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Original article

BTYNB, an inhibitor of RNA binding protein IGF2BP1 reduces proliferation and induces differentiation of leukemic cancer cells

Alam Jamal [a], Mahmood Hassan Dalhat [a], Sadaf Jahan [b], Hani Choudhry [a,c], Mohammad Imran Khan [a,c,*]

[a] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[b] Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Majmaah University, Al-Majmaah, Saudi Arabia
[c] Centre of Artificial Intelligence for Precision Medicines, King Abdulaziz University, Jeddah 21589, Saudi Arabia

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ABSTRACT

Leukemia is a group of diseases characterized by altered growth and differentiation of lymphoid or myeloid progenitors of blood. The existence of specific clusters of cells with stemness-like characteristics like differentiation, self-renewal, detoxification, and resistance to apoptosis in Leukemia makes them difficult to treat. It was recently reported that an oncofetal RNA binding protein, insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), maintains leukemic stem cell properties. BTYNB is an inhibitor of IGF2BP1 that was shown to affect the biological functions of IGF2BP1 however, the effect of BTYNB in Leukemia is not properly established. In this study, we assessed the effect of BTYNB on leukemic cell differentiation and proliferation. We performed cell viability assay to assess the effect of BTYNB in leukemic cells. We then assessed cell morphology of the leukemic cells treated with BTYNB. Further, we conducted an apoptosis assay and cell cycle assay. We found the cell viability of leukemic cells was significantly decreased post treatment with BTYNBs. Further, a noticeable morphological change was observed in BTYNB treated leukemic cells. BTYNB treated leukemic cells showed increased cell death and cell cycle arrest at S-phase. Evidence from the upregulation of *BAK* and *p21* further confirmed apoptosis and cycle arrest. The gene expression of differentiation genes such as *CD11B*, *ZFPM1*, and *KLF5* were significantly upregulated in BTYNB treated leukemic cells, therefore, confirming cell differentiation. Collectively, our study showed inhibition of IGF2BP1 function using BTYNB promotes differentiation in leukemic cells.

1. Introduction

Cancer is a disease that arises due to abnormalities in cell division and differentiation. So, there is a possibility that through induction of differentiation in cancer cells we can treat cancers (Spira and Carducci, 2003). The malignant potential of cancer cells can be controlled by forcing them to differentiate and arrest their growth. Many blood cancers, including leukemia, initiate in the bone marrow and produce a significant number of aberrant blood cells. Blasts or leukemia cells are the term for these immature blood cells. Symptoms may include an increased risk of infections, bone soreness, fatigue and bleeding and bruising. A deficiency of healthy blood cells causes these symptoms to manifest (Corces et al., 2016).

Acute myeloid leukemia (AML), Acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) are the four common types of this disease. Similar to the normal blood system, acute myeloid leukemia (AML) is a hierarchically organized disease defined by poor differentiation of myeloid (Döhner et al., 2015). Long-term clonal proliferation in AML is caused by leukemia stem cells (LSC), just like long-term hematopoietic stem cells (LT-HSC) keep blood production going for life. (Thomas and Majeti 2017). An estimated 21,450 people (11,650 men and 9800 women) had an AML diagnosis in 2019. About 2.3 million people have leukemia in 2015, and it was

* Corresponding author at: Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
E-mail addresses: ajamal0015@stu.kau.edu.sa (A. Jamal), mdalhatdalhat@stu.kau.edu.sa (M. Hassan Dalhat), S.jahan@mu.edu.sa (S. Jahan), hchoudhry@kau.edu.sa (H. Choudhry), mikhan@kau.edu.sa (M. Imran Khan).
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responsible for 353,500 fatalities (2016) Acute myeloid leukemia is responsible for the largest proportion (62 %) of leukemia-related deaths (Health, 2021). The most prevalent cancer type is Leukemia In children and one of the most prevailing in adults with impaired growth and differentiation capabilities of lymphoid or myeloid progenitors of blood (Ferlay et al., 2019).

Radiation therapy, Chemotherapy, targeted therapy and transplant of bone marrow are all potential components of treatment, along with palliative care and supportive care (Cordo et al., 2021). In cases of leukemia, the prognosis is contingent on the patient's age and the specific kind of the disease (Hoggatt et al., 2016). Leukemia and other inoperable malignancies can be treated by using differentiation therapy and it can also be used as a preventive treatment for cancer (Gutteridge and Halliwell 2000). Consequently, treatment via inducing differentiation is gaining increasing interest due to its possible effectiveness and lower toxicity. Despite the fact that other agents like dimethyl sulfoxide (DMSO) and vitamin D3 hydroxyl have been studied for a long time, all-trans retinoic acid has been the differentiation agent that has been the most thoroughly examined and clinically evaluated to treat acute promyelocytic leukemia patients (Spira and Carducci, 2003; Latagliata et al., 1997). Despite significant advances in treatment and better survival rates, chemotherapeutic resistance is the main reason of illness return. In adults and children, the five-year reappearance rate for acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) is reported to be 20–30 % in children and 70 % in adults (Döhner et al., 2017). At three years 30 % patients without treatment with chronic myeloid leukemia (CML) have a molecular relapse (Hernández-Boluda et al., 2018). The existence of cells clusters with stem cell traits like ability to renew itself, unable to differentiate, detoxification, drug efflux and defiance to apoptosis that make them difficult to treat is linked to the return of leukemia and other malignancies. So, there is a possibility that through differentiation therapy we can treat cancers (Spira and Carducci, 2003).

