DOI: 10.31276/VJSTE.66(1).90-95

Expression of several deubiquitinase and tyrosine phosphatase genes involved in inflammatory response in lymphoma

Nguyen Hoang Giang[1], Nguyen Trong Ha[2], Nguyen Thi Xuan[1*]

[1]Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Street, Nghia Do Ward, Cau Giay District, Hanoi, Vietnam
[2]103 Hospital, Vietnam Military Medical University, 261 Phung Hung Street, Phuc La Ward, Ha Dong District, Hanoi, Vietnam

Received 30 January 2023; revised 24 April 2023; accepted 28 April 2023

Abstract:

Lymphoma represents a heterogeneous group of cancers affecting the lymphatic system, encompassing non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL). The majority of NHL cases originate from B cells. HL is characterized by the presence of rare Hodgkin and Reed-Sternberg cells. Within the context of the inflammatory response, two SH2 domain-containing protein tyrosine phosphatases (SHPs), namely *SHP-1* and *SHP-2*, serve as negative regulators. Deubiquitinating enzymes (DUBs) play a crucial role in inhibiting NF-κB activation in response to various stimuli. This study focuses on the DUBs *OTUB1*, *OTUB2*, and *Cezanne*. Blood samples were collected from 50 NHL patients, 26 HL patients, and 50 healthy individuals. Quantitative RT-PCR was employed to assess the mRNA expression of *OTUB1, OTUB2*, *Cezanne, SHP-1*, and *SHP-2*, while ELISA was used to determine IL-6 and CA125 concentrations. The results revealed that the mRNA level of *OTUB1* was significantly downregulated in NHL patients but not in HL patients. Notably, Cezanne expression was downregulated in lymphoma patients, with significantly lower levels observed in HL compared to NHL patients. Furthermore, *SHP-1* mRNA expression was significantly lower in the NHL group compared to the HL group or healthy individuals. Conversely, *SHP-2* gene expression was upregulated in NHL patients but remained unchanged in HL patients. In conclusion, these findings highlight significant differences in the expressions of *DUB* and *SHP* genes and the inflammatory response in lymphoma patients. This study provides a foundation for further investigation into the roles of DUBs and tyrosine phosphatases in regulating the functional activation of lymphoma cells.

Keywords: **CA125, deubiquitinating enzymes, IL-6, lymphoma, tyrosine phosphatases.**

Classification numbers: **3.4, 3.5**

1. Introduction

Lymphoma is a group of cancers affecting the lymphatic system, involving cells of the immune system. Lymphoma initiates when healthy B cells, T cells, or natural killer (NK) cells in the lymphatic system undergo alterations and uncontrolled growth, leading to the formation of tumours. It is comprised of two main subtypes: NHL and HL [1]. NHL is a lymphoproliferative disorder, with the majority originating from B cells, while the remainder arises from T lymphocytes or NK cells [2]. Approximately 90% of all lymphomas are NHL, with the remaining 10% classified as HL [3]. HL is a rare form of cancer characterized by the presence of rare Hodgkin and Reed-Sternberg cells (HRS) that originate from a subtype of B cells and are typically found in the bone marrow and peripheral blood [4, 5]. Several studies have indicated that lymphoma patients, particularly those with poor outcomes, exhibit elevated serum levels of carbohydrate antigen 125 (CA-125), a commonly used tumour biomarker, although it is more commonly associated with ovarian cancer [6, 7].

DUB genes, including OTU domain-containing ubiquitin aldehyde binding protein *OTUB1*, *OTUB2*, and *OTUD7B* (*Cezanne*), play pivotal roles in regulating the expression of various signal transduction pathways, such as NF-κB [8-10], by removing specific types

*Corresponding author: Email: xuannt@igr.ac.vn

of polyubiquitin chains from substrate proteins. This activity leads to the induction of various cellular biological processes. Recent research has demonstrated the involvement of *OTUB1* and *OTUB2* in modulating the development of solid cancers, including breast, liver, and lung cancers [9, 11, 12]. Mice lacking *OTUB1* exhibit abnormal responses in CD8 T cells and NK cells [13]. *Cezanne* inactivation has been associated with disease progression and poor prognosis in hepatocellular carcinoma [14]. Despite the well-established effects of DUBs on the activation of solid cancer cells, their impact on the biological activation of lymphoma cells remains largely unexplored.

