Clin. Lab. 2023;69:XXX-XXX

ORIGINAL ARTICLE

Effects of Ruxolitinib on Myeloproliferative Neoplasms via the Negative Regulators

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SUMMARY

Background: **We evaluated the JAK2V617F mutation and p-JAK2, SOCS-1, SHP-1 expression in JAK2V617F positive myeloproliferative neoplasms (MPNs) patients and the role of JAK/STAT pathway in human erythroleukemia (HEL) cells, which had JAK2V617F mutation.**
Methods: **Protein expression of p-JAK2, SOCS-1, SHP-1 in bone marrow biopsies (BMBs) were detected by immunohistochemical staining methods. Cell apoptosis and cell cycle were detected by flow cytometry and Caspase 3/7 assay kits.**
Results: **1. The p-JAK2, SOCS-1, and SHP-1 expressions were significantly different between JAK2V617F positive MPN and control patients ($p < 0.01$); 2. After being treated for 3 months, the p-JAK2, SOCS-1, and SHP-1 expressions were significantly different compared with newly diagnosed patients ($p < 0.01$). 3. HEL cell viabilities were significantly different after being treated with different concentrations of ruxolitinib. Ruxolitinib had a significant effect on the cell apoptosis, viability, and the protein activity of caspase-3 and -7 of HEL cells. 3. The mRNA and protein expressions of JAK2 and the protein expression of p-JAK2 were gradually decreased ($p < 0.01$, $p < 0.05$), while the mRNA and protein expressions of SOCS1 and SHP1 were gradually increased (all $p < 0.01$).**
(Clin. Lab. 2023;69:xx-xx. DOI: 10.7754/Clin.Lab.2022.220442)

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Manuscript accepted May 11, 2022

KEYWORDS

ruxolitinib, myeloproliferative neoplasms, JAK2V617F mutation, SOCS-1, SHP-1

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of clonal disorders, and characterized by the abnormal proliferation of mature cells in the peripheral blood. The patients always have an increased risk of thrombosis and progression to acute myeloid leukemia. JAK2V617F is a common mutation in JAK2 kinase which can lead to constitutive activation of the Janus kinase-signal transducer and activator of transcription (JAK/STAT) pathway in BCR-ABL1-negative MPN [1,2]. The genetic-pathologic characteristics provided the progression of MPN [3,4]. The discovery of the JAK2V617F mutation gave hope for the targeted

therapy in MPNs. Ruxolitinib is a potent and selective JAK1/JAK2 inhibitor, that is able to reduce the proinflammatory state and, consequently, to reduce symptom burden and cachexia and clonal myeloproliferation with possible leukemic transformation. It was approved for the treatment of intermediate and high-risk IPSS myelofibrosis, which showed reduction in spleen volume, amelioration in quality of life, and improvement in symptoms [5-7].

The JAK/STAT signaling pathway is involved in cell growth and differentiation [8,9]. The JAK/STAT pathway is stringently regulated at several steps by 3 main families of proteins: the SOCS (suppressor of cytokine signal transduction), the tyrosine phosphatases (SHP-1 and SHP-2), and the PIAS (protein inhibitor of activated STATs), particularly SOCS-1 and SHP-1 [10], all of which are known as a negative feedback regulator to inhibit JAK signaling [11]. Until now, little is known about SOCS-1 and SHP-1 genes expression in MPN cells. Moreover, the relationship between ruxolitinib and these two JAK/STAT suppressor genes is not well documented. Therefore, we examined the expression of SOCS-1 and SHP-1 in JAK2V617F positive MPN in bone marrow biopsy. Meanwhile, we intended to study the effect of ruxolitinib on the proliferation, apoptosis, cell cycle, and expression of SOCS-1 and SHP-1 in JAK2V617F positive human erythroleukemia cells (HEL cells). Thus, we want to offer a new drug for targeting negative feedback regulators, which could be important to the development of new therapeutics in patients with MPN in the future.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Institutional Ethics Committee of Baoding No.1 Hospital, and written informed consent was obtained from all participants.

Patients and bone marrow samples

Forty-eight bone marrow biopsies (BMBs) of newly diagnosed JAK2V617F positive MPN patients (22 males and 26 females; median age: 52 years, range: 31-72) were included in Hebei General Hospital and No. 1 Hospital of Baoding from July 2012 through August 2016. The JAK2 exon 12 mutations, calreticulin (CALR), thrombopoietin receptor (MPL), and chromosome karyotype analyses were examined. Diagnosis and clinical data of all the patients (21 PV cases, 14 ET cases, and 13 PMF cases) were evaluated and the morphological, immunological, molecular biological and bone marrow biopsy pathological features were classified according to the rationale for revision and proposed changes of the WHO diagnostic criteria [12]. Twenty-four BMBs isolated from patients with anemia were included as controls (10 males and 14 females; median age: 48 years, range: 28 - 67). This study was performed according to the regulations of the No. 1 Hospital of Baoding ethics committee. The registration number was 20120701, and all patients gave their informed consent.