Multiple reports suggest that LSC resistance is mediated by pathways that are kinase independent (Corbin et al., 2011; Hamilton et al., 2012). Different cues from the bone marrow microenvironment (BMM) and modifications in intrinsic cell-regulatory systems both contribute to LSC persistence in the BM (Agarwal et al., 2017). Supported by diverse niches, HSCs in the BM are distributed in several anatomical regions that govern stem cell destiny. A variety of cell types, such as endothelial cells, mesenchymal stromal cells (Pinho et al., 2013), osteoblasts, adipocytes, and megakaryocytes, may make up a niche. Inflammatory cytokines and chemokines are significantly altered during CML formation, which may promote LSCs (Zhang et al., 2012).

The IGF2 mRNA-binding protein (IGF2BP) family is an example of an RNA-binding protein (RBP) which has a crucial role in regulating tumor and stem cell destiny (Hattori et al., 2016). Evidence from CLIP (crosslinking immunoprecipitation) experiments indicates a multitude of IGF2BP target mRNAs, most of which are redundant (Conway et al., 2016). The 'oncogenic' potential of cancer is present in all IGF2BP paralogues (Lederer et al., 2014) However, only IGF2BP1 exhibits robust conserved oncogenic potential in cancer-derived cell lines (Gutschner et al., 2014). IGF2BP1 is a protein also called as ZBP1, VICKZ1, CRD-BP or IMP1 which bind to RNA found in oncofetal cells that is involved in firmness, translation and transportation of RNA (Degrauwe et al., 2016). IMP1 protects target mRNAs against endoribonucleases and microRNAs which would otherwise degrade them (Elcheva et al., 2009). IGF2BP1 played a major role in this by preventing the degradation of MYC mRNA (Köbel et al., 2007).There are six canonical RNA-binding domains in IGF2BP1 in which four domains are K homology (KH) and two motifs which are involve in recognition of RNA (Wächter et al., 2013). Instead the truth that the RRM domains of IGF2BP1 may have a role in IMP1 -RNA complexes stability in a target-dependent way (Nielsen et al., 2004) in vitro studies revealed that the majority of binding to RNA occurred via KH domains (Farina et al., 2003). The KH1/2 domain is important for IMP1-RNA complex stability. In vitro the domain of KH1/2 could for example control IMP1 binding to cis-determinants in the ACTB 3′-UTR and more notably the MYC-CRD (coding region stability determinant) RNA (Bell et al., 2013). Recent structural investigations of the human IGF2BP1 KH3/4 domain revealed the creation of an pseudo-dimer of antiparallel conformation in which KH3 and KH4 both engage the targeted RNA (Chao et al., 2010). The KH domains specifically the KH3–4 di-domain are required for binding of IMP1 to mRNAs target in an N6-methyladenosine (m6A)-dependent way according to our information with KH domains identifying the mRNAs' consensus GG (m6A) C sequence (Huang et al., 2018a; Huang et al., 2018b). IGF2BP1 is a protein which bind to m6A that has above three thousand mRNA transcript targets (Dominissini et al., 2012). These mRNAs' m6A modifications are essential for IGF2BP1 targeting to mRNAs like *MYC* as well as IMP1-mediated mRNA expression control. Furthermore, some IGF2BP1 co-factors like ELAV-like RNA binding protein 1 (ELAVL1) probably bound by IMP1 and shield m6A-containing mRNAs from breakdown thereby promoting their expression (Huang et al., 2018a; Huang et al., 2018b). They are mostly active during embryogenesis, but adult tissues have little or no expression and are frequently activated again in malignancy. IGF2BP1 influences leukemia cell proliferation and tumorigenic potential via the *MYB* and *HOXB4* self-renewal regulators as well as aldehyde dehydrogenase ALDH1A1 (Elcheva et al., 2020). While in solid tumors IGF2BPs have been extensively studied for their tumorigenic and metastatic qualities but in leukemia there are little information about their role (Huang et al., 2018a; Huang et al., 2018b). Overexpression of IGF2BP1 and 3 has been associated to rearrangements of ETV-RUNX1 and MLL in specific kinds of B-cell acute lymphoblastic leukemia (B-ALL) (Palanichamy et al., 2016). IGF2BP1 has also been found to arbitrate the tumor related effects of LIN28B in AML cells (Zhou et al., 2017).

Inhibitors of IGF2BP1-mediated cell signaling would thus arise as a possible method to cure the cancer and IGF2BP1 has been identified as one of the best therapeutic targets to cure the cancer. Recently, however, its inhibitory impact in tumor growth and metastasis has been discovered (Hamilton et al., 2015). So, it's important to make sure the procedures that led to the seemingly contradictory results (Wang et al., 2016).

2-[(5-bromo-2-thienyl) methylene]amino benzamide (BTYNB) is a small molecule which inhibit IGF2BP1. BTYNB was reported to prevent stemness (Degrauwe et al., 2016). Furthermore, BTYNB prevents IGF2BP1 binding to mRNA of c-*Myc* in a strong and selective manner. BTYNB also decreases the activity of nuclear transcriptional factors-kappa B (NF-κB) via downregulating TrCP1 mRNA. Collectively, although BTYNB is reported to prevent stemness, reduce cell proliferation and mitigate carcinogenesis no information reported the effect of BTYNB in cell differentiation. Therefore, in our study we assessed whether BTYNB induces differentiation in leukemic cells.

