JIMA Vol. 74, No. 3 (Aug 2022) pp. 95-106.

Original

Stauprimide suppresses proliferation and survival via inhibition of IRF4 expression and activation of JNK in multiple myeloma cells

Kazuki KIYOHARA and Shigeki ITO

Hematology and Oncology, Department of Internal Medicine,
School of Medicine, Iwate Medical University, Yahaba, Japan

(Received on January 13, 2022 & Accepted on February 2, 2022)

Abstract

Multiple myeloma (MM) is a malignancy that develops from the uncontrolled proliferation of plasma cells. Although recent advances in drug development have significantly improved the overall survival rate of patients with MM, it remains incurable due to its eventual relapse. Therefore, the development of new agents is critical. Stauprimide is a staurosporine analog that inhibits the nuclear localization of the transcription factor non-metastatic cells 2 (NME2) in embryonic stem cells, thereby suppressing c-Myc transcription. Although it has anti-tumor effects in various cancer cell lines, its effects on MM cells have not been elucidated. We found that stauprimide suppressed proliferation through cell cycle arrest at the G2/M phase and induced apoptosis through poly ADP-ribose polymerase (PARP) activation in KMS-28PE and RPMI 8226 cells. Stauprimide suppressed interferon regulatory factor 4 (IRF4), which was followed by a c-Myc expression in KMS-28PE cells. Furthermore, stauprimide inhibited protein kinase Cα (PKC α) constitutive phosphorylation and induced c-Jun N-terminal kinase (JNK) activation and transient c-Jun expression in KMS-28PE cells. These results indicated that stauprimide suppresses proliferation and induces apoptosis by inhibiting IRF-4 expression and PKC α phosphorylation, and via JNK activation in myeloma cells. Stauprimide should be further evaluated as a novel agent for MM.

Key words : *stauprimide, IRF4, JNK, protein kinase C, multiple myeloma*

I. Introduction

Multiple myeloma (MM) is a B-cell malignancy characterized by unrestricted growth of monoclonal plasma cells, resulting in the synthesis of nonfunctional immunoglobulins or immunoglobulin chains. Although the introduction of novel drugs, including proteasome inhibitors, immunomodulatory drugs, and monoclonal antibodies, has improved clinical outcomes, the disease eventually relapses in most patients [1, 2]. Therefore, the identification of novel molecular targets and the development of new therapeutic agents are urgently required to improve the prognosis of patients with MM.

Interferon regulatory factor 4 (IRF4) is a transcription factor that regulates plasma cell differentiation and class-switch recombination [3]. It is essential for myeloma cell proliferation

Corresponding author: Shigeki Ito
shigei@iwate-med.ac.jp

and survival. Shaffer et al. showed that the knockdown of IRF4 using RNA interference is toxic to myeloma cells. They also identified the transcription factor Myc as a direct target of IRF4 in MM cells and discovered that IRF4 and Myc form a positive autoregulatory loop in MM cells [4]. A recent study reported that IRF4 antisense oligonucleotides impaired myeloma cell survival and suppressed IRF4 and c-Myc expression [5]. Furthermore, c-Myc is a member of the gene family that codes for helix-loop-helix leucine zipper transcription factors. It plays a crucial role in regulating cell growth, proliferation, survival, and metabolic adaptation [6] in various cancers, including leukemia and lymphoma [7]. In MM, c-Myc target genes are activated in approximately two-thirds of patients, excluding patients with monoclonal gammopathy of undetermined significance, indicating a pivotal role of c-Myc in myeloma pathogenesis [8]. A recent study reported that ablating c-Myc with small hairpin RNAs or small molecule inhibition induced MM cell death [9]. Previous studies revealed that the survivin inhibitor, YM155, suppressed the proliferation and survival of MM cells by promoting c-Myc proteasomal degradation and that a natural diterpenoid, oridonin, also induced MM cell apoptosis by triggering c-Myc degradation [10, 11]. Thus, both c-Myc and IRF4 are potential therapeutic targets for MM. Despite numerous efforts, both molecules remain undruggable.

Stauprimide is a staurosporine analog that interacts with the Myc transcription factor non-metastatic 2 (NME2), and inhibits its nuclear localization, resulting in c-Myc downregulation in embryonic stem cells [12]. Additionally, a recent study confirmed that it performs the same function in renal cancer cells [13]. The study also demonstrated that stauprimide, a small molecule that inhibits tumor growth in rodent xenograft models, targets Myc transcription in Myc-driven tumors. However, its effect on MM cells remains unclear. In this study, we demonstrated that stauprimide induces apoptosis via c-Jun N-terminal kinase (JNK) activation and cell cycle arrest at the G2/M phase and suppresses IRF4 expression followed by c-Myc and constitutive phosphorylation of PKCα in human myeloma cells.

