

**MINISTRY OF EDUCATION
AND TRAINING**

**VIETNAM ACADEMY OF SCIENCE
AND TECHNOLOGY**

**GRADUATE UNIVERSITY OF SCIENCE AND
TECHNOLOGY**



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**Study on the Regulatory Role of Certain Deubiquitinase Genes in
Dendritic Cell and Progenitor Cell Function in Patients with
Acute Leukemia**

SUMMARY OF DISSERTATION IN BIOTECHNOLOGY

Major: Biotechnology

Code: 9420201

Ha Noi - 2026

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy of Science and Technology.

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INTRODUCTION

Acute leukemia is a type of blood cancer characterized by the rapid proliferation and accumulation of immature white blood cells in the blood and bone marrow. This abnormal growth impairs normal hematopoiesis, leading to anemia, thrombocytopenia, and immune suppression, thereby increasing the risk of infection and hemorrhage. The disease comprises two main subtypes: acute lymphoblastic leukemia and acute myeloid leukemia. Although treatments such as chemotherapy, hematopoietic stem cell transplantation, and immunotherapy have improved prognoses, issues such as relapse, drug resistance, and immune dysregulation remain significant therapeutic challenges.

Dendritic cells are specialized antigen-presenting cells that regulate immune responses by processing and presenting antigens to T lymphocytes. However, in acute leukemia, malignant cells can impair dendritic cell function through the secretion of immunosuppressive cytokines, thereby fostering an environment conducive to cancer cell survival.

Various signaling pathways such as NF- κ B, JAK/STAT, MAPK, PI3K/AKT/mTOR, and Wnt/ β -catenin are closely linked to the proliferation, drug resistance, and immune evasion of leukemic cells. Notably, genes encoding deubiquitinases, such as *A20* and *CYLD*, function as negative regulators of inflammatory and immune signaling pathways. Nevertheless, the specific roles of these genes in modulating the immune function of dendritic cells and immature cells within the acute leukemia microenvironment have not yet been fully elucidated. For the reasons mentioned above, the thesis titled "Study on the regulatory role of certain deubiquitinase genes in the function of dendritic cells and immature cells in patients with acute leukemia" was conducted.

Research objectives

- To characterize the clinical and preclinical features, inflammation-related gene expression, and immune phenotypes in patients with acute leukemia;

- To determine the regulatory roles of specific genes encoding deubiquitinase enzymes (*A20*, *CYLD*) and associated intracellular signaling pathways such as NF- κ B, JAK/STAT, MAPKp38, and Wnt in the activity of dendritic cells and immature cells in patients with acute leukemia.

Research content

- To characterize the clinical and paraclinical features, expression of inflammation-related genes (genes encoding deubiquitinase enzymes and signaling genes), and immune phenotypes in patients with acute leukemia;
- To evaluate the associations among clinical and paraclinical features, expression of inflammation-related genes, and immune phenotypes in patients with acute leukemia;
- To determine the regulatory role of genes encoding deubiquitinase enzymes regarding the function of immature cells in patients with acute leukemia;
- To determine the regulatory role of signaling genes regarding dendritic cell activity in patients with acute leukemia upon exposure to the active compounds Malloapelta C and Doxorubicin.

Chapter 1. LITERATURE OVERVIEW

1.1. Acute leukemia

1.1.1. Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (BCLC) is a hematologic malignancy characterized by the uncontrolled proliferation of immature B or T lymphocytes in the bone marrow and lymphoid organs. BCLC is highly genetically heterogeneous, featuring numerous chromosomal abnormalities and gene rearrangements that directly influence pathogenesis, treatment response, and prognosis. According to NCCN guidelines, patients are stratified into risk groups intermediate and poor based on gene mutations and chromosomal profiles.

1.1.2. Acute myeloid leukemia

Acute myeloid leukemia (BCTC) is a malignancy characterized by the uncontrolled proliferation of myeloid blasts in the bone marrow and peripheral blood, resulting in the impairment of normal hematopoiesis. An BCTC diagnosis is established when the blast percentage is $\geq 20\%$. According to ELN and NCCN classifications, patients are stratified into favorable, intermediate, and adverse risk groups, a process that plays a crucial role in guiding treatment and determining prognosis.

1.2. Deubiquitinase gene

CYLD and *A20 (TNFAIP3)* are key deubiquitinases involved in the regulation of immune signaling, inflammation, and oncogenesis by controlling ubiquitination processes. *CYLD* belongs to the USP family and selectively hydrolyzes Lys63- and M1-linked polyubiquitin chains, thereby regulating signaling pathways such as NF- κ B, Wnt, JNK, and p38 MAPK through the deubiquitination of intermediate proteins like TRAF2, TRAF6, and TAK1. *A20* belongs to the OTU family and possesses a unique structure featuring an N-terminal deubiquitinase domain and a C-terminal E3 ubiquitin ligase domain; this allows it to simultaneously remove Lys63-linked ubiquitin chains and attach Lys48-linked chains to RIP1, resulting in RIP1 degradation and the inhibition of NF- κ B activation. Dysfunction of these deubiquitinases can lead to sustained NF- κ B activation, increased secretion of pro-inflammatory cytokines, and the promotion of malignant cell survival.

1.3. Dendritic cell

Dendritic cells (DC) are professional antigen-presenting cells that play a central role in bridging innate and adaptive immunity. DCs are widely distributed in antigen-contacting tissues such as skin, respiratory and digestive mucosa; After antigen capture, DCs migrate to lymphoid organs to present antigens through MHC I/II, activating and directing T cell differentiation and supporting B cell responses. In cancer, factors from the

tumor can inhibit DC maturation, promote Treg/Th17 and create an immunosuppressive environment, contributing to helping tumors evade immune responses.

1.4. Certain chemicals that regulate cellular activity

Doxorubicin is an anthracycline antibiotic widely used to treat various solid tumors and hematologic malignancies; its primary mechanisms involve DNA intercalation, topoisomerase II inhibition, and the generation of reactive oxygen species leading to DNA damage and apoptosis though it is also associated with cardiotoxicity and bone marrow suppression. Malloapelta C is a natural diterpenoid isolated from the *Mallotus* plant that exhibits potent anticancer activity with low IC₅₀ values across multiple cell lines, likely through the inhibition of NF- κ B and the induction of apoptosis. SB203580 is a selective p38 MAPK inhibitor frequently used as a research tool to elucidate the role of p38 MAPK in inflammation, cellular stress, and cancer progression. Everolimus, a rapamycin derivative, selectively inhibits mTORC1, thereby disrupting the cell cycle and suppressing proliferation and angiogenesis; it is widely employed in targeted therapies. Wnt-C59 is a potent inhibitor of the Wnt pathway acting via the inhibition of the Porcupine enzyme and demonstrates significant potential for suppressing tumor progression and modulating Wnt/ β -catenin signaling. Nifuroxazide is a nitrofurantoin derivative with intestinal antibacterial activity that has also been identified as a potent inhibitor of STAT3 signaling, blocking Tyr705 phosphorylation by inhibiting JAK2 and Tyk2.