2. Materials and methods

2.1. Cell culture and treatment

RPMI 1640 medium (Bis Biotech, KSA) consists of 1 % penicillin streptomycin and 10 % fetal bovine serum was used to cultivate human HL60 and K562 cells (ATCC, USA). All cells were cultured at 37 °C with 5 % CO_2 in a humidified incubator. The cells in this investigation were grown to 60–70 % confluence before treated

with BTYNB. Cells were treated with different doses of IGF2BP1 inhibitor, BTYNB for 24 h.

2.2. Cell viability assay

MTT (3[4,5dimethylthiazol2yl]2,5 diphenyl tetrazolium bromide) was used for determination of cells viability. Cells were cultured in 96 wells plate for 24 h followed by treatment with BTYNB. After 24 h of treatment with BTYNB, MTT assay was measured according to manufacturer's instruction. Briefly, 10 µl of MTT solution (5 mg/mL, MOLEQULE-ON, NZ) was added to each well and then incubated at 37 °C for 3 h. Then the contents with MTT from the wells were removed and replaced with 100 µl dimethyl sulfoxide (DMSO) and incubated for 1/2h at 37 °C. Absorbance was measured using Bio Tek® microplate reader and data analysis was conducted with Gen5™ software. IC_{50} values were obtained by version 8.0 Graph Pad Prism.

2.3. Apoptosis assay

An apoptosis assay was performed using Annexin V-FITC and propidium iodide (PI). Briefly, cells treated with and without BTYNB were harvested and washed three times with phosphate buffer saline (PBS). Washed cells were resuspended in 200 µl binding buffer (PBS solution containing 2 % FBS) and stained with 50 µl Annexin V-FITC and 4 µl PI followed by incubation for 30 min. The assay was measured at 10,000 cells per event in triplicate for each sample using Amnis flow cytometer.

2.4. Cell cycle assay

After 24 h treatment with BTYNB, treated were assessed for cell cycle assay. Briefly, BYTNB treated cells and control were harvested and washed three times with PBS. Washed cells were fixed with 70 % iced cold ethanol and incubated at −20 °C for 30 min. Fixed cells were then centrifuged at 5000 rpm for 5 min to remove ethanol and subsequently washed two time with PBS before finally resuspending the cells in PBS. Cells were stained with 5 µl PI and incubated for 30 min. Cell cycle assay was performed at 10,000 cells per event in triplicate per sample using Amnis flow cytometer.

2.5. Real time quantitative PCR

Pure link RNA isolation kit (Haven Scientific, KSA) was used for total RNA extraction from BTYNB treated cancer cells and their corresponding controls. The complementary DNA (cDNA) was produced via high-capacity cDNA reverse transcription kit (Applied Biosystems, USA). To study the changes in genes expression of BTYNB treated and untreated cells. RT qPCR was done using cDNA in triplicate and changes in expression of different genes were determined. The list of primers used for this study is shown in Table 1.

2.6. Bioinformatic analysis

Selection of leukemic cells HL60 and K562 was done using the human protein atlas database (https://www.proteinatlas.org/) in cell lines with high *IGF2BP1* mRNA expression. The network analysis of cell differentiation genes was performed with String database (https://string-db.org/) and for correlation analysis of IGF2BP1 and cell differentiation genes was conducted in Onco database (http://oncodb.org/survival_plot_nonv.html).

Table 1
Primer list for q-RT PCR.

Gene	Forward primers	Reverse Primers
HBG1	GGAAGATGCTGGAGGAGAAACC	GTCAGCACCTTCTTGCCATGTG
HBG2	GGAAGATGCTGGAGGAGAAACC	GTCAGCACCTTCTTGCCATGTG
CD11b	ACTGGGCTGGTGGAGTCTTT	AACATCGCTACCAGGCCGAT
CEBPA	AGGAGGATGAAGCCAAGCAGCT	AGTGCGCGATCTGGAACTGCAG
GATA1	CCCAAGCTTCGTGGAACTCT	AGGCGTTGCATAGGTAGTGG
GATA2	CAGCAAGGCTCGTTCCTGTTCA	ATGAGTGGTCGGTTCTGCCCAT
GATA3	ACCACAACCACACTCTGGAGGA	TCGGTTTCTGGTCTGGATGCCT
HK3	CATCGTGGACTTCCAGCAGAAG	CTTGGTCCAGTTCAGGAGGATG
KLF5	GGAGAAACGACGCATCCACTAC	GAACCTCCAGTCGCAGCCTTC
KRAS	CAGTAGACACAAAACAGGCTCAG	TGTCGGATCTCCCTCACCAATG
MYB	GGGAACAGATGGGCAGAAATCG	GCTGGCTTTTGAAGACTCCTGC
ZFPM1	TTCGTGTGCCTGATCTGCCTGT	GTTGGTGACCAAGTGGCTGTAG
p21	AGGTGGACCTGGAGACTCTCAG	TCCTCTTGGAGAAGATCAGCCG
p16	CTCGTGCTGATGCTACTGAGGA	GGTCGGCGCAGTTGGGCTCC
BAK	TTACCGCCATCAGCAGGAACAG	GGAACTCTGAGTCATAGCGTCG
BOK	ACGCCTGGCTGAGGTGTGCG	AGGAACGCATCGGTCACCACAG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

2.7. Statistical analysis

All data are recorded in triplicates and presented as mean ± SEM. The student 2-tailed *t* test was used to calculate statistical significance. *P*-value of $\leq$0.05 was considered as statistically significant. Statistical analysis was conducted with Graph Pad Prism 8.0.1.

