

ACTIVATION OF SH2 DOMAIN-CONTAINING PROTEIN TYROSINE PHOSPHATASE AND INFLAMMATORY EXPRESSION IN PSORIASIS

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SUMMARY

Psoriasis is a chronic autoimmune disease characterized by abnormal proliferation and differentiation of keratinocytes and infiltration of inflammatory cells into the site of inflammation. Plaque psoriasis is the most common type of psoriasis, affecting up to 80–90% of psoriasis cases. Among inflammatory cells, myeloid dendritic cells or Langerhans cells are mainly activated cells during the pathogenesis of psoriasis to induce activation and differentiation of naive T cells into T helper cells (Th)1 and Th17 cells. SH2 domain-containing protein tyrosine phosphatase (SHP) is a negative regulator of the phosphorylation of several proteins involved in cellular differentiation, growth and activation. Chronic inflammation promotes tumor progression, which is characterized by the release of carcinogenic antigens, including alpha-fetoprotein (AFP) and cancer antigen 125 (CA125) into blood and urine. They are common tumor markers to serve as predictors of cancer development and survival of cancer patients. To this end, blood samples of 103 psoriasis patients and 46 healthy subjects were collected. The mRNA expressions of *SHP1* and *SHP2* were examined by using quantitative RT-PCR and the serum levels of IL-6, TNF- α , IFN- γ , IL-17A, AFP and CA125 by ELISA. As a result, the mRNA level of *SHP1* was higher expressed, whereas the level of *SHP2* was unaltered in the patient group compared to the control individuals. Importantly, psoriasis patients had CA125 level higher than the clinical cutoff 35U/mL was 15.6%, while healthy individuals had CA125 level lower than 35U/mL. In addition, the serum TNF- α and IL-17A concentrations were significantly increased in the patient group. In conclusion, the results indicated the significant differences in expression of *SHP1* gene and inflammatory response in psoriasis patients. This study further hint for investigations on the functional role of SHP1 in regulating activation of immune cells present in psoriasis patients.

Keywords: AFP, CA 125, Cytokine, Psoriasis, SHP

INTRODUCTION

Psoriasis is an immune-mediated systemic inflammatory disease characterized by abnormal proliferation and differentiation of keratinocytes and infiltration of inflammatory cells, mainly including T lymphocytes, macrophages, and neutrophils into the site of

inflammation (Talaee *et al.*, 2017). The identification sign of psoriasis includes red, itchy and scaly patches, which appears on the knees, elbows, trunk and scalp (Duffin *et al.*, 2008). The life-long disease affects large skin surfaces and then uncontrollably causes keratinocyte proliferation, and affects approximately 2% of the world's population

(Kim *et al.*, 2017). Plaque psoriasis is the most common type of psoriasis, affecting up to 80–90% of psoriasis cases (Kim *et al.*, 2017).

Excessive activation of adaptive immune response is associated with risk of developing psoriasis, in which leukocytes and keratinocytes secrete cytokines, including TNF- α , IFN- γ , IL-1 β , and IL-6 to cause chronic inflammation (Lowe *et al.*, 2007; Nograla *et al.*, 2011). Among inflammatory cells, myeloid dendritic cells or Langerhans cells are mainly activated cells during the pathogenesis of psoriasis to induce activation and differentiation of naive T cells into T helper cells (Th)1 and Th17 cells (Lowe *et al.*, 2007; Nograla *et al.*, 2011). Th1 cells produce cytokines such as IL-6, TNF- α and IFN- γ , while IL-17 is released by Th17 cells (Miossec, 2009).

The secretion of inflammatory cytokines was induced by activation of antigen-triggered immune cells through several signaling pathways, such as nuclear factor kappa-light-chain-enhancer of activated B (NF- κ B) and SH2 domain-containing protein tyrosine phosphatases (SHPs) (Christophi *et al.*, 2009). SHPs are negative regulators of the phosphorylation of several proteins involved in cellular differentiation, growth and activation as well as inflammatory gene expression (Haque *et al.*, 1998). The expression of *SHP1* is down-regulated in leukemias and lymphomas, resulting from DNA methylation of its promoter (Takeuchi *et al.*, 2011; Li *et al.*, 2014). Differently, *SHP2* is also considered as a negative regulator of JAK/STAT pathway (You *et al.*, 1999) and higher expressed in cancer patients (Jiang *et al.*, 2013).