Chapter 2. MATERIAL AND METHODS

2.1. Study subjects

The study was conducted on a group of subjects comprising 63 patients with acute myeloid leukemia, 57 patients with acute lymphoblastic leukemia, and 50 healthy individuals.

2.2. Methodologies

- *Sample collection*
- *Disease prognosis classification*
- *Isolation of peripheral blood mononuclear cells (PBMCs)*
- *Isolation and culture of dendritic cells from PBMCs*
- *RNA extraction, cDNA synthesis, and RT-qPCR*
- *Plasmid transformation*
- *Flow cytometry*
- *ELISA*
- *Cell migration*
- *Data analysis*

2.3. Experimental diagram

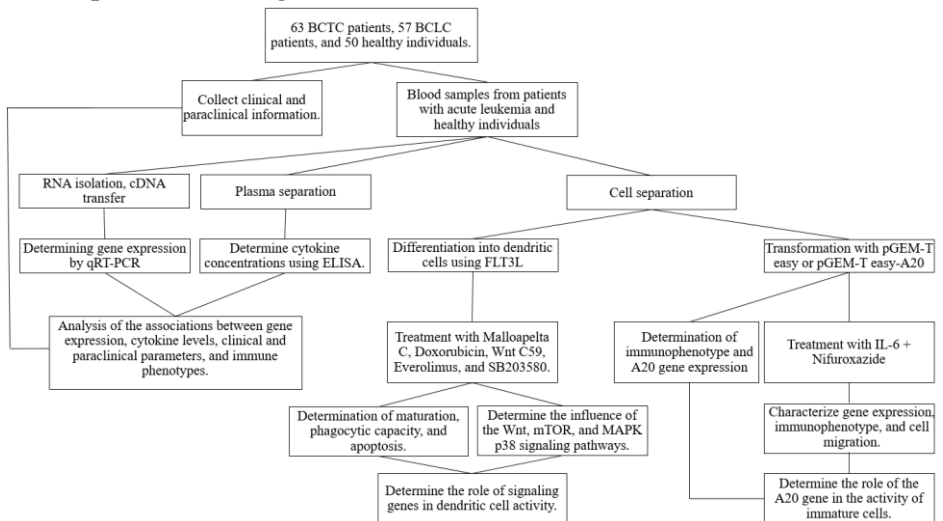


Figure 2.1. Research diagram

Chapter 3. RESULTS

3.1. Analysis of clinical and paraclinical characteristics in patients with acute leukemia.

3.1.1 Clinical and paraclinical characteristics of patients with BCTC

Table 3.1: Clinical and paraclinical characteristics of patients with BCTC.

Index	Normal range	Patient group of BCTC			
		Good prognosis (n=14)	Intermediate prognosis (n=34)	Poor prognosis (n=15)	p-value
Age		31.21 ± 20.80	50.53 ± 21.28	58.47 ± 15.62	0.004
Creatinine (µmol/L)	62- 120	61.00 (54.00 - 83.00)	88.50 (69.50 - 99.00)	81.00 (72.00 - 92.00)	0.048
Total Bilirubin (µmol/L)	≤17	7.90 (6.00 - 9.60)	11.10 (7.875 - 14.250)	9.60 (7.00 - 11.70)	0.041
Indirect Bilirubin (µmol/L)	≤12.7	6.10 (4.95 - 8.15)	8.75 (6.40 - 11.250)	7.20 (5.50 - 8.80)	0.021
Number of red blood cells with nuclei (G/L)	0	0 (0 - 0.01)	0.04 (0 - 0.20750)	0.020 (0 - 0.040)	0.006
White blood cell count (G/L)	3.5- 10.5	10.13 (4.52 - 24.12)	33.75 (6.58 - 81.38)	6.01 (4.66 - 19.03)	0.015
Number of lymphocytes (G/L)	1.2- 3	1.82 (1.15 - 5.305)	3.7 (2.07- 6.305)	1.74 (1.16 - 2.58)	0.016

Analysis of Table 3.1 shows that the mean age increased progressively from the good prognosis group to the poor prognosis group ($p = 0.004$). The favorable prognosis group exhibited significantly lower levels of creatinine, total bilirubin, and indirect bilirubin compared to the other

two groups ($p < 0.05$). Conversely, nucleated red blood cell counts were elevated in the intermediate and poor prognosis groups ($p = 0.006$), leukocyte counts differed significantly among the groups ($p = 0.015$), and lymphocyte counts were markedly lower in the poor prognosis group ($p = 0.016$).

3.1.2 Clinical and paraclinical characteristics of BCLC patients

Table 3.2: Clinical and paraclinical parameters of patients with BCLC.

Index	Normal ranger	Patient group of BCLC		p-value
		Intermediate -prognosis (n=36)	Poor prognosis (n=21)	
Age		28.78 $\pm$ 20.61	40.00 $\pm$ 18.04	0.044
Glucose (mmol/l)	3.9-6.4	6.4 (5.3-7.7)	5.3 (5-6.3)	0.044
LDH (U/L)	230-460	1078 (477-2718)	2230 (1538.25-4824)	0.013
White blood cell count (G/L)	3.5-10.5	10.01 (2.45-48.66)	65.58 (22.27-127.4)	0.003
Neutrophil count (G/L)	1 -7.5	1.84 (0.54-5.82)	4.39 (2.55-10.24)	0.004
Number of lymphocytes (G/L)	1.2-3	2.44 (1.31-7.14)	6.74 (3.24-14.5)	0.001

The results in Table 3.2 show that the poor-prognosis group had a significantly higher mean age ($p = 0.044$). The poor-prognosis group also exhibited lower glucose levels ($p = 0.044$) and markedly elevated LDH levels ($p = 0.013$). Notably, leukocyte, neutrophil, and lymphocyte counts were all sharply higher in the poor-prognosis group compared to the intermediate-prognosis group ($p < 0.01$).