3. Results

3.1. BTYNB inhibits cell proliferation in leukemic cells

First, we assessed the human protein atlas database (https://www.proteinatlas.org/) for cell lines with high expression of *IGF2BP1*. The IGF2BP1 expression was noticed to be remarkably high in leukemic cell lines such as HL60 and K562 (Fig. 1A). As a result, we chose the HL60 and K562 cell lines to assess IGF2BP1 on cell proliferation using BTYNB, a small molecule inhibitor of IGF2BP1 (Fig. 1B). Leukemic cells HL60 and K562 were treated with different doses of BTYNB and subjected to MTT assay. The viability of BTYNB treated leukemic cells revealed a dose dependent reduction in viability of cell. The half maximal inhibitory concentration (IC_{50}) was 21.56 µM and 6.76 µM for HL60 and K562 respectively (Fig. 1C). Cell morphology of BTYNB treated leukemic cells was examined and a difference in shape and cell number was observed in leukemic cells treated with and without BTYNB (Fig. 1D). Therefore, the results here showed that leukemic cells are sensitive to BTYNB in a dose dependent manner and BTYNB reduces cell proliferation through decreased cell number and change in cell shape.

3.2. BTYNB induces cell death and cell cycle arrest in leukemic cells

Since we noticed remarkable change in cell proliferation and cell shape we then ask if BTYNB induces apoptosis and cell cycle arrest. Leukemic cells post treated with BTYNB were subjected to Annexin V/PI staining to determine the quantitative amount of apoptosis (Fig. 2A). The percentages of dead cells in BTYNB treated HL60 and K562 were 5.34 % and 2.65 % respectively, indicating that BTYNB has mild effect on apoptosis in leukemic cells. BTYNB treated K562 showed cell cycle arrest at S-phase, however, no effect was observed in HL60 cells (Fig. 2B). To further evaluate whether BTYNB has impact on cell death, we performed gene expression analysis of cell death related genes such as *BAK, BOK, BAX, p16* and *p21*. Interestingly, remarkable upregulation of *BAK* and *p21* was observed leukemic cells post treated with BTYNB (Fig. 2C).