Similar to DUBs, SH2 domain-containing protein tyrosine phosphatases (SHPs) are crucial for a wide range of cellular processes in response to external stimuli. Two protein tyrosine phosphatases (PTPs), *SHP-1* and *SHP-2*, are recognised as negative regulators of the inflammatory response, acting via NF-κB and STAT signalling pathways [15]. Mice lacking *SHP-1* display myelin deficiency due to increased inflammatory mediators [16]. In leukaemia and lymphoma, *SHP-1* expression is often inactivated as a result of DNA methylation of its promoter region [17]. Conversely, *SHP-2* levels are elevated in leukaemia and diffuse large B-cell lymphoma [18, 19]. In the context of solid cancers, *SHP-2* serves as a negative regulator of interferon production, diminishing cytotoxic effects in fibroblast cells [20].

This study aims to investigate the mRNA expressions of DUBs, SHPs, and the inflammatory response in Vietnamese lymphoma patients.

2. Materials and methods

2.1. Patients and control subjects

Peripheral blood samples were collected from 76 untreated patients diagnosed with two types of lymphomas: 50 NHL and 26 HL, at the National Hospital of Haematology and Blood Transfusion and the 103 Military Hospital in Hanoi, Vietnam. The control group consisted of 50 healthy subjects. None of the individuals in the control group were on medication or had a known acute or chronic disease. All patients and volunteers provided written consent to participate in the study. Personal care and experimental procedures were conducted in accordance with Vietnamese laws governing human welfare and were approved by the Ethical Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology. All experimental protocols involving human subjects adhered to the Helsinki Declaration of 1975, as revised in 2008.

2.2. RNA extraction and real-time RT-PCR

Total mRNA was isolated using the Qiashredder and RNeasy Mini Kit from Qiagen, following the manufacturer's instructions. For first-strand cDNA synthesis, 1 µg of total RNA in 12.5 µl of DEPC-treated H_2O was mixed with 1 µl of oligo-dT primer (500 µg/ml, Invitrogen) and heated for 2 min at 70°C. Quantitative real-time PCR using the LightCycler System (Roche Diagnostics) was employed to determine transcript levels of *SHP-1* and *SHP-2*, using the following primers (Table 1).

Table 1. List of primers used for RT-PCR.

Genes	Direction	Primer sequence
GAPDH	Forward	5'-GGAGCGAGATCCCTCCAAA-3'
GAPDH	Reverse	5'-GGCTGTTGTCATACTTCTCAT-3'
OTUD7B	Forward	5'-ACAATGTCCGATTGGCCAGT-3'
OTUD7B	Reverse	5'-ACAGTGGGATCCACTTCACATTC-3'
OTUB-1	Forward	5'-ACAGAAGATCAAGGACCTCCA-3'
OTUB-1	Reverse	5'-CAACTCCTTGCTGTCATCCA-3'
OTUB-2	Forward	5'-CTCACGTCGGCCTTCATCA-3'
OTUB-2	Reverse	5'-GCCATGGGCTCTACTTCGT-3'
SHP-1	Forward	5'-GCCCAGTTCATTGAAACCAC-3'
SHP-1	Reverse	5'-GAGGGAACCCTTGCTCTTCT-3'
SHP-2	Forward	5'GAGAGCAATGACGGCAAGTCT-3'
SHP-2	Reverse	5'-CCTCCACCAACGTCGTATTTC-3'

PCR reactions were conducted in a final volume of 20 µl, comprising 2 µl of cDNA, 2.4 µl of $MgCl_2$ (3 µM), 1 µl of primer mix (0.5 µM of both primers), 2 µl of cDNA Master SybrGreen I mix (Roche Molecular Biochemicals), and 12.6 µl of DEPC-treated water. Amplification of the target DNA involved 40 cycles of 95ºC for 10 s, 62ºC for 10 s, and 72ºC for 16 s, each with a temperature transition rate of 20ºC/s, a secondary target temperature of 50ºC, and a step size of 0.5ºC. Subsequently, melting curve analysis was conducted at 95ºC for 0 s, 60ºC for 10 s, and 95ºC for 0 s to ascertain the melting temperature of primer dimers and specific

PCR products. The ratio between the respective gene and corresponding GAPDH was calculated for each sample using the $2^{-\Delta CT}$ method.

