JQ1 synergize with anti-CD47 antibody to enhance the function of macrophages and repress the progression of Burkitt lymphoma

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Abstract

Background: M2 macrophages, as the most prominent immune cells in Burkitt lymphoma (BL), are promising targets and anti-CD47 antibody could enhance the phagocytosis but be unable to eradicate tumor cells. JQ1, a C-MYC inhibitor, may enhance the function of macrophages and be a choice for combination therapy.

Methods: The effect JQ1, on the expression of CD47 was measured. Then the synergy of JQ1 and anti-CD47 antibody was measured using phagocytosis assays. The effect of JQ1 on the polarization of macrophages was also detected. Finally, the efficiency and safety of JQ1 and anti-CD47 antibody combination therapy was explored in a groin orthotopically implanted Raji tumor model.

Results: JQ1 could suppress the expression of CD47 on the surface of BL cells, thus synergize with anti-CD47 antibody to enhance the phagocytosis of macrophages. JQ1 could polarize macrophages from M2 to M1 while inhibiting the proliferation, inducing the apoptosis and blocking the cell cycle of BL cells. Finally, JQ1 and anti-CD47 antibody combination therapy could

repress the progression of BL in NOD/SCID mice.

Conclusions: Macrophages may be a promising target in BL and JQ1 combined with anti-CD47 antibody may be a potential therapeutic choice, providing theoretical basis for the use of this new targeted immunotherapy in clinical practice.

Keywords: Macrophage; Targeted immunotherapy; Anti-CD47 antibody; JQ1; Burkitt lymphoma

Introduction

Burkitt lymphoma (BL) is highly curable in children relying on short duration, high-intensity chemotherapy regimens. However, these regimens are hard to apply in older and immunosuppressed patients because of the side effects. Novel agents are in urgent demand to decrease the treatment-related toxicity while maintaining the high cure rates. Macrophages play an important role in tumor microenvironment (TME), attracting increasingly more attention and become hotspot. M1-polarized macrophages inhibit the progression of tumor while M2-polarized macrophages promote immune escape. In the latest studies, M2 was found to be the most prominent component in TME of BL and was crucial to the development of lymphoma [1]. Therefore, macrophages may be a promising target in the exploration of novel agents or approaches.

CD47 is a "don't eat me" marker overexpressed on the surface of numerous tumor cells. Anti-CD47 antibody can block the binding of CD47 and SIRPα on macrophages, thus recovering phagocytosis of macrophages [2]. Although anti-CD47 antibody can repress the progression of tumor, especially lymphoma, it alone is unable to eradicate tumor cell [3-5]. Combination with other drugs is a good way to enhance the effect of anti-CD47 antibody.

JQ1 is a small molecular inhibitor that can repress the progression of a majority of tumors by downregulating MYC transcription. Many signal pathways are involved in subsequent downregulation of MYC-dependent genes and they interweave into a complicated network, thus various compensatory transcription mechanisms also evolved, leading to the resistance to JQ1 [6]. As the resistance to JQ1 appeared increasingly often, some studies have revealed the mechanism of resistance to JQ1, which varies in different tumor cells [7-11]. To improve the efficacy of JQ1, people try to combine JQ1 with other drugs and the regimens

are destined to differ in different tumors. At the same time, TME is vital in overcoming drug resistance but few studies were done about the effect of JQ1 on immune cells, let alone macrophages.

Exploration on the effect of JQ1 on macrophages will undoubtedly help to discover better combination therapy, especially in BL.

In the latest studies, JQ1 was found to inhibit the expression of CD47 in T-cell acute lymphoblastic leukemia cell line [12]. Being impressed by this discovery, we supposed that JQ1 could synergize with anti-CD47 antibody to enhance the phagocytosis of macrophages in BL, thus inhibit the growth of tumor. This is the first research to explore the effect of JQ1 and anti-CD47 antibody combination therapy from the perspective of macrophage, laying foundation for the development of new drugs targeted in macrophages.

Materials and methods

Cell Lines and cell culture

Raji was purchased from American Type Culture Collection (ATCC). RAW264.7 murine macrophages were supplied by Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. THP1 human acute monocytic leukemia cells were supplied by Cancer Center, The First Bethune Hospital of Jilin University. All the cells were cultured in RPMI1640 medium (Hyclone, UT) containing 10% fetal bovine serum (FBS) (Gibco, USA), streptomycin (50 UmL^{-1}) and penicillin (50 UmL^{-1}), at 37 ℃ in a 5% CO_2 atmosphere.