A recent study showed that patients with severe psoriasis are more likely to die from cancer due to the chronic inflammatory nature of the disease and the use of immunosuppressive medications and UV therapies (Pouplard *et al.*, 2013). Chronic inflammation promotes tumor development and progression, which is characterized by the release of carcinogenic antigens, including

alpha-fetoprotein (AFP) and cancer antigen 125 (CA 125) into blood and urine. They are common tumor markers recommended for clinical use in the diagnosis of several cancers including ovarian, gastric and liver cancers to serve as predictors of cancer development and survival of cancer patients (Malati, 2007; Sharma, 2009). CA1 25 is elevated in the majority of patients with epithelial ovarian cancer (Gubbels *et al.*, 2010). The enhanced CA 125 level is also reported to be poor outcome in patients with lymphoma and leukemia (Prochazka *et al.*, 2012; Wu *et al.*, 2016). AFP is a glycoprotein mostly produced in liver and yolk sac during the early stage of fetal development and reduces to 1 ng/ml in healthy adults after birth. The increased level of AFP is related to liver cancer, malignant tumors from stomach, pancreas, and reproductive system (Wang, Wang, 2018).

Little is known about the involvement of SHP expression and level of CA 125 and AFP in psoriasis patients. In this study, mRNA expression of *SHP* genes and inflammatory reactions in Vietnamese patients with psoriasis were determined.

MATERIALS AND METHODS

Patients and control subjects

Blood samples of 103 unrelated patients with psoriasis were collected at the Thien Phu Duong Traditional Medical Clinic, Hanoi, Vietnam. The diagnosis of psoriasis was based on the 2016 WHO criteria (World Health Organization, 2016), including sharply demarcated round-oval erythematous plaques with loosely adherent silvery-white scales, especially affecting the elbows, knees, lumbosacral area, intergluteal cleft, and scalp. A control group comprised of 46 healthy volunteers. No individuals in the control population took any medication or suffered from any known acute or chronic disease. All patients and volunteers gave written consent to participate in the study. Person care and experimental procedures were performed

according to the Vietnamese law for the welfare of humans and were approved by the Ethical Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology.

RNA extraction and real-time RT-PCR

Total mRNA was isolated using the Qiashredder and RNeasy Mini Kit from Qiagen according to the manufacturer's instructions. For cDNA first-strand synthesis, 1 µg of total RNA in 12.5 µl DEPC-H₂O was mixed with 1 µl of oligo-dT primer (Invitrogen) and heated for 2 min at 70°C. To determine transcript levels of *SHP-1*, *SHP-2* and *GAPDH*, the quantitative real-time PCR with the LightCycler System (Roche Diagnostics) was applied. The following primers were used: *SHP-1* primers: 5'- GCC CAG TTC ATT GAA ACC AC-3' (forward) and 5'- GAG GGA ACC CTT GCT CTT CT-3' (reverse); *SHP-2* primers: 5'- GAGAGCAATGACGGCAAGTCT-3' (forward) and 5'- CCTCCACCAACGTCGTA-TTTC-3' (reverse) and *GAPDH* primers: 5'- GGAGCGAGATCCCTCCAAA-3' (forward) and 5'-GGCTGTTGTCATACTTCTCAT-3' (reverse). PCR reactions were performed in a final volume of 20 µL containing 2 µL cDNA, 2.4 µL MgCl₂ (3 µM), 1 µL primer mix (0.5 µM of both primers), 2 µL cDNA Master SybrGreen I mix (Roche Molecular Biochemicals), and 12.6 µL DEPC-treated water. The target DNA was amplified during 40 cycles of 95°C for 10 s, 62°C for 10 s, and 72°C for 16 s, each with a temperature transition rate of 20°C/s, a secondary target temperature of 50°C, and a step size of 0.5°C. Melting curve analysis was performed at 95°C, 0 s; 60°C, 10 s; 95°C, 0 s to determine the melting temperature of primer dimers and the specific PCR products. The ratio between the respective gene and corresponding GAPDH was calculated per sample according to the $\Delta\Delta$ cycle threshold method.

Measurements of cytokines and tumor markers

Blood samples obtained from psoriatic patients and healthy subjects by venipuncture

were centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored at -20°C until tested for enzyme-linked immunosorbent assay (ELISA). Serum levels of TNF- α , IL-6, IL-17A, IFN- γ , CA 125, and AFP were measured by using ELISA kits (Thermo Fisher Scientific) according to the manufacturers' instructions.

