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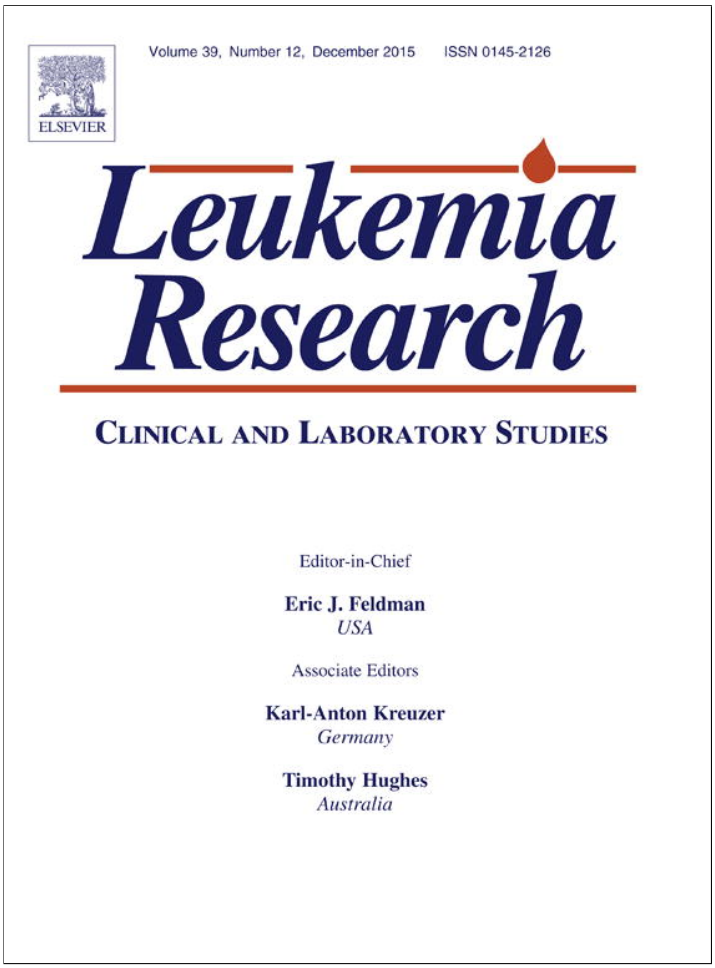

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YM155 suppresses cell proliferation and induces cell death in human adult T-cell leukemia/lymphoma cells

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ABSTRACT

Adult T-cell leukemia (ATL) is an aggressive malignancy of peripheral T cells infected with human T-cell leukemia virus type 1 (HTLV-1). The prognosis of patients with aggressive ATL remains poor because ATL cells acquire resistance to conventional cytotoxic agents. Therefore, development of novel agents is urgently needed. We examined the effects of YM155, sepantronium bromide, on cell proliferation and survival of ATL or HTLV-1-infected T-cell lines, S1T, MT-1, and MT-2. We found that YM155 suppressed cell proliferation in these cells and induced cell death in S1T and MT-1 cells. Both real-time quantitative polymerase chain reaction and immunoblot analyses showed suppression of survivin expression in S1T, MT-1, and MT-2 cells. In addition, we observed the cleavage of caspase-3 and poly(ADP-ribose) polymerase in YM155-treated S1T and MT-1 cells, indicating that YM155 induces caspase-dependent apoptosis in these cells. To clarify the mechanism of drug tolerance of MT-2 cells in terms of YM155-induced cell death, we examined intracellular signaling status in these cells. We found that STAT3, STAT5, and AKT were constitutively phosphorylated in MT-2 cells but not in S1T and MT-1 cells. Treatment with YM155 combined with the STAT3 inhibitor S3I-201 significantly suppressed cell proliferation compared to that with either YM155 or S3I-201 in MT-2 cells, indicating that STAT3 may play a role in tolerance of MT-2 cells to YM155 and that STAT3 may therefore be a therapeutic target for YM155-resistant ATL cells. These results suggest that YM155 presents potent antiproliferative and apoptotic effects via suppression of survivin in ATL cells in which STAT3 is not constitutively phosphorylated. YM155 merits further investigation as a potential chemotherapeutic agent for ATL.