Reagents

JQ1 was purchased from Changchun Sanbang Biomedical Technology Co., Ltd. B6H12.2 cells at logarithmic growth phase were harvested and injected into abdominal cavity of BALB/C mice, and then ascites was collected and Protein G affinity chromatography was used to purify ascites, getting anti-CD47 antibody at the concentration of 2.6mg/mL. The preparation of antibody was completed by ChinaPeptides Co., Ltd.

Animals

Male NOD/SCID mice at 5 weeks of ages were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The animal experiments were revised and approved by the Animal Care and Use committee of Jilin University, and all animals received care in accordance with national guidelines and requirement for the Care and Use of Laboratory Animals of Jilin University. Mice were euthanized for signs of distress or before the maximum allowable tumor size of 3500 mm^3. Then these animal corpses were preformed harmless treatment when the experiments finished.

Viability, apoptosis, cell cycle analysis

Cells were plated at 5000 cells per well of 96-well plates. Then different concentrations of JQ1 was added and cells were treated for 72 h. After that, each well was added with 10 μL of CCK-8 reagent. Eventually, cell viability was analyzed by calculating in a Tecan Spark Multifunctional enzyme marker at the wavelength of 450 nm. GI50 values for each cell line were calculated as the concentration of compound giving a 50% reduction in cell number relative to the RPMI1640 control.

Cells at the density of 2.78×10^4/mL were treated with JQ1 at different concentrations. Then the cells were washed once with PBS and stained with 5 μL of Annexin V-FITC/PI staining solution (Nanjing Keygen Biotech, China) in 500 μL of 1× binding buffer. After a 15-minute incubation at room temperature, the cells were analyzed by using a BD FACS Calibur flow cytometer and FlowJo VX. The percentage of viable cells (low annexin VFITC/low PI), early apoptotic cells (high annexin V-FITC/low PI), and late apoptotic/necrotic cells (high annexin V-FITC/high PI) was determined.

The cell cycle distribution was detected according to the instruction of manufacturer and cell number and cell cycle data were obtained by using a BD FACS Calibur flow cytometer. DNA content histograms were analyzed by using ModFit LT 3.2 Software (Verity).

Cell surface expression

Cells at the density of 2.78×10^4/mL were treated with JQ1 at different concentrations. Then the cells were washed once with PBS and stained with FITC-conjugated anti-CD47 antibody. Control staining was done using isotype-matched IgG and secondary

antibodies alone. After 30 minutes of inoculation, the data was obtained using a BD FACS Calibur flow cytometer and was analyzed using FlowJo VX.

Quantitative RT-PCR

THP1 cells were matured into macrophages with 150 ng/ml PMA and further into M2 with 20 ng/mL IL-4/IL-13. RAW264.7 cells were induced into M2 with 20 ng/mL IL-4. Then macrophages were treated with JQ1 at the concentration of 10 nmol/L. The RNA was collected to administer quantitative RT-PCR and the supernatant was collected to administer enzyme-linked Immunosorbent assay.

Total RNA from cells was isolated with RNAprep Pure Cell/Bacteria Kit (TIANGEN BIOTECH(BEIJING) CO., LTD). RNA was reverse-transcribed to single-stranded cDNA by the PrimeScript RT-PCR Kit(Takara, Japan) and Sybr green-based qPCR was performed using human primers to *ACTIN*, *C-MYC*, *CD47*, *CCL3*, *TNF-α*, *CCL17*, *CCL22* and murine primers to *actin*, *c-myc*, *cd47*, *pd-l1*, *nos2*, *cxcl10*, *arg-1*, *mrc-1*. The primer sequence are listed below. The expression of mRNA levels was normalized to Actin (dCt = Ct gene of interest − Ct Actin). Relative mRNA expression was calculated by the ethod (ddCt = $2^{\wedge -(dCt\ sample\ -\ dCt\ control)}$).

