene in leukemia cells. CCNC silencing increased cell migration in leukemia cells, as shown in Figure 3. A greater number of leukemia cells were found in the siCCNC transfected sample compared to the siControl transfected sample. This means the increased number of CCNC silenced cells have migrated from (-) FBS to (+) FBS. We repeated the experiment four times and had the same result. Therefore, our results show that inhibiting the expression of CCNC enhanced cell migration and may lead to decreased leukemia patient survival.

Conclusion

This study aimed to identify novel genes deleted in leukemia patients that could serve as important biomarkers for the disease. The study identified ten such genes by analyzing genomic data, and one of them, CCNC, was investigated further for its functional role in leukemia. In addition, the study aimed to understand how the deletion of CCNC affects the migration ability of leukemia cells and whether it could be used as a potential biomarker for leukemia prognosis.

As shown in Table 1, we have found ten novel genes deleted in leukemia patients that make them important biomarkers for leukemia. In Figure 1, the agarose gel represents how CCNC siRNA transfection significantly decreases the normalized expression level of CCNC, indicating successful silencing of CCNC gene expression. Figure 2 shows our concept of migration assay using transwell to measure the cell migration ability toward the environment with richer nutrients. Finally, Figure 3 demonstrates the result in which silencing CCNC increases the migration of the leukemia cells. Overall, the deletion of CCNC acts as a novel biomarker for leukemia, as it contributes to enhanced cancer cell migration and eventually decreased chance of survival.

Nevertheless, we acknowledge some limitations to our experiment. First, eight other gene deletions in chromosome 6 other than CCNC are significantly enriched in deceased leukemia patients. Therefore, we need to analyze the function of the other deleted genes. Second, only one type of leukemia cell line was used, which was Jurkat. Therefore, we need to verify our results using different types of leukemia cancer cell lines. Also, we only investigated the functional role of CCNC on cell migration. Investigation into the functional role in cell proliferation, cell invasion, and chemoresistance is also needed. Finally, we only performed an *in vitro* experiment and must complete a mouse experiment to verify our results further.

The findings of our study have significant implications for the diagnosis and treatment of leukemia. Our identification of CCNC deletion as a novel biomarker for leukemia patients provides an easier and more accurate diagnosis method using genomic information, leading to a more precise prognosis prediction. Furthermore, personalized treatments based on genomic information can increase the efficacy of cancer therapy drug and minimize the side effects.

Additionally, our study highlights the association between CCNC deletion and poor prognosis in leukemia patients. The deletion of CCNC increases cancer cell migration and promotes metastasis. Thus, our findings suggest that targeting CCNC could be a potential therapeutic strategy to prevent metastasis and improve the survival rate of leukemia patients.

Overall, our study contributes to advancing the understanding of the genetic mechanisms underlying leukemia, which can significantly impact the development of diagnosis and treatment options for patients. In addition, by identifying novel biomarkers and elucidating their functional roles, we pave the way for developing personalized medicine that considers each patient's unique genetic makeup, leading to more effective and targeted treatments.

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Sooah Yoo is a current high school junior at St. Mark's School, MA. As a student who enjoys diving into scientific research, she aims to major in biology and medical science, pursuing a passion for neuroscience and genetics. She is willing to continue her research on cancer and leukemia in the future to improve human health.

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