Cell culture and main reagents

The human erythroleukemia cell (HEL) line (Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Beijing, China) was cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), glutamine (2%), and streptomycin (100 µg/mL) at 37°C in a humidified atmosphere with 5% CO_2. Antibodies of p-JAK2 (Tyr 1007/Tyr 1008), SOCS-1, SHP-1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Chemicals were purchased from Qiagen Biotechnology (Gaithersburg, MD, USA) and Sigma (St. Louis, MO, USA). Cell Counting Kit-8 was purchased from Dojindo Chemical Research (Tokyo, Japan). Caspase-Glo 3/7 assay systems were purchased from Promega (USA).

Immunohistochemistry analysis

The BMB samples were analyzed by immunohistochemical method. Formalin-fixed, paraffin-embedded sections of BMBs from newly diagnosed MPN patients and control subjects were deparaffinized in xylene and hydrated in graded alcohols. Antigen retrieval was performed with citrate buffer at 95°C for 20 minutes.

To evaluate the expression of p-JAK2, SOCS-1 and SHP-1, the BMBs samples were stained with mouse monoclonal antibody specific for p-JAK2, SOCS-1, SHP-1. Then the samples were incubated with peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulin. All staining procedures used an isotypic antibody as negative control sample. The cell cytoplasm was stained with brown color or brown-appearing intracytoplasmic granules as the positive one, the positive rate was measured. Expression of p-JAK2, SOCS-1, and SHP-1 was assessed by counting the positively stained cells in three fields at 400 x magnification, and then calculating the mean for one field and the positive rate.

Cell viability by Cell Counting Kit-8 (CCK-8) assay

In order to explore the effect of ruxolitinib on the cell viability of HEL cells, the CCK-8 assay was used. The collected cells were plated into 96-well plates at the density of 5,000 cells/100 µL medium/well after the cells were treated with or without different concentrations of ruxolitinib (0 nmol/L, 50 nmol/L, 100 nmol/L, 250 nmol/L, 500 nmol/L, and 1,000 nmol/L). HEL cells were harvested to determine zero absorbance values. The cells were cultured for 0, 24, 48, and 72 hours. The half maximal inhibitory concentration (IC50) value for ruxolitinib was measured. Optical density (OD) was measured at 450 nm, and cell viability rate was calculated as (OD experimental group - OD blank) / (OD control group - OD blank group) × 100%. All the experiments were repeated three times.

Cell cycle analysis

HEL cells (at least 1×10^5 cells) were cultured for 0, 24, 48, and 72 hours for cell cycle analysis. The cells were collected, washed with PBS, fixed with 70% ethanol, and placed at 4°C overnight at each time-point. Then the cells were treated with 10 mg/mL of RNase for 30 minutes, stained with propidium iodide (PI) and placed at 4°C for 15 minutes. The cell cycle was measured by FACS analysis. The data were retrieved using a BD FACSCanto II flow cytometer and analyzed using the FlowLogic software (Chromocyte, Sheffield, UK).

Hoechst33342 accumulation assay fluorescent functional assays

Hoechst33342 was used to evaluate the effect of ruxolitinib. The collected HEL cells were plated into 96-well plates at the density of 5,000 cells/100 µL medium/well. The collected cells were washed twice with PBS, stained with 10 µg/mL of Hoechst33342 for 15 minutes and examined at 350 nm under a fluorescence microscope. The cells which have apoptosis characteristics were counted in 10 microscopic fields (200 cells per field). Each assay was repeated at least three times.

Caspase-3/7 activities detected by Caspase 3/7 assay systems

The collected HEL cells were plated into 96-well plates at the density of 5,000 cells/100 µL medium/well. Plates containing cells were removed from the incubator and equilibrated to room temperature. To each well, 100 µL of Caspase 3/7 reagent was added to each well containing 100 µL of blank, negative control cells or treated cells in culture medium. Contents in wells were mixed using a plate shaker at 300 - 500 rpm for 30 seconds and then incubated at room temperature for 3 hours. The luminescence of each sample was measured under a fluorescence microscope. Each assay was repeated at least three times.