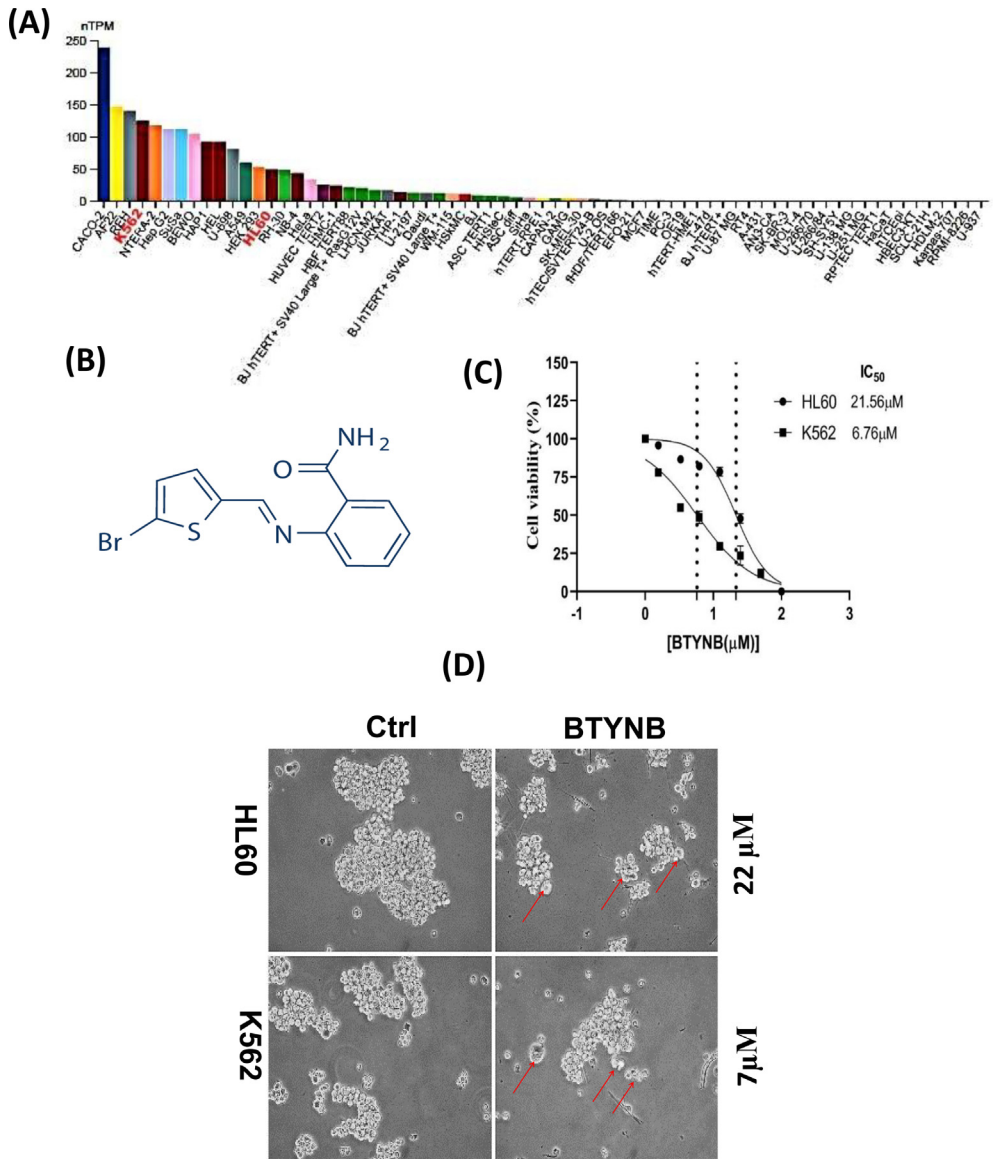

Fig. 1. Impact of BTYNB on leukemic cancer cells (A) IGF2BP1 gene expression profile in leukemic cells retrieved from the human protein atlas (B) Structure of BTYNB (C) IC_{50} of BTYNB treated HL60 and K562 cells after 24 h (D) Morphological changes of HL60 and K562 cells induced by BTYNB. The representative pictures of K562 and HL60 with or without BTYNB treatment are presented. The morphological changes were observed by microscopy (magnifications 40×) taken by a digital charge-coupled device (CCD).

Overexpression of *BAK* and *p21* were shown to be associated with different cancer types, therefore, supporting the results that BTYNB induces apoptosis and cell cycle arrest in leukemic cells.

3.3. BTYNB induce differentiation of leukemic cells

Previously it was reported that IGF2BP1 promotes stemness in leukemia, therefore, we asked whether inhibiting IGF2BP1 function could induce cell differentiation. Changes in cell size and cell shape from morphological examination showed possibility of cell differentiation in BTYNB treated leukemic cells illustrated in Fig. 1D. To further confirm cell differentiation in BTYNB treated leukemic cells gene expression analysis of cell differentiation genes such as *CD11B* also known as Integrin alpha M *(ITGAM),* GATA binding protein-2 *(GATA2),* GATA binding protein-3 *(GATA3),* Zinc finger protein FOG family member 1 *(ZFPM1),* hemoglobin subunit ϒ-1 *(HBG1),* hemoglobin subunit ϒ-2 *(HBG2),* CCAAT/ Enhancer binding protein-α *(CEBPA),* hexokinase 3 *(HK3),* and Kruppel-like factor 5 *(KLF5)* was performed (Fig. 3). With the aid of String database (https://string-db.org/) the cell differentiation genes were stratified into groups (Fig. 3). Notably, CD11B which has important role in myeloid differentiation was observed to be consistently upregulated in both BTYNB treated cells, however, ZFPM1which is also known as friend of *GATA1* is cofactor of *GATA1* transcription factor which in turn activate or represses hundreds of genes expression during erythroid development showed significant upregulation in treated BTNB treated HL60 only (Fig. 3A). Additionally, remarkable upregulation was recorded in KLF5 in both HL60 and K562 (Fig. 3B). The KLF5 is a member of the Kruppel-like Factors (KLF) gene family which are a group of transcription factors. To date, the most well-established target gene of KLF5 is Transforming growth factor β (TGFβ) and its receptors. TGFβ and KLF5 cooperatively regulate a wide range of cellular processes such as cell differentiation and proliferation. The KLF5 has a very important role in stem cell differentiation and act as a transcriptional factor and forms a complicated transcriptional regulatory network. No