II. Materials and Methods

1. Reagents and antibodies

Stauprimide was purchased from Cayman Chemical (Ann Arbor, MI). A stock solution was prepared by dissolving in dimethyl sulfoxide (DMSO), and working solutions were prepared in RPMI 1640 medium from this stock solution. Cells incubated with DMSO (maximum concentration) alone were treated as controls. Antibodies against myeloid cell leukemia-1 (Mcl-1), a cellular inhibitor of apoptosis protein-1 (cIAP-1), B-cell lymphoma-extra-large (Bcl-xL), cleaved caspase-3, cleaved poly ADP-ribose polymerase (PARP), c-Myc, interferon regulatory factor 4 (IRF4), phospho-extracellular signal-regulated kinase 1/2 (Erk1/2) (Thr202/Tyr204), Erk1/2, phospho-JNK (Thr183/Tyr185), protein kinase C α (PKC α), JNK and β-actin antibodies were obtained from Cell Signaling Technology (Beverly, MA). Santa Cruz Biotechnology (Santa Cruz, CA) supplied the phosho-PKCα (Ser657) and c-Jun antibodies. We purchased X-linked inhibitor of apoptosis protein (XIAP) antibodies and NME2 antibodies from BD Biosciences (San

Diego, CA) and Abcam (Cambridge, UK), respectively.

2. Cell lines and culture

Human MM cell lines (KMS-28PE and RPMI 8226) were purchased from JCRB Cell Bank (Tokyo, Japan) and cultured in RPMI 1640 (Gibco, Carlsbad, CA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Hyclone, Logan, UT), 100 U/mL penicillin, and 100 μg/mL streptomycin (Life Technologies, Grand Island, NY). Cells were cultured at 37℃ in 5% CO_2.

3. Cytotoxicity assay

KMS-28PE and RPMI 8226 cells were seeded in a 24-well plate at a density of 1×10^6 cells per well with DMSO as a control, and 0.5 μM, 1 μM, and 10 μM stauprimide as the study samples for 24 and 48 h. Cell viability was determined using the trypan blue exclusion method with a Countess II FL automated cell counter, according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA). The proliferation rate was defined as the percentage of surviving cells in each group compared with that in the untreated group.

4. Apoptosis assay

Cells (1×10^6/mL) were treated with DMSO, 0.5, 1, and 5 μM stauprimide in the presence of RPMI 1640 supplemented with 10% FBS for 24 h. Apoptosis detection was performed using the APC Annexin V staining (BioLegend, San Diego, CA) and a flow cytometer (FACSCalibur, BD Biosciences).

5. Cell cycle assay

We performed a cell cycle assay using a cell cycle assay solution (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions. Briefly, after being cultured in the absence or presence of stauprimide (1 or 5 μM) for 24 h, the KMS-28PE and RPMI 8226 cells were prepared as a cell suspension (5×10^5 cells/mL) in a 1.5 mL microcentrifuge tube. Furthermore, to examine the time course of the cell cycle distribution, the KMS-28PE cells were cultured in the presence of 5 μM stauprimide for 0, 6, 12, 18, 24 h. They were then centrifuged at 300 × g for 5 min, and the supernatant was discarded. After being washed once with 0.5 mL PBS, the cells were re-suspended in 0.5 mL PBS supplemented with 0.1% bovine serum albumin and incubated with 5 μL of the cell cycle assay solution at 37℃ for 15 min in the dark. We then analyzed the cell cycle using FACSCalibur.

6. Immunoblotting

The KMS-28PE and RPMI 8226 cells were washed twice with cold PBS after being treated with RPMI 1640 supplemented with 10% FBS in the presence or absence of stauprimide. We then prepared cell lysates using ice-cold RIPA lysis buffer (Santa Cruz Biotechnology) with 5 mM NaF, 0.5 mM sodium orthovanadate, and 1% protease inhibitors, incubated for 30 min at 4℃, and then centrifuged at 10,000 rpm for 10 min, with supernatants retained. The cell lysates were eluted by incubating them for 5 min at 95℃ in sodium dodecyl sulfate sample buffer (Bio-Rad Laboratories, Hercules, CA). The samples were loaded into a 4%–12% Tris-glycine gel (Bio-Rad Laboratories) and transferred to a polyvinylidene difluoride membrane (GE Healthcare, Buckinghamshire, UK). We conducted immunoblotting assays according to the instructions provided by the manufacturers of the antibodies. Blots were visualized using horseradish peroxidase-