2.3. Cytokine quantification

Serum samples were isolated from the blood samples of NHL and HL patients as well as healthy subjects, and these samples were stored at -20˚C until utilised for ELISA. IL-6 and CA-125 concentrations were determined using ELISA kits from Thermo Scientific, following the manufacturer's protocol.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 20 (IBM, USA). Differences between the control and patient groups were assessed for significance using a non-parametric Mann-Whitney U test. All statistical tests were two-sided, and the level of significance was set at $p<0.05$.

3. Results and discussion

3.1. Analysis of DUB gene expression in lymphoma patients

In terms of DUB gene expression, our findings revealed that the mRNA level of *OTUB1* was significantly lower in NHL patients compared to the control group, while no significant difference was observed in HL patients (Fig. 1A). In contrast, the expression of *OTUB2* remained unchanged in both NHL and HL groups (Fig. 1B). Importantly, we noted suppressed expression of *Cezanne* in both NHL and HL groups, with a notably more significant decrease in HL compared to NHL patients.

The downregulation of *Cezanne* in both NHL and HL groups and of *OTUB1* in NHL patients represents a novel discovery in this study. These findings suggest a pivotal role for *OTUB1* and *Cezanne* in influencing the development of lymphoma cells. Previous studies have primarily associated the effects of *OTUB1*, *OTUB2*, and *Cezanne* with the regulation of cellular processes in solid cancers [9, 11, 12, 14].

3.2. Expression level of SHP genes in lymphoma patients

To evaluate the activation of the SHP signalling pathway, we observed that mRNA expression of *SHP-1* was significantly reduced in the NHL group, while it remained unaltered in the HL group compared to healthy individuals (Fig. 2A). Intriguingly, the level of *SHP-1* was markedly lower in NHL compared to HL patients. This aligns with previous reports of reduced *SHP-1* expression in various cancers [17, 21], where *SHP-1* deficiency leads to impaired apoptosis and diminished treatment response [22]. In contrast, gene expression of *SHP-2* was elevated in NHL patients but exhibited no significant change in HL patients (Fig. 2B). Overexpression of *SHP-2* has also been detected in patients with leukaemia [19]. Consequently, the distinct roles of *SHP-1* and *SHP-2* in different cancer cell types, with their opposing effects, warrant further investigation into the molecular mechanisms governing the function of NHL cells.

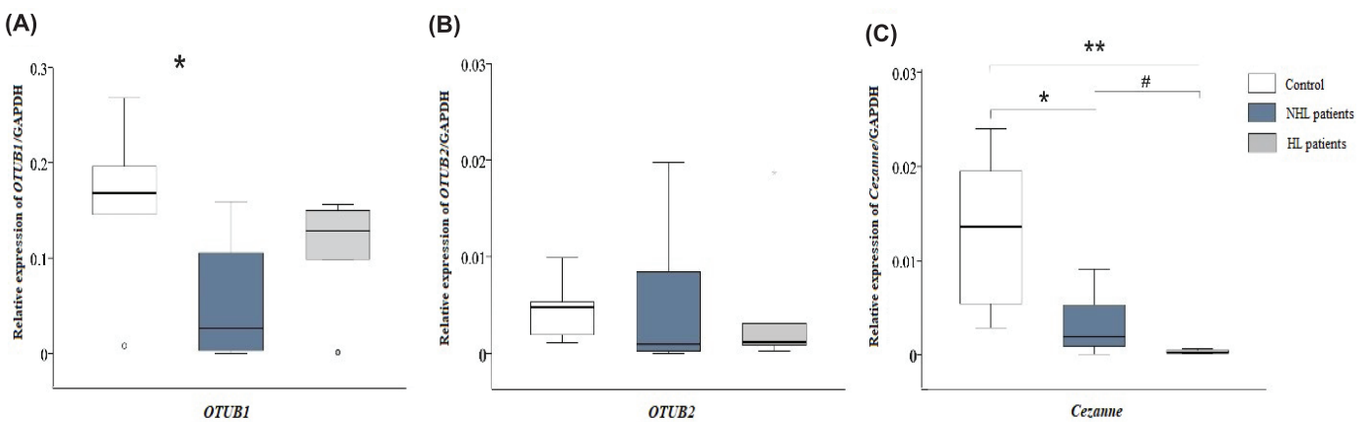

Fig. 1. Box plot analysis of *OTUB1* (A), *OTUB2* (B), and *Cezanne* (C) gene expression in NHL (blue bar), HL (grey bars) patients, and the control group (white bar). *($p<0.05$) and **($p<0.01$) indicate a significant difference between control and patient groups and #($p<0.05$) indicates a significant difference between NHL and HL groups (Mann-Whitney U test).

