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Effect of CD20 signaling pathway on lymphoma cell proliferation, invasion and related protein IDO/AHR expression

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Abstract

CD20 is a transmembrane receptor, highly expressed in more than 90% of lymphocytes. Our research aims to probe CD20 signaling pathway in the proliferation, invasion and expression of related proteins IDO/AHR. OCI-Ly3 cells were with CD20 or inhibitor rituximab, and apoptosis, cell cycle, proliferation index (PI) were detected by flow cytometry. Cell invasion and migration detection using Transwell. Western blot detection of IDO/AHR levels. Comparing to blank control group, the percentage of S in the cell cycle of the cells treated with CD20 decreased significantly, and the PI increased significantly. With the extension of the culture time, the S ratio and PI further increased significantly, the cell motility and infiltration ability were significantly enhanced, and the IDO/AHR protein level increased. Inhibition of CD20 signaling pathway (rituximab) can hamper the cells, reduce cell invasion or migration, and the protein expression of IDO/AHR in cells is significantly reduced. Correlation analysis showed that the expression of CD20 and IDO/AHR showed a negative correlation. CD20 take part in the occurrence and development of lymphoma. Its expression mainly regulates the occurrence and development of lymphoma by affecting the proliferation and invasion of lymphoma cells. Our results show that CD20 can mediate IDO/AHR and affect lymphoma. Mechanism research.

Keywords: CD20; lymphoma; IDO/AHR.

Practical Application: Our study demonstrates that CD20 signaling participates in the regulation of lymphoma cell proliferation and invasion. However, whether CD20 signaling also exerts similar roles in patients with lymphoma remains to be further investigated.

1 Introduction

DLBCL is a more studied lymphoma, accounting for 30%-40% of non-Hodgkin's lymphomas and 37% of B-cell lymphomas (Bettelli et al., 2021; Opinto et al., 2020; Xu-Monette et al., 2020). The treatment of DLBCL contains with R-chop (Rituximab (R), cyclophosphamide (C), doxorubicin (H), vincristine (O), prednisone (P)) chemoimmunotherapy regimen And/or radiotherapy of the involved part. In recent years, several studies have demonstrated the anti-cancer properties of Cheddar cheese (Rafiq et al., 2020) or synbiotic sheep milk ice cream (Balthazar et al., 2021), indicating that they might be a novel agent for the treatment of cancers. The success of the new treatment regimen has greatly extended the survival time of DLBCL patients. However, rituximab included in the R-CHOP regimen has completely changed the management of DLBCL since it was approved. Part of conventional induction and maintenance therapy, however, the CD20 monoclonal antibody rituximab is not effective in a small number of patients. It is particularly important to find suitable biomarkers (Bojarczuk et al., 2019; Khan et al., 2022). Although the treatment of the disease has improved in recent years, the 5-year survival rate in Europe was 42% from 1997 to 1999 and 55% from 2006 to 2008 (Ciavarella et al., 2019). Many patients still do not respond or respond poorly to treatment, and their prognosis is poor. Some cancers show persistent proliferation signals and/or are not sensitive to growth inhibitory factors, so some patients have limited treatment effectiveness. Similarly, tumors can escape the killing of immune cells through immune escape, thereby aggravating tumors, including focal invasion and distant metastasis, these factors seriously affect mortality (Han et al., 2019; Nowakowski & Czuczman, 2015).

IDO exerts an immunoregulation effect (Chen et al., 2021; Lee et al., 2017). IDO activity may take part in regulating the immune response of antigen-presenting cells, as an effective measure to help escape the immune system. Several types of cancer use tryptophan decomposing enzyme, TDO. Inhibition of CD8+ T cell aromatic hydrocarbon receptor (AHR) take part in tumor occurrence, progression, invasion and metastasis (Campesato et al., 2020). Additionally, AHR is predicted to have an effect in the cancer microenvironment. In many cancers, increased IDO1 and TDO activities and kynurenine levels are correlated to tumor grade and poor prognosis. Therefore, the activity of enzymes has a significant correlation with cell growth, but data on their prognostic effects on hematological tumors are lacking. Studies have shown that IDO is highly expressed in lung cancer tissues, and its expression is related to lymph node metastasis or survival rate (Labadie et al., 2019). IDO is expressed in 59 cases of laryngeal carcinoma and 58 cases of hypopharyngeal carcinoma, and the expression is closely related

