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The Signal Transducer and Activator of Transcription 5B (STAT5B) Gene Promotes Proliferation and Drug Resistance of Human Mantle Cell Lymphoma Cells by Activating the Akt Signaling Pathway

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Mantle cell lymphoma (MCL) is a high-grade B-cell lymphoma with poor prognosis. Fludarabine is used alone or in combination for relapsed and advanced-stage MCL. The expression of the signal transducer and activator of transcription 5B (STAT5B) gene is associated with tumorigenesis in solid tumors, but its role in MCL remains unknown. The aims of this study were to investigate the role of STAT5B in GRANTA-519 human mantle cell lymphoma cells and drug resistance.

Material/Methods: GRANTA-519 human mantle cell lymphoma cells were cultured with and without 10 μM fludarabine dephosphorylated 9-β-D-arabinofuranosyl-2-fluoroadenine, (2-F-araA) or 10 μM 4-hydroperoxycyclophosphamide (4-HC). The MTT assay assessed cell proliferation. Flow cytometry was used to investigate the cell cycle in MCL cells treated with the specific inhibitor of the Akt pathway, LY294002, and assessed cell cycle and cell apoptosis. Western blot was used to detect the expression levels of p-Akt/Akt and STAT5B/p-STAT5B. The gene expression profiles of lymph node (LN)-derived MCL cells were compared with peripheral blood (PB)-derived lymphocytes using bioinformatics and hierarchical cluster analysis. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed to determine the expression of the marker of proliferation Ki-67 (MKI67) gene.

Results: STAT5B was significantly upregulated in LN-derived MCL cells compared with PB lymphocytes. Increased expression of STAT5B was associated with increased MCL cell proliferation and reduced cell apoptosis and was associated with drug resistance and activation of Akt.

Conclusions: STAT5B promoted cell proliferation and drug resistance in human MCL cells by activating the Akt signaling pathway.

MeSH Keywords: Apoptosis • Cell Proliferation • Drug Resistance • Lymphoma, Mantle-Cell • STAT5 Transcription Factor

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Background

Mantle cell lymphoma (MCL) is a high-grade B-cell lymphoma with poor prognosis and is associated with t(11;14) (q13;q32) translocation and overexpression of cyclin D1 [1]. In Western countries, MCL represents between 3–10% of all cases of non-Hodgkin lymphomas (NHL) [2]. Current standard chemotherapy treatment for MCL may be ineffective as the lymphoma cells can sequester in lymph nodes where they respond to signals from accessory cells that facilitate cell survival, progression, and drug resistance, resulting in residual disease and relapse [3]. Relapse in patients with MCL is accompanied by a low median overall survival (OS) of between 4–6 years [3]. Therefore, there is a need for novel and effective therapeutic agents to improve the outcome for patients with MCL.

Signal transducer and activator of transcription (STAT) proteins include a family of latent transcription factors STAT1, STAT2, STAT3, STAT4, STAT5A/5B, and STAT6, which act as cytoplasmic signaling proteins and nuclear transcription factors [4]. The STAT proteins can be activated via tyrosine phosphorylation and participate in the regulation of gene expression in several cell types. STAT5 consists of two highly homologous isoforms, STAT5A and STAT5B, which have a role in tumor growth, metastasis, and apoptosis in several types of solid tumor [5,6]. STAT5A was initially identified as a mammary gland factor that can upregulate milk protein expression under prolactin induction [7] and has been shown to have a role in the pathogenesis of breast cancer [8] and prostate cancer [9]. At the amino acid level, STAT5B is highly homologous with STAT5A and is associated with the physiological processes of human tumor cells, including the growth of lung cancer cells [10], tumorigenesis and development in glioblastoma multiforme [11], and venous invasion, tumor progression, and the reduced prognosis in hepatocellular carcinoma [12], and increased tumor stage in colorectal carcinoma [13]. Although several studies have identified the role for STAT5B in the pathogenesis of several types of solid tumor, the biological role of STAT5B in the pathogenesis and prognosis of MCL remains unknown.

Therefore, the aims of this study were to investigate the role of STAT5B in GRANTA-519 human mantle cell lymphoma cells and drug resistance. The expression levels of STAT5B were detected in MCL cells from lymph nodes (LNs) and peripheral blood (PB) lymphocytes. The effects and mechanism of overexpression of STAT5B were investigated on cell proliferation, apoptosis, survival, and drug resistance were investigated.

Material and Methods

Cell culture and treatment

The human mantle cell lymphoma (MCL) cell line, GRANTA-519, was cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 15% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37°C and in 5% CO_2 culture conditions. The GRANTA-519 cells were cultured in six-well culture plates with medium alone or containing 10 μM fludarabine dephosphorylated 9-β-D-arabinofuranosyl-2-fluoroadenine, (2-F-araA) or 10 μM 4-hydroperoxycyclophosphamide (4-HC). The cell viability of GRANTA-519 cells at 24–72 h after treatment was measured using flow cytometry.

Transfection of overexpression plasmids

Cell clones that overexpressed STAT5B were established by subcloning STAT5B cDNA into the pcDNA3.1/V5-His TOPO vector. A sequencing technique was applied to verify transfection. To produce stable cell lines, GRANTA-519 cells were infected with FuGENE HD transfection reagent, according to the manufacturer's protocol, then the stably transfected clones were selected in the presence of the aminoglycoside geneticin, or G418 (800 μg/ml). Following the separation of individual clones, the cell lysate was harvested and STAT5B protein expression was detected by Western blot.