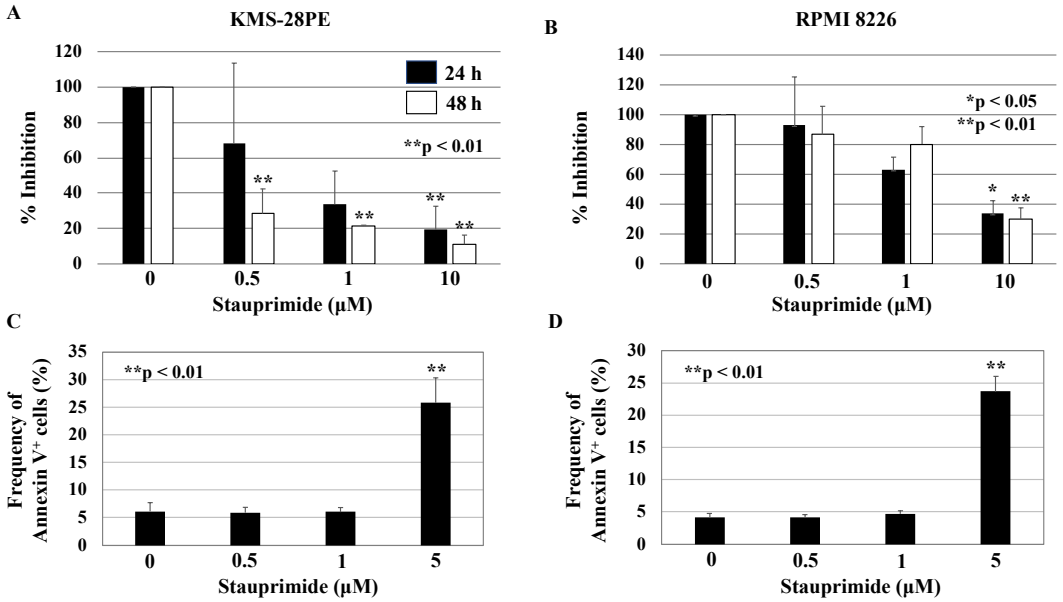

Fig. 1. Stauprimide-mediated growth inhibition and apoptosis in multiple myeloma cells.
(A) KMS-28PE and (B) RPMI 8226 cells were cultured in 24-well tissue culture plates treated with and without stauprimide at the indicated concentrations for 24 and 48 h. Cell viability was measured using the trypan blue exclusion method with Countess II FL automated cell counter. Data are expressed as the mean percentage of the control cells representing the average of triplicate samples. Error bars represent one standard deviation (SD) from the mean of the triplicate wells. The results are representative of three independent experiments. (C) KMS-28PE and (D) RPMI 8226 cells were cultured in 24-well tissue culture plates for 24 h in the absence or presence of stauprimide at the given concentrations. The degree of apoptosis was measured using flow cytometric analysis with annexin V staining. Data represent the average of triplicate samples, and error bars represent one SD from the mean of the triplicate samples. The results are representative of two independent experiments ($^{*}p < 0.05$, $^{**}p < 0.01$).

conjugated secondary antibodies and an enhanced chemiluminescence detection system (ECL plus; GE Healthcare). We incubated the membranes in stripping buffer (Pierce, Rockford, IL) to re-probe with other antibodies, according to the manufacturer's instructions. To comapre the c-Myc protein levels, mean intensities of c-Myc and β-actin were measured using ImageJ software. Then, each c-Myc expression level relative to control (0 μM) were caluculated as c-Myc protein expression.

7. Statistical analysis

We conducted the statistical analysis using JMP11 (SAS Institute, Cary, NC). The data obtained were expressed as mean ± standard deviation (SD). Statistical differences between test groups were analyzed using the one-way analysis of variance (ANOVA) with the Bonferrini post-test. p-values < 0.05 were considered statistically significant.

III. Results

1. Stauprimide inhibits proliferation and induces apoptosis in myeloma cell lines

We initially examined the effect of stauprimide treatment and discovered that it significantly inhibited the proliferation of KMS-28PE and RPMI 8226 cells after 24 h

and 48 h (Fig. 1A, B). However, the sensitivity level of these cell lines differed, with KMS-28PE cells being more sensitive to stauprimide than RPMI 8226 cells. Next, we examined the frequency of cell death using flow cytometric analysis with annexin V staining of cells treated or not treated with stauprimide (0.5, 1, and 5 μM) for 24 h. Because the autofluorescence signal of stauprimide in treated cells overlapped fluorescence signals of propidium iodide or 7-aminoactinomycin D (7-AAD), we performed the analysis using only APC annexin V staining. The number of annexin V+ cells in the presence of 5 μM stauprimide was significantly higher than in the absence or presence of 0.5 and 1 μM stauprimide ($p < 0.01$, Fig. 1C, D). These results suggested that stauprimide suppressed cell proliferation and induced apoptosis in KMS-28PE and RPMI 8226 cells.