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to tumor stage. AHR originates from mesenchymal tissue and is a cytoskeletal component (Hoshi et al., 2020). Its abnormally elevated expression can cause the destruction of cytoskeletal proteins, which are more effective for exercise. Kaplan-Meier survival analysis showed that AHR has important prognostic potential. The positive expression of AHR predicts higher disease-free survival (DFS) and overall survival (OS) in LC patients. It is suggested that the positive expression of AHR indicates the poor prognosis of patients with recurrence and overall survival (Cheong & Sun, 2018; Liu & Zhai, 2021).

CD20 antigen is a 35kd transmembrane highly hydrophobic glycosylated phosphoprotein. Its structure is encoded by the MS4A1 gene. CD20 has a specific role in the differentiation and growth of healthy mature B cells at the pro-B stage and in chronic lymphocytic leukemia. It regulates the gradual regulation of B cells from the resting state (G0) G1 through cell activation, and the gradual regulation of the cell cycle from S phase to mitosis. It is part of the cell surface complex that regulates calcium ion transport (Holmgaard et al., 2013; Santos & Lima, 2017). Our research aims to explore the role of CD20 in DLBCL and provide help for clinical practice decision-making.

2 Materials and methods

2.1 Cells and reagents

OCI-Ly3 cells (Institute of Medicine, Academy of Chinese Medical Sciences), CD20 (Suomeng Biology, China), Transwell chamber (BD, USA), Fetal Bovine Serum (FBS), RPMI-1640 Medium (Gibco, USA), Rabbit Anti-Human IDO, AHR, β-actin antibody and hrp-labeled IgG (China Zhongshan Golden Bridge)

2.2 Equipment

Incubator (hot), CO2 incubator (Sanyo, Japan); inverted microscope (Nikon, Japan), cold high-speed centrifuge (Beckman, USA), -80 °C refrigerator (Sanyo, Japan).

2.3 Cell culture OCI-Ly3 cell line

DMEM: 10% fetal bovine serum, 5% CO2, humidity, 37 °C. After digestion with 0.25% trypsin, the cells were seeded in a 24-well plate. Then replace the medium with 10% fetal bovine serum. Cells are cultured to 70%-80% confluence. Change 1% fetal bovine serum and incubate continuously for 24 h.

2.4 Cell transfection and grouping

Dilute Lip 2000 and CD20 with Opti-MEM and incubate at room temperature for 5 minutes, then mix Lip 2000 with them. After standing, gently mix and continue culturing for 72 hours to collect cells.

CD20 group: log-growth OCI-Ly3 cells were cultured until they are fully attached. Add CD20 10 ng/mL and act for 6 h, 12 h, 24 h respectively. Rituximab group: Store OCI-Ly3 cells in the log growth phase in the CD20 group until they are fully attached. Add 20 μmol/L rituximab at the same time point and incubate for 6 h, 12 h or 24 h. Blank control group: Normally cultured OCI-Ly3 cells and CD20 group OCI-Ly3 cells were continuously incubated in RPMI-1640 medium for 6 h, 12 h, 24 h.

2.5 Flow cytometry analysis

Cell cycle, proliferation index and apoptosis rate of cell line OCI-Ly3. After all cells have grown completely, the cells are digested, and the supernatant is removed by centrifugation. The cells were collected at a concentration of 1X109/L, resuspended and fixed in 75% cold ethanol. Mix 1 mL of cell suspension with PI fluorescent dye and incubate in the dark for 30 min. The cell proliferation index (PI) and apoptosis rate were detected by flow cytometry.

2.6 Transwell method to detect cell migration and invasion

OCI-Ly3 invasion test: Dilute the matrix gel and mix it with serum-free medium, spread it in the room, and use it the next day. Add cells (1×106/mL) to each well, containing 200 μL of 0.2% BSA. The lower chamber is filled with 1300 μL culture medium.