Cell viability and proliferation assay

GRANTA-519 cells were seeded into 96-well culture plates (1×10^4 cells/well), and were incubated in 10% FBS. Then, 5 mg/mL of MTT reagent dissolved in phosphate-buffered saline (PBS) was added into each well and incubated at 37°C for 4 h. The optical density (OD) value at 570 nm was measured using a SpectraMax 340PC microplate reader (Molecular Devices, Sunnyvale, CA, USA). Cell proliferation was measured by the MTT dye uptake method, as previously described [14].

Cell cycle and apoptosis analysis

The cell cycle was analyzed by flow cytometry using propidium iodide (PI) to stain the cell nuclei [15]. GRANTA-519 cells with different treatments were washed with ice-cold PBS supplemented with 5 mM EDTA, then incubated in staining buffer containing 0.2 mg/ml RNase A at room temperature for 30 min. At the end of incubation, the cells were analyzed using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) equipped with CellQuest software (Becton Dickinson, Franklin Lakes, NJ, USA). Cell apoptosis was determined using staining with Annexin V- fluorescein isothiocyanate (FITC) and PI for 15 min at room temperature in a dark environment. Cells were then were washed and resuspended in binding

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buffer. Samples were analyzed using a FACScan flow cytometer (Becton-Dickinson FACScan, San Jose, CA, USA). The percentage of early apoptotic cells (Annexin-V-positive, PI-negative) and late apoptotic cells (Annexin-V-positive, PI- positive) were quantified by fluorescence microscopy.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNAs were extracted from the GRANTA-519 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Quantification of isolated RNA was performed using real-time reverse transcription PCR (RT-qPCR) with Universal SYBR Green Master Mix (Bio-Rad, Hercules, CA, USA), as previously described [16]. The cDNA was synthesized from total RNA using first strand cDNA synthesis kits (Bioneer Corp., Daejeon, Korea). RT-PCR amplification was performed using cDNA and the following conditions: denaturation at 95°C for 30 s; annealing at 55°C for 30 s; and extension at 72°C for 30 s. β-actin was served as an internal control.

Western blot

The total cell lysate was harvested, as previously described [17]. Briefly, 30 μg of extracted protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto polyvinylidene fluoride (PVDF) membranes, and then probed with primary and secondary antibodies according to the manufacturers' instructions. The primary antibodies used in the immunoblot included: p-STAT5B (1: 1000), STAT5B (1: 1000), cyclin D1 (1: 1000), IGF-1 (1: 1000), Bcl-2 (1: 1000), Bcl-xL (1: 1000), cPARP (1: 1000), p-AKT (1: 1000), AKT (1: 1000), β-actin (1: 2000), and secondary horseradish peroxidase (HRP)-conjugated antibodies (1: 5000). Blots were recorded using a LAS-4000 imaging system (Fujifilm, Minato, Tokyo, Japan) and by chemiluminescence (Thermofisher Scientific, Waltham, MA, USA), and quantified using Multi Gauge software version 3.1 (Fujifilm, Minato, Tokyo, Japan).

Hierarchical cluster analysis

GeneChip® operating software (GCOS), version 1.4 for microarray analysis (Affymetrix, Santa Clara, CA, USA), was used to assay the signal intensity on the hybridized gene chip. The signal-intensity values of the probe sets were transformed with an adaptive variance-stabilizing, quantile-normalizing transformation using GeneLogic Workshop of Low-Level Analysis of Affymetrix GeneChip Data, 2001, software (*http://abs.cit.nih.gov/geneexpression.html*). The overexpression of gene sets was identified from the online database available on the Gene Set Enrichment Analysis (GSEA) website (*http://www.broadinstitute.org/gsea/*) using GSEA version 2.04. Enriched genes between lymph node (LN)-derived MCL cells and peripheral blood (PB) lymphocytes were identified from 1,000 permutations of the phenotype labels.

Statistical analysis

Data were presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's t-test or one-way analysis of variance (ANOVA). All statistical analysis was performed using GraphPad Prism software version 5 (GraphPad Software, La Jolla, CA, USA). A P-value <0.05 was considered to be statistically significant.

Results

Heatmap of the differentially expressed genes (DEGs) between lymph node (LN)-derived mantle cell lymphoma (MCL) cells and peripheral blood (PB) lymphocytes

Hierarchical cluster analysis identified a total of 366 differentially expressed genes (DEGs) that were verified between the sources of MCL. To confirm that the genes identified were DEGs, a further data analysis with expression levels of at least two-fold difference between LN-derived MCL cells and PB lymphocytes was performed. Then, the top 100 DEGs between the two types of cells were screened and presented in the heatmap (Figure 1), of which, 68 genes were significantly upregulated in LN-derived MCL cells and 32 genes were downregulated in PB lymphocytes. Among the upregulated genes, STAT5B was the main one.