2. Stauprimide induces cell cycle arrest in the G2/M phase in myeloma cell lines

Next, we examined whether stauprimide affected the cell cycle of myeloma cells. When KMS-28PE cells were treated with 1 μM and 5 μM stauprimide for 24 h, the percentage of G2/M phase cells significantly increased from 11.1 ± 1.0% in the control group to 14.8± 0.8 % in samples treated with 1 μM stauprimide and 37.3 ± 1.8% with 5 μM stauprimide ($p < 0.05$, $p < 0.01$, respectively, Fig. 2A, B). Conversely, the percentage of G1 phase cells decreased from 45.6% ± 0.1% in the control cells to 5.9 ± 0.4% in the group treated with 5 μM stauprimide ($p < 0.01$, Fig. 2A, B). When RPMI 8226 cells were treated with 1 μM and 5 μM stauprimide for 24 h, the percentage of G2/M phase cells increased from 17.7 ± 1.0% in the control group to 22.0 ± 0.8% in the group treated with 1μM stauprimide and 44.0 ± 0.9% in the group treated with 5 μM stauprimide ($p < 0.01$, Fig. 2C, D). In contrast, the percentage of G1 phase cells significantly decreased from 53.7 ± 3.1% to 9.5 ± 0.1% when treated with 5 μM stauprimide for 24 h ($p < 0.01$, Fig. 2C, D). We then examined the time course of cell cycle distribution in KMS-28PE cells treated with 5 μM stauprimide. As shown (Fig. 2E), the percentage of G2/M phase cells significantly increased from 17.8% at 0 h to 24.0% at 6 h, 47.1% at 12 h, 48.9% at 18 h, and 51.2% at 24 h in KMS-28PE cells treated with 5 μM stauprimide. Additionally, the percentage of sub G1 phase cells increased from 3.0% at 0 h to 9.6% at 18 h and 9.9% at 24 h.

3. Stauprimide inhibits IRF4 protein expression followed by c-Myc in KMS-28PE cells

As stauprimide suppresses c-Myc expression by inhibiting nuclear localization of NME2, we analyzed for similar effects in MM cells (Fig. 3A, B). Treatment with 10 μM stauprimide for 24 h inhibited c-Myc protein expression in KMS-28PE and RPMI 8226 cells. Alternatively, treatment with 5μM stauprimide for 24 h suppressed IRF4 expression in both cell lines; however, the effect was more prominent in KMS-28PE compared to RPMI 8226 cells. Next, we analyzed the nuclear and cytoplasmic localization of NME2 in KMS-28PE cells using immunoblot. However, the nuclear localization of NME2 was not observed in either stauprimide-treated or untreated cells, despite a high amount of NME2 being detected in the cytoplasm (data not shown). Additionally, treatment with at least 5 μM stauprimide for 24 h triggered PARP in both cell lines (Fig. 3A).