Statistics

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

RESULTS AND DISCUSSIONS

Expression levels of SHP1 and SHP2 genes

Firstly, we examined mRNA levels of *SHP1* and *SHP2* genes, which are crucial negative regulators of inflammatory gene expression via NF- κ B and STAT signalling pathways (You *et al.*, 1999; Christophi *et al.*, 2009). In this study, we showed for the first time that the expression level of *SHP-1* in psoriasis patients was observed a 3.2-fold increase as compared to healthy individuals (Fig. 1A). In contrast, recent studies reported that expression of *SHP-1* is decreased in patients with leukemias and lymphomas (Takeuchi *et al.*, 2011; Li *et al.*, 2014). This suggested that activation of *SHP-1* in psoriasis and blood cancers was different from each other. The evidence might further hint for investigating on molecular mechanism involved in regulation of SHP pathway on the development of psoriasis.

Unlike *SHP1* expression, as shown in Fig. 1B, the expression level of *SHP2* gene did not differ between patient and control groups. Another study indicates elevated *SHP-2* gene expression in cancer patients (Jiang *et al.*, 2013). Enhanced expression of the *SHP-2* gene is caused by a point mutation at the terminal end of the N-terminus in leukemias (Pandey *et al.*,

2017), whereas reduced expression of *SHP-2* gene is reported in multiple myeloma patients

due to DNA methylation (Beldi-Ferchiou *et al.*, 2017).

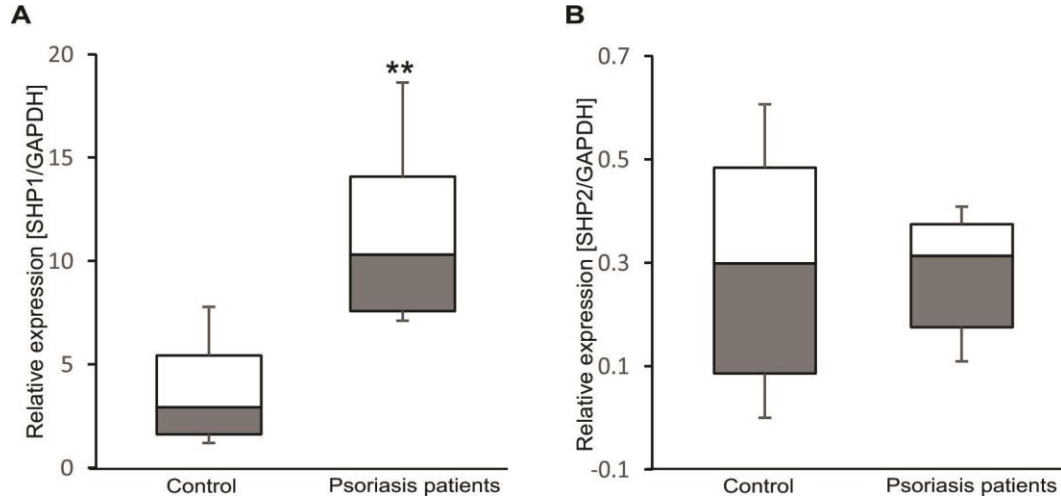


Figure 1. Box plot analysis of *SHP1* (A) and *SHP2* (B) gene expressions in control and patient groups. GAPDH was used as a reference gene for relative quantification. ** (p < 0.01) indicates significant difference between control and patient groups.

Cytokine profile in psoriasis patients

Psoriasis is known as a chronic inflammatory disease, therefore, assessment of serum cytokine concentrations was performed by using ELISA. The results showed that the

levels of TNF- α , IL-6, IL-17A, and IFN- γ in the patient group were found higher than that in the control group, in which TNF- α and IL-17A levels in psoriasis patients were significantly higher than in healthy subjects (Fig. 2).