The expression and activity of STAT5B was increased in LN-derived MCL cells

Validation of the initial findings of upregulated STAT5B gene expression in LN-derived MCL cells was performed with Western blot, which measured the protein levels of STAT5B and its phosphorylation status in MCL cells. The protein levels of both STAT5B and p- STAT5B in LN-derived MCL cells were significantly greater than that in PB lymphocytes ($p<0.05$) (Figure 2A). Also, the relative expression levels of STAT5B target genes, including cyclin D1, IGF-1, Bcl-2, and Bcl-xL, were significantly upregulated in LN-derived MCL cells ($p<0.05$ and $p<0.01$, respectively) (Figure 2B). These findings showed that the expression and activity of STAT5B was upregulated in LN-derived MCL cells when compared with the PB lymphocytes.

Overexpression of STAT5B promoted cell proliferation in GRANTA-519 human MCL cells *in vitro*

To investigate the role of STAT5B in MCL cells, transfection using a plasmid vector encoding STAT5B was performed to produce overexpression clones in GRANTA-519 human MCL

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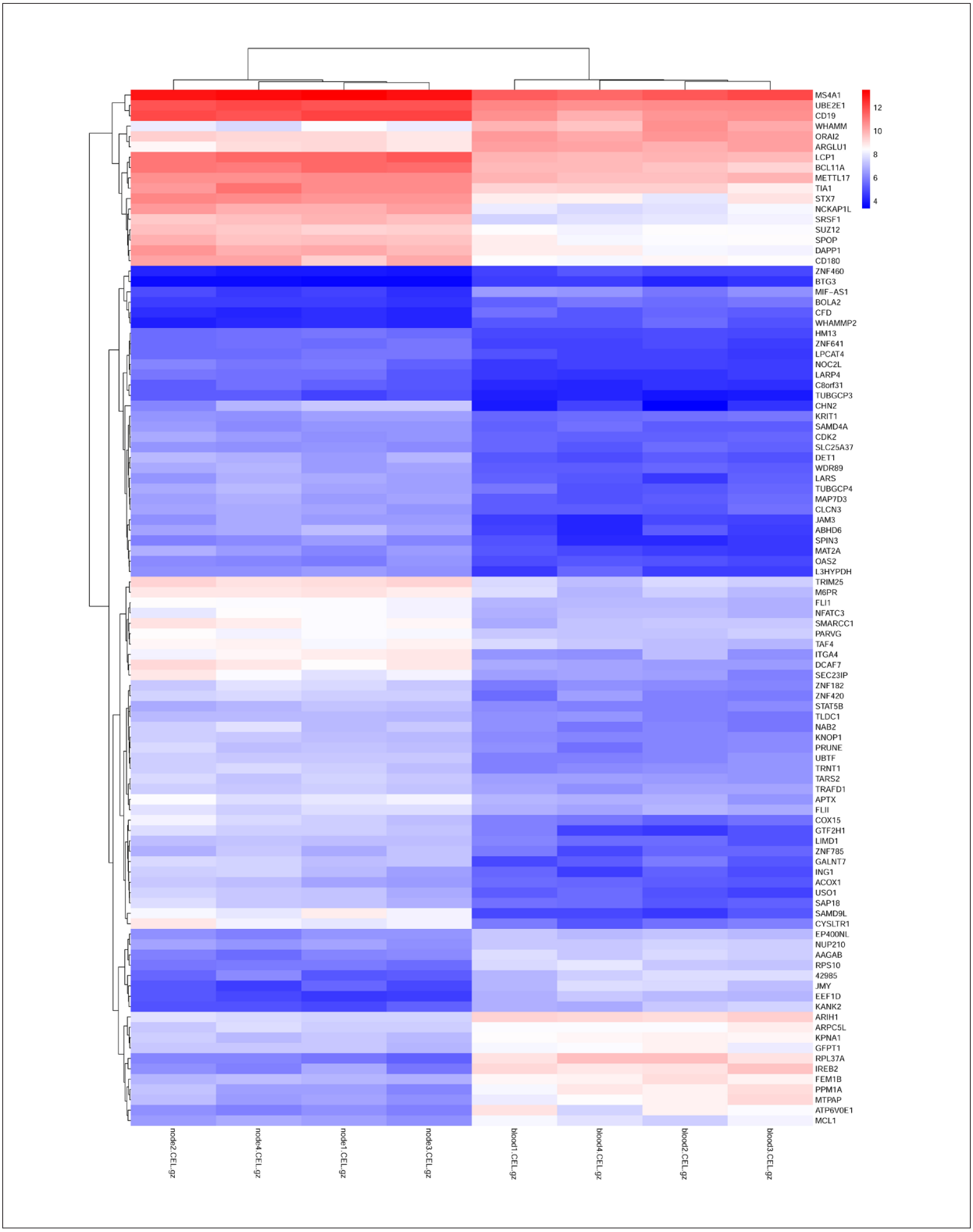

Figure 1. Heatmap of the top 100 differentially expressed genes (DEGs) between the lymph node (LN)-derived mantle cell lymphoma (MCL) cells and peripheral blood (PB)-derived cells (>two-fold change). The upregulated genes are shown in red, while the downregulated genes are shown in green. Each column represents an individual tissue sample. Gene names are noted on the right.