Furthermore, we analyzed the effects

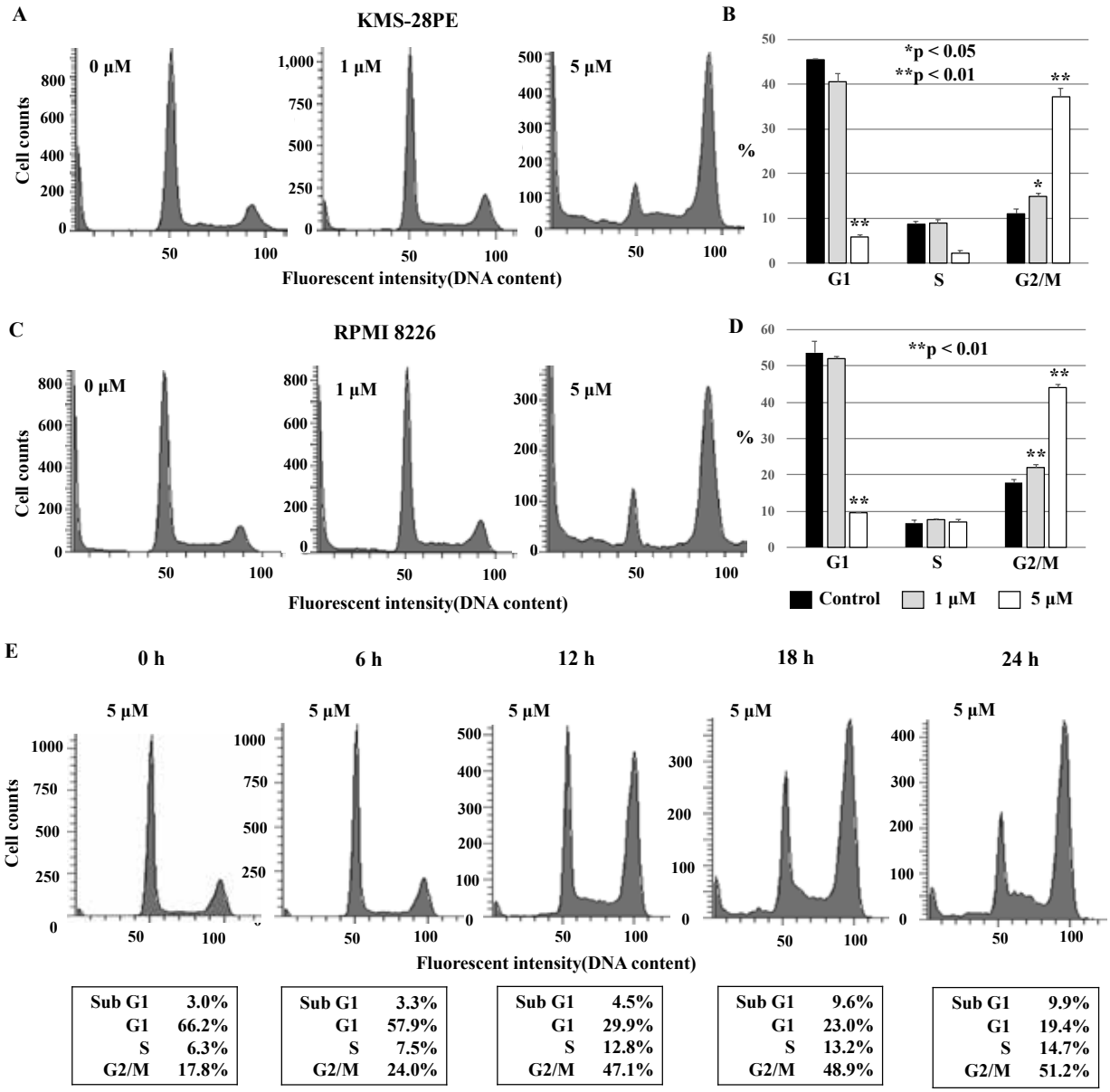

Fig. 2. Effects of stauprimide on the cell cycle in KMS-28PE and RPMI 8226 cells.

KMS-28PE and RPMI 8226 cells were cultured in 24-well tissue culture plates with and without stauprimide at the given concentrations for 24 h. The frequency of G1, S, and G2/M phases was measured using flow cytometric analysis with the Cell Cycle Assay Solution Deep Red staining. (A) Representative cell cycle histograms of KMS-28PE cells showing the frequency of G1, S, and G2/M phases. (B) Data represent the average of triplicate samples, and error bars represent one SD from the mean of triplicate wells. (C) Representative cell cycle histograms of RPMI 8226 cells depicting the frequency of G1, S, and G2/M phases. (D) Data represent the average of triplicate samples, and error bars represent one SD from the mean for triplicate wells. The results are representative of two independent experiments ($^{*}p < 0.05$, $^{**}p < 0.01$). (E) KMS-28PE cells were cultured in 24-well tissue culture plates with 5 μM of stauprimide for 0, 6, 12, 18, and 24 h. The frequency of sub G1, G1, S, and G2/M phases was measured using flow cytometric analysis. Representative cell cycle histograms and each frequency of Sub G1, G1, S, and G2/M phase are shown.