编号	引物名称	序列(5' to 3')
1	人ACTIN（Forward）	CCTAGAAGCATTTGCGGTGG
	人ACTIN（Reverse）	GAGCTACGAGCTGCCTGACG
2	人CCL3（Forward）	CAGCCAGGTGTCATTTTCCT
	人CCL3（Reverse）	TCAGGCATTCAGTTCCAGGT
3	人TNF-α（Forward）	TAGCCCATGTTGTAGCAAACC
	人TNF-α（Reverse）	ATGAGGTACAGGCCCTCTGAT
4	人CCL17（Forward）	ACTGCTCCAGGGATGCCATCGTTTTT
	人CCL17（Reverse）	ACAAGGGGATGGGATCTCCCTCACTG
5	人CCL22（Forward）	TGATTACGATCCGTTACCGTCT
	人CCL22（Reverse）	CCTGAAGGTTAGCAACACCAC
6	人C-MYC（Forward）	AATGTCAAGAGGCGAACACAC
	人C-MYC（Reverse）	ATTGTTTTCCAACTCCGGGAT
7	人CD47（Forward）	GGCAATGACGAAGGAGGTTA
	人CD47（Reverse）	ATCCGGTGGTATGGATGAGA
8	鼠ACTIN（Forward）	GTGCTATGTTGCTCTAGACTTCG
	鼠ACTIN（Reverse）	ATGCCACAGGATTCCATACC
9	鼠NOS2（Forward）	AAACCCCTTGTGCTGTTCTC
	鼠NOS2（Reverse）	ATACTGTGGACGGGTCGATG
10	鼠CXCL10（Forward）	AAGTGCTGCCGTCATTTTCT
	鼠CXCL10（Reverse）	CCTATGGCCCTCATTCTCAC
11	鼠Arg-1（Forward）	GCAGAGGTCCAGAAGAATGG
	鼠Arg-1（Reverse）	AGCATCCACCCAAATGACAC
12	鼠Mrc-1（Forward）	GGAGGCTGATTACGAGCAGT
	鼠Mrc-1（Reverse）	CATAGGAAACGGGAGAACCA
13	鼠C-MYC（Forward）	ATCTGCGACGAGGAAGAGAA
	鼠C-MYC（Reverse）	ATCGCAGATGAAGCTCTGGT
14	鼠CD47（Forward）	TGCGGTTCAGCTCAACTACTG
	鼠CD47（Reverse）	GCTTTGCGCCTCCACATTAC
15	鼠PDL1（Forward）	GCTCCAAAGGACTTGTACGTG
	鼠PDL1（Reverse）	TGATCTGAAGGGCAGCATTTC

Enzyme-linked Immunosorbent assay

The enzyme-linked Immunosorbent assay was conducted according to the instruction of manufacturer (Lanpaibio.com) and the data was analyzed using ELISACalc software.

Phagocytosis assays

Target (lymphoma cells) were stained with CFSE, and effector (matured THP1 cells) were stained with Hoechst. Then they were co-cultured in a 4:1 ratio for two hours while treated by different concentrations of anti-CD47 antibody. Phagocytosis was assessed with confocal microscopy (Carl Zeiss AG). For confocal microscope images, engulfment was judged by looking for fragments of target cell stain contained within an effector cell. Phagocytosis index is number of phagocytosing macrophages/total number macrophages per 100 macrophages on field.

Tumor challenge and treatment experiments

The Burkitt lymphoma model was prepared by subcutaneously injecting 5×10^6 Raji cells into the right groin of the mice on day 0 of experiments. Tumor volume approximately reaching 400 mm^3 on day 10 post tumor implant, the mice were randomly divided into groups and then treated following the therapy regimen. Anti-CD47 antibody dissolved in phosphate buffered saline (pH 7.4) was injected intraperitoneally at the dose of 200 μg/d. JQ1 was administered by intraperitoneally injection at the dose of 30 mg/kg twice a day. Treatment was started on day 10 and stopped on day 24 of experiments. The tumor volume was measured every second day with a caliper, using the formula tumor volume = a $\times$ b^2/2, where a and b are the major and minor axes of the tumors. Mice were sacrificed at designed time points for analysis. Eyeballs were extracted and blood was got to detect liver function and renal function.

Statistical analysis

All data were expressed as means ± S.E.M. Statistical significance was analyzed by using Student's t test. The analyses were performed with GraphPad Prism 7.0 statistical software (GraphPad software, San Diego, Calif). *p < 0.05 was considered to

indicate statistical significance, **p < 0.01 was highly significant difference and ***p < 0.001 extremely significant difference. All experiments were performed at least three times, n refers to biological replicate.