3.2. Assessment of immunophenotype in patients with acute leukemia

3.2.1. Results of immunophenotypic analysis in patients with BCTC

Flow cytometry analysis (Figure 3.1A–B) revealed a significant increase in the proportions of activated $CD56^+CD40^+$ and $CD56^+CD44^+$ NK cells compared to the control group. Concurrently, $CD4^+CD25^+FoxP3^+$ regulatory T cells were markedly elevated in patients. Notably, the $CD13^+CD117^+$ myeloid progenitor population showed a substantial increase, whereas general T-cell activation markers remained unchanged.

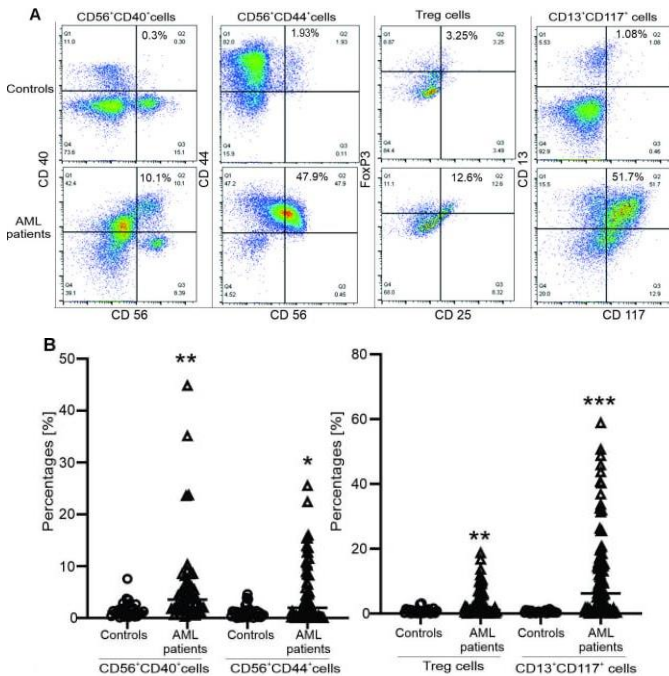


Figure 3.1: Immune profile of BCTC patients and healthy controls.

3.2.2 Results of immunophenotypic assessment in BCLC patients

Flow cytometry analysis (Figure 3.2) revealed a significant increase in the proportion of activated NK cells exhibiting the $CD56^+CD44^+$ phenotype in BCLC patients compared to the healthy control group. Concurrently, the Treg population identified by the

CD3⁺CD4⁺CD25⁺FoxP3⁺ phenotype—also showed a marked increase in the patient group. In contrast, markers reflecting the general activation status of T cells, including CD4⁺CD25⁺ and CD4⁺CD44⁺, did not differ significantly between the two groups.

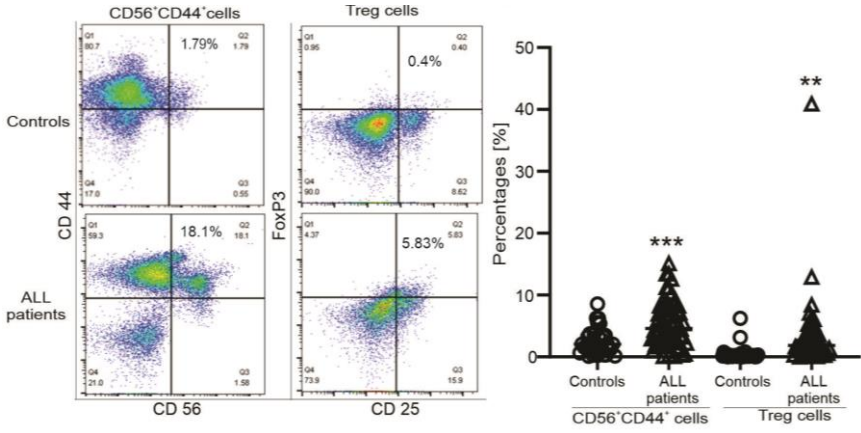


Figure 3.2: Cytokine concentrations and immune phenotypes in BCLC patients.

3.3. Association of *A20* and *CYLD* gene expression with immunological, clinical, and paraclinical characteristics in patients with acute leukemia.

3.3.1. Association between *A20* and *CYLD* gene expression and immune phenotype in BCTC patients.

The results indicated that *CYLD* gene expression did not affect the immunophenotypic characteristics of BCTC patients. The proportions of cells belonging to the lymphoid lineage—including total lymphocytes and CD3⁺, CD8⁺, CD10⁺, CD19⁺, CD20⁺, and CD79a⁺ cells—were significantly lower in the low-*A20* group compared to the high-*A20* group (Figure 3.3A). Conversely, myeloid lineage markers such as MPO⁺, CD33⁺, and CD13⁺CD117⁺ were significantly elevated in this group.

