TUSC3 Expression in Childhood Acute Lymphoblastic Leukemia Patients in Baghdad, Iraq

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer world-wide. This disease is characterized by wide-range of recurrent genetic aberrations that have an impact on the disease initiation and patient’s outcome. This study was set to investigate the expression of TUSC3, tumor suppressor candidate 3, in a set of 31 cases diagnosed with ALL at the Child’s Central Teaching Hospital, Baghdad- Iraq using qPCR technique. Down-regulation of TUSC3 was observed in the majority (17/31(64.5%) of the studied ALL cases, while the rest of the patients (14/31(35.5%) have shown normal to unregulated TUSC3 expressionsuggesting a key role for this gene in the diseases biology. The expression level of TUSC3 gene could be investigated further, in large scale studies, for its diagnosis and prognosis value, especially within the different cytogenetic subtypes of ALL.

Keywords: [acute lymphoblastic leukemia, TUSC3 expression, qPCR]


Introduction:

Acute lymphoblastic leukemia (ALL) is a bone marrow neoplastic marked by acute proliferation of lymphoid hematopoietic progenitors. This leads to the accumulation of immature-functionally impaired white leukemic cells (blasts) in the bone marrow and peripheral blood as well replacing normal hematopoietic components, with predominance of B-ALL lineage(Woo et al., 2014). ALL is the most common childhood cancer which accounts for approximately 80% of pediatric leukemia’s, however, this disease represents only 20% of adult acute leukaemia’s(Board, 2019). In a number of developed countries, intensive chemotherapy utilizing contemporary therapies along with palliative care have raised the cure rate for children with ALL up to 90% (Ghaffiar et al., 2019, Devi and Allenidekania, 2019), however, only half of that successive cure rate have been achieved in adult ALL(Stock et al., 2019).
As a heterogeneous disease, ALL is characterised by a wide-range of genetic and epigenetic alterations (Mullighan, 2012, Gabriel et al., 2015). These including recurrent genetic aberrations with known prognostic value such as ETV6/RUNX1, BCR/ABL1 (Bartram et al., 2019, Moorman, 2012). In addition to large number leukaemia-associated molecular changes that have been investigated for their potential utility as biomarkers for the disease diagnosis and prognosis. In this respect, researchers efforts have mainly concentrated on alteration in cancer-related genes including those with oncogenic and tumor suppression potentials. Moreover, genes that are believed to have a role in drug resistance are of a great research interest (Zhao, 2016). Identifying such molecular signature that could be used to stratified leukemia patients into different risk groups would help to guide the treatment options and intensification. Accordingly, low risk leukaemia patients could spare of high doses of chemotherapy to minimize the short/long-terms side effects associated with chemotherapies.

*TUSC3* is the tumor suppressor candidate 3, (previously termed N33), a gene frequently reported to be methylated and down regulated in different types of solid tumor(Yu et al., 2017, Sheng et al., 2018). *TUSC3* maps at 8p22, a region also concurrently deleted and or mutated over multiple types of epithelial malignancies( Huang et al., 2009).It is thought that *TUSC3* may act as plasma membrane Mg2+ transporter and has essential role in the vertebrate embryonic development(Zhou and Clapham, 2009).It has been also suggested that *TUSC3* can act as a subunit of the endoplasmic reticulum-bound oligosaccharyltransferase (OST) complex, with having an oxidoreductase action. In this context, *TUSC3* defect can disrupt the N-glycosylation process(Horak et al., 2014).