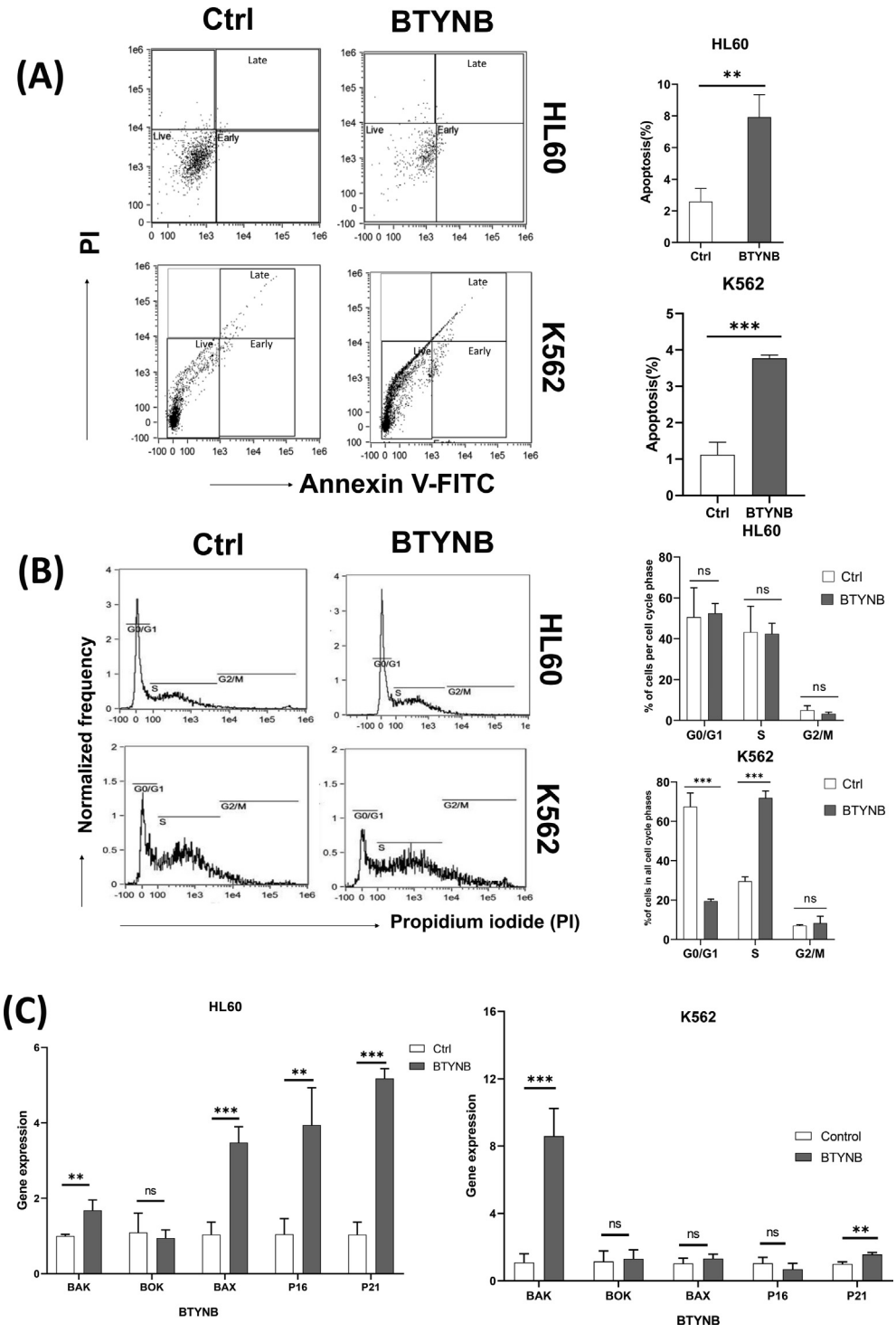

Fig. 2. Cell death and cell cycle arrest in BTYNB treated leukemic cells. (A) HL60 and K562 after 24 h BTYNB treatment. Bars represent the average number of death cells vs live cells in cancer cells treated with 21 μM and 7 μM BTYNB for 24 h. (B) Cell cycle assay of BTYNB treated HL60 and K562 cells 24 h posttreatment. Each bar represents mean ± SEM (n = 3). (C) Expression of cell death related genes BAK, BOK, BAX, p16 and p21 in HL60 and K562. Error bars are presented as mean ± SEM, and p values as $^{*}p$ <.05; $^{**}p$ <.01; $^{***}p$ <.001; ns: non-significant.

significant difference (p > 0.05) was observed in *HBG1* and *HBG2* expression in both BTYNB treated cell lines (Fig. 3C). Collectively, upregulation of CD11B, and KLF6 confirmed that BTYNB induces cell differentiation in leukemic cells.

The correlation analysis of IGF2BP1 and all studied cell differentiation genes was performed using Onco database (https://oncodb.org/survival_plot_nonv.html) (Fig. 4). Negative correlation was seen in IGF2BP1 vs all cell differentiation biomarker with the most