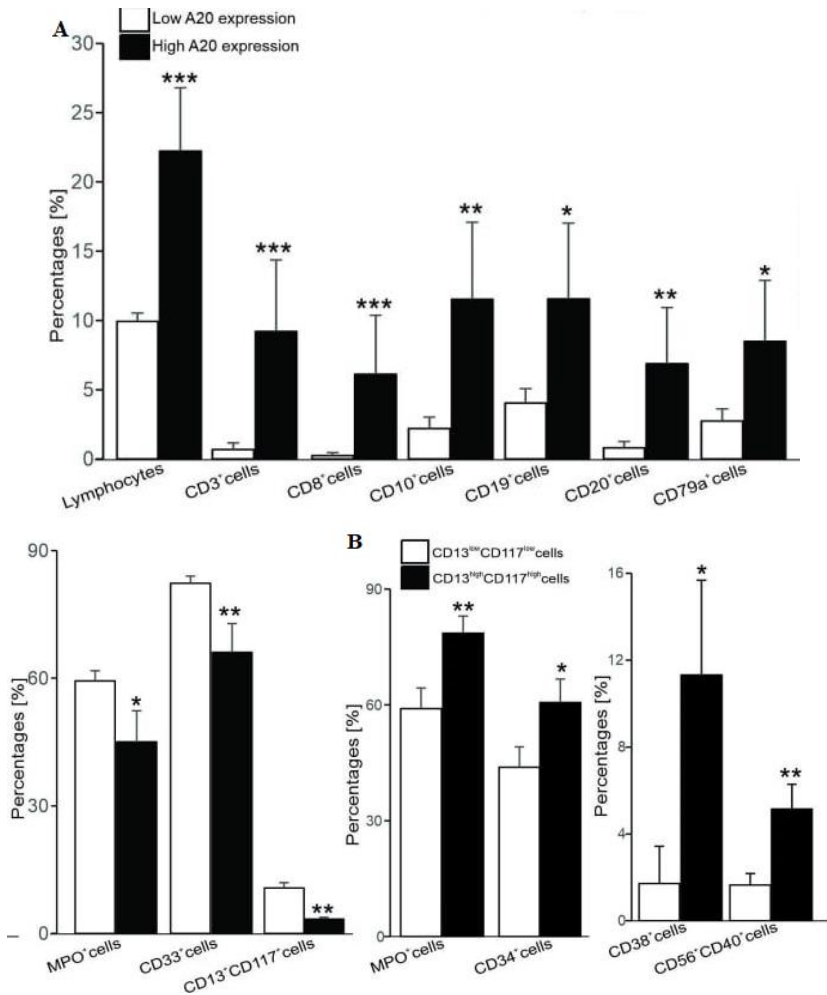


Figure 3.3: The relationship between A20 gene expression and the immune phenotype in BCTC patients.

3.3.2. Association of A20 and CYLD gene expression with clinical and paraclinical characteristics in patients with BCTC

Table 3.3: Correlation between A20 gene expression and clinical and paraclinical characteristics.

Index	A20 gene expression		<i>p</i> -value
	Low-expression group (n=47)	High-expression group (n=16)	
Albumin (g/L)	38,81 (26,6 - 51,1)	48,7 (32,9–64,6)	0,029
Platelet count	51 (31 -115.5)	28 (12.5 -61.5)	0,039

The results indicate that the expression level of the *CYLD* gene does not affect clinical or paraclinical parameters in BCTC patients. Meanwhile, the expression level of the A20 gene shows an association with albumin levels and platelet counts ($p<0.05$).

3.3.3. Association between A20 and *CYLD* gene expression and immune phenotype in BCLC patients.

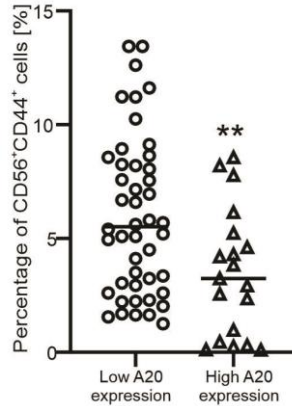


Figure 3.4: The chart shows the proportion of CD56+CD44+ cells in BCLC patients with low and high A20 expression.

Similar to the BCTC patient group, the expression level of the *CYLD* gene did not influence immunophenotypic characteristics in BCLC patients upon analysis. The results showed that patients with low A20 expression levels exhibited a significantly higher number of CD56+CD44+ cells compared to those with high A20 expression levels (Figure 3.4).

3.3.4. Association of A20 and CYLD gene expression with clinical and paraclinical characteristics in BCLC patients.

Table 3.4: Relationship between A20 and CYLD gene expression to clinical and paraclinical characteristics in BCLC patients

Index		Number of lymphocytes (G/L)	Ure (mmol/L)
Reference threshold		1-3	2.5 -7,5
Gene expression A20/ GAPDH	Low-expression group (n=39) Median (Q1-Q3)	3,33 (1,71 – 8,02)	5,3 (3,95 – 6,7)
	High-expression group (n=18) Median (Q1-Q3)	7 (2,03 – 10,29)	7,25 (6,15 – 11,93)
	<i>p</i> -value	0,39	0,012
Gene expression CYLD/ GAPDH	Low-expression group (n=48) Median (Q1-Q3)	3,93 (2,24 – 9,05)	5,4 (4,2 – 6,8)
	High-expression group (n=9) Median (Q1-Q3)	1,3 (0,63- 4,61)	8,7 (3,15 –17,2)
	<i>p</i> -value	0,032	0,36

Analysis of the relationship between A20 gene expression and clinical parameters revealed that patients with high A20 expression had higher urea levels compared to the low-expression group ($p = 0.012$). Regarding the CYLD gene, patients with high CYLD expression tended to have lower lymphocyte counts than those with low expression, a difference that was statistically significant ($p = 0.032$). Additionally, this group exhibited higher urea levels compared to the low-expression group ($p > 0.05$).

3.4. Evaluating the role of the A20 gene in regulating immature cell activity in acute leukemia.

3.4.1. The role of the A20 gene in regulating immune cell proliferation in acute leukemia.

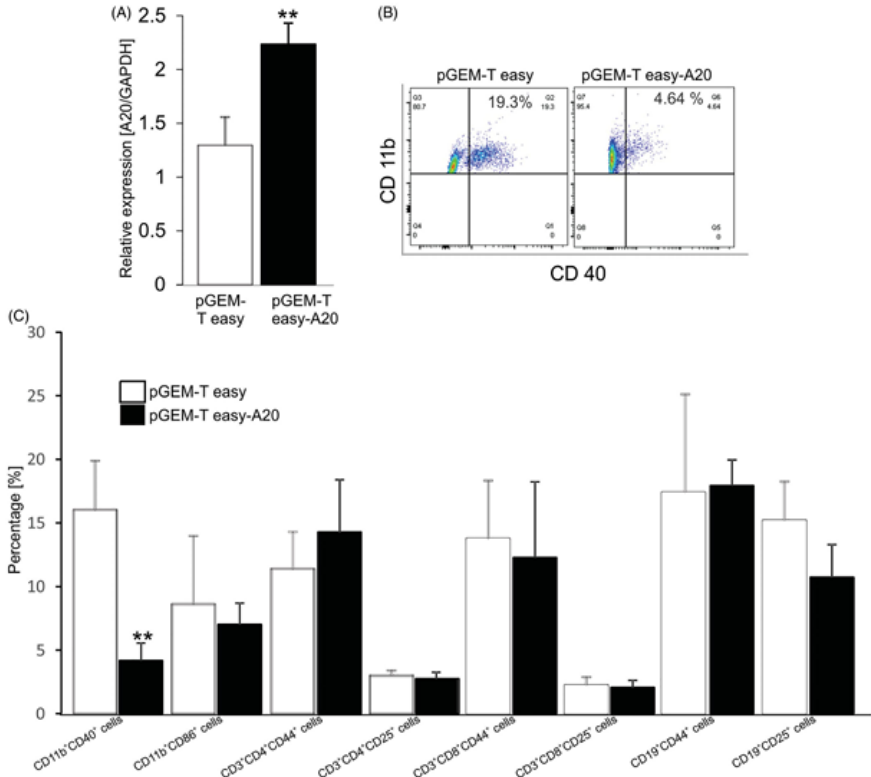


Figure 3.5: The effect of A20 on the immune phenotype in patients with acute leukemia

RT-qPCR results, as shown in Figure 3.5, confirm that pGEM-T easy-A20 significantly increases A20 expression. Flow cytometry analysis reveals that increased A20 expression markedly reduces the proportion of CD11b⁺CD40⁺ cells, while having no effect on other activated immune cell populations.

