**KCNMA1 gene expression a promising biomarker in childhood acute lymphoblastic leukaemia**

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**Abstract**

Amplification and over expression of ion channels have been suggested to involve in tumourgenesis. This study was set to assess the expression level of KCNMA1 gene, encodes for calcium-activated potassium channel, in a set of childhood acute lymphoblastic leukaemia (ALL) patients in Iraq-Baghdad. Relative expression utilising RT-PCR technique was used to estimate the KCNMA1 gene expression in 31 cases diagnosed with childhood ALL. The results showed that the vast majority (77.42) of the investigated ALL cases have over expression of KCNMA1. While only 22.58 of the cases express normal to low levels of KCNMA1 gene. Overall, the present study results suggest an oncogenic role for KCNMA1 gene expression in childhood ALL and this would be assessed further in large scale studies to investigate its prognostic and therapeutic potential utility.

**Key words:** KCNMA1 gene expression, ion channels, childhood ALL

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**Introduction**

Haematological malignancies represent serious life threat affecting individuals within all the different age categories, from infants to an elderly people. In addition to the short and long-term side effects associated with the toxicity of chemotherapies used to treat such malignancies that affect the quality of life of leukaemia survivors. For this reason, patients’ stratification based on their predictive biomarkers could spare standard risk cancer patients from unnecessary high toxicity that usually allocated for high risk cancer patients. This would help with minimizing the accompanied treatment side-effects and could constitute the basis of cancer personalized medicine(Gegechkori, et al. 2017). Understanding the underlying cancer-associated biological alterations is essential for the developing of disease-specific diagnostic and prognostic biomarkers. Within this context, a key role has been suggested for ions channels in cancer biology recently (Huang and Jan 2014; Pethö, et al. 2019). These channels are integral plasma membrane proteins regulating the transport of ions with a great pharmacological value as a primary target of approximately 13% of the known medications that used to treat different human diseases(Cheng, et al. 2019). Ions channels are known to have role in the vascular and nervous systems physiology and pathology (Oyrer, et al. 2018; Zhu, et al. 2018). However, the involvement of ions channels in driving malignant cell behaviour has been supported by several lines of evidence lastly. It is believed that the dysregualition of the expression such channels could altered cell cycle progression and confer proliferative advantage for the transformed cells (Ningaraj, et al. 2003). KCNMA1 is maps to 10q22-q23 and
encodes for calcium-activated potassium channel subunit alpha-1. In normal cells, it controls cell volume and other cellular processes including apoptosis. It is also play an essential role in modulating vascular smooth muscle tone and the release of synaptic neurotransmitter (Bailey, et al. 2019). On the other hand, studies have shown that KCNMA1 amplification associated with several types of malignancies including glioma (Bury, et al. 2013), breast (Khaitan, et al. 2009), ovarian (Samuel, et al. 2016), with an association with the diseases progression and poor prognosis. The findings obtained from these studied suggested an oncogenic role for KCNMA1 expression within the aforementioned types of tumours. Moreover, KCNMA1 is shown to be amplified/over expressed in both primary samples and cell line of prostate cancer while blocking the B-K channel using iberei toxin inhibitor resulted in growth reduction in PC-3 prostate cancer cell line (Bloch, et al. 2007; Bloch, et al. 2004). However, it seems that KCNMA1 has a tissue-specific role in cancer biology. This is was evident when KCNMA1 is down regulated and hypermethylated in a number of other malignancies. Basile and colleagues have reported down-regulation of KCNMA1 expression due to CpG island promoter in colorectal cancer (Basile, et al. 2019). Similar to this observation, another study has reported decreased KCNMA1 expression in gastric carcinoma cell lines (Ma, et al. 2017). These evidence collectively suggest a tumour suppressor role for KCNMA1 in the aforementioned malignancies. In haematological malignancies, a novel gene fusion RUNX1–KCNMA1 was identified by Grossmann and colleagues in acute myeloid leukaemia (Grossmann, et al. 2011). While recurrent KCNMA1 amplifications were reported by Schiffman and colleagues in paediatric leukaemia (Schiffman, et al. 2008). Similarly, the expression of HERG, a membrane potassium channel, showed to be associated with increase relapses and shorter survival in acute myeloid leukaemia (Pillozzi, et al. 2007). Knockout KCNMA1 resulted in significant reduction in the proliferation of rat osteoblast suggesting a key role for this gene expression in cell proliferation and differentiation (Hei, et al. 2016). Osteoblast could be a leukaemia initiating clone and alterations of its biological characteristics is shown to induce aggressive acute myeloid leukaemia (Frisch and Calvi 2014).

This study was set to investigate KCNMA1 gene expression levels in a set Iraqi childhood acute lymphoblastic leukaemia patients (ALL, which the most common childhood cancer that accounts for 30% of paediatric malignancies). The KCNMA1 gene expression levels would be assessed for their association with some of the ALL prognostic features including age and gender.