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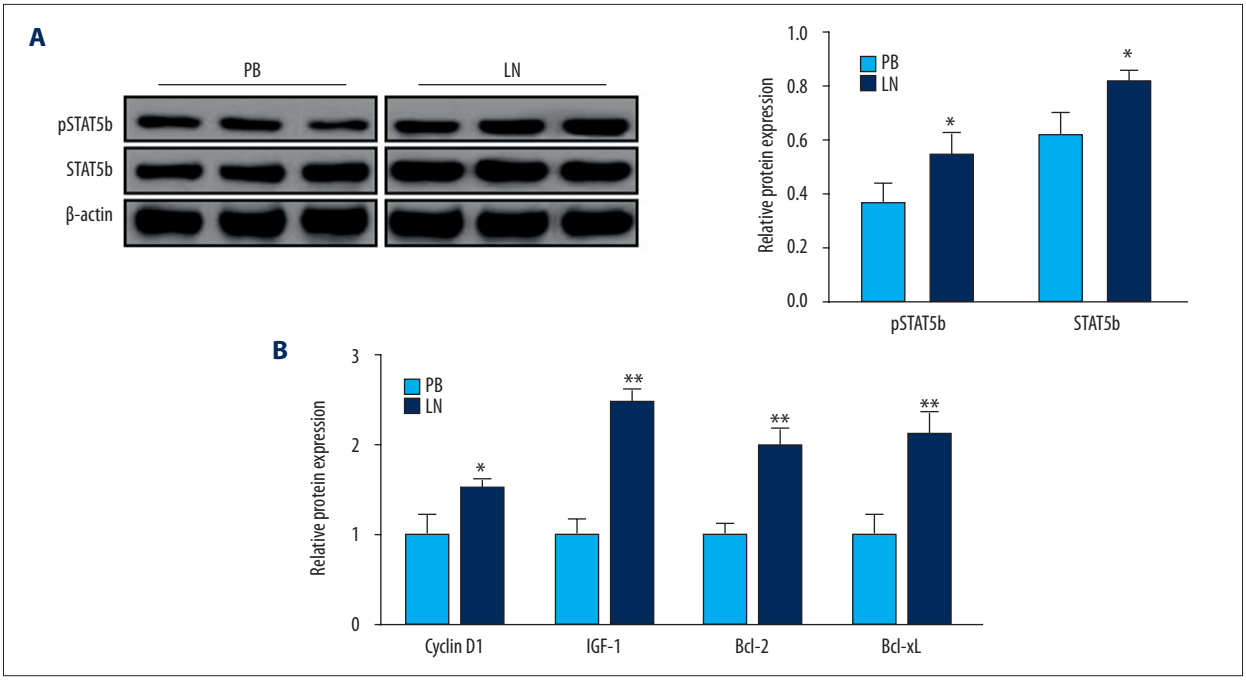

Figure 2. The expression and activity of STAT5B was increased in the lymph node (LN)-derived mantle cell lymphoma (MCL) cells. (**A**) Western blot shows the protein expression of STAT5B and p-STAT5B. (**B**) Western blot shows the protein expression of cyclin D1, IGF-1, Bcl-2, and Bcl-xL. * p<0.05, ** p<0.01.

cells *in vitro*. As shown in Figure 3A, clones transfected with STAT5B showed significantly increased expression levels of STAT5B (p<0.05), and unsuppressed STAT5A expression, demonstrating the successful establishment of the STAT5B overexpression plasmid.

The marker of proliferation Ki-67 (MKI67) gene is a cellular marker for proliferation [18], and in this study, MKI67 expression was significantly increased at the mRNA level compared with the control group with the use of STAT5B overexpression clones (p<0.05) (Figure 3B), supporting the inhibition of STAT5B overexpression on MCL cells growth *in vitro*. Consistent with these findings, the MTT assay also showed that the use of the STAT5B clone contributed to the significant and time-dependent increase in proliferation of GRANTA-519 cells (p<0.01) (Figure 3D). Flow cytometry was used to determine the effects of STAT5B on the cell cycle distribution of the GRANTA-519 MCL cell line. Compared with the sham-transfected cells, the number of cells in G0/G1 phase was significantly reduced after treatment with STAT5B alone (p<0.01) (Figure 3C), indicating that STAT5B treatment could induce cell cycle arrest in G0/G1 phase, which may have explained the role of STAT5B overexpression in promoting the proliferation of MCL cells. These findings supported that the overexpression of STAT5B facilitated the growth of MCL cells.

Overexpression of STAT5B reduced MCL cell apoptosis

Carcinogenesis is commonly accompanied by the deregulation of apoptosis [19], thus the induction of apoptosis is a common approach for anticancer therapies [20]. Therefore, the onset of apoptosis in MCL was evaluated to determine whether STAT5B overexpression resulted in MCL cell apoptosis when compared with sham-transfected cells, using staining with Annexin-V and propidium iodide (PI). As shown in Figure 4A, the rate of early and late apoptosis in sham-transfected cells were 19.9% and 37.3% respectively, which were higher than those in the STAT5B clone-transfected cells, which were 0.802% and 15.4%, respectively. This finding indicated that increased expression of STAT5B had an inhibitory effect in apoptosis in MCL.

Expression of the key apoptosis-related proteins, Bcl-2 and cleaved PARP

Western blot showed that STAT5B resulted in a significantly increased level of Bcl-2 (p<0.05) and PARP cleavage (p<0.01) in GRANTA-519 human mantle cell lymphoma cells (Figure 4B), which was consistent with the previous findings. These finding indicated that STAT5B suppressed MCL cell apoptosis by maintaining the level of anti-apoptotic protein Bcl-2 and cleavage of PARP.