3.4.2. The role of the A20 gene in regulating STAT signaling and immature leukocyte migration.

The results showed that IL-6 reduced A20 gene expression and stimulated the activation of STAT1 and STAT3, but did not significantly affect STAT5 or STAT6 (Figure 3.6). The IL-6-induced increase in

STAT1/STAT3 activation was inhibited by the upregulation of A20, demonstrating that A20 plays a negative regulatory role in the STAT pathway within an inflammatory context. However, in cell migration assays, IL-6 enhanced the migratory capacity of immature cells stimulated by CXCL12, and alterations in A20 expression did not significantly affect this process.

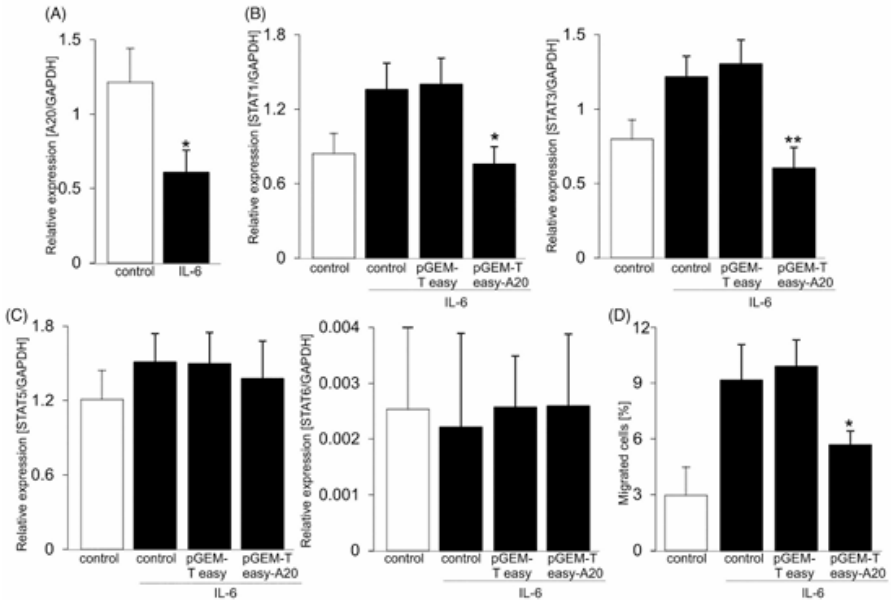


Figure 3.6: Effect of A20 on STAT signaling and immature leukocyte migration

3.4.3. The role of STAT signaling in the regulation of immature leukocyte migration.

The results shown in Figure 3.7 indicate that IL-6 markedly increases the proportion of CD11b⁺CD40⁺ cells while promoting the migratory capacity of myeloid and immature cells. However, these effects are significantly attenuated by the inhibition of STAT signaling using Nifuroxazide, demonstrating that IL-6-mediated effects are partially dependent on the STAT pathway. Notably, STAT inhibition does not alter

the impact of A20 expression levels on immature cell migration, suggesting that A20 does not directly regulate migration via the IL-6/STAT axis but primarily functions to control the immune activation state of myeloid cells.

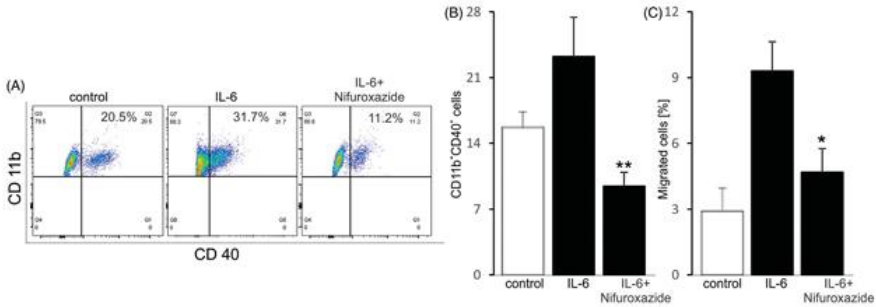


Figure 3.7: The role of STAT signaling in the regulation of leukocyte progenitor activation

3.5. The role of inflammation-related genes in regulating dendritic cells in acute leukemia patients under the influence of Doxorubicin and Malloapelta C.

RT-qPCR analysis revealed that Mal C markedly upregulated *mTOR* and *MAPK p38* mRNA expression in both groups (Figure 3.8), with a particularly strong effect observed in patients. In contrast, Dox primarily upregulated the expression of genes associated with the Wnt pathway, including Wnt1 and Wnt3. Wnt1 expression increased sharply in DCs from healthy individuals upon Dox treatment, whereas only a slight increase was noted in patients. Wnt3 expression increased in both groups following treatment with either Mal C or Dox. While both Mal C and Dox upregulated mTOR in healthy individuals, only Mal C elicited this effect in patients. No significant changes were observed in the expression of ERK2 and AKT1 following treatment.