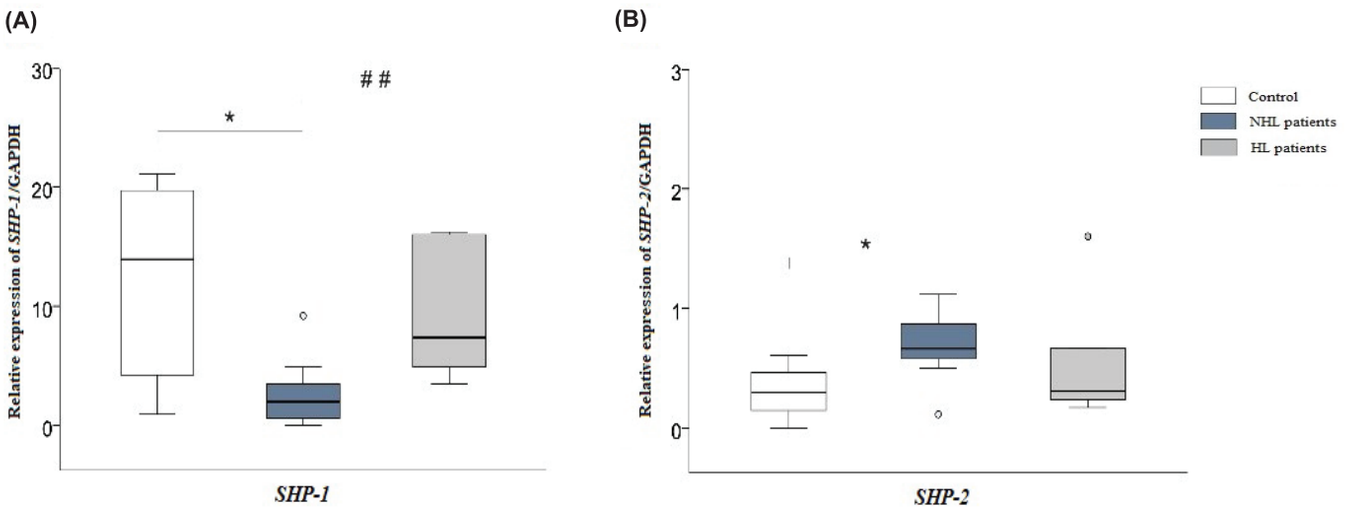

Fig. 2. Box plot analysis of *SHP-1* (A) and *SHP-2* (B) gene expression in NHL (blue bar), HL (grey bars) patients, and the control group (white bar). *(p<0.05) indicates a significant difference between control and patient groups and #(p<0.01) indicates a significant difference between NHL and HL groups (Mann-Whitney U test).

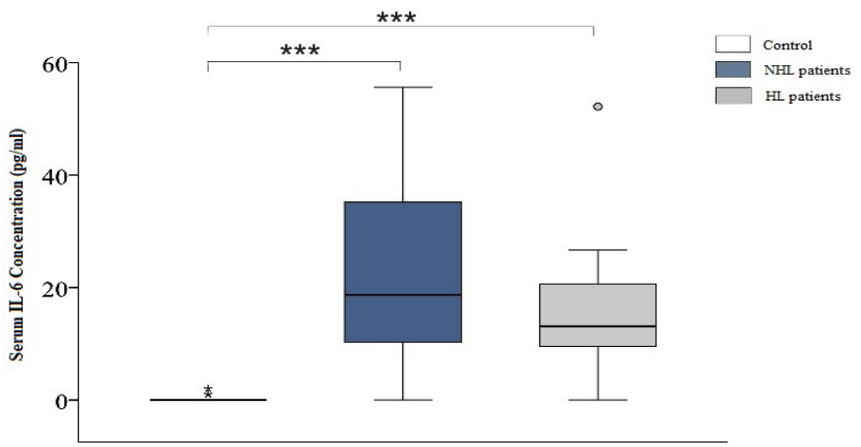

Fig. 3. Box plot analysis of IL-6 serum level in NHL (grey bar), HL (blue bar), and the control group (white bar). ***(p<0.001) indicates a significant difference between control and patient groups (Mann-Whitney U test).