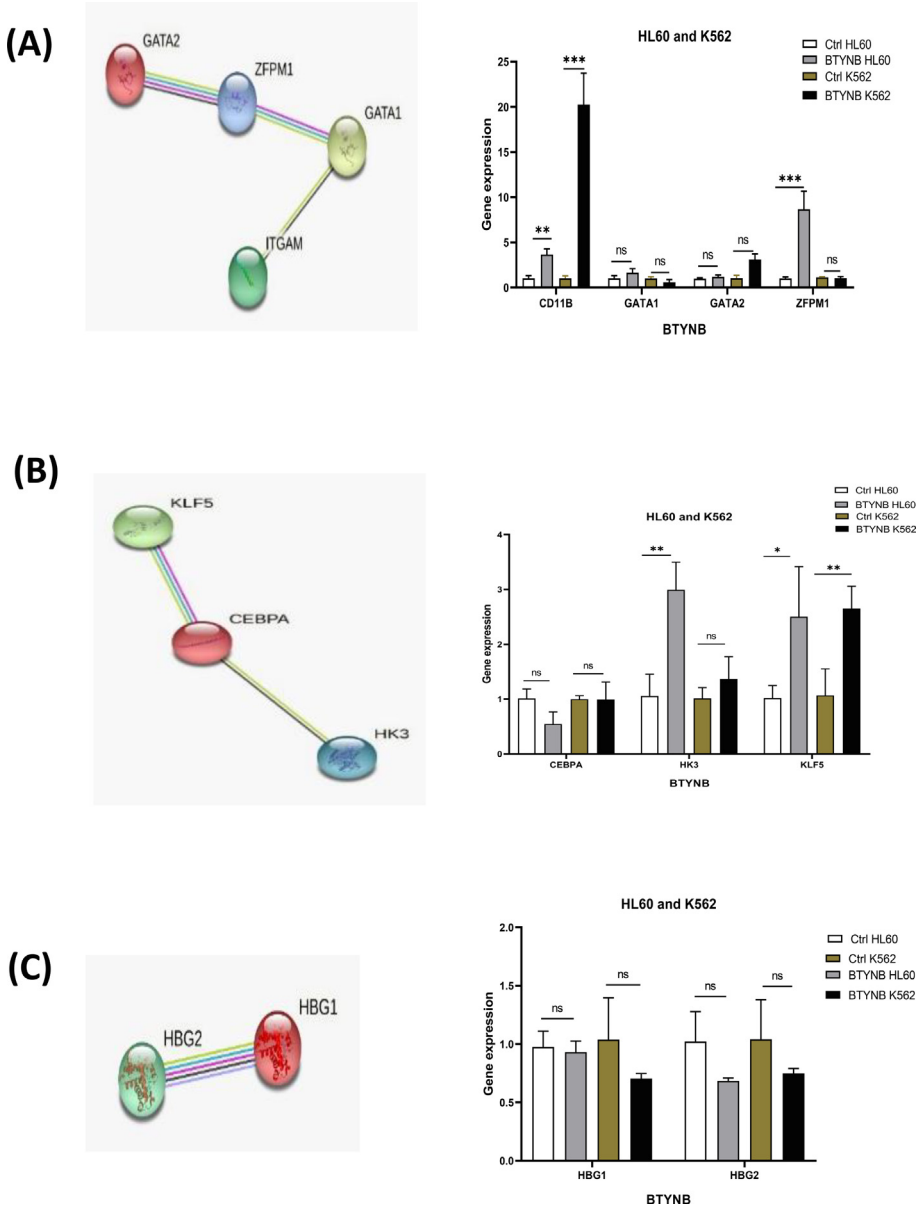

Fig. 3. Expression of differentiation related genes ZFPM1, CD11B, HK3, KLF5, CEBPA, HBG1, HBG2, GATA2 and GATA3. Error bars are presented as mean ± SEM and p values as $^{*}p$ <.05; $^{**}p$ <.01; $^{***}p$ <.001; ns: non-significant.

negative correlation seen in *ITGAM* (*CD11B*) at Pearson correlation (r) of −0.1991 followed by *GATA2* (r = −0.1968) and *KLF5* (r = −0.1036). Although all results showed negative correlation, only GATA2 showed significant difference.

Mechanistically, cell differentiation is negatively regulated by IGF2BP1, and therefore, targeting IGF2BP1 function with BTYNB leads to elevation cell differentiation biomarkers which in turn induces cell differentiation (Fig. 5).

4. Discussion

Leukemia refers to a group of cancers of the blood that often start in the bone marrow and result in the production of huge numbers of blood cells that are aberrant. Leukemia stem cells (LSC) are responsible for long-term clonal proliferation. The oncogenic potential of cancer is present in all IGF2BP paralogues. However, only IGF2BP1 exhibits robust conserved oncogenic potential in cancer-derived cell lines. IGF2BP1 played a main role in this by preventing the degradation of MYC mRNA. The IMP1 is very important as it regulate stem cell and tumor destiny and its increased expression in different types of malignancies is related to a poor diagnosis (Degrauwe et al., 2016). The IGF2BP1-mediated cell signaling Inhibitors would thus arise as a possible method to cure cancer, and IGF2BP1 has been recognized as the most potential therapeutic target for the treatment of cancer. Even with the conserved control of leukemic cell characteristics like invasion, proliferation, metastasis, migration and conserved IGF2BP1 effector pathways and target RNAs in leukemia are unknown (Müller et al., 2020) (Rosenfeld et al., 2019). IGF2BP1 has been shown to enhance stability of mRNA in various models of tumor and stem cells in earlier research. Controlling mRNA turnover by inhibiting microRNA-dependent downregulation of target mRNAs encoding pro-oncogenic factors is a recurrent feature of this regulation (Müller et al., 2018). The developing cancer-related mRNAs c-*MYC, MAPK4*, *CD44, PTEN, ACTB* and *MKI67* have been discovered in several in vivo and in vitro investigations.