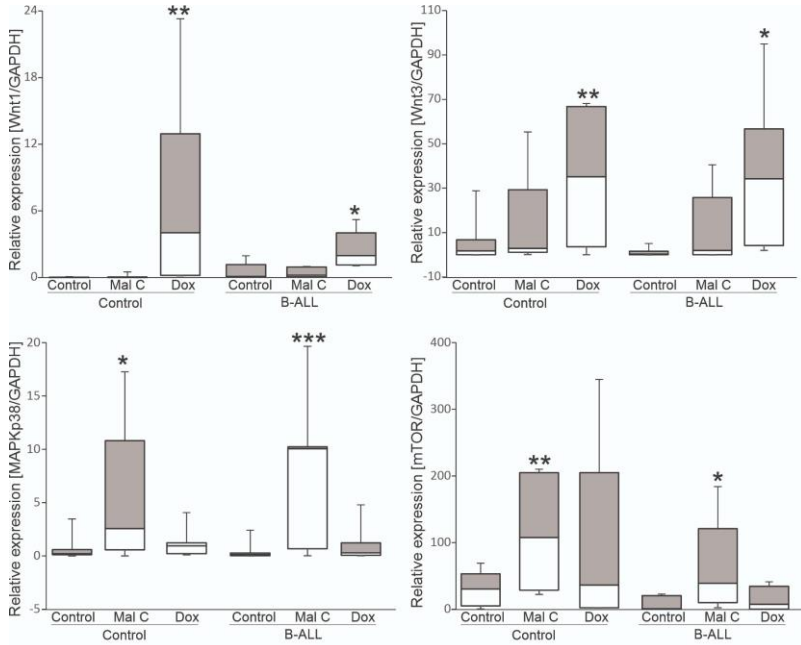


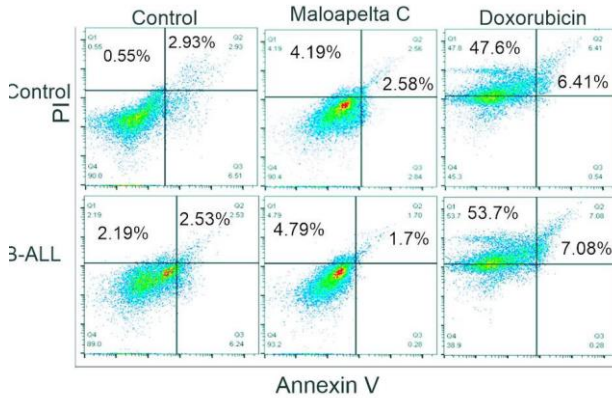
Figure 3.8: Effects of Dox and Mal C on the expression of mTOR, MAPKp38, and Wnt signaling molecules in healthy individuals and BCLC patients.

3.5.1. Effects of Doxorubicin and Malloapelta C on dendritic cell maturation, apoptosis, and phagocytosis in BCLC patients.

The results shown in Figure 3.9 indicate that both Mal C and Dox significantly affect DC maturation, phagocytic capacity, and apoptosis. In healthy individuals, Mal C (20 μ M) and Dox (5 μ M) significantly increased the CD40⁺CD86⁺ and CD11c⁺CD123⁺ DC populations as well as the Annexin V⁺/PI⁺ cell fraction; consequently, these concentrations were selected for subsequent experiments. Cell viability analysis revealed that Mal C caused only a slight increase in the necrosis rate (Annexin V-PI⁺) in both groups, whereas Dox exhibited strong cytotoxicity, markedly increasing both apoptosis and necrosis. These findings suggest that Mal C is

not a potent cytotoxic agent but primarily acts by modulating DC function, in contrast to the pronounced cytotoxic effects of Dox.

A



B

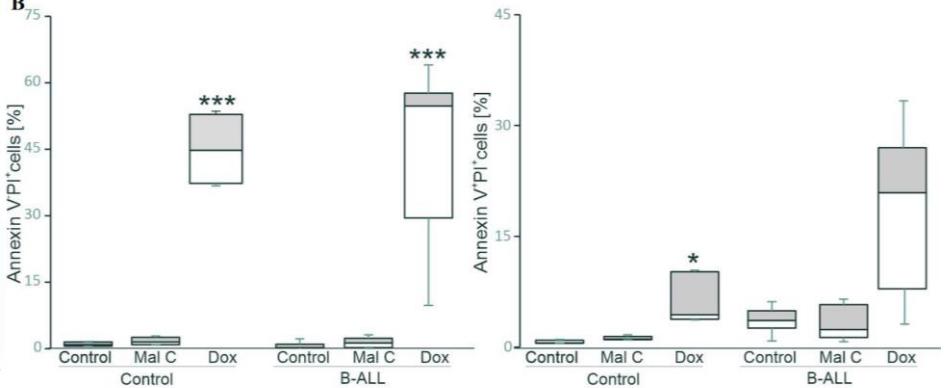


Figure 3.9: Effects of Dox and Mal C on DC cell function from healthy individuals and BCLC patients.

Next, the phagocytic capacity of DC populations ($CD11c^+CD123^-$, $CD11c^+CD123^+$, and $CD11c^-CD123^+$) from healthy individuals and BCC patients was evaluated following treatment with Mal C and Dox. The results shown in Figure 3.10 indicate that Mal C markedly enhanced the phagocytic capacity of $CD11c^+CD123^+$ DCs in both groups—particularly in

BCC patients, where the rate nearly doubled compared to the control (rising from 31.6% to 72.4%)—demonstrating that Mal C promotes DC differentiation and phagocytic function. In contrast, Dox caused a sharp reduction in the CD11c⁺CD123⁺ DC population in patients (dropping to 16.6%), reflecting cytotoxicity and the inhibition of DC formation.

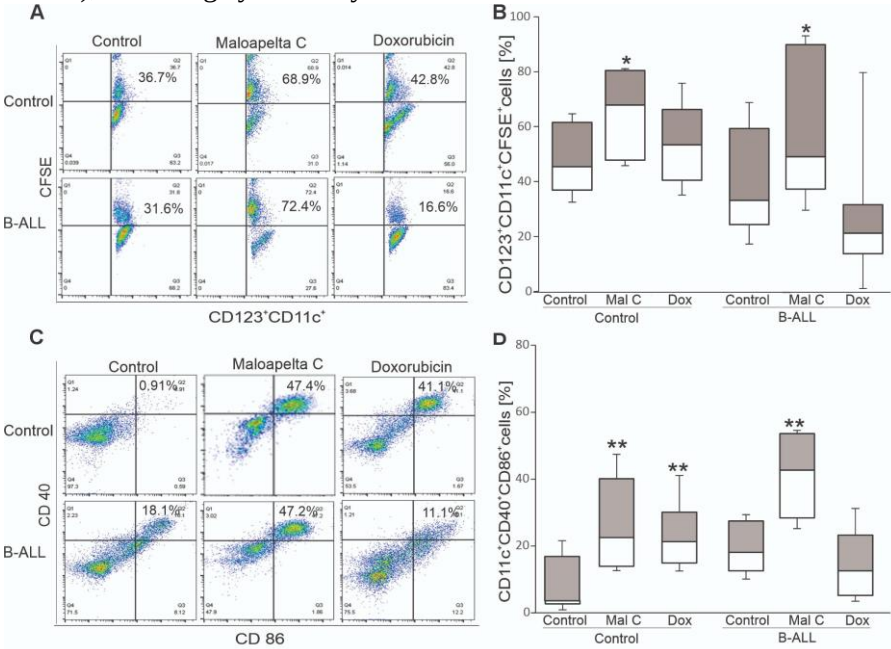


Figure 3.10: Effects of Dox and Mal C on phagocytosis and maturation of dendritic cells in healthy individuals and BCLC patients.