Results

JQ1 could decrease the expression of CD47

In order to investigate the effect of JQ1 in BL. Human BL cell line Raji was used. JQ1 can influence the transcription of multiple genes and MYC gene is the most frequently investigated. We detected the effect of JQ1 on the relative mRNA level of MYC in Raji using RT-PCR. JQ1 could decrease the transcription of MYC obviously in Raji (Figure 1A). The mRNA level of CD47 in was also detected, and JQ1 could decrease the transcription of CD47 (Figure 1B). Flow cytometry was also used to detect the effect of JQ1 on the expression of CD47 on the surface of Raji and the results were consistent with mRNA level (Figure 1C).

JQ1 could synergize with anti-CD47 antibody to increase the phagocytosis of macrophages

We had proved JQ1 could decrease the expression of CD47. CD47, as we know, could repress the phagocytosis of macrophages. To explore whether JQ1 could help recover the phagocytosis of macrophages. We treated Raji with JQ1, and then Raji cells were stained with CFSE and macrophages were stained with Hoechst. After two hours of co-culture, Raji cells had not been phagocytosed, which were floated in supernatant, were taken away. Raji cells had been phagocytosed were inside the macrophages, which were adherent cells. Then confocal microscopy was used to detect cells and phagocytic index was calculated. The representative confocal laser scanning microscopy (CLSM) images were shown (Figure 1D). Both JQ1 and anti-CD47 antibody could enhance the phagocytosis of macrophages and anti-CD47 antibody was more effective (Figure 1E). Their combination treatment was more effective than any other group (Figure 1E).
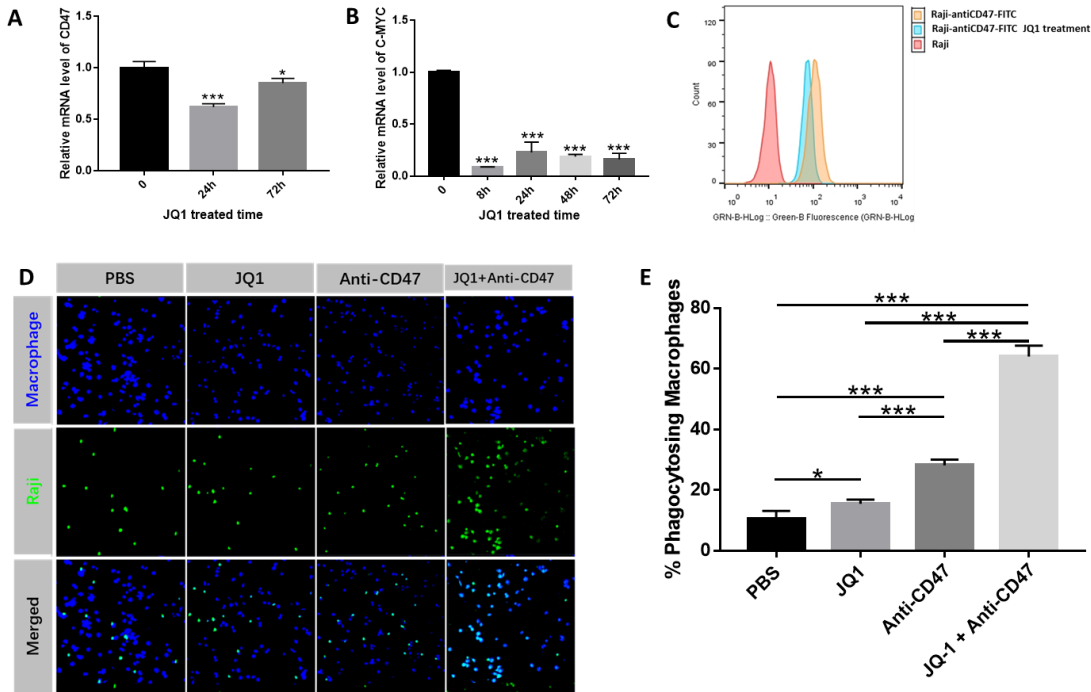
Figure 1. JQ1 synergize with anti-CD47 antibody to enhance macrophage phagocytosis. (A-B) The relative level of CD47 (A) and C-MYC(B) in Raji cells after being treated by JQ1(10 μM) for different times. (C) The expression of CD47 on Raji cells being treated by JQ1 (10uM) or not was measured by staining the cells with anti-human CD47 mAb conjugated to FITC. (C-D) Target Raji cells being treated by JQ1 or not were stained with CFSE, and macrophages with Hoechst in confocal microscopy before co-incubating for 2 hours at 4:1 ratio with PBS or anti-CD47 antibody B6H12 at 10 μg/ml. Phagocytosis was assessed by measuring percentage of double positive macrophages. Representative confocal laser scanning microscopy (CLSM) images were presented as (C) and quantification of the results were shown in (D). Bars are mean ±SEM (n= 3) (*, p < 0.05; **, p < 0.01; ***, p < 0.001, all compared with 0h group).