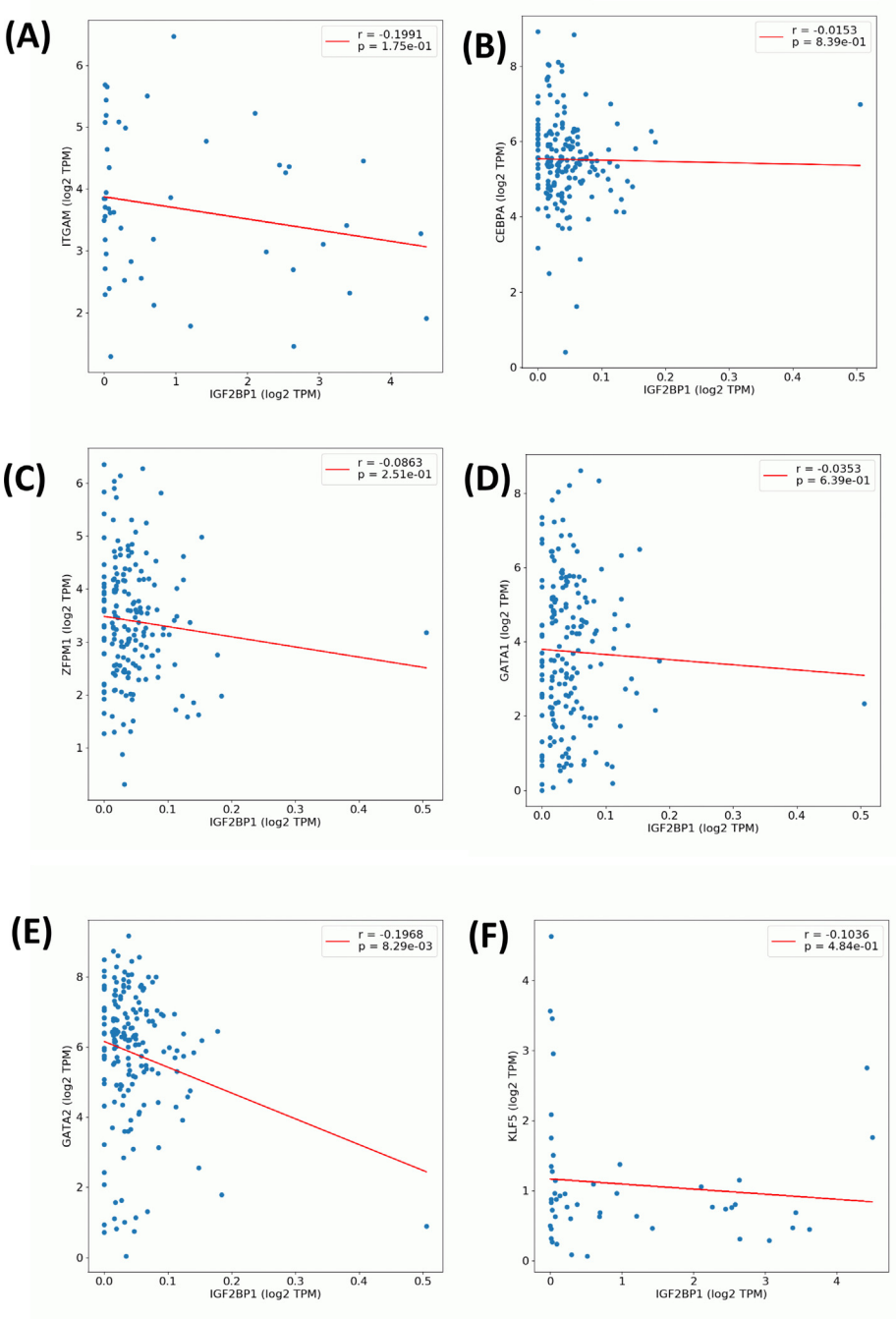

Fig. 4. Correlation analysis of cell differentiation genes in Leukemia. IGF2BP1 vs all cell differentiation genes. (A) ITGAM (CD11B). (B) CEBPA. (C) ZFPM1. (D) GATA1. (E) GATA2. (F) KLF5. Data were retrieved from Onco database (http://oncodb.org/survival_plot_nonv.html).

IMP1 has been found to have crucial roles in cell growth and proliferation in non-cancer and tumor tissues including migration, apoptosis, invasion, and adhesion of tumor cell through modulating those mRNAs (Bell et al., 2013). As a result, IGF2BP1 is regarded as one of the best therapeutic targets to treat cancer, with inhibitors of IMP1-arbitrated signaling of cell emerging as a viable method for treatment of leukemia. BTYNB (BTYNB IGF2BP1. Inhibitor) inhibits binding of IGF2BP1 to c-Myc mRNA in a strong and selective way.

But to date there is no report that BTYNB induces differentiation in leukemic cells. For the first time our study confirms that BTYNB induces differentiation in leukemic cells by modulating the genes that play a very important role in cellular differentiation like *CD11B (ITGAM), ZFPM1,* and *KLF5* according to our findings. The other important thing that we found in our research work is that BTYNB slightly induces apoptosis in leukemic cells through the upregulation of the pro-apoptotic gene called *BAK* and cell cycle arrest through *p21*. Take together our results showed that targeting

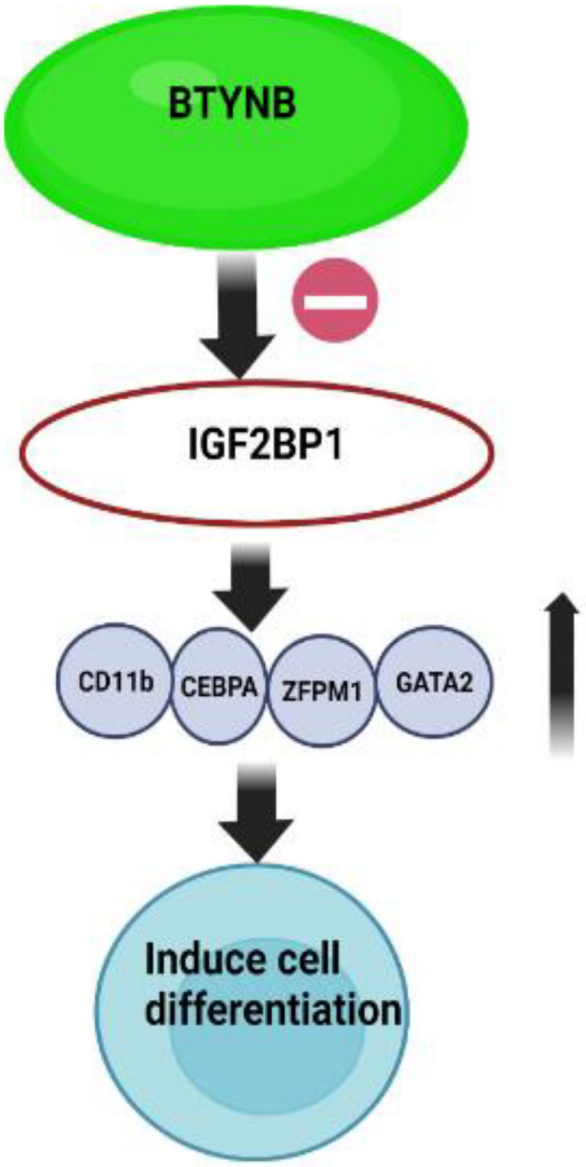

Fig. 5. A schematic diagram for the possible mechanism of action of BTYNB induced differentiation of leukemic cells.

IGF2BP1 with BTYNB induces cell differentiation by overexpressing of *CD11B (ITGAM) ZFPM1,* and *KLF5.*

5. Conclusion

We provided novel insight into the role of IGF2BP1 in cell differentiation and hence targeting IGF2BP1 with BTYNB induces cell differentiation together with reduced cell proliferation. Therefore, IGF2BP1 is a promising therapeutic target for treatment of leukemia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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