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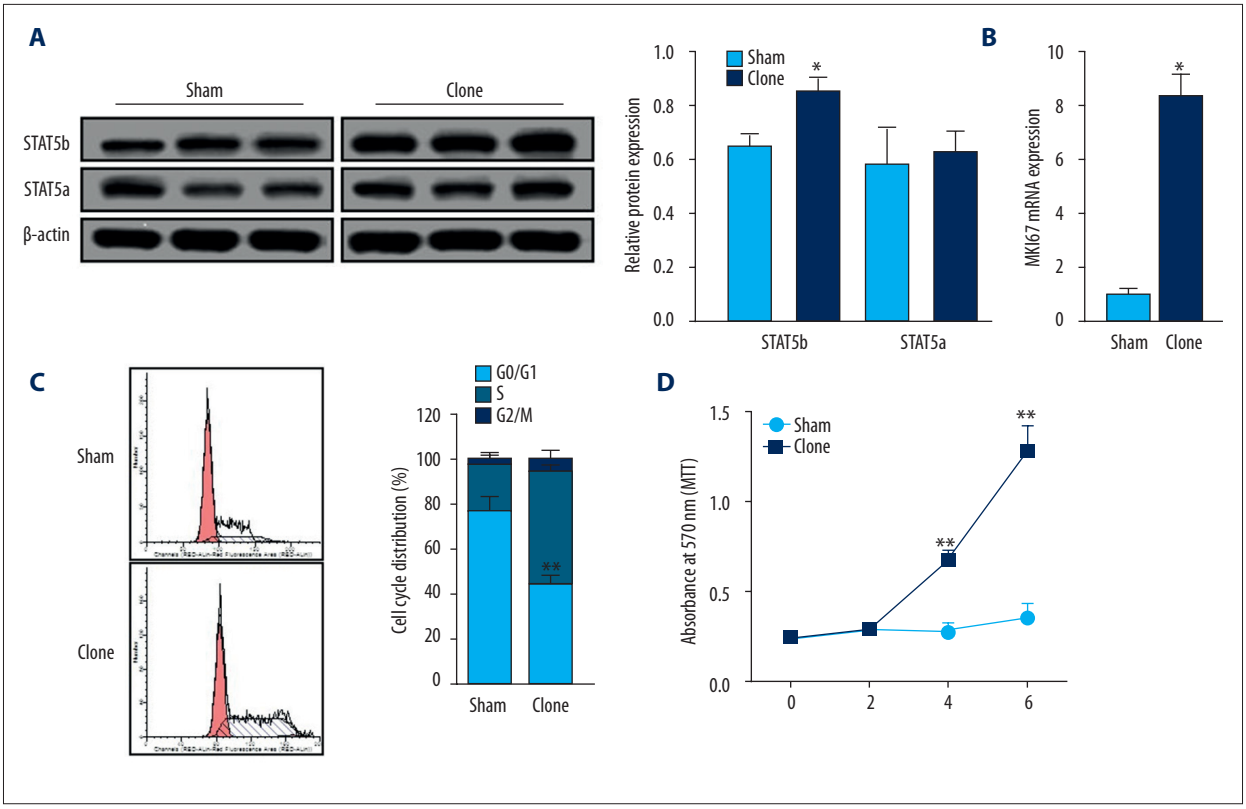

Figure 3. Increased expression of STAT5B promoted the proliferation of mantle cell lymphoma (MCL) cells. GRANTA-519 human mantle cell lymphoma (MCL) cells were transfected with the sham or the STAT5B overexpression clone. (**A**) Western blot shows the protein expression of STAT5B and STAT5A. (**B**) Quantitative reverse transcription polymerase chain reaction (RT-qPCR) shows the mRNA expression of the marker of proliferation Ki-67 (MKI67) gene. (**C**) Flow cytometry analysis shows the cell cycle distribution. (**D**) MTT assay shows the optical density (OD) value at 570 nm of MCL cells after incubation with the sham or STAT5B overexpression clone at 0, 2, 4, and 6 h. * p<0.05, ** p<0.01.

Effects of increased expression of STAT5B on drug sensitivity

GRANTA-519 human mantle cell lymphoma cells treated with fludarabine dephosphorylated 9-β-D-arabinofuranosyl-2-fluoroadenine, (2-F-araA) and 4-hydroperoxycyclophosphamide (4-HC) showed time-dependent reduced cell viability (p<0.01), indicating high drug cytotoxicity for fludarabine. However, overexpression of STAT5B resulted in a significant increase in cell viability at 48 h and 72 h compared with the untreated cells (p<0.01) (Figure 4C), indicating that STAT5B increased MCL cell survival following fludarabine treatment. In 4-HC-treated cells, this effect was increased (p<0.01), as shown in Figure 4D. These findings indicated that STAT5B could enhance drug resistance of MCL cells.