Real-time quantitative PCR (FQ-PCR) analysis

Cells were collected after having been treated with or without different concentrations of ruxolitinib (0 nmol/L, 50 nmol/L, 100 nmol/L, 250 nmol/L, 500 nmol/L, and 1,000 nmol/L). Total RNA was extracted from the different cells using the Trizol reagent (Promega, USA). The resultant cDNA was subjected to FQ-PCR. PCR reaction was carried out in a volume of 25 µL with the SYBR Green I PCR reagent kit (Qiagen) and a light cycler fluorescent quantitative amplification analyzer (Bio-Rad, Hercules, CA, USA). PCR was performed for an initial denaturation at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of two-step reactions: 95°C for 15 seconds, 60°C for 1 minute. Relative quantification of gene expression was calculated by the method as follows: to amplify the target gene and ß-actin gene, the Ct value was determined. The ratio of target mRNA to ß-actin mRNA was calculated by the formula $\triangle\triangle Ct = 2\text{-}\triangle\triangle Ct$. The levels of quantitative gene expression of JAK2V617F, JAK2, and JAK2V617F/JAK2 mutant allele burden were calculated based on the standard curves which were generated by plasmid standards (Genechem Biotech, Beijing, China). The probe (Genechem Biotech, Beijing, China) was used for quantification. MGB-Probe: 5'-FAM-TCA CAA GCA TTT GGT TTT-MGB3', JAK2V617F reverse: 5'-CCA GAA TAT TCT CGT CTC CAC TGA A-3'. The specific primer sequences of each target gene are shown in Table 1. Each assay was repeated three times.

Western blot analysis of protein expression

HEL cells were collected from each different group, and western blot analysis was performed for detecting the expression level of each target protein. The protein concentration was measured by Coomassie brilliant blue kit (Kang Chen Biotechnology, Beijing, China). The cell extract was loaded onto 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and run at 100 V for 2 hours. Cell proteins were transferred to nitrocellulose (NC) membrane. The membrane was blocked with 5% skim milk in TBS with 0.1% Tween 20 for 1 hour and washed and incubated with the primary antibody at 4°C overnight. Then the membranes were washed and incubated with the secondary antibody, imaged with ECL Plus. The bound immunoglobulins were removed from the membranes by washing twice, each time for 15 minutes at room temperature in restore western blot stripping buffer. Protein levels were measured using a fluorescent scanner and quantity software for analysis (Bio-Rad, Hercules, CA).

Statistical analysis

Statistical analyses were completed with SPSS 19.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Data are shown as mean ± SD. Comparison between variables was performed using Student's *t*-test or one-way analysis of variance (ANOVA) or *q*-test. Correlation analysis used Spearman's rank correlation analysis. A p-value < 0.05 was considered statistically significant.

RESULTS

JAK2V617F mutant allele burden and expression levels of p-JAK2, SOCS-1, SHP-1, protein in bone marrow biopsy specimens of JAK2V617F positive MPN patients

As shown in Table 2, in 48 MPN patients with JAK2-V617F mutation, the mean JAK2V617F/JAK2 mutant allele burden was 57.33% ± 20.82%, while the JAK2-V617F/JAK2 mutant allele burden was 0% in the control group. The p-JAK2, SOCS-1 and SHP-1 protein were detected by immunohistochemistry method and expressed in cytoplasm of bone marrow cells. In the MPN patients, the mean expression of p-JAK2, SOCS-1, and SHP-1 protein were 74.01% ± 23.19%, 18.08% ± 7.90%, and 25.23% ± 13.62%, respectively. Compared

Table 1. Primer sequences for FQ-PCR.

Primer	Sequence
JAK2	F: 5′-CAGCAAGTATGATGAGCAAGCTTT-3′
	R: 5′-TGAACCAGAATATTCTCGTCTCCAC-3′
SOCS-1	F: 5′-ACCAGGTGGCAGCCGACAAAT-3 ′
	R: 5′-GCGAACGGAATGTGCGGAAGT-3′
SHP-1	F: 5′-CAGAAGCAGGAGGTGAAGAAC-3′
	R: 5′GATGTAGTTGGCATTGATGTAGTC-3′
β-actin	F: 5′-GCGGACATCCGCAAAGAC-3′
	R: 5′-AAAGGGTGTAACGCAACTAA-3′

Table 2. The expression of JAK2V617F allele burden, p-JAK2, SOCS-1, and SHP-1 in MPN patients and control patients.

Group	Number	(JAK2V617F/JAK2) %	p-JAK2+ (%)	SOCS-1 (%)	SHP-1 (%)
Newly diagnosed group	48	57.33 ± 20.82	74.01 ± 23.19 *	18.08 ± 7.90 *	25.23 ± 13.62 *
Control group	24	0	42.29 ± 15.78	51.68 ± 18.33	47.97 ± 20.11

*** - MPN patients versus control patients, $p < 0.01$.**

Table 3. Caspase-3 and -7 protein activity change of HEL cells after being treated with different concentrations of ruxolitinib for 12 or 24 hours.