Subjects and Methods

ALL cases and healthy controls

This pilot study was conducted quantify the KCNMA1 gene expression on 31 subjects (17 females and 14 males) diagnosed with childhood ALL at the Child’s Central Teaching Hospital, Baghdad- Iraq. The participant’s mean of age was 7.07 year ranged from 0.5 to 14 years. Peripheral blood samples were collected from the participants according to the ethical consideration and the hospital ethical committee and verbal patient’s consent during the period of November 2018 to July 2019. In addition to the 31 ALL cases, peripheral blood samples were also collected from five apparently healthy children to serve as age-matched controls.

RNA extraction and KCNMA1 gene expression estimation using q-PCR
For the estimation of KCNMA1 gene expression, RNA was extracted from the collected peripheral blood samples of the ALL cases and the age-matched healthy controls using Direct-zol™ RNA MiniPrep, R205-(ZYMO RESEARCH / USA). To check the extracted RNA purity and concentrations, all of RNA sample were nanodroped. This was followed by cDNA synthesis for the extracted RNA samples using the PrimeScript™ RT reagent Kit according to the manufacturer’s protocol. Relative quantification method was utilized to assess KCNMA1 gene expression using RT-PCR. The following primer set was used for the amplification of target gene: KCNMA1-forward: CGAGGATGAAGAAGACCATGA and KCNMA1-reverse: GGTTCATCCATTTGTTGGAG. While the primer sequence for the reference gene was as the following: GAPDH-forward: GCTCATTTCCTGTATGACAC and the GAPDH-reverse: CTGTGAGGAGGGGAGATTCA. All qPCR amplifications were performed 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 in addition to the use of linear correlation regression using http://vassarstats.net/ web site.

**Results and Discussion**

Approximately more than one third of childhood cancers are attributed to acute lymphoblastic leukemia. Beside it is considered as a leading cause of cancer-related death worldwide, short and long term side-effects of cancer treatment have significant impact in reducing the life quality of cancer survivors. Identifying of cancer biomarkers that influence the disease course, especially those with prognostic value, is of a great research interest to provide clinicians with information that would add the treatment decision and the disease management. A key aspect of the malignant phenotype that has been highlighted recently is the involving of ions channels in tumorigenesis. Based on that, the present pilot study was set to investigate the gene expression levels of KCNMA1, plasma membrane calcium-activated potassium channel, in a set of ALL in Iraq-Baghdad. The results showed that the vast majority (77.42) of the investigated ALL cases have KCNMA1 gene over expression. That is to say that approximately more than three quartiles of the studied childhood ALL patients showed increased KCNMA1 expression while only less than one quartile (22.58) of the cases express normal to low levels of KCNMA1 gene (Figure1). With the least mentioned sub group of patients, only three samples showed to have relatively low expression levels of KCNMA1 (12.67, 35.85 and 47.30, figure 2 and 3). On the other hand, the KCNMA1 gene expression levels did not correlate the investigated well-known childhood ALL prognostic markers, age at diagnosis (r²=0.0367) and gender (t-Test, P≤ 0.05), suggesting potential independent role for KCNMA1in childhood ALL.
The present study results strongly support the recent several lines evidence of the oncogenic role of KCNMA1, as calcium-activated potassium channel, in tumorigenesis especially in solid tumors (Bloch, et al. 2004; Khaitan, et al. 2009). After it has been shown to be correlated with a number of different types of tumors, KCNMA1 showed to be over expressed in the vast majority of the investigated ALL cases in the present study. This suggests a potential key role for this gene in the biology of childhood ALL. The obtained results are in line with the suggested role for ion channels in promoting cellular transformation through their influence on the cell volume that impacts cell cycle regulation and cellular proliferation (Pethö, et al. 2019). Our study results propose that may be a selective pressure to maintain KCNMA1 expression in the majority of the studied ALL cases required to promote the leukaemia phenotype. Further studies are recommended targeting KCNMA1 knockdown/ expression modulating to validate the potential oncogenic impact of this gene using a large panel leukaemia cell lines. After it has been shown that modulating ion channel could protect mice from leukaemia, ions channels, including KCNMA1, representing promising therapeutic targets for the development of effective anti leukemic drugs. Large scale studies also are needed to assess the potential association of KCNMA1 expression with the other childhood ALL prognostic markers including the cytogenetic subtypes, chemo-resistance, and metastasis and relapse site. In addition to investigate the utility of KCNMA1 expression levels for ALL risk-stratification and treatment optimization.
Conclusion: Over all, the present study results suggest potential key role for KCNMA1 expression in childhood ALL biology when the vast majority of the investigated cases showed over expression for this gene. This would be assessed further in large scale studies for its prognostic and therapeutic utility.

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