2.7 Western blot

Take the cell homogenate and collect the supernatant protein with cold RIPA lysis buffer (50 μL) after centrifugation. The protein was frozen at -80 °C, put into buffer and mixed with cell protein, boiled and separated by electrophoresis, transferred to PVDF membrane, and blocked overnight at 4 °C. Add anti-E-cadherin, Vimentin or β-actin primary antibody (1:100), incubate for 2 h, wash 4 times. Add antibody (1:100), incubate for 1h, and rinse 3 times. The film is then washed and exposed for image analysis.

2.8 RT-PCR

Take the cells after successful transfection, extract DNA, add appropriate amount of agarose in TAE solution, heat, add nucleic acid, mix well, place in electrophoresis tank, and add miR-199 and β-actin primers. 95 °C 5min, 94 °C 30s, 40 cycles, 72° C 10min, 35cycles. The primer sequences are shown in Table 1.

2.9 Data processing

SPSS17.0 software was used for data processing, expressed as mean standard deviation (SD), and evaluated by analysis of variance. $p < 0.05$ indicates a difference.

3 Results

3.1 Comparison of cell cycle, proliferation index and apoptosis index

Compare each group OCI-Ly3 cell cycle, PI and apoptosis rate. The cells proliferated most vigorously after treatment in the CD20 group, as shown in Figures 1A and 1B. The results showed that comparing to the blank control group, the CD20 group significantly increased the S-phase percentage and PI at 6 h, 12 h and 24 h ($p < 0.05$). The rituximab group was evidently lower than PI ($p < 0.05$). With the extension of the treatment time, the S-comparison and PI values of the CD20 group gradually

Table 1. Primer sequence.

Primer		Primer sequence	length(bp)
CD20	F-	5'-GATCGAAGCTAGCAGAGAGTACTTTAG-3'	25
	R-	5'-GAGCTAGAGCTCGCAGAGAGTATATAT-3'	
β-actin	F-	5'-AAGAAGCACATCAGAGAGTGTTCTTGG-3'	22
	R-	5'-GATGAATCGTCTTGGGGCGAAATAAAC-3'	

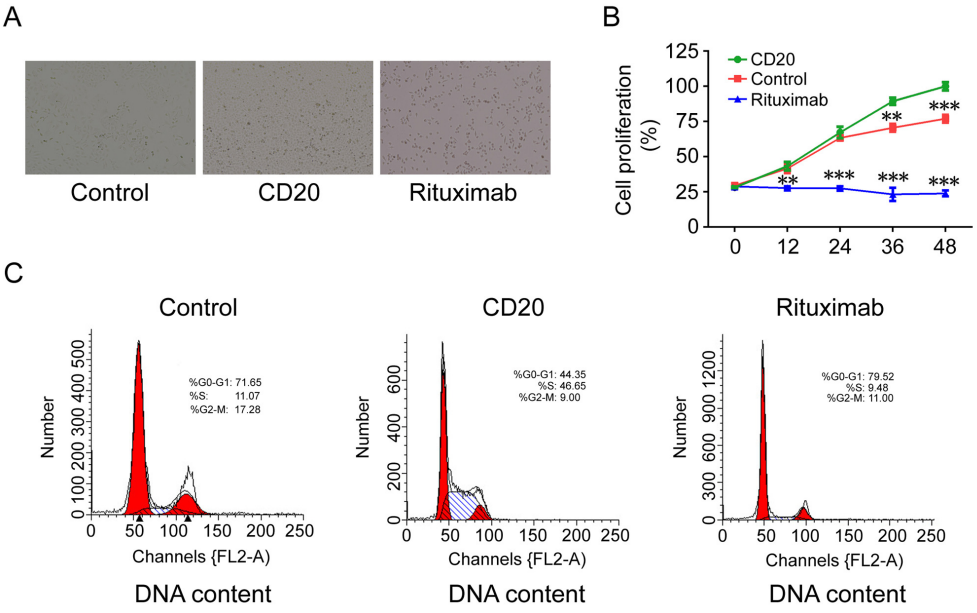

Figure 1. Comparison of cell cycle, proliferation index, and apoptosis index. (A) The cell proliferation diagram (B) MTT detection of cell proliferation and expression (C) Cell cycle and apoptosis. (The magnification is 1:100, ***p < 0.001 vs. the control group).

increased, while the rituximab S-comparison and PI values decreased ($p < 0.05$, Figure 1C).