Treating time *t*/h	Control group	Ruxolitinib c_B/(nmol·L^{-1})			
		50	100	250	500
12	0.145 ± 0.010	0.340 ± 0.023 **	0.410 ± 0.028 **	0.455 ± 0.031 **	0.523 ± 0.035 **
24	0.151 ± 0.011	0.357 ± 0.031 **, ΔΔ	0.451 ± 0.034 **, ΔΔ	0.534 ± 0.036 **, ΔΔ	0.589 ± 0.039 **, ΔΔ

**** - $p < 0.01$ vs. control group, ΔΔ - $p < 0.01$ vs. 12 hours group.**

Table 4. The effect of different concentrations of ruxolitinib on cell cycle of HEL cells.

Group	Control group	Ruxolitinibc_B/(nmol·L^{-1})			
		50	100	250	500
G_0/G_1	45.2 ± 3.0	67.4 ± 3.6 **	73.1 ± 3.6 **	76.3 ± 3.9 **	80.4 ± 3.7 **
G_2/M	28.0 ± 2.1	8.4 ± 1.1 **	4.6 ± 0.9 **	4.0 ± 0.8 **	3.6 ± 0.4 **

**** - $p < 0.01$ vs. control group.**

with the control group, there was a significant difference between the two groups ($p < 0.01$) (see Table 2, Figure 1).

There was a significant positive correlation between SOCS-1 or SHP-1 and the amount of mutant JAK2-V617F alleles in the newly diagnosed group (Spearman's $r = -0.649$, $p < 0.05$, and $r = -0.695$, $p < 0.05$, respectively) by Spearman's correlation analysis method.

Cell viability assay

The effects of different concentration of ruxolitinib on HEL cell viability were studied by CCK-8 assay. The

Table 5. The expression level of JAK2, SOCS-1, and SHP-1 mRNA in HEL cells after being treated with different concentrations of ruxolitinib (nmol/L) for 48 hours.

mRNA	0	50	100	250	500	F value	p-value
JAK2	0.429 ± 0.038	0.371 ± 0.027	0.249 ± 0.030	0.192 ± 0.028	0.141 ± 0.025	60.84	< 0.01
Socs-1	0.092 ± 0.009	0.153 ± 0.016	0.211 ± 0.022	0.237 ± 0.021	0.261 ± 0.031	34.20	< 0.01
Shp-1	0.089 ± 0.008	0.146 ± 0.012	0.188 ± 0.022	0.251 ± 0.027	0.354 ± 0.031	87.23	< 0.01