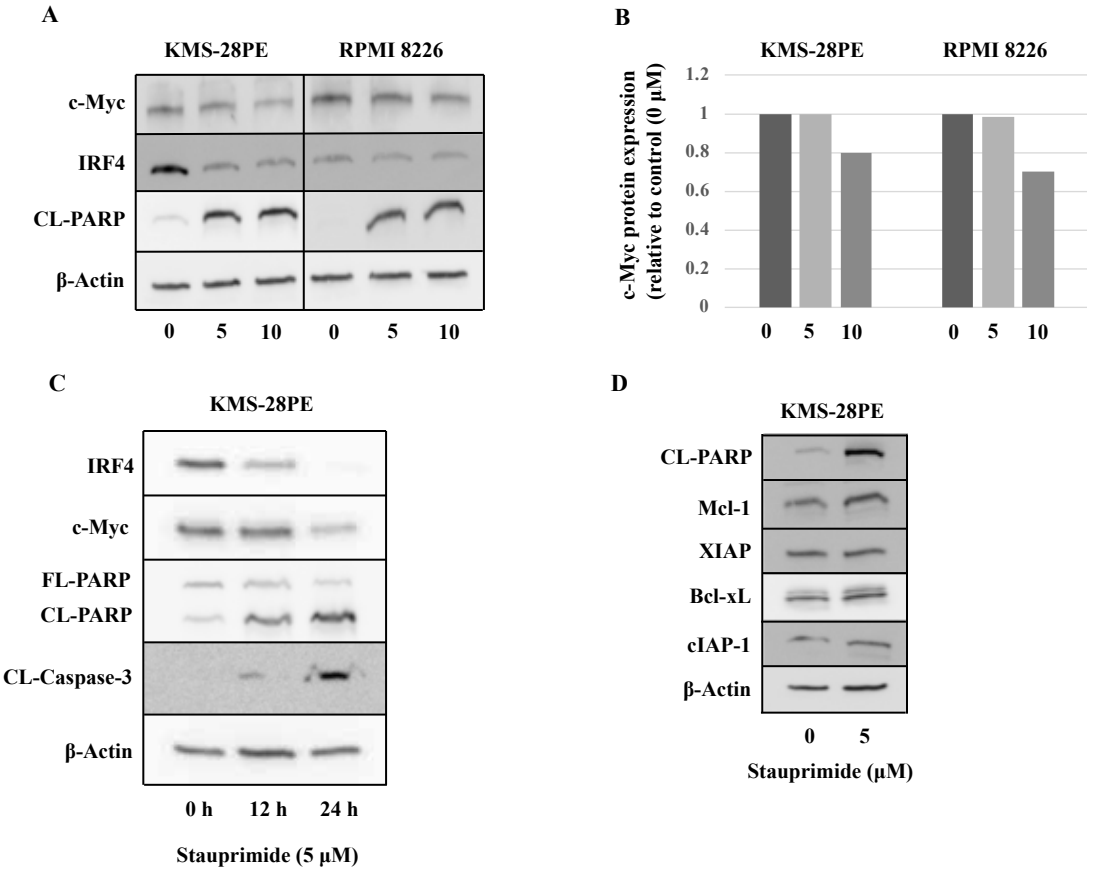

Fig. 3. Stauprimide suppresses c-Myc and IRF4 expression and activates PARP in KMS-28PE and RPMI 8226 cells.
(A) KMS-28PE and RPMI 8226 cells were treated with stauprimide (5 or 10 μM) for 24 h. Immunoblotting was performed with specific antibodies against c-Myc, IRF4, cleaved PARP, and β-actin. (B) c-Myc protein expression level relative to β-actin were measured using ImageJ. (C) KMS-28PE cells were treated with 5 μM stauprimide for 0, 12, and 24 h. Specific antibodies against c-Myc, cleaved PARP, cleaved caspase-3, and β-actin were used for immunoblotting. (D) KMS-28PE cells were cultured in the absence or presence of stauprimide (5 μM) for 24 h. Immunoblotting was performed with specific antibodies against cleaved PARP, Mcl-1, XIAP, Bcl-xL, cIAP-1, and β-actin. All the immunoblots shown are representative of two independent experiments. CL-PARP; cleaved PARP, FL-PARP; full-length PARP, CL-Caspase-3; cleaved Caspase-3.

of stauprimide on the time course of IRF4 and c-Myc expression, as well as caspase-3 and PARP activation in KMS-28PE cells. As shown in (Fig. 3C), stauprimide inhibited IRF4 expression followed by c-Myc. Also, treatment with 5 μM stauprimide for at least 12 h induced PARP and caspase-3 activation in KMS-28PE cells.

To better understand the mechanism by which stauprimide-induced apoptosis, we examined the effects of stauprimide on the expression of representative anti-apoptotic proteins. However, we did not observe any suppressive effect on anti-apoptotic proteins including Mcl-1, XIAP, Bcl-xL, and cIAP-1 (Fig. 3D).