3.2 Invasion and migration of 2 cells

Our research results show that comparing to the blank control group, the invasion and migration capabilities of the CD20 group are evidently enhanced. In the rituximab group, the ability of invasion (Figure 2A) or migration (Figure 2B) was sobviously reduced ($p < 0.05$, Figure 2).

3.3 IDO/AHR level

Our research results show that, comparing to the blank control group, IDO and AHR protein in the CD20 group was significantly increased, while the IDO and AHR protein in the rituximab group were obviously reduced ($p < 0.05$) (Figure 3). It shows that CD20 mediates the expression of IDO and AHR.

3.4 CD20 can negatively regulate IDO and AHR

After interfering with CD20 by si-RNA interference technology, RT-PCR detected IDO and AHR, and found that the expression of IDO and AHR decreased significantly. As shown in Figure 4A, the correlation analysis of the three shows that the expression of IDO and CD20 is present. Negative correlation, similarly, the expression of AHR and CD20 shows a negative correlation, as shown in Figure 4B and 4C.

3.5 Discussion

CD20 is a tyrosine kinase receptor composed of an extracellular domain, a transmembrane domain and an intracellular membrane. Abnormal CD20 (gene mutation, rearrangement or overexpression) is related to abnormal expression in the body. Once CD20 is over-activated in the body, it can activate multiple downstream genes through signal transduction and induce abnormal gene expression, thereby promoting malignant cell proliferation and inhibiting cell apoptosis. Accelerate the invasion/migration of malignant tumors and promote angiogenesis (Krem & Gopal, 2015; Wu et al., 2013). Invasion and migration are the basic differences between malignant tumor cells and normal tumor cells. They are the unique biological characteristics of malignant tumor cells. They are the pathological basis for tumor recurrence, metastasis, gradual deterioration and death. Our results show that the CD20 agonist and antagonist rituximab were used to analyze the effect of CD20 on OCI-Ly3 cells. The percentage of S phase and PI in the CD20 group elavated evidently. The percentage of S phase and PI in the rituximab group were significantly reduced at all time points. With the extension of the treatment time, the S phase percentage and PI value of CD20 cells gradually increased, while the S phase percentage and PI value of the rituximab group

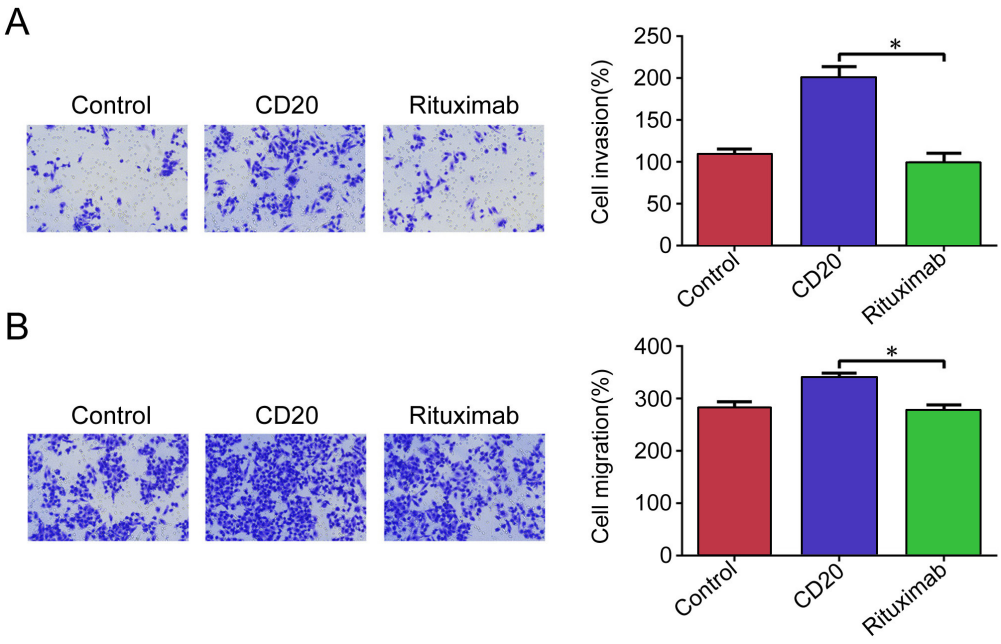

Figure 2. Cell invasion and migration. (A) The cell proliferation diagram (B) The MTT detection of cell proliferation and expression (C) Cell cycle and apoptosis. (The magnification is 1:400, ***p < 0.001 vs. control group).