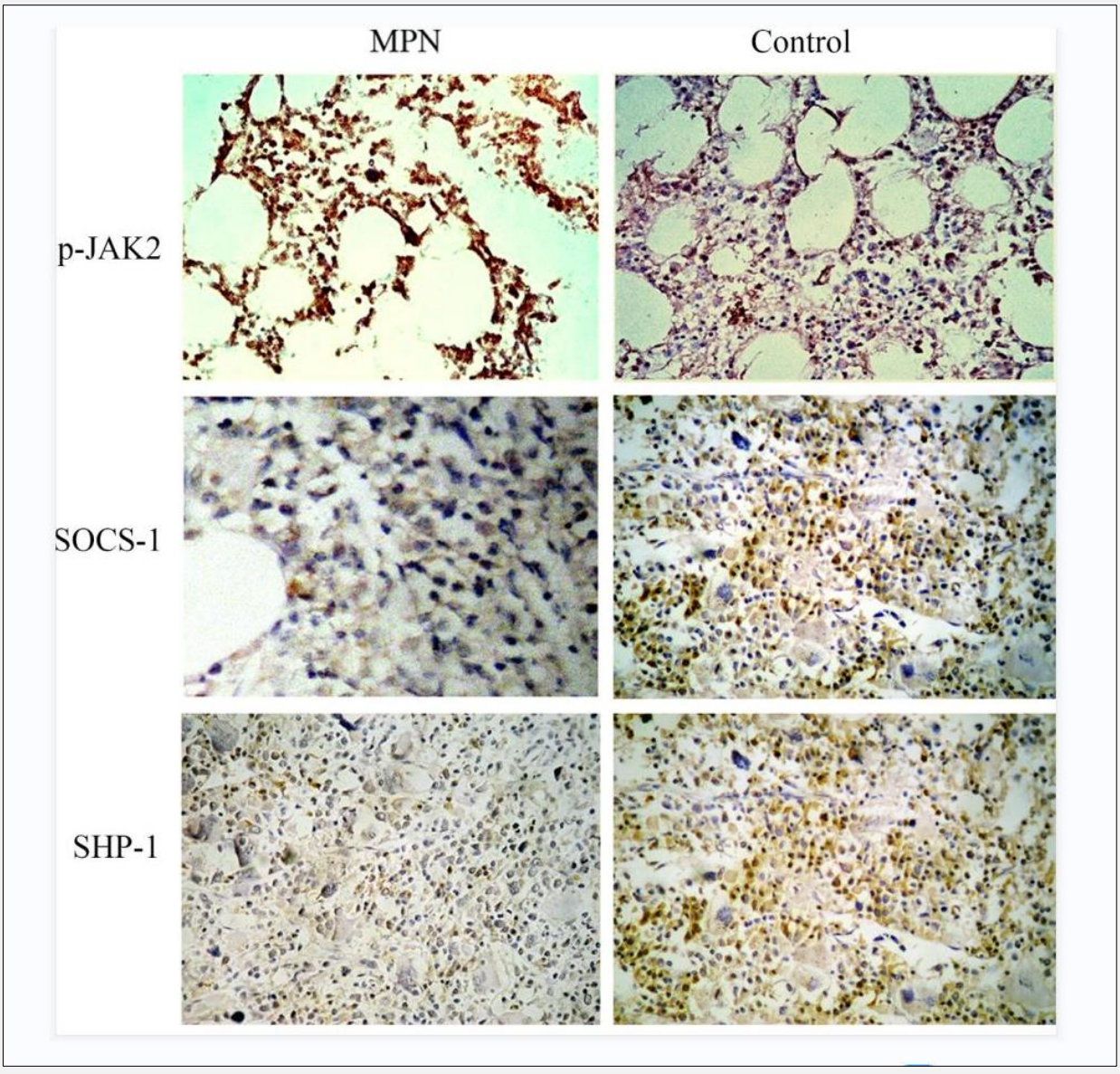

Figure 1. The expression of p-JAK2, SOCS-1, and SHP-1 in MPN patients and control patients (×400).

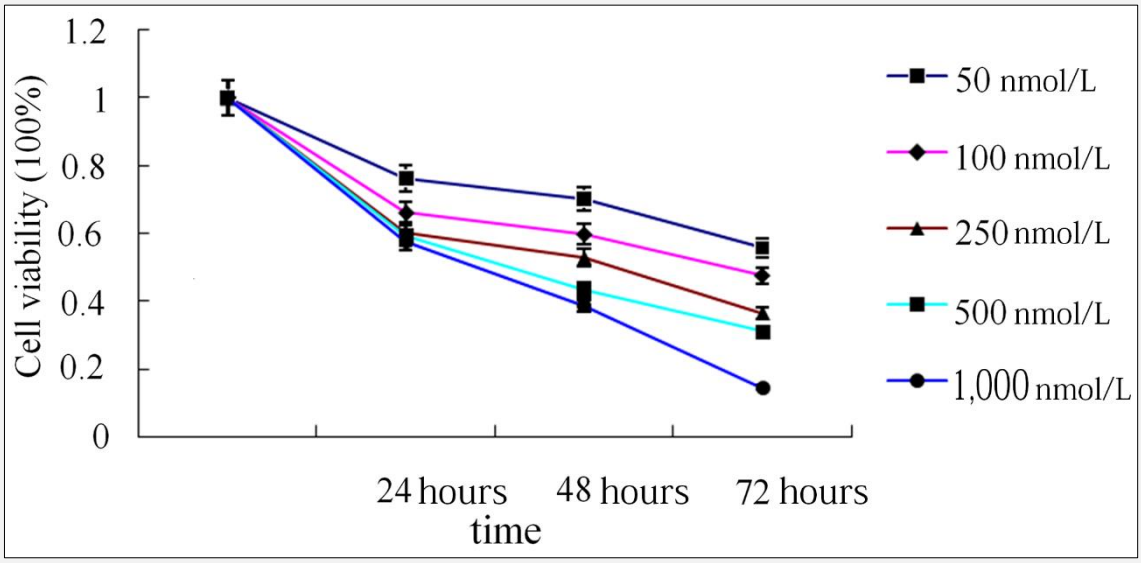

Figure 2. HEL cell viability was detected by CCK-8 assay after being treated with ruxolitinib.