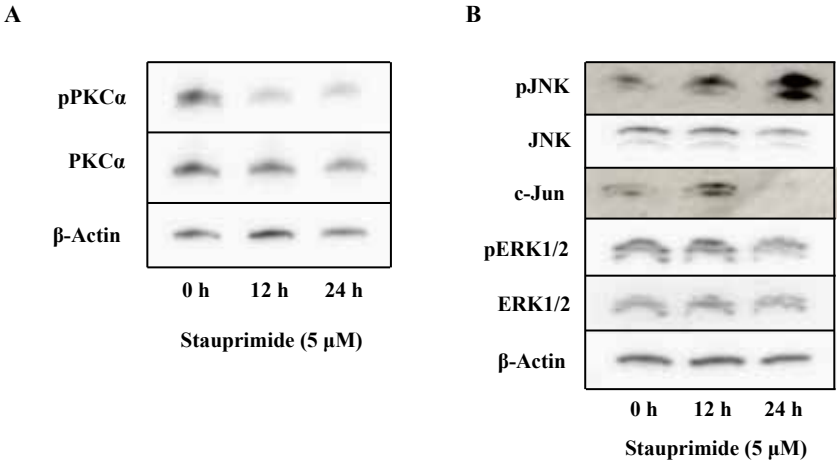

Fig. 4. Stauprimide suppresses PKC α phosphorylation and activates JNK/Jun pathway in KMS-28PE cells.
KMS-28PE cells were treated with 5 μM stauprimide for 0, 12, and 24 h. (A) Immunoblotting was performed with specific antibodies against phospho-PKCα, PKCα, and β-actin. (B) Immunoblotting was performed with specific antibodies against phospho-JNK, JNK, c-Jun, phosphor-Erk1/2, Erk1/2, and β-actin. All the immunoblots shown are representative of two independent experiments.

4. Stauprimide suppresses PKC α phosphorylation and induces JNK activation in KMS-28PE cells

We investigated the mechanism by which stauprimide induces apoptosis. Stauprimide is a staurosporine analog, and staurosporine is a potent inhibitor of PKC, a serine-threonine kinase involved in signal transduction in response to growth factors, hormones, and neurotransmitters [14]. A recent report showed that PKC inhibitor induces JNK-dependent apoptosis of human myeloma cell lines and primary MM cells [15]. We initially examined if stauprimide suppresses PKC α phosphorylation in KMS-28PE cells. We found that treatment with 5 μM stauprimide for 12 h inhibited PKC α constitutive phosphorylation (Fig. 4A). Additionally, treatment with stauprimide induced JNK phosphorylation at Thr183/Tyr185 in KMS-28PE cells (Fig. 4B). Because the phosphorylated JNK phosphorylates and transactivates c-Jun, we analyzed the c-Jun protein expression. Although c-Jun was significantly induced 12 h after treatment with stauprimide, the expression was transient (Fig. 4B). However, stauprimide did not affect Erk1/2 phosphorylation (Fig. 4B).

IV. Discussion

In this study, we demonstrated for the first time that stauprimide dose-dependently inhibited cell proliferation and induced apoptosis in KMS-28PE and RPMI 8226 cells (Fig. 1). This finding was consistent with previous studies on other cancer cell types, including colorectal, cervical, and liver cancers [16-18]. However, the frequency of apoptotic cells did not increase while cell proliferation was suppressed when treated with 1 μM stauprimide for 24 h. These results indicated that treatment with stauprimide

at lower concentrations results in cell cycle arrest in MM cells. To test this hypothesis, we investigated the effect of stauprimide on the cell cycle of these cells and found that it significantly induced cell cycle arrest at the G2/M phase in the presence of 1 μM stauprimide in KMS-28PE and RPMI 8226 cells (Fig. 2). Additionally, the G2/M cell cycle arrest was more prominent with 5 μM stauprimide. The mechanism by which stauprimide induces cell cycle arrest at the G2/M phase has not been elucidated. Stauprimide has been reported to bind to NME2 and inhibit its nuclear localization, resulting in the suppression of c-Myc transcription in embryonic stem and cancer cells [12, 13]. NME2 overexpression was reported in many cancer cell lines and cancer tissues, such as chronic myeloid leukemia and giant cell tumor [19, 20]. Furthermore, a proteomic study revealed NME2 overexpression in the primary MM cells of patients [21], indicating that NME2 plays a significant role in MM pathophysiology. Li et al. recently demonstrated that the downregulation of NME2 expression using small interfering RNA (siRNA) could arrest the cell cycle in the G2/M phase in osteosarcoma cells [22], thus suppressing MM cell proliferation at least in part. We examined whether stauprimide treatment inhibits nuclear localization and protein expression level of NME2 in KMS-28PE cells. However, we did not detect its nuclear localization regardless of the treatment and reduction of NME2 protein expression level after the treatment (data not shown). These findings indicate that cell cycle arrest at the G2/M phase induced by stauprimide is not associated with translocation and protein expression level of NME2 in KMS-28PE cells. The precise mechanism of the G2/M cell cycle arrest induced by stauprimide should be examined in future studies.