decreased. It is suggested that CD20 can promote the protein synthesis and mitosis of OCI-Ly3 cells, mainly by promoting the G1/S transitional proliferation of cells, and continuously activate NF-κB and cyclin D1 through the PI3K/Akt/IκBα pathway. On the other hand, the CD20 antagonist rituximab group can inhibit the protein synthesis of OCI-Ly3 cells and inhibit cell division and proliferation (Leleu et al., 2008; Wallace & Reagan, 2021). The invasion and migration ability of OCI-Ly3 cells in the rituximab group was lower than that of the blank control group. These data suggest that CD20 can enhance the invasion and migration of OCI-Ly3 cells, while the CD20 antagonist rituximab can inhibit the invasion and migration of OCI-Ly3 cells. Previous studies have shown that CD20 can affect the occurrence and development of prostate cancer by regulating downstream signal transduction pathways. Blocking the CD20 gene can inhibit related functions. Literature has shown that CD20 can affect the activity of colorectal cancer cells, and its main mechanism is to cause the up-regulation of protein, and enhance the ability of cell invasion. This effect can be reversed by reducing the expression of CD20 (Tuijnenburg et al., 2020; Zhou et al., 2014), which is consistent with our results.

EMT is widely distributed in a variety of pathophysiological processes, and mainly promotes the focal infiltration and distant metastasis of malignant tumors. Its main feature is loss of epithelial polarity, which is characteristic of mesenchymal cells. Previous studies have predicted the change of epithelial cell phenotype and the acquisition of fibroblast-like phenotype are early signs of malignant tumor invasion and metastasis (Lyu et al., 2021). The complete EMT process includes three stages: firstly, the degree of cell malignancy is closely related to the maturity of fibroblasts; secondly, epithelial cell markers are down-regulated through signal transduction pathways, while mesenchymal

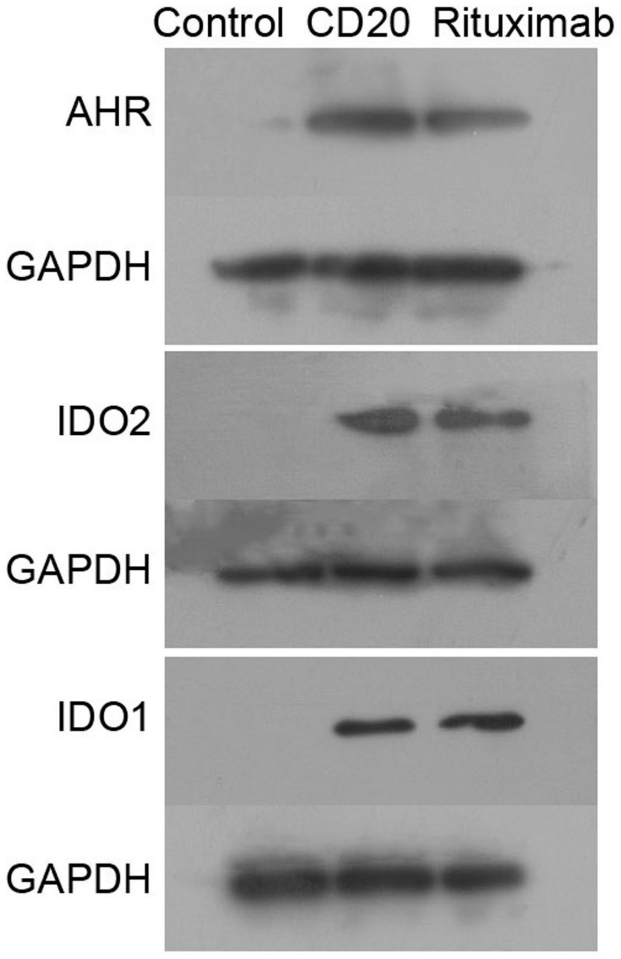

Figure 3. The level of IDO and AHR.