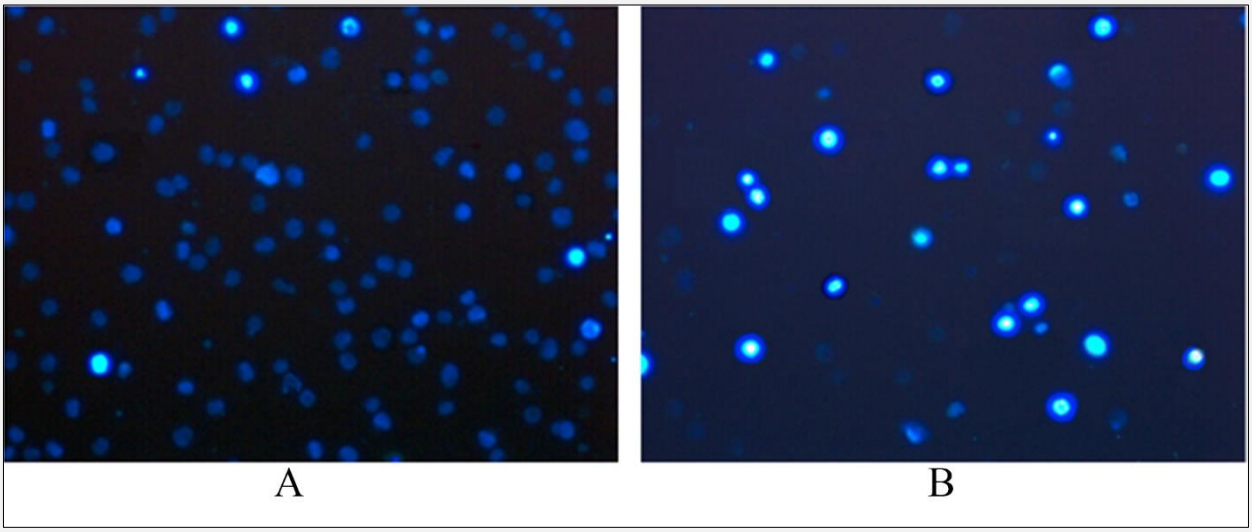

Figure 3. Apoptosis was detected by Hoechest staining in HEL cells after being treated with ruxolitinib for 48 hours.

A - control group.
B - 100 nmol/L ruxolitinib treatment group.

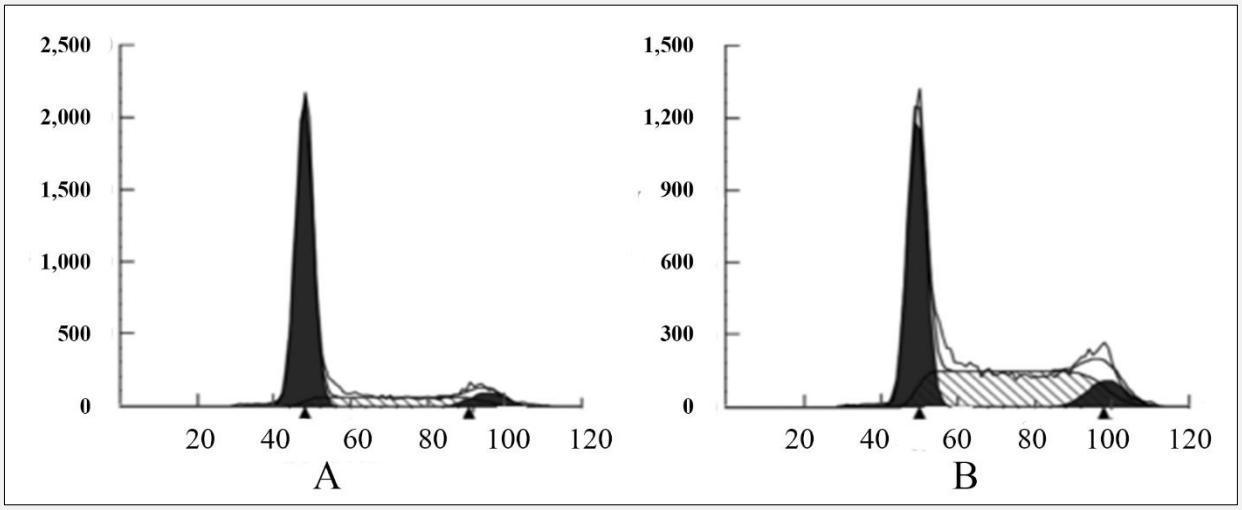

Figure 4. Cell cycle was detected by flow cytometry after HEL cells were treated with different concentrations of ruxolitinib.

A - Control group.
B - 100 nmol/L ruxolitinib treatment group.