To better comprehend the mechanism by which stauprimide inhibits cell proliferation and induces apoptosis in MM cells, we used immunoblotting to assess the effect of stauprimide on c-Myc and IRF4 protein expression, both of which are crucial transcription factors for myeloma cell survival, as well as apoptosis-associated proteins. Stauprimide inhibited IRF4 and c-Myc protein expression while activating PARP in KMS-28PE and RPMI 8226 cells (Fig. 3A). We concentrated on KMS-28PE cells because they were more sensitive to stauprimide than RPMI 8226 cells. Interestingly, stauprimide inhibited IRF4 protein expression followed by c-Myc in KMS-28PE cells (Fig. 3C), indicating its potential as an IRF4 inhibitor rather than c-Myc in MM cells. Furthermore, stauprimide activated caspase-3 in KMS-28PE cells (Fig. 3C), indicating that stauprimide caspase-dependently induces apoptosis at least in part. Alternatively, stauprimide exhibited no effect on expression levels of anti-apoptotic proteins, including Bcl-xL, Mcl-1, XIAP, and cIAP-1 in KMS-28PE cells (Fig. 3D). Because the mechanism by which stauprimide-induced apoptosis has not been fully elucidated, we focused on a protein kinase C (PKC) signal transduction pathway, which is a molecular target of staurosporine. PKC is a serine/threonine kinase responsible for signal transduction in response to growth factors, hormones, and neurotransmitters [23]. Sharkey et al. demonstrated that the PKC inhibitor PKC412 induced apoptosis of MM cell

lines and primary MM cells with variable efficacy [15]. Additionally, PKC412 induced c-Jun expression and the JNK inhibitor suppressed c-Jun expression and abrogated PKC412-induced apoptosis. In this study, we found that stauprimide suppressed constitutive PKC α phosphorylation in KMS-28PE cells (Fig. 4A). Additionally, stauprimide-induced persistent JNK phosphorylation and transient c-Jun expression in KMS-28PE cells (Fig. 4B). Recently, Zhang et al. demonstrated that IRF4 promotes MM cell proliferation via the JNK pathway [24]. The study reported that silencing IRF4 could inhibit MM cell proliferation and induce apoptosis, partly by activating the JNK/Jun pathway. These findings imply that stauprimide-induced apoptosis is associated with JNK activation by inhibiting the PKC α pathway and IRF4 expression. Further study is warranted to understand the precise mechanism of apoptosis induced by stauprimide in MM.

Conclusively, stauprimide inhibits proliferation and induces apoptosis in MM cells by suppressing IRF4 expression and the PKC α transduction pathway in MM cells. Stauprimide should be further evaluated as a novel agent for MM.

Acknowledgments

We thank Ms. Yuki Seki for her technical assistance. We thank Enago for English language editing.

Funding

This study was supported by a research grant (20113 [S.I.]) from the Japanese Society of Hematology.

Author contributions

K.K. and S.I. performed experiments, designed research, wrote the paper, and discussed the manuscript.

Conflict of interest: The authors have no conflict of interest to declare.

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岩手医誌 74 巻，3 号（令和 4 年 8 月）95-106 頁.

スタウプリミドは骨髄腫細胞株において IRF4 の発現抑制と JNK の活性化を介して 増殖と生存を抑制する

清原千貴，伊藤薫樹

岩手医科大学医学部，内科学講座血液腫瘍内科分野

(Received on January 13, 2022 & Accepted on February 2, 2022)

要旨

多発性骨髄腫は，難治性の形質細胞性腫瘍であり，治癒が困難であるため新規薬剤の開発が求められている．スタウプリミドは転写因子 non-metastatic cells 2 と結合し，c-Myc の転写を抑制する．c-Myc は骨髄腫細胞の生存に重要な転写因子であるためスタウプリミドの抗骨髄腫細胞効果を検討した．骨髄腫細胞株 KMS-28PE と RPMI 8226 において，スタウプリミドは増殖を抑制しアポトーシスを誘導した．また，細胞周期解析では G2/M 期停止を誘導した．KMS-28PE ではインターフェロン制御因子 4（IRF-4）および c-Myc の発現を抑制し，caspase 3 の活性化を認めた．さらに，protein kinase C α（PKCα）のリン酸化を阻害し，c-Jun N-terminal kinase（JNK）の活性化と c-Jun の発現を誘導した．以上から，スタウプリミドは骨髄腫細胞において，IRF-4 の発現および PKCα のリン酸化を抑制，JNK の活性化を介して増殖を抑制し，カスパーゼ依存性にアポトーシスを誘導することが示唆された．