3.5.2. The role of the Wnt signaling pathway in the effects of Doxorubicin on dendritic cell maturation and apoptosis.

The results showed that Dox markedly increased the proportion of necrotic Annexin V-PI⁺ cells in both healthy individuals and BCC patients (Figure 3.11), whereas the combination of Dox and Wnt C59 significantly reduced this proportion and almost completely eliminated the Annexin V-PI⁺ population. Furthermore, Dox notably increased the proportion of

mature $CD40^+CD86^+$ dendritic cells in healthy individuals, but this effect was inhibited in the presence of Wnt C59.

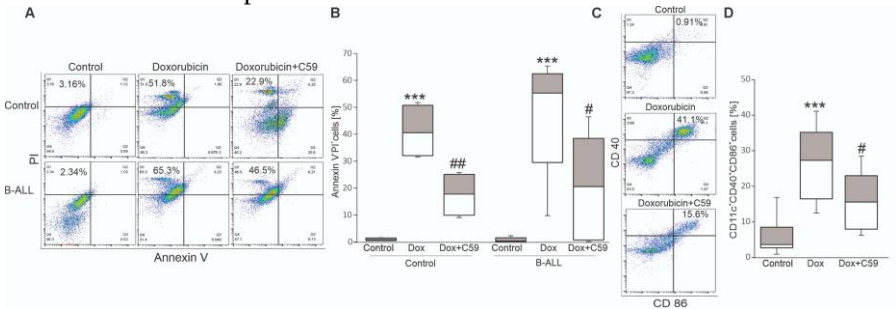


Figure 3.11: The influence of the Wnt pathway on dendritic cell maturation and apoptosis in healthy individuals and BCLC patients.

3.5.3. Roles of mTOR and MAPKp38 signaling pathways in the effects of *Malloapelta C* on dendritic cell maturation and phagocytosis.

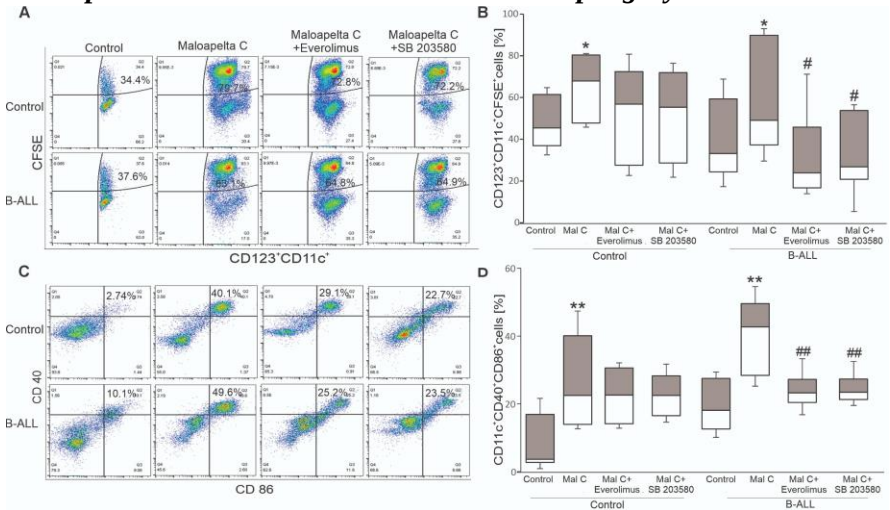


Figure 3.12: The influence of mTOR and AMPK/p38 pathways on dendritic cell maturation and phagocytosis in healthy individuals and BCLC patients.

Results Figure 3.12 shows that Mal C strongly increases the phagocytic ability of $CD11c^+CD123^+$ DC cells in BCC patients, however

this effect is clearly attenuated when adding Everolimus or SB203580. Similarly, Mal C significantly promoted DC (CD40⁺CD86⁺) maturation in BCC patients, but this increase was strongly reduced by mTOR or MAPKp38 inhibition. In contrast, in healthy people, inhibitors only slightly reduce the effect of Mal C and do not cause significant changes.

CHAPTER 4. DISCUSION

4.1. The effect of A20 on immature leukocyte activity in acute leukemia.

Research findings indicate that A20 regulates the activation state of myeloid cells in acute leukemia by reducing the CD11b⁺CD40⁺ population. CD40 is a critical co-stimulatory molecule involved in activating the NF- κ B and MAPK signaling pathways, thereby promoting inflammatory and immune responses. The downregulation of CD40 mediated by A20 suggests that this gene limits the pro-inflammatory activation state of myeloid cells. This aligns with previous studies showing that reduced A20 expression leads to excessive myeloid cell activation and increased secretion of inflammatory cytokines. Notably, A20 primarily affects the myeloid population without significantly impacting T and B lymphocytes, suggesting a selective immunoregulatory role for this gene in acute leukemia.

Furthermore, IL-6 was observed to enhance STAT1 and STAT3 activation while simultaneously reducing A20 expression in leukemic blasts. Upregulation of A20 can inhibit IL-6-induced STAT activation, indicating that A20 acts as a negative regulator of the IL-6/STAT signaling axis. However, although A20 effectively inhibits STAT signaling, it cannot fully reverse cell migration induced by IL-6 and CXCL12. This suggests that the migration of leukemic blasts depends not only on STAT but also involves other signaling pathways, such as CXCL12/CXCR4, PI3K/Akt, and MAPK.

Additionally, treatment with Nifuroxazide a STAT inhibitor significantly reduced the CD11b⁺CD40⁺ population and cell migration, confirming the central role of IL-6/STAT signaling in regulating the activation and migration of leukemic blasts. These results indicate that A20 contributes to controlling the inflammatory environment and the activity of leukemic cells by regulating IL-6/STAT signaling, thereby suggesting the potential of A20 as an immunomodulatory target in acute leukemia.

4.2. The role of dendritic cell-regulating signaling genes in acute lymphoblastic leukemia patients exposed to Doxorubicin and Malloapelta C.

Research findings indicate that Mal C and Dox modulate dendritic cell (DC) function through distinct signaling mechanisms. Mal C primarily activates the mTOR and MAPKp38 pathways, whereas Dox preferentially triggers Wnt signaling, reflecting two different modes of immunomodulation and cytotoxicity.