The role of STAT5B on MCL cells was dependent on the activation status of Akt

The Akt signaling pathway has been previously shown to have key roles in cell survival, cell proliferation, and apoptosis in cell lines. In this study, the levels of Akt were measured in GRANTA-519 human MCL cells that overexpressed STAT5B. Western blot showed increased phosphorylated Akt in STAT5B-treated cells compared with controls, which was also shown by the increased ratio of p-Akt/Akt in the cells in the clones (p<0.05) (Figure 5A), indicating that STAT5B overexpression promoted the activation of Akt. The specific inhibitor of the Akt pathway, LY294002, was used to treat the MCL cells and assessed the changes in cell cycle, cell apoptosis, and cell viability. As shown in Figure 5B, the use of the specific inhibitor of the Akt pathway, LY294002, inhibited the progression of cell cycle, resulting in the accumulation of cells in G0/G1 phase (p<0.01), and the decreased the proportion of cells in the S-phase. Also, the proportion of apoptotic cells was significantly increased in the LY294002 treated-cells compared with the vehicle-treated cells (p<0.01) (Figure 5C). These findings supported that the inhibition of Akt could increase apoptosis of MCL cells by reducing the progression of the cell cycle. Finally, cell proliferation activity was measured when treated with LY294002 and 2-F-araA using the MTT assay. Treatment with LY294002 after 48 and 72 h resulted in significant reduction in cell viability

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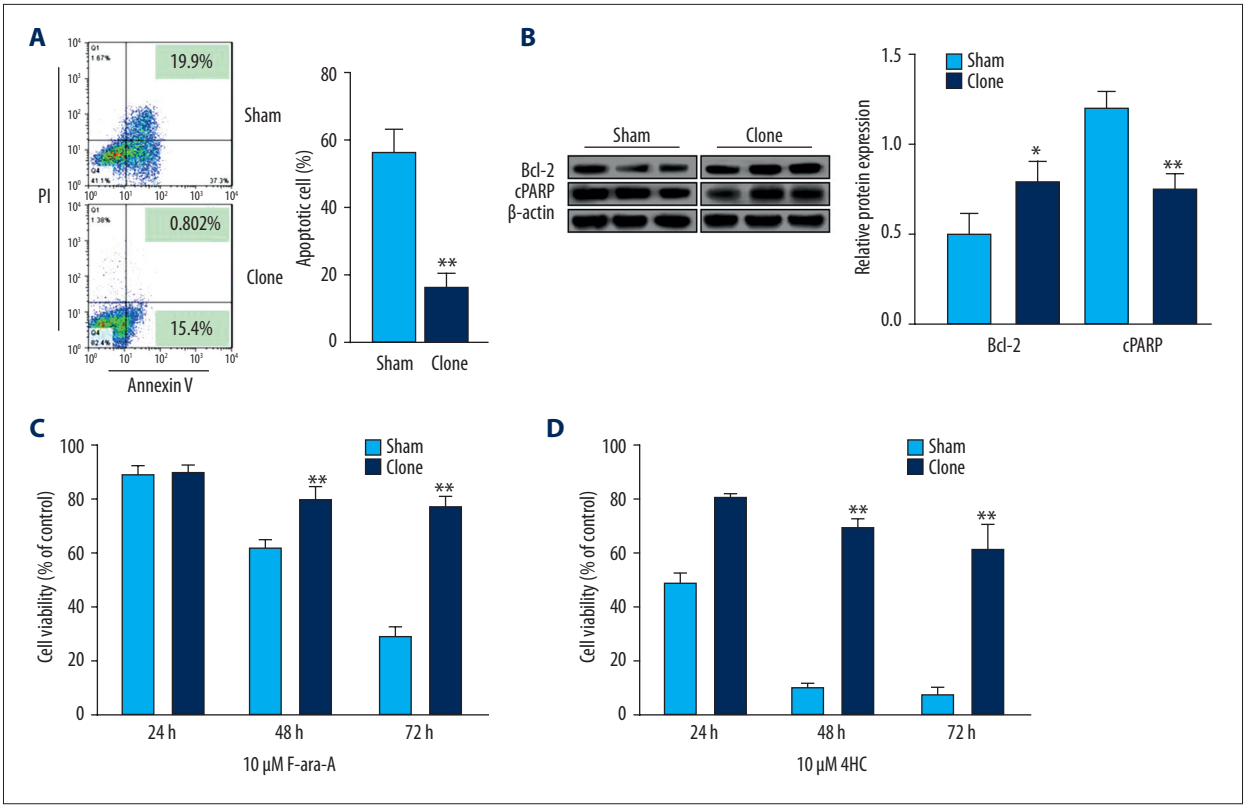

Figure 4. Overexpression of STAT5B reduced mantle cell lymphoma (MCL) cell apoptosis and cell survival. GRANTA-519 human mantle cell lymphoma (MCL) cells were cultured with medium or medium containing fludarabine dephosphorylated 9-β-D-arabinofuranosyl-2-fluoroadenine, (2-F-araA) or 10 μM 4-hydroperoxycyclophosphamide (4-HC), then transfected with the sham or the STAT5B overexpression clone. (**A**) Flow cytometry analysis shows cell apoptosis. (**B**) Western blot shows the protein expression of Bcl-2 and cPARP. (**C**) The MTT assay shows the cell viability of MCL cells at 24, 48, and 72 h after treatment with 10 μM 2-F-araA. (**D**) The MTT assay shows the cell viability of MCL cells at 24, 48, and 72 h after treatment with 10 μM 4-HC. * p<0.05, ** p<0.01.

compared with treatment with 2-F-araA (p<0.01) (Figure 5D), indicating the role of Akt in the regulation of MCL cell drug resistance. Therefore, the effects of STAT5B on MCL cells were partly dependent on the activation status of Akt.