JQ1 could induce the polarization of macrophages from M2 to M1

Macrophages could be further polarized into M1 and M2. In tumor immune microenvironment, M1 was considered to eradicate tumor cells and M2 was considered to induce the growth and invasion of tumor cells. The transformation from M2 to M1 has become a research focus. Here, RT-PCR, flow cytometry and Elisa were used to analyze the change on the mRNA level of associated genes, the expression level of surface marker, the secretory level of associated cytokine, respectively. Macrophages were induced into M2 with IL-4 alone or in combination with IL-13. In human macrophages, after being treated by JQ1, the relative mRNA level of human M1-associated genes, CCL3 and TNF-α, increased, while M2-associated genes, CCL17 and CCL22, decreased (Figure 2A-2D). The secretory level of human M1-associated cytokine, TNF-α, increased and M2-associated

cytokine, IL-10 decreased (Figure 2E, 2F). In murine macrophages, JQ1 increased the relative mRNA level of murine M1-associated genes, NOS2 and CXCL10, and decreased M2-associated genes, Arg-1 and Mrc-1 (Figure 2G-4J). The secretory level of murine M2-associated cytokine, IL-10, decreased while the percentage of F4/80(+) CD206(+) cells increased (Figure 2K-4L). So JQ1 induced the polarization of M2 to M1.
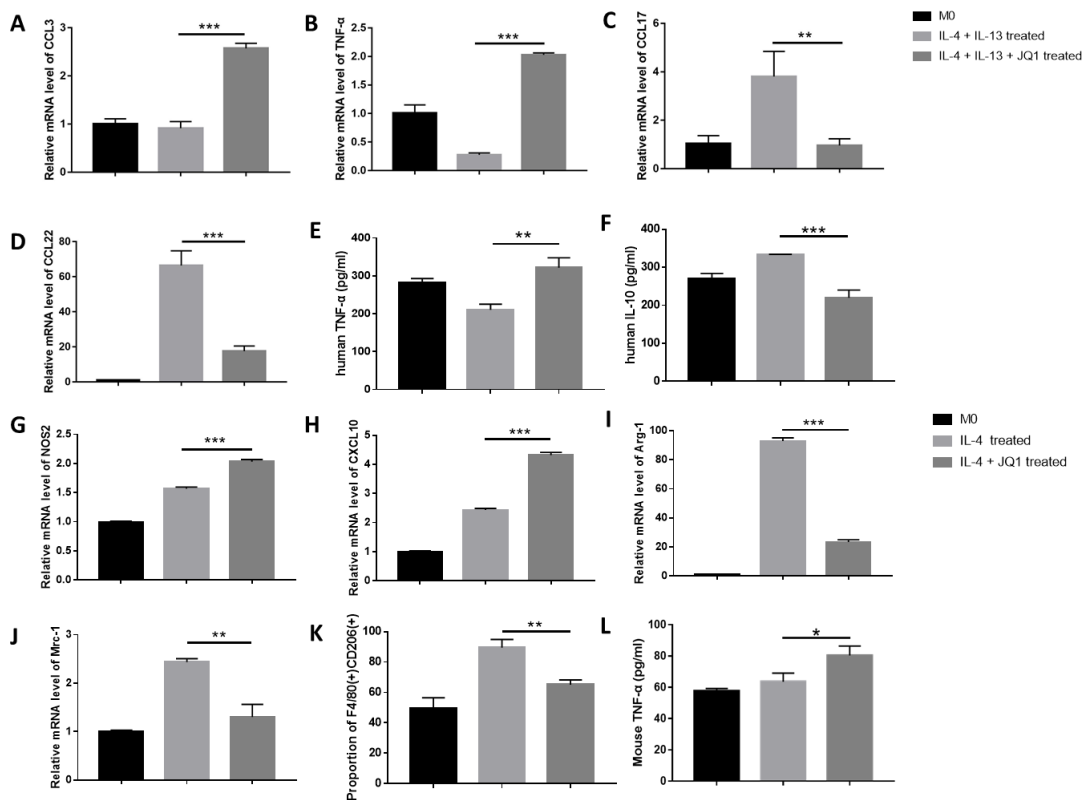