Mal C upregulates mTOR and MAPKp38 expression, consistent with the roles of these pathways in promoting DC maturation, antigen presentation, and immune activation. This activation is accompanied by increased CD40/CD86 expression and enhanced phagocytic capacity without significant cytotoxicity, suggesting that Mal C functions primarily as an immunomodulator rather than a direct cytotoxic agent. In contrast, Dox upregulates Wnt1/Wnt3 expression while simultaneously inducing marked apoptosis and necrosis in DCs. This aligns with the role of Wnt/ β -catenin signaling in stress responses and the regulation of cell survival.

Mal C significantly increases the proportion of mature CD40⁺CD86⁺ DCs and phagocytic CD123⁺CD11c⁺ DCs, particularly in BCLC patients, demonstrating a capacity to restore DC immune function within the leukemic microenvironment. Conversely, Dox exerts potent cytotoxicity on DCs—evidenced by a marked rise in Annexin V⁺PI⁺ and Annexin V-PI⁺ cells—and impairs DC maturation and phagocytic ability in

BCLC patients. These results indicate that while Dox can stimulate DC maturation in healthy individuals, its cytotoxic effects predominate within the pathological environment of BCLC.

Treatment with the Wnt inhibitor C59 markedly reduces Dox-induced apoptosis and DC maturation, demonstrating that Wnt signaling plays a central role in both processes. Furthermore, the inhibitors Everolimus and SB203580 significantly impaired Mal C-induced DC phagocytosis and maturation, confirming the pivotal roles of mTOR and p38 MAPK in regulating DC function.

Collectively, the results demonstrate that Mal C promotes DC maturation and functional activation via mTOR/p38 MAPK signaling without causing significant toxicity, whereas Dox primarily activates Wnt signaling, leading to apoptosis and DC functional impairment in BCLC patients. These findings suggest the potential of Mal C as an immunomodulator to restore DC function and enhance anti-tumor immune responses in acute lymphoblastic leukemia.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. Clinical and preclinical characteristics, expression of inflammation-related genes, and immune phenotypes in patients with acute leukemia have been determined.

- a) Clinical and preclinical characteristics of patients with acute leukemia were identified. Clinically, the majority of patients with acute myeloid leukemia (BCTC) and acute lymphoblastic leukemia (BCLC) exhibited classic bone marrow failure syndromes (anemia, hemorrhage, infection); BCTC was notably characterized by extramedullary infiltration (gingival hypertrophy, skin lesions), whereas BCLC was predominantly characterized by lymphoid infiltration syndromes (lymphadenopathy, hepatosplenomegaly, and bone pain). In BCTC, elevated white blood cell

and lymphocyte counts were observed in the intermediate-prognosis group. Additionally, levels of IL-6 and TNF- α , as well as the proportions of NK cells, Treg cells, and CD13+CD117+ cells, were increased, while TGF- β levels were decreased compared to healthy individuals. In BCLC, the poor-prognosis group exhibited higher LDH, white blood cell, and lymphocyte counts compared to the intermediate-prognosis group. Concurrently, levels of IL-6, TNF- α , and IL-35, along with the proportions of NK and Treg cells, were elevated, whereas TGF- β levels were reduced compared to the healthy control group.

b) The expression patterns of genes encoding deubiquitinase enzymes specifically A20 were determined to be closely associated with the regulation of the inflammatory microenvironment and immune phenotypes; meanwhile, CYLD expression was also reduced in leukemia patients, though no definitive link to hematological parameters or cytokine levels was established. These findings indicate that A20 and CYLD play distinct regulatory roles in patients with acute leukemia.

c) Acute leukemia patients with low A20 expression exhibited low counts of lymphoid-lineage cells—including total lymphocytes and CD3+, CD8+, CD10+, CD19+, CD20+, and CD79a+ cells—alongside markedly elevated counts of myeloid-lineage cells (such as MPO+, CD33+, and CD13+CD117+ cells) compared to patients with high A20 expression.

2. The regulatory roles of A20, CYLD, and specific intracellular signaling pathways associated with the functions of leukemic blasts and dendritic cells in acute leukemia patients were identified.

a) The role of A20 in regulating the activation of myeloid-lineage cells and leukemic blasts was determined; A20 functions by inhibiting IL-6-dependent STAT1/STAT3 signaling, reducing the CD11b⁺CD40⁺ cell population, and restricting blast migration, thereby contributing to the control of immune activation.

b) The roles of mTOR and MAPK/p38 signaling pathways in regulating dendritic cell function in acute leukemia patients were identified; activation of these pathways by Malloapelta C enhanced dendritic cell maturation and phagocytosis without compromising cell viability.

RECOMMENDATIONS

1. Increase sample sizes in future studies to ensure adequate representation of disease subgroups (based on age, gender, disease stage, etc.), thereby enhancing the study's accuracy and reliability as well as the generalizability of the findings;
2. Conduct further research on the impact of genes encoding deubiquitinase enzymes on the function of other immune cell types in patients with acute leukemia.

NEW FINDINGS OF THE THESIS

1. The role of the A20 gene in influencing the function of immature cells in patients with acute leukemia has been determined.
2. The roles of specific signaling pathways—including mTOR, MAPKp38, and Wnt—in regulating dendritic cell activity in patients with acute leukemia upon exposure to the active compounds Malloapelta C and Doxorubicin have been determined.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Le Thuy Ha, **Nguyen Hoang Giang**, Nguyen Linh Toan, Nguyen Van Giang, Can Van Mao, Nguyen Quoc Nhat, Tran Dang Quan, Nguyen Huy Hoang, Ngo Thu Hang, Nguyen Thi Xuan., 2025. Cross-Sectional Study: Associations of A20 and Cezanne with Leukocyte Accumulation in B-Cell Acute Lymphoblastic Leukemia. *Medicina (Kaunas)*, 61(7):1166. doi: 10.3390/medicina61071166. PMID: 40731796; PMCID: PMC12299410.
2. **Hoang Giang Nguyen**, Xuan Nghia Vu, Thi Trang Do and Thi Xuan Nguyen., 2026. Expression of immunophenotype, inflammatory response and chromosomal abnormality in Acute Lymphoid Leukemia. *Vietnam Journal of Biotechnology*, 24(1), 13–27. doi: <https://doi.org/10.15625/vjbt-24015>
3. **Hoang Giang Nguyen**, Xuan Nghia Vu, Hong Hanh Ha and Thi Xuan Nguyen., 2026. Expression of several inflammation-related genes, immunophenotype and chromosome abnormalities in Acute Myeloid Leukemia. *Vietnam Journal of Biotechnology*, , 24(2),147-160 doi: <https://doi.org/10.15625/vjbt-23474>