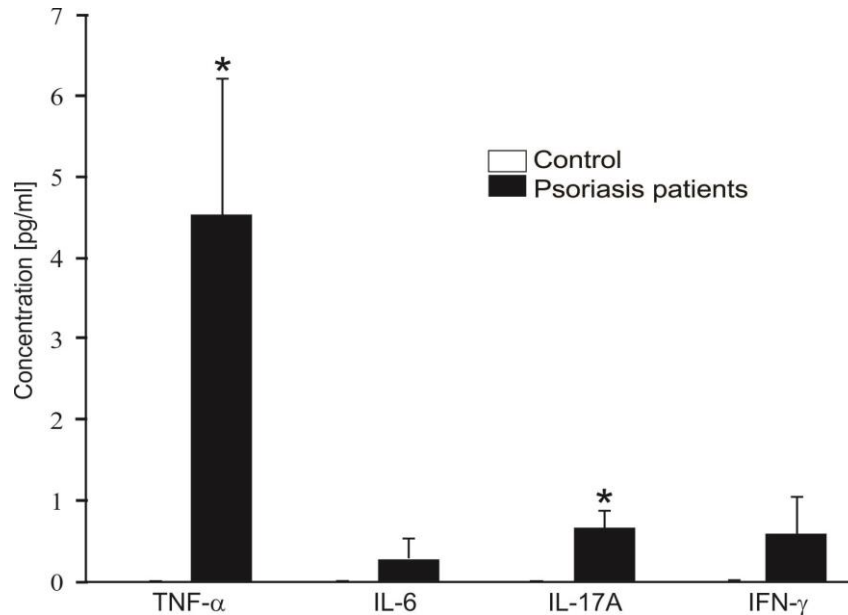


Figure 2. TNF- α , IL-6, IL-17A, and IFN- γ concentrations in control and patient groups. *(p < 0.05) indicates a statistically significant difference between control and patient groups.

In agreement, the role of TNF- α in modulating psoriasis is indicated in recent investigations that the use of anti-TNF- α antibody has proved to have disease-reducing activity in psoriasis and appears to be well tolerated (Tobin *et al.*, 2005). Several genetic investigations reported that alterations of TNF- α gene is related to susceptibility to psoriasis (Wang, Zhou, 2021). Besides, the promoting role of IL-17A on cell proliferation and abnormal differentiation of keratinocytes is also revealed (Lai *et al.*, 2012). IL-17A contributes to skin barrier disruption by downregulating the expression of keratinocyte differentiation markers such as filaggrin (Gutowska-Owsiak *et al.*, 2012). Hence, the elevated expression of these cytokines might be linked to activation of SHP1 in psoriasis patients.

Expression of tumor antigens in psoriasis patients

Finally, we determined the levels of two

tumor antigens CA 125 and AFP in the serum by ELISA. The results showed that CA 125 level was significantly higher in the patient group than healthy individuals, whereas the level of AFP was slightly increased in the patient group, but not reaching to the significant difference (Fig. 3). Importantly, psoriasis patients had CA 125 level higher than the clinical cutoff 35U/mL was 15.6%, while healthy individuals had CA 125 level lower than 35U/mL, pointing out that psoriasis might be a risk factor for developing cancer.

Similar to *SHP1* expression, the elevated expression of CA 125 in psoriasis patients was revealed for the first time. Recently, the enhanced CA 125 level is indicated to be poor outcome in patients with lymphoma and leukemia (Prochazka *et al.*, 2012; Wu *et al.*, 2016). CA 125 is expressed in all tissues but leukocytes and related to the risk for psoriasis in a previous study (Huffmeier *et al.*, 2009).

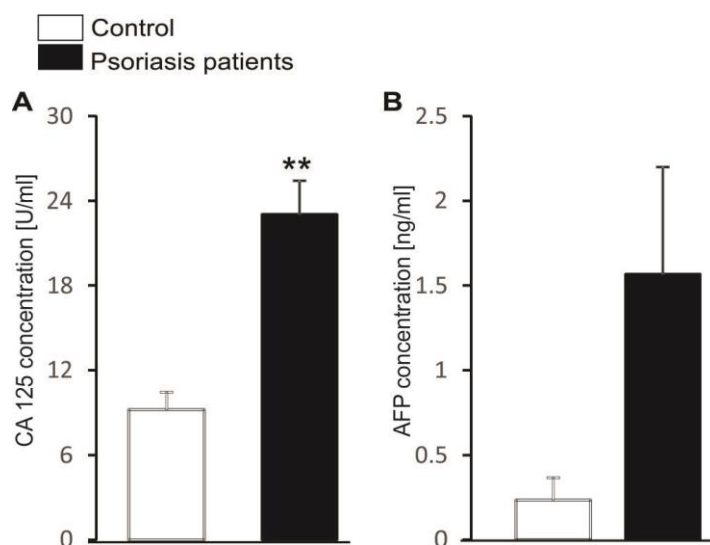


Figure 3. Box plot analysis of CA 125 (A) and AFP (B) concentrations in psoriasis patients and controls. ** ($p < 0.01$) indicates a statistically significant difference between control and patient groups.

CONCLUSION

Our results indicated different expressions of *SHP-1* and *SHP-2* genes, cytokines and tumor antigens in psoriasis patients, in which the increased expressions of *SHP-1* gene and CA125 were relatively novel. In addition, levels of TNF- α and IL-

17A were also found higher in these patients. The results suggested that a functional correlation between SHP-1 activation and inflammatory expression in psoriasis patients need to be investigated.

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