Discussion

The present study included hierarchical cluster analysis of the gene expression profiles of peripheral blood (PB)-derived lymphocytes compared with lymph node (LN)-derived mantle cell lymphoma (MCL) cells, and identified a total of 366 differentially expressed genes (DEGs) from the MCL cells. The STAT5B gene was significantly upregulated in LN-derived MCL cells. Western blot confirmed upregulation of the expression of STAT5B and its relative target genes. A plasmid vector encoding STAT5B overexpression clones were transfected into GRANTA-519 human MCL cells cultured *in vitro*, and the results showed that STAT5B overexpression promoted MCL cell proliferation and reduced cell apoptosis. Investigation of the mechanisms involved in the expression of STAT5B showed that the effects of STAT5B on MCL cells were associated with the activation status of Akt.

In previously published studies, studies on MCL involved lymph nodes or normal lymphoid tissues. Hofmann et al. [21] and Islam et al. [22] identified several DEGs in MCL when compared with normal lymphoid tissues. Also, a total of 106 DEGs including 63 upregulated and 43 downregulated genes were previously screened in MCL cells compared with naïve B-cells [6]. However, these previous studies are unable to identify whether the differences were due to the transformed MCL or the tumor microenvironment. In the present study, all DEGs were compared between LN-derived MCL cells and PB lymphocytes and were identified using hierarchical cluster analysis, which suggested that microenvironment has a significant impact on the progression of MCL. Among these differentially expressed genes, STAT5B was significantly upregulated, indicating its involvement in the pathogenesis of MCL. Western blot showed that the protein levels of both STAT5B

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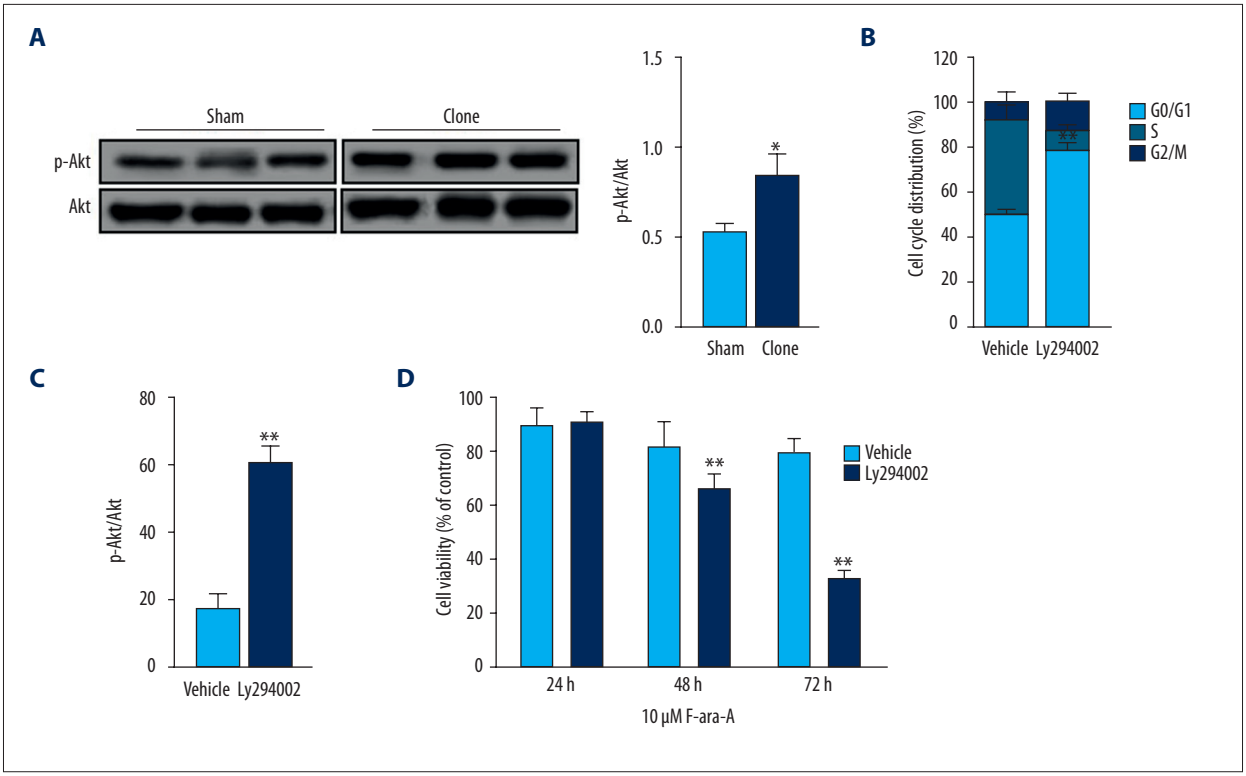

Figure 5. The function of STAT5B in GRANTA-519 human mantle cell lymphoma cells was dependent on the activation status of Akt. (**A**) Western blot showing the protein levels of p-Akt and Akt in GRANTA-519 human mantle cell lymphoma cells transfected with the STAT5B overexpression clone or sham, and the histogram shows the relative value of p-Akt and Akt. (**B**) Flow cytometry analysis showing the cell cycle distribution in GRANTA-519 human mantle cell lymphoma cells treated with the specific inhibitor of the Akt pathway, LY294002, or vehicle. ** p<0.01. (**C**) Flow cytometry analysis shows the percentage of apoptotic GRANTA-519 human mantle cell lymphoma cells treated with the specific inhibitor of the Akt pathway, LY294002, or vehicle. ** p<0.01. (**D**) The MTT assay shows the cell viability of GRANTA-519 human mantle cell lymphoma cells at 24, 48 and 72 h after treated with LY294002 or vehicle and 10 μM 2-F-araA. * p<0.05, ** p<0.01.

and p-STAT5B in LN-derived MCL cells were significantly increased when compared with PB-derived lymphocytes. Also, the relative expression levels of several related target genes activated by the STAT cascade were detected, including cyclin D1, IGF-1, Bcl-2, and Bcl-xL.