3.3. Serum IL-6 level in lymphoma patients

A recent study [23] has demonstrated the involvement of SHP molecules in the regulation of cytokine IL-6 in cancer cells, with higher levels of IL-6 reported in lymphoma patients [24]. The activation of *SHP-1* has also been associated with IL-6 release in prostate cancer cells [23]. Similarly, our findings indicate a significant increase in serum IL-6 concentration in both NHL and HL patient groups (Fig. 3).

3.4. Serum CA125 level in lymphoma patients

The secretion of IL-6 has been linked to an elevated level of CA125 by cancer cells [25]. Consequently, we examined the serum CA125 levels in NHL, HL, and control groups using ELISA. The results revealed a significant increase in serum CA125 concentration in both NHL and HL groups, with a notably higher level in HL compared to NHL patients (Fig. 4). CA125 has previously been identified as a marker associated with the development and pathogenesis of lymphoma and ovarian cancer [6, 7]. In this study, we additionally observed that the frequency of lymphoma patients with CA125 levels exceeding the clinical cutoff of 35 UI/ml was higher in HL (48.14%) than in NHL (32%) patients. Notably, patients with CA125 levels ≥35 UI/ml tend to have lower survival rates than

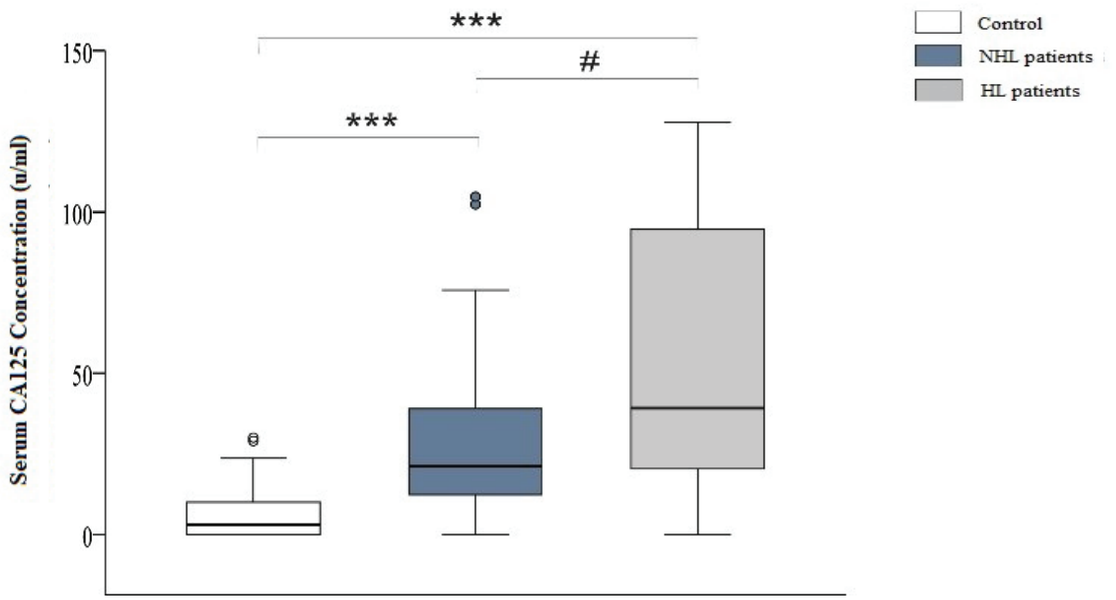

Fig. 4. Box plot analysis of serum CA125 level in NHL (grey bar), HL (blue bar), and the control group (white bar). ***(p<0.001) indicates a significant difference between control and patient groups and #(p<0.05) indicates a significant difference between NHL and HL groups (Mann-Whitney U test).

those with normal CA125 levels before any treatment [6]. These findings suggest a potential relationship between the level of CA125 and the modulatory activation of SHP pathways in lymphoma cells.

Additionally, we investigated the potential association between the expressions of *DUB* and *SHP* genes and the inflammatory response in lymphoma patients. However, no significant associations were observed.