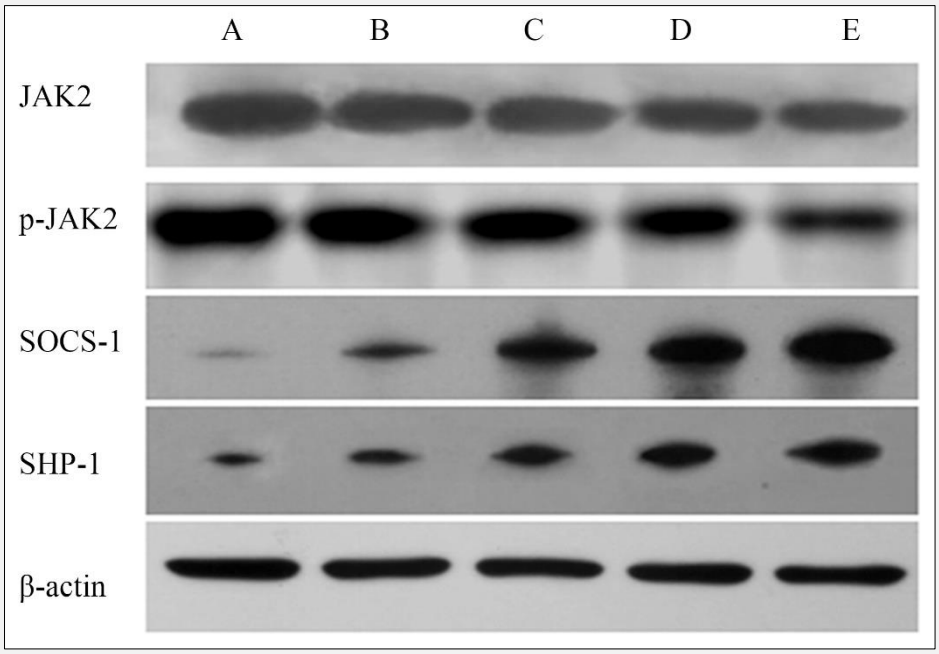

Figure 5. Protein expressions of JAK2, p-JAK2, SOCS-1, and SHP-1 in HEL cells after being treated with different concentrations of ruxolitinib.
A - 0 nmol/L, B - 50 nmol/L, C - 100 nmol/L, D - 250 nmol/L, E - 500 nmol/L by western blot method.

cell viability curve is shown in Figure 2. Different concentrations of ruxolitinib had a significant effect on the cell viability of HEL cells. The cell viability decreased as the concentration of ruxolitinib increased. The activity of HEL cells treated by the same concentration of ruxolitinib was decreased gradually with increasing time. The cell viability rate was 70.14% ± 3.21%, 59.71% ± 3.46%, 52.05% ± 2.88%, 43.31% ± 2.16%, and 38.56% ± 2.08%, respectively, after treatment with 50, 100, 250, 500, and 1,000 nmol/L ruxolitinib 48 hours late ($p < 0.05$).

Apoptosis of HEL cells

After HEL cells were treated with 100 nmol/L ruxolitinib for 48 hours, the effect on apoptosis was analyzed by Hoechst fluorescence staining. Ruxolitinib induced typical apoptosis of HEL cells, while the untreated HEL cells displayed excellent growth state. The nonapoptotic cells were light blue and rounded following Hoechst 33342 staining (Figure 3A), but the apoptotic cells were bright blue (Figure 3B) and exhibited cell shrinkage and detachment with nuclear condensation, nuclear margination, lobular nuclear, debris-like and fragmentation. The rate of bright blue cells in 100 nmol/L ruxolitinib group was 49.2% ± 1.80%, which was statistically significantly higher compared with that of the control group (10.02% ± 1.40%, $p < 0.05$).

Effect of ruxolitinib on caspase-3 and -7 protein activity

After HEL cells were treated with different concentrations of ruxolitinib for 12 or 24 hours, the dosage dependent relationship existed in the protein activity of caspase-3 and -7 ($p < 0.01$) (Table 3).

G0/G1 cell cycle arrest of HEL cells induced by ruxolitinib

After HEL cells were treated with different concentrations of ruxolitinib for 0, 24, 48 or 72 hours, cell cycle analysis showed that the percentage of HEL cells in G0/G1 phase increased significantly at each time point and each concentration from low to high by FCM. The G0/G1 phase distribution of HEL cells treated with 100 nmol/L ruxolitinib for 48 hours was increased significantly compared to that of control group (73.1% ± 3.6% vs. 45.2% ± 3.0%) (Figure 4, Table 4).

Effect of different concentrations of ruxolitinib on the expression of p-JAK2, SOCS-1, and SHP-1 mRNA in HEL cells

After HEL cells were treated with different concentrations of ruxolitinib for 48 hours, the effect on the mRNA expression was analyzed by FQ-PCR analysis. As the concentration of ruxolitinib increased, the expression level of JAK2 mRNA decreased and SOCS-1 and SHP-1 increased gradually (see Table 5, $p < 0.01$). These results indicate that ruxolitinib negatively regulates the mRNA expression of JAK2 mRNA. High concentrations of ruxolitinib can inhibit the transcription of JAK2 mRNA and enhance the expression of SOCS-1 and SHP-1 mRNA in HEL cells.

Effect of different concentrations of ruxolitinib on the expression of p-JAK2, SOCS-1, and SHP-1 protein in HEL cells by western blot

As shown in Figure 5, the expression level of JAK2 and p-JAK2 protein decreased and SOCS-1 and SHP-1 protein increased gradually when the concentration of ruxolitinib increased. β-actin was used as a loading control. The level of actin, a common internal protein, was not changed in the different concentrations of ruxolitinib treated cells. This is similar to high concentrations of ruxolitinib inhibiting JAK2 mRNA in HEL cells and increasing the transcription of SOCS-1 and SHP-1.