Figure 2. JQ1 can induce the polarization of macrophages from M2 to M1. THP1 cells were matured into macrophages with 150 ng/ml PMA and further into M2 with 20 ng/mL IL-4/IL-13. RAW264.7 cells were induced into M2 with 20 ng/mL IL-4. The relative mRNA level of human M1-associated genes, CCL3 (A), TNF-α(B), and M2-associated genes, CCL17 (C), CCL22 (D). The secretory level of human M1-associated cytokine, TNF-α (E) and M2-associated cytokine, IL-10 (F). The relative mRNA level of murine M1-associated genes, NOS2 (G), CXCL10 (H), and M2-associated genes, Arg-1 (I), Mrc-1 (J). The expression of murine M2-associated surface marker F4/80, CD206 (K) and the secretory level of mouse M1-associated cytokine, TNF-α (L). Bars are mean $\pm$ SEM (n= 3) (*, $p < 0.05$; **, $p < 0.01$).

Burkitt lymphoma cells were sensitive to JQ1

CCK-8 assay was used to detect the effect of JQ1 on the proliferation of Raji after being treated by JQ1 from the concentration of 0 to 1600nM for 72 h. JQ1 could dose-dependently inhibit the proliferation of Raji (Figure 3A). The cells were Annexin V-FITC/PI double stained and the proportion of apoptosis was tested by flow cytometry. The percentage of cells in Q2 and Q4

quadrants, which represented early apoptosis and late apoptosis respectively, was obviously increased after being treated by JQ1 (Figure 3C). JQ1 could dose-dependently induce the apoptosis of Raji. The cell cycle distribution was also detected by flow cytometry, and the percentage of cells in G0/G1 phase was dose-dependently increased after being treated by JQ1 in Raji (Figure 3B), so these Raji could be arrested in G0/G1 phase.

JQ1 and anti-CD47 antibody combination therapy could repress the progression of Burkitt lymphoma