4. Conclusions

Our results have revealed significant differences in the expressions of *DUB* and *SHP* genes and the inflammatory response in lymphoma patients. Specifically, the expressions of *SHP*, *OTUB1*, and *Cezanne* genes appear to be sensitive to the activation of NHL cells. Furthermore, the altered expressions of *Cezanne*, IL-6, and CA125 concentrations are dependent on the specific cell types of lymphoma. This study provides valuable insights and warrants further investigation into the roles of DUBs and tyrosine phosphatases in regulating the functional activation of lymphoma cells.

CRediT author statement

Nguyen Hoang Giang, Nguyen Trong Ha: Conceptualisation and Design, Acquisition of data, Analysis and Interpretation of data; Nguyen Thi Xuan: Conceptualisation and Design, Acquisition of data, Analysis and Interpretation of data, Writing.

ACKNOWLEDGEMENTS

This research is funded by the Vietnam Academy of Science and Technology (VAST) under grant number TDTBG0.05/21-23.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] R.L. King, E.D. Hsi, W.C. Chan, et al. (2023), "Diagnostic approaches and future directions in Burkitt lymphoma and high-grade B-cell lymphoma", *Virchows Arch*, **482(1)**, pp.193-205, DOI: 10.1007/s00428-022-03404-6.

[2] C. Küçük, X. Hu, B. Jiang, et al. (2015), "Global promoter methylation analysis reveals novel candidate tumor suppressor genes in natural killer cell lymphoma", *Clin. Cancer Res.*, **21(7)**, pp.1699-1711, DOI: 10.1158/1078-0432.CCR-14-1216.

[3] E.N. Mugnaini, N. Ghosh (2016), "Lymphoma", *Prim. Care*, **43(4)**, pp.661-675, DOI: 10.1016/j.pop.2016.07.012.

[4] A.A. Ibraheem, F.M. Mottaghy, M.E. Juweid (2023), "PET/CT in Hodgkin lymphoma: An update", *Semin. Nucl. Med.*, **53(3)**, pp.303-319, DOI: 10.1053/j.semnuclmed.2022.10.006.

[5] S.M. Ansell (2015), "Hodgkin lymphoma: Diagnosis and treatment", *Mayo. Clin. Proc.*, **90(11)**, pp.1574-1583, DOI: 10.1016/j.mayocp.2015.07.005.

[6] F. Russo, S. Lastoria, G. Svanera, et al. (2007), "Long-term follow-up study on the role of serum CA-125 as a prognostic factor in 221 newly diagnosed patients with Hodgkin's lymphoma", *Leuk Lymphoma*, **48(4)**, pp.723-730, DOI: 10.1080/10428190601183710.

[7] G. Wei, Z. Yuping, W. Jun, et al. (2006), "CA125 expression in patients with non-hodgkin's lymphoma", *Leuk Lymphoma*, **47(7)**, pp.1322-1326, DOI: 10.1080/10428190600580882.

[8] K. Enesa, M. Zakkar, H. Chaudhury, et al. (2008), "NF-kappaB suppression by the deubiquitinating enzyme Cezanne: A novel negative feedback loop in pro-inflammatory signaling", *J. Biol. Chem.*, **283(11)**, pp.7036-7045, DOI: 10.1074/jbc.M708690200.

[9] Z.L. Gu, J. Huang, L.L. Zhen (2020), "Knockdown of otubain 2 inhibits liver cancer cell growth by suppressing NF-kappaB signaling", *Kaohsiung J. Med. Sci.*, **36(6)**, pp.399-404, DOI: 10.1002/kjm2.12187.

[10] F. Mulas, X. Wang, S. Song, et al. (2020), "The deubiquitinase OTUB1 augments NF-kappaB-dependent immune responses in dendritic cells in infection and inflammation by stabilizing UBC13", *Cell Mol. Immunol.*, **18(6)**, pp.1512-1527, DOI: 10.1038/s41423-020-0362-6.

[11] M.F. Baietti, M. Simicek, L.A. Asbagh, et al. (2016), "OTUB1 triggers lung cancer development by inhibiting RAS monoubiquitination", *EMBO Mol. Med.*, **8(3)**, pp.288-303, DOI: 10.15252/emmm.201505972.