DISCUSSION

Currently, JAK2V617F mutation is a genetic diagnostic criterion for MPNs. Several studies had shown the JAK2V617F allele burden varied and in part contributed to the clinical course of MPNs [13] in MPN patients. The increase of JAK2V617F allele burden was correlated with prognostic variables and was likely to have a major impact on disease phenotype in MPN patients [14,15]. Otherwise, in MPNs, the aberrant and constitutive activation of the JAK/STAT pathway regulating genes might be involved in this abnormality [16-18]. SOCS-1 and SHP-1 proteins are known to suppress the JAK/STAT signaling pathway as negative regulators [19]. SOCS-1 is induced by cytokine activation of the JAK/STAT pathway and acts as a negative feedback regulator to inhibit JAK signaling [11]. SOCS protein family is important in the molecular regulation of cytokine signaling. SOCS-1 was the most important class of cytokine-inducible genes [20]. SHP-1 is a tumor suppressor gene and expressed primarily in hematopoietic stem cells [21,22]. In normal cells, SHP-1 negatively regulates JAK/STAT signaling. JAK or STAT signaling pathway can be activated by the loss of SHP-1 suppressor function in cancer cells [23].

Thus, in our study, we investigated of JAK2V617F mutated alleles and the expression of SOCS-1, SHP-1 protein in newly diagnosed JAK2V617F positive MPN patients. We found that in Chinese MPN patients, the JAK2V617F mutation burden and p-JAK2 protein expression were significantly higher than that in control group. Meanwhile, in the bone marrow biopsy specimens of the patients, the expressions of SOCS-1 and SHP-1 protein were decreased significantly compared to that in control group. In the newly diagnosed group, there was a significant positive correlation between SOCS-1 or SHP-1 and the amount of mutant JAK2-V617F alleles, suggesting the involvement of a common mechanism underlying their inactivation during MPNs.

Ruxolitinib is a potent JAK1/JAK2 inhibitor [24-26]. It competes with adenosine triphosphate binding in the

JAK2 catalytic site. Inhibition of JAK provokes a downstream hypophosphorylation of STAT [27,28]. In the present study, the ability of ruxolitinib to inhibit the apoptosis and proliferation of HEL cells was observed. Our data indicated that ruxolitinib had a significant effect on the cell viability, apoptosis, and the protein activity of caspase-3 and caspase-7 of HEL cells. The proliferation of the cells treated by different concentrations of ruxolitinib was significantly inhibited. This data demonstrates ruxolitinib induced apoptosis and reduced cell proliferation through a dose-dependent mechanism in HEL cells. Taken together, the results from the present study showed that ruxolitinib played an important role in regulating cell apoptosis and proliferation. Furthermore, same as previous study [29], the results also showed that the cell cycle of ruxolitinib treated cells was arrested at the G0/G1 phase. It is indicated that ruxolitinib induced apoptosis by blocking the HEL cell cycle at the G0/G1 phase. But unlike several previous studies, in RPMI8226 and U266 cells, after treatment with ruxolitinib and bortezomib, higher number of cells in sub-G0 phase were found, and in three acute myeloid leukemia cell lines, JAK inhibitors alter the cycle by arresting cells in different phases [30,31]. These different results maybe because the effect of JAK2 inhibitors on cell cycle level in different cell lines are associated not only with activity within JAK family, but also presumably with other off-target activities.

In this study, as aforementioned, we found significantly decreased negative regulators of SOCS-1 and SHP-1 expression in MPN compared with controls. Using RT-PCR and western blot, we could study the gene expression profile of SOCS-1 and SHP-1 in HEL cells. Our data show that ruxolitinib strongly increases mRNA and protein levels, revealing that the capacity of ruxolitinib to induce SOCS1 and SHP1 is dose-dependent. Remarkably, ruxolitinib induces a simultaneous up regulation of SOCS1 and SHP1 in all assays, indicating that co-regulation of both negative regulators may occur.

In conclusion, in newly diagnosed JAK2V617F positive MPN patients, the expressions of SOCS-1 and SHP-1 protein were decreased. Ruxolitinib played multiple roles in inhibiting the proliferation and apoptosis of HEL cells through down-regulating the activity of JAK/STAT signaling pathway by up-regulating SOCS-1 and SHP-1 gene expression.

Source of Funds:

This research was supported by the Program of the Fundamental Research of Shenzhen Science and Technology Innovation Commission (Grant No. JCYJ202103241 41007017); Supported by the Scientific Research Project of Medical and Health Institutions in Longhua of Shenzhen (Grant No. 2020034).

Declaration of Interest:

The authors declare that there are no conflicts of interest.

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