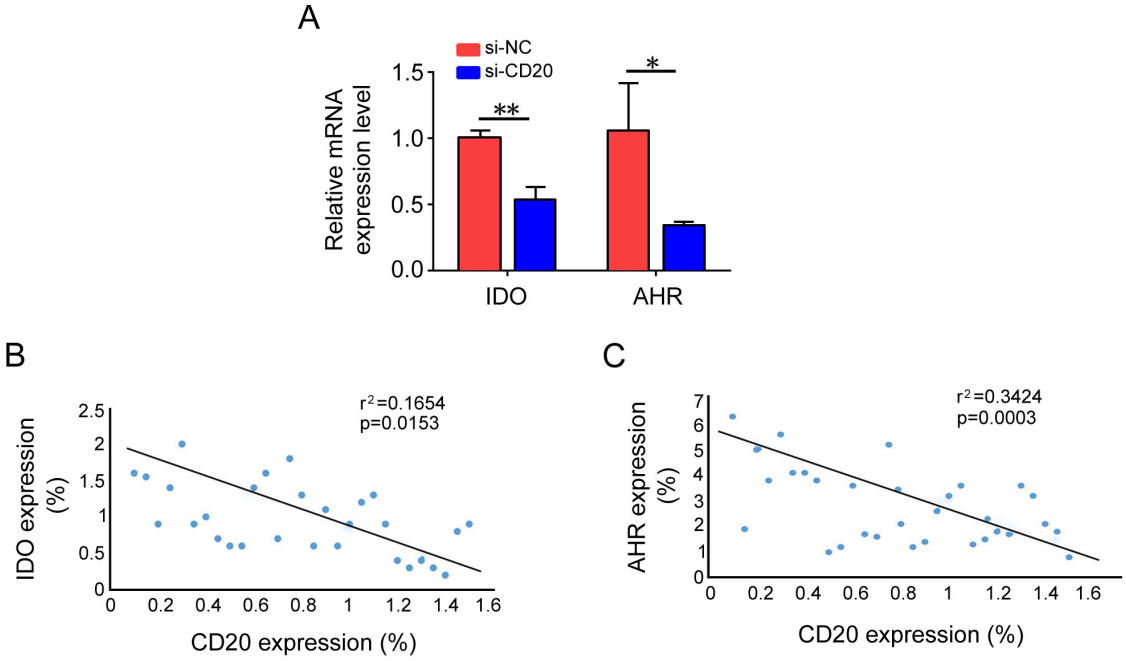

Figure 4. CD20 can negatively regulate the expression of IDO and AHR. Figure A shows the RT-PCR detection of mRNA expression, and Figures B and C show the correlation analysis. (*p < 0.05 vs. the control group).

markers are up-regulated; finally, the extracellular matrix Gradually degradation allows cells to migrate to focal tissues and even distant metastasis (Chen et al., 2008). Studies have shown that EMT activates the mechanism of cell survival, including immune escape and epigenetic reprogramming. Therefore, when combined with CD20-targeted drugs, therapeutic inhibition of these pathways will slash cancer (Li et al., 2018). There are also some interesting studies. The decrease in the number of cells expressing E-cadherin (E-cadherin loss) is replaced by the cells expressing nuclear N-cadherin. The above results are similar to previous results, and nuclear N-cadherin is associated with poor prognosis (Ouled Dhaou et al., 2020; Tooulou et al., 2015). Similarly, we have only conducted a detailed analysis of the CD20 signaling pathway's effects on lymphoma cell proliferation, invasion and the expression of related proteins IDO/AHR, but the mechanism of action is still unknown. Therefore, we will focus on future work. this.

4 Conclusion

CD20 is an important regulatory factor in lymphoma. Its expression mainly regulates the occurrence and development of lymphoma by affecting the proliferation and invasion of lymphoma cells. Further studies have shown that CD20 can regulate IDO/AHR and mediate the study of lymphoma.

Conflict of interest

None.

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