[12] U. Karunarathna, M. Kongsema, S. Zona, et al. (2016), "OTUB1 inhibits the ubiquitination and degradation of FOXM1 in breast cancer and epirubicin resistance", *Oncogene*, **35(11)**, pp.1433-1444, DOI: 10.1038/onc.2015.208.

[13] X. Zhou, J. Yu, X. Cheng, et al. (2019), "The deubiquitinase OTUB1 controls the activation of $CD8^+$ T cells and NK cells by regulating IL-15-mediated priming", *Nat. Immunol.*, **20(7)**, pp.879-889, DOI: 10.1038/s41590-019-0405-2.

[14] J.H. Wang, W. Wei, Z.X. Guo, et al. (2015), "Decreased *Cezanne* expression is associated with the progression and poor prognosis in hepatocellular carcinoma", *J. Transl. Med.*, **13**, DOI: 10.1186/s12967-015-0396-1.

[15] J. Zhang, F. Zhang, R. Niu (2015), "Functions of SHP-2 in cancer", *J. Cell. Mol. Med.*, **19(9)**, pp.2075-2083, DOI: 10.1111/jcmm.12618.

[16] G.P. Christophi, M. Panos, C.A. Hudson, et al. (2009), "Macrophages of multiple sclerosis patients display deficient *SHP-1* expression and enhanced inflammatory phenotype", *Lab. Invest.*, **89(7)**, pp.742-759, DOI: 10.1038/labinvest.2009.32.

[17] Y. Li, L. Yang, Y. Pan, et al. (2014), "Methylation and decreased expression of *SHP-1* are related to disease progression in chronic myelogenous leukaemia", *Oncol. Rep.*, **31(5)**, pp.2438-2446, DOI: 10.3892/or.2014.3098.

[18] C. Voena, C. Conte, C. Ambrogio, et al. (2007), "The tyrosine phosphatase *SHP-2* interacts with NPM-ALK and regulates anaplastic lymphoma cell growth and migration", *Cancer Res.*, **67(9)**, pp.4278-4286, DOI: 10.1158/0008-5472.CAN-06-4350.

[19] R. Xu (2007), "*SHP-2*, a novel oncogenic tyrosine phosphatase and potential therapeutic target for human leukemia", *Cell Res.*, **17(4)**, pp.295-297, DOI: 10.1038/cr.2007.18.

[20] M. You, D.H. Yu, G.S. Feng (1999), "*SHP-2* tyrosine phosphatase functions as a negative regulator of the interferon-stimulated Jak/STAT pathway", *Mol. Cell Biol.*, **19(3)**, pp.2416-2424, DOI: 10.1128/MCB.19.3.2416.

[21] S. Takeuchi, M. Matsushita, M. Zimmermann, et al. (2011), "Clinical significance of aberrant DNA methylation in childhood acute lymphoblastic leukemia", *Leuk. Res.*, **35(10)**, pp.1345-1349, DOI: 10.1016/j.leukres.2011.04.015.

[22] K. Nakata, Y. Suzuki, T. Inoue, et al. (2011), "Deficiency of *SHP-1* leads to sustained and increased ERK activation in mast cells, thereby inhibiting IL-3-dependent proliferation and cell death", *Mol. Immunol.*, **48(4)**, pp.472-480, DOI: 10.1016/j.molimm.2010.10.001.

[23] H. Tassidis, Z. Culig, A.G. Wingren, et al. (2010), "Role of the protein tyrosine phosphatase *SHP-1* in Interleukin-6 regulation of prostate cancer cells", *Prostate*, **70(14)**, pp.1491-1500, DOI: 10.1002/pros.21184.

[24] I. Dlouhy, X. Filella, J. Rovira, et al. (2017), "High serum levels of soluble interleukin-2 receptor (sIL2-R), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF) are associated with adverse clinical features and predict poor outcome in diffuse large B-cell lymphoma", *Leuk. Res.*, **59**, pp.20-25, DOI: 10.1016/j.leukres.2017.05.014.

[25] K. Fan, C. Yang, Z. Fan, et al. (2018), "MUC16 C terminal-induced secretion of tumor-derived IL-6 contributes to tumor-associated Treg enrichment in pancreatic cancer", *Cancer Lett.*, **418**, pp.167-175, DOI: 10.1016/j.canlet.2018.01.017.