To evaluate the effect of JQ1 and anti-CD47 antibody combination treatment, we conducted the regimen in Raji orthotopic Burkitt lymphoma model (Figure 3D). Both JQ1 and anti-CD47 antibody significantly delayed the tumor growth as compared to the control group while JQ1 had a better effect (Figure 3E). Importantly, an enhanced tumor repression was observed in the combination therapy group, indicating anti-CD47 antibody improved the therapeutic efficacy of JQ1. However, no regimen could block the increase of tumor volume, demanding further improvement of the combination therapy regimen. No body weight loss more than 20% was observed in any group (Figure 3G). No adverse event was found in liver function, renal function or histomorphology of important organs (Figure 3H).
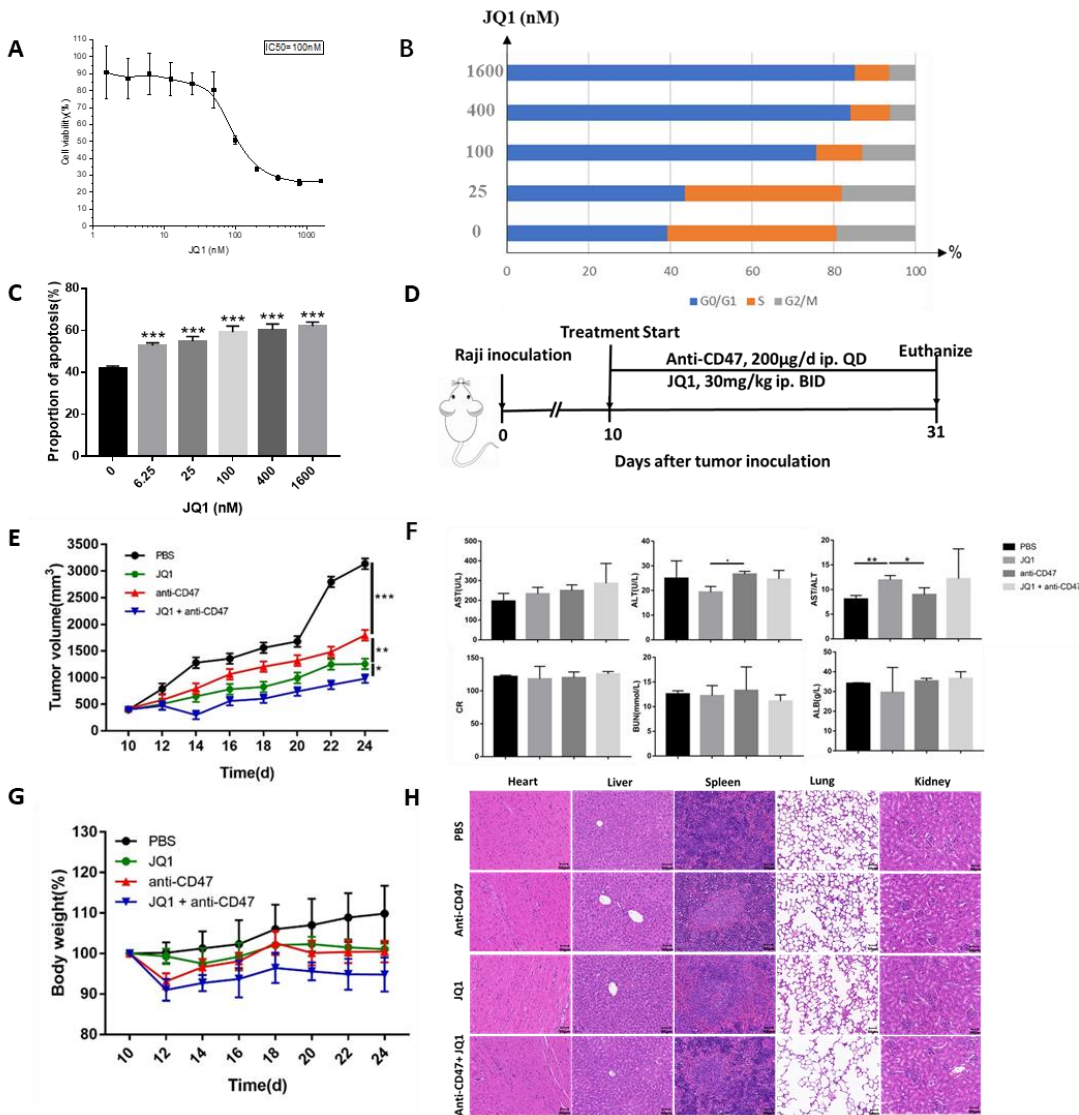
Figure 3. Combined treatment with JQ1 and anti-CD47 antibody had better antitumor efficacy than monotherapy in a groin orthotopically implanted Raji tumor model with no obvious side effects. (A) Dose–response curve of Raji treated with JQ1 for 72 h. (B) The cell cycle distribution of Raji being treated by increased concentration of JQ1. (C) The change on proportion of apoptosis as Raji being treated by increased concentration of JQ1 (all compared with control group). (D) Treatment scheme. (E) Tumor volumes and (G) body weight change rates of Raji tumor-bearing mice after being administrated with PBS, JQ1 30 mg/kg twice a day, anti-CD47 antibody 200ug/d, or JQ1 30 mg/kg twice a day + anti-CD47 antibody 200ug/d. Average and individual Raji tumor growth curves are illustrated. (F) The effect of different therapeutic regimens on the liver function and renal function. (H) The effect of different therapeutic regimens on the morphology of different organs. Values were shown as the mean ± SEM (n= 3) (*, p < 0.05; **, p < 0.01).

Discussion

Macrophage was a promising target to regulate TME and the enhancement of its function become the hotspot. The precise

function of macrophages in Burkitt lymphoma is still unclear, but M2 was found to be prominent in TME and may have potential

oncogenic effect, which makes macrophages an attractive target [1,13].

JQ1 is the first drug that has been identified to inhibit the expression of C-MYC gene, an important oncogene, and had been thought to be a breakthrough in the field of tumor therapy. However, studies have indicated frequent resistance to JQ1 appeared in numerous tumor cells, so the exploration of resistance mechanism and combination therapy regimen have become the new hotspot [7-11]. The combination therapy focusing on the function of macrophages is few.