Furthermore, *TUSC3* dysregulation has been identified in ovarian tumors and its expression level was found to be a tumor grade dependent (Pils et al., 2005). Although *TUSC3* role in cell’s transformation is not fully understood, repression of *TUSC3* that locates in the endoplasmic reticulum, in an OTS, can modify the endoplasmic reticulum response and induce epithelial mesenchyme transition in ovarian cancer cells (Kratochvílová et al., 2015). Additionally *TUSC3* showed to rise the proliferation, migration and invasion in prostate cancer cell that lacked *TUSC3* expression (Horak et al., 2014). *TUSC3* is one of the genes whose expression correlates with survival in solid tumors. Pils and colleagues have identified frequent hypermethylation (33% in ovarian cancer compared to 0% in healthy controls) and decreased expression of *TUSC3* in ovarian cancer cell line and patients samples using MSP method. DNA methylation of *TUSC3* was significant and an independent prediction marker of the disease free survival as well as the total survival in ovarian cancer patients(Pils et al., 2013).

However, a little known regarding the prognostic role of *TUSC3* in hematological malignancies. Only orphan study by Mullighan and his colleagues has reported mutations of *TUSC3* in 8%(2/23) of paired diagnosis and relapse ALL cases, suggesting a potential role in relapse prediction in this type of leukemia(Mullighan et al., 2011).Here the expression level of *TUSC3* gene was investigated for its potential association with the disease biology in a set of childhood acute lymphoblastic leukemiapatiens in Iraq.

**Subjects and Methods**
Blood samples collection from the studied ALL cases and controls

For the extraction and purification of RNA, peripheral blood (PBL) samples were collected from thirty one childhood ALL patients (including 17 (54.8%) females and 14 (45.2%) males. All patients were diagnosed at the Child’s Central Teaching Hospital, Baghdad- Iraq. Blood samples were collected according to the ethical consideration and the hospital managers approvals during the period of November 2018 to July 2019. The patient’s age ranged from 0.5 to 14 years (mean of age 7.07 years). In addition to five peripheral blood samples that were collected from age matched healthy controls.

RNA extraction and TUSC3 gene expression using real time-PCR

To estimate the expression levels of TUSC3 in the studied ALL cases, RNA was extracted from the ALL’s peripheral blood samples. The extraction of RNA was done using Direct-zol™ RNA MiniPrep, R205- (ZYMO RESEARCH / USA). Following the RNA extraction, samples were nanodroped to verify the concentration and the purity of the extracted RNA. Thereafter, the cDNA synthesized by conversion the RNA to cDNA from the extracted RNA samples using thePrimeScript™ RT reagent Kit. The expression of TUSC3 gene was quantified by the real time PCR technique using the following primer set:

*TUSC3*-forward: ATGTTCACTGCTTCCTCAGCC
*TUSC3*-reverse: GCAGAGTTCTGTTGTGAGCTG. While GAPDH was used as a reference gene with the following primer sequence: GAPDH-forward: GCTCATTTCTGGTATGACAAC, and the GAPDH-reverse: CTGTGAGGAGGGAGGATTCA. All qPCR amplifications were performed in triplicate, with a final volume of 10μl of each. These reactions included 20 ng of cDNA, 300 ng of primer mix (forward + reverse), 5 μl of Syber Green and 4.25 μl of distilled water. Comparative Ct method was used to analyze the obtained real time PCR data (Livak and Schmittgen, 2001). The results were presented and analyzed using excels data analysis software.

Results and Discussion

Acute lymphoblastic leukemia is one of the most common childhood cancers world-wide. In this disease the outcome largely depends on patient’s stratification into different risk groups based on their age, WBC count and the disease associated genetic abnormalities. Based on the current WHO classification of B-lymphoblastic leukemia, this disease is categorized into seven genetic subtypes including t(9;22)(q34;q11.2)/BCRABL1, MLL/11q23 translocations, (12;21)(p13;q22)/ETV6-RUNX1, t(1;19)(q23;p13.3)/TCF3-PBX1, t(5;14)(q31;q32)/IGH@-IL3, hyperdiploidy. Understanding the disease’ underlying molecular genetic abnormalities would help to further refine the known risk stratification and offer the opportunity for the development of new therapeutic targets. This study was set to investigate the expression levels of TUSC3, a tumorsuppressor candidate 3, in acute lymphoblastic leukemia patients in Iraq-Baghdad.
The present study results showed that the majority (64.5%) of the studied ALL cases have down regulation of TUSC3 gene expression (Figure 1, 3). This included more than half of these cases (17/31 (54.8%)) have TUSC3 gene expression below the levels of 50% (figure 2). While only about one third (11/31 (35.5%)) of the investigated ALL cases have had normal to up regulated TUSC3 expression levels (Figure 1).