1. Introduction

Adult T-cell leukemia (ATL) is a peripheral T lymphocytic malignancy infected with human T-cell leukemia virus type 1 (HTLV-1) [1–3]. Although several therapeutic approaches have been used, aggressive ATL, including acute and lymphoma types as well as the chronic type with poor prognostic factors, has a poor prognosis with a median survival duration of 13 months because of its resistance to conventional chemotherapy [4]. The poor prognosis and frequent relapse of patients highlight the need for development of new approaches and novel therapeutic targets for ATL. A recent study reported on gene expression profiling in ATL cells [5]. In the study, leukemia cells were characterized by a striking increase in genes linked to the cell cycle (CDC2, cyclin B), hypercalcemia (RANKL, PTHLH), tyrosine kinase signaling (SYK, LYN) pathways, and anti-apoptosis (BIRC5/survivin) factors. Notably, transfection of ATL cells with survivin small hairpin RNA (shRNA) decreased survivin expression and cell viability [5]. Survivin is a member of the inhibitor of apoptosis protein (IAP) family and has been implicated in both suppression of apoptosis and regulation of mitosis [6,7]. Molecular mechanism by which survivin acts in the cells is not fully elucidated. Survivin acts as a subunit of the chromosomal passenger complex, including aurora kinase B, inner centromere protein antigens and borealin, and is essential for proper chromosome segregation and cytokinesis [8]. On the other hand, the role of survivin in the inhibition of apoptosis has a similar degree of complexity, connecting to multiple parallel pathways that regulate gene expression, protein–protein interactions and mitochondrial function. Regarding protein–protein interactions, survivin exhibits parallel interactions with other members of the IAP gene family. Assembly of the survivin-X-linked inhibitor of apoptosis protein (XIAP) complex in vivo abolishes XIAP-associated anti-apoptotic

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function [9]. In the cytosol, survivin associates with the hepatitis B X-interacting protein (HBXIP), and this complex, but either protein alone, binds caspase 9 and inhibits mitochondrial apoptosis [10].

Because survivin is upregulated in many human tumors and plays a key role in cancer progression and drug resistance, survivin is considered to be a potential therapeutic target in cancers, including ATL [5,11,12].

YM155 is a novel small molecule that suppresses transactivation of survivin and induces apoptosis in various cancer cells regardless of p53 status [13]. Phase I and II trials with YM155 have reported on its safety and tolerability in patients with melanoma, prostate cancer, non-small cell lung cancer, and lymphomas [14–17]. In a murine model using human ATL cells, YM155 alone and in combination with anti-CD52 monoclonal antibody alemtuzumab (Campath-1H) was recently shown to have therapeutic efficacy; thus, prolonging survival [18]. However, the exact cytotoxic mechanism of YM155 remains unclear in ATL. Here, we report that YM155 induces caspase-dependent apoptosis at least in part by the transcriptional repression of survivin and that STAT3 phosphorylation may be associated with drug tolerance of ATL or HTLV-1-infected T-cells to YM155.

2. Materials and methods

2.1. Reagents and antibodies

YM155 was purchased from Selleck Chemicals (Houston, TX). S3I-201 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Caspase-3 inhibitor Z-DEVD-FMK was purchased from R&D Systems (Minneapolis, MN). Antibodies against phospho-specific anti-STAT3 (Tyr^{705}), STAT5 (Tyr^{694}), and AKT (Ser^{473}), as well as anti-STAT3, STAT5, cellular inhibitor of apoptosis protein (cIAP)-1, cIAP-2, myeloid cell leukemia sequence (Mcl)-1, XIAP, cleaved poly(ADP-ribose) polymerase (PARP), cleaved caspase-3 and β-actin were obtained from Cell Signaling Technology (Beverly, MA). Antibodies against Bcl-2 were purchased from Santa Cruz Biotechnology (Dallas, TX). Antibodies against Tax were purchased from abcam (Cambridge, UK).

2.2. Cell lines

Human ATL cell lines S1T and MT-1 were kindly provided by Dr. Kozako (Fukuoka University, Fukuoka, Japan) and purchased from JCRB Cell Bank (Osaka, Japan), respectively. Both S1T and MT-1 cells are non-Tax-producing T-cell lines established from peripheral mononuclear cells of ATL patients [19,20]. MT-2 cells were kindly provided by Dr. Kibata (Hayashibara Biochemical Laboratory, Okayama, Japan); these cells were derived from normal human leukocytes transformed by leukemic T cells of a patient with ATL [21]. These cells were cultured in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco), and 2 mM of glutamine (Gibco) in a 5% CO_2 incubator at 37 °C.

2.3. Cell proliferation assay and cell death assay

After cultivation of cells (2.5×10^5/mL) in RPMI 1640 medium supplemented with 10% FBS in the presence or absence of YM155 or S3I-201 for 24 h and 48 h, cell proliferation was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

For the apoptosis assay, one million cells were incubated in 4 mL of RPMI 1640 medium with 10% FBS with or without 50 nM of YM155 for 24 h and 48 h without medium exchange. Two hundred thousand cells were stained with annexin V and propidium iodide (PI) according to the manufacturer's instruction (BD Biosciences, San Diego, CA). The percentage of annexin V-positive and/or PI-positive cells was analyzed by FACS calibur (Becton Dickinson, LA). To confirm caspase-dependent apoptosis by YM155, we treated each cell lines with 10 nM of YM155 and/or 100 μM of caspase-3 inhibitor Z-DEVD-FMK for 48 h, and cell proliferation and cell death were measured by the MTT and apoptosis assays, respectively.