In our study, C-MYC inhibitor JQ1 could decrease the expression of CD47 on the surface of BL cell, thus synergize with anti-CD47 antibody to enhance the phagocytosis of macrophages. JQ1 could also induce the polarization of macrophages from M2 to M1, so the anti-tumor function of macrophages was amplified to be better. The direct killing effect of JQ1 to BL cells was identified, too. Therefore, the anti-tumor effect of JQ1 and anti-CD47 antibody combination therapy combine immunoenhancement with direct killing. Finally, we identified JQ1 and anti-CD47 antibody combination therapy could repress the growth of human Burkitt lymphoma in NOD/SCID mice with no obvious side effects. Because of the difference of species, we could only establish human lymphoma model in immune deficient mice. We used anti-human CD47 antibody to treat human Burkitt lymphoma in NOD/SCID mice, so anti-human CD47 antibody enhanced the phagocytosis of murine macrophages. On one hand, the binding affinity of human CD47 to murine macrophages was almost tenfold stronger than syngeneic human CD47-SIRPα interaction, so the therapeutic efficacy of anti-CD47 antibody here may be enhanced compared to that in lymphoma patients [14]. On the other hand, the B cells, T cells and NK cells in NOD/SCID mice were deficient, so the effect of anti-CD47 antibody was underestimated since the deficiency of ADCC and FcR-dependent cytotoxicity. Maybe the therapeutic efficiency could be more accurate when the human anti-CD47 antibody was detected in mice with established human immune system, which demanded to be proved in the future. Undoubtedly, the most precise outcome can only be got through clinical trials.

In this new targeted immunotherapy regimen, the detailed association between direct killing and immunoenhancement is unclear, and the precise mechanism of polarization regulation of JQ1 demands more exploration. TAM receptors (Tyro3, Axl and MerTK) family is a classified family, which play essential roles in macrophage polarization and phagocytosis. When tumor cells

are induced to apoptosis, phosphatidylserine (PS) is exposed at cell surface. In the presence of Ca^2+ ions, Gas6 and Protein S can bind TAM receptor and bridge the receptor to PS. Following the tethering, cytoskeletal rearrangements induce phagocytosis. Then nuclear receptors are engaged in response to an increase in metabolic demand from the ingested cellular components, which is associated with transcription of genes inducing M2 polarization [15]. In Burkitt lymphoma, MerTK was identified only expressed in macrophages, not lymphoma cells. And the phagocytosis of apoptotic cells by macrophages was MerTK-dependent. Most significantly, lymphoma growth was markedly inhibited in $Mertk^{-/-}$ mice, indicating the oncogenic effect of MerTK-dependent apoptotic-cell clearance [13]. Maybe the oncogenic effect is associated with M2 polarization following phagocytosis, which demands further study. PI3K/Akt pathway is one of the most well-studied TAM receptors signaling pathways in macrophages, which has been proved to be closely associated with macrophage polarization, while Akt1 induce M2 polarization and Akt2 induce M1 polarization [16,17]. In the latest studies, JQ1 was proved to inhibit the expression of PI3K [18]. In our study, JQ1 could induce the apoptosis of BL cells and enhance the phagocytosis of macrophages, which may be bridged by TAM receptors activation. JQ1 could also induce the polarization from M2 to M1, which may be associated with the inhibition of PI3K/Akt pathway. Considering the important role of TAM/PI3K/Akt signaling pathway in the phagocytosis and polarization of macrophages, this pathway may be the key to target in macrophages in the TME of BL, which demands further exploration.

Conclusions

Macrophages are promising target to regulate tumor microenvironment especially in Burkitt lymphoma where M2 is prominent. We proposed a novel targeted immunotherapy regimen, JQ1 and anti-CD47 combination therapy, which has been proved to not only enhance the phagocytosis of macrophages but also induce the polarization from M2 to M1. The anti-lymphoma effect and safety of the regimen was also identified in Burkitt lymphoma established in NOD/SCID mice, laying foundation for the use of this regimen in clinical practice. The precise mechanism of JQ1 influencing the function of macrophages is still unclear and TAM/PI3K/Akt signaling pathway may be the key. More effort is demanded to explore the extent to which macrophage

influencing the development of lymphoma and the exact mechanism, which may provide direction for the exploration of novel agents targeted in macrophages.

Acknowledgements

Not applicable.

Abbreviations

BL: Burkitt lymphoma; TME: tumor microenvironment; CLSM: confocal laser scanning microscopy; ATCC: American Type Culture Collection; PS: phosphatidylserine.

Authors' contributions

Ou Bai, wantong Song designed the study. Shunan Wang, Wei Guo, Yangzhi Zhao, Zhihe Liu performed the experiment and wrote the manuscript. Jia Li, Xin Wan analyzed the data and performed figures and tables. All authors read and approved the final manuscript.

Fundings

Availability of data and materials

The data in this study is available from the corresponding author on request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Hospital of Jilin University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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