Cyclin D1 expression can be upregulated by recognition of a conserved element in the promoter of STAT [23]. Bcl-xL overexpression has been shown to be capable of initiating the mutations of STAT5B in T-cell acute lymphoblastic leukemia (ALL) [24]. Bcl-2 is a critical effector of the mammalian apoptotic signaling cascade [25] and an important regulator of tumorigenesis [26]. Also, lymphoid malignancies usually have high expression levels of BCL2 gene expression, and these genes were all significantly upregulated in the MCL cells from the LN. Therefore, it is possible that the expression and activity of STAT5B were greater in LN-derived MCL cells than in PB-derived lymphocytes. Also, other members of the STAT family, except for STAT5B have been previously shown to be activated in MCL. Lai et al. reported the activation of STAT3 in a fraction of MCL tumors detected by frozen tissue samples by Western blot [27]. Also, Yared et al. [28] showed that STAT3 was activated in MCL cells, and the overall frequency in this series was up to 47%.

Findings from previously published studies have shown that STAT5B has multiple functions, including cellular proliferation, cell differentiation [29], cell survival [30], cell cycle regulation [31], and metastasis [32]. Kataoka et al. [33] found that expression of STAT5B protein was associated with cell proliferation and progression of intraductal papillary mucinous neoplasms of the pancreas. Also, STAT5B knock-out mice showed a significantly reduced survival and growth of hematopoietic cells from lymphoid and myeloid lineages [34]. Recent studies have shown that STAT5B has a critical regulatory role in many hematologic malignancies, an functional significance of STAT5B mutations have been shown in large granular lymphocytic (LGL) leukemia [35].

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In the present study, a plasmid vector encoding STAT5B overexpression clones was transfected into GRANTA-519 cells to identify the potential role of endogenous STAT5B in MCL cells and showed that the overexpressed STAT5B promoted MCL proliferation by inducing cell cycle arrest in G0/G1 phase and suppressed cell apoptosis. Previous studies have shown that STATs may be implicated in cell proliferation by mediating the expression of cell cycle regulatory genes, such as the cyclins, and early and immediate genes, including c-myc and c-fos [4]. However, the findings of the present study were also consistent with the findings from a study supporting the underlying role of STAT5 as an oncogene in human cancers [36]. Also, STAT5 has been shown to be associated with chemoresistance in human tumors. Sumiyoshi et al. [34] found that STAT5B knockdown significantly reduced gemcitabine chemoresistance in pancreatic cancer cells. Based on these previous findings, in this study, the effects of overexpression of STAT5B on MCL cell drug sensitivity was studied by treating MCL cells with fludarabine dephosphorylated 9-β-D-arabinofuranosyl-2-fluoroadenine, (2-F-araA) or 4-hydroperoxycyclophosphamide (4-HC), and showed significant enhancement of activity in cells treated with both drugs, compared with the control cells, suggesting that STAT5B could significantly reduce the drug resistance of MCL cells.

The Akt pathway has a role in the regulation of cell survival, proliferation, and apoptosis in multiple cell lines [37]. Previous studies on the role of AKT in MCL have supported that the persistent activation of phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) is associated with the pathogenesis of MCL [38]. Also, constitutive Akt Ser 473 phosphorylation has also been identified in many cases of MCL and in MCL cell lines [17]. Based on these previous findings, the regulatory role of STAT5B on MCL cells was believed to be most likely to be associated with Akt. This study showed that increased phosphorylation levels of Akt were found in STAT5B-treated, which identified that increased expression of STAT5B could promote the activation of Akt. Further experiments showed that increased apoptosis of MCL cells caused by downregulated Akt reduced the progression of the cell cycle and that Akt had a role in drug resistance. Igney and Krammer showed that several upregulated genes in the PI3K/AKT signaling pathway were present in MCL cells when compared with naïve B-cells, and they proposed that PI3K/AKT signaling activation could be a mechanism in apoptosis resistance in MCL that had potential clinical relevance [39]. The findings from the present study are supported by findings from previous studies and have shown that the role of STAT5B in MCL depends on the activation status of Akt.

Conclusions

The findings of this study showed increased expression and activity of STAT5B in lymph node-derived mantle cell lymphoma (MCL) cells. Cell culture of GRANTA-519 human MCL cells showed that overexpression of STAT5B was associated with increased MCL cell proliferation, inhibition of apoptosis, and enhanced drug resistance. The functions of STAT5B on MCL cells were dependent on the activation status of Akt. The results of this *in vitro* and gene network analysis study support the need for further studies on the role of STAT5B expression in clinical samples from patients with MCL, including prospective studies to evaluate the associations with patient prognosis and response to therapy.

Conflict of interest

None.

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