![Figure 1: The number of ALL cases with down or upregulated TUSC3 expression levels.](image-url)
No significant differences (P ≤ 0.05) in \textit{TUSC3} gene expression were observed from the comparison between the different genders of the studied ALL cases (mean of TUSC3 expression fold changes $2^{\Delta \Delta ct}$ for the males and females were 1.227 and 1.234 respectively). Similarly, the comparison between different age groups (0-<2 years vs. 2-10 vs. > 10-14 years) did not show significant differences in the expression levels of \textit{TUSC3}(TUSC3 expression fold changes $2^{\Delta \Delta ct}$ were 1.742, 1.207 and 0.815 respectively).

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{\textit{TUSC3} expression levels in the studied ALL cases}
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\includegraphics[width=\textwidth]{figure2.png}
\caption{\textit{TUSC3} expression fold change in the studied ALL cases. Each blue square represent the expression level of one of the ALL studied cases. The figure shows that the bulk of the studied ALL cases have reduced \textit{TUSC3} expression.}
\end{figure}

Over all, the decreased \textit{TUSC3} expression in the majority (64.5\%) of the studied ALL cases suggesting a key role for this gene in the diseases biology. This result fit well with the suggested tumor suppression role for \textit{TUSC3} in other different types of cancer (Kratochvílová et al., 2015, Yu et al.,...
2017). However, the tumor suppression activity of TUSC3 seems to be a leukemia-specific and associated with a specific subtype of ALL as a significant number of the cases (35.5%) exhibited normal to increased levels of TUSC3 expression. The maintained TUSC3 expression in this sub-set of ALL cases may offer proliferative advantages for the leukemic clone. In this regard, large scale studies on genetically well characterized cohort of ALL patients are recommended to establish the association between TUSC3 expression and the different subtypes of ALL.

A number of studies have reported mutation and/or down-regulation of TUSC3 in several types of solid tumors. The tumor suppression activity of this gene has been suggested based on a number of findings. One of these evidence came from the fact that TUSC3 is located at 8p22, a region frequently mutated in different types of epithelial tumors (Kratochvílová et al., 2015); thus TUSC3 has been suggested to have a tumor suppressor role in these malignancies. Additionally, homologous deletion of TUSC3 was identified in a metastatic prostate carcinoma (Horak et al., 2014). Recurrent loss of region encodes to the TUSC3 had been also observed in pancreatic adenocarcinoma (Birnbaum et al., 2011). Furthermore, TUSC3 dysregulation has been identified in ovarian tumors and its expression was found to be a tumor grade dependent (Pils et al., 2005). TUSC3 expression also found to be down regulated in favorable histology Wilms tumor (FHWT) and showed to have a significant influence relapse stage of this disease (Huang et al., 2009).

Additionally, promoter hypermethylation have been suggested as an alternative mechanism for the loss of TUSC3 expression. In this regard, TUSC3 promoter hypermethylation was observed in the serum of prostate cancer patients (Horak et al., 2014). In this context, reactivation of TUSC3 expression, possibly through the use of demethylation agents, could be investigated as a potential therapeutic target.

**Conclusion**

Overall, the study has identified significant reduction in TUSC3 gene expression in the majority of the studied acute lymphoblastic leukemia patients. While the rest of the studied ALL patients (14/31(35.5%) have shown unregulated TUSC3 expression suggesting a key role for this gene in the diseases biology. The expression level of TUSC3 gene could be investigated further for its association with disease specific subtypes, especially within the different cytogenetic subtypes of ALL, for its diagnostic and prognostic potentials.

**References**