2.4. Synergy assays

The effects of drug combinations were evaluated using Chou–Talalay median effect analysis [22]. Cells were treated with each drug alone and in combination in two independent 96-well plates, and proliferation was measured by the MTT assay. The Chou–Talalay method was employed to calculate combination index (CI), with the CI values of <0.9 considered as evidence of synergy; 0.9–1.1, additive effects; CI >1.1, anatagonism [22].

2.5. cDNA synthesis and real-time transcription-PCR quantification

The total RNAs were isolated using the RNeasy Mini kit (QIAGEN Sciences, MD). RNA (1 μg) was reverse transcribed using a first-strand cDNA synthesis kit (QIAGEN Sciences, MD). The first-strand cDNA was assayed by real-time reverse transcription (RT)-PCR (Light-Cycler, Roche Applied Science, Indianapolis, IN) according to a technical brochure of the company. GAPDH internal control primers were purchased from Nihon Gene Research Laboratories Inc. (Sendai, Japan). SYBR green (Roche Diagnostics, Indianapolis, IN) was used as a probe and the ratio of survivin to GAPDH was calculated.

2.6. Immunoblotting

After being treated with RPMI 1640 medium supplemented with 10% FBS in the presence or absence of YM155 or S3I-201 for the indicated periods, the cells were washed twice in cold PBS. Cell lysates were then prepared using ice-cold lysis buffer (0.5% NP-40, 10 mM Tris base, 200 mM NaCl, 10% glycerol, 5 mM NaF, 0.5 mM sodium orthovanadate, pH 7.4) with 1% protease inhibitor cocktail (EMD Biosciences, San Diego, CA), incubated for 30 min at 4 °C and then centrifuged at 4066 × *g* for 10 min, and the supernatants were retained. Cell lysates were treated with SDS sample buffer (Pierce, Rockford, IL) for 5 min at 95 °C. Samples were loaded onto a 12% SDS-polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA). After the electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane (GE Healthcare, Buckinghamshire, UK). Immunoblotting was then performed according to the manufacturer's instructions. Blots were visualized using horseradish peroxidase-conjugated secondary antibodies and an enhanced chemiluminescence detection system (ECL plus; GE Healthcare, Buckinghamshire, UK). To reprobe with other antibodies, membranes were incubated in stripping buffer (Pierce, Rockford, IL) according to the manufacturer's instructions.

2.7. Statistical analysis

The data obtained are expressed as means ± standard deviation (SD). Differences between tests groups were analyzed by Wilcoxon test. *P*-values < 0.05 were considered statistically significant. We used JMP® 11 (SAS Institute Inc., Cary, NC, USA) for statistical analysis.

3. Results

3.1. YM155 suppresses cell proliferation in all cell lines and induces cell death in S1T and MT-1 cells but not in MT-2 cells

We examined whether YM155 suppresses the proliferation of S1T, MT-1, and MT-2 cells. As shown in Fig. 1A, YM155 treatment inhibited the proliferation of these cells in a dose-dependent manner. Each half maximal inhibitory concentration (IC50) was approximately 30 nM, 50 nM, and not reached in S1T, MT-1, and MT-2 cells treated with YM155 for 24 h, respectively. In addition, it was approximately 10 nM, 30 nM, and 500 nM in S1T, MT-1, and MT-2 cells treated with YM155 for 48 h, respectively. The complete inhibitory effect was not observed in MT-2 cells treated with YM155 even at the highest concentration (10 μM) for 48 h. Because S1T and MT-1 cells were much more sensitive to YM155 compared to MT-2 cells according to the results from proliferation assay, we used 50 nM of YM155 in the following experiments. We next examined whether YM155 induced cell death in these cells. The frequency of cell death was evaluated using flow cytometric analysis with annexin V and PI staining of cells treated in the presence or absence of 50 nM YM155 for 24 h and 48 h. The frequency of annexin V^+ and/or PI^+ cells in the presence of YM155 was significantly higher than that in the absence of YM155 in S1T and MT-1 cells (Fig. 1B and C). In contrast, no increase in annexin V^+ cells was observed in MT-2 cells by 50 nM of YM155 treatment, indicating the tolerance of MT-2 cells to YM155-induced cell death.

3.2. YM155 suppresses survivin expression and induces caspase-dependent apoptosis

We next examined the effects of YM155 on the expression of anti-apoptotic proteins in S1T, MT-1, and MT-2 cells. We examined whether YM155 induces transcriptional repression of survivin. As shown in Fig. 2A, treatment with YM155 (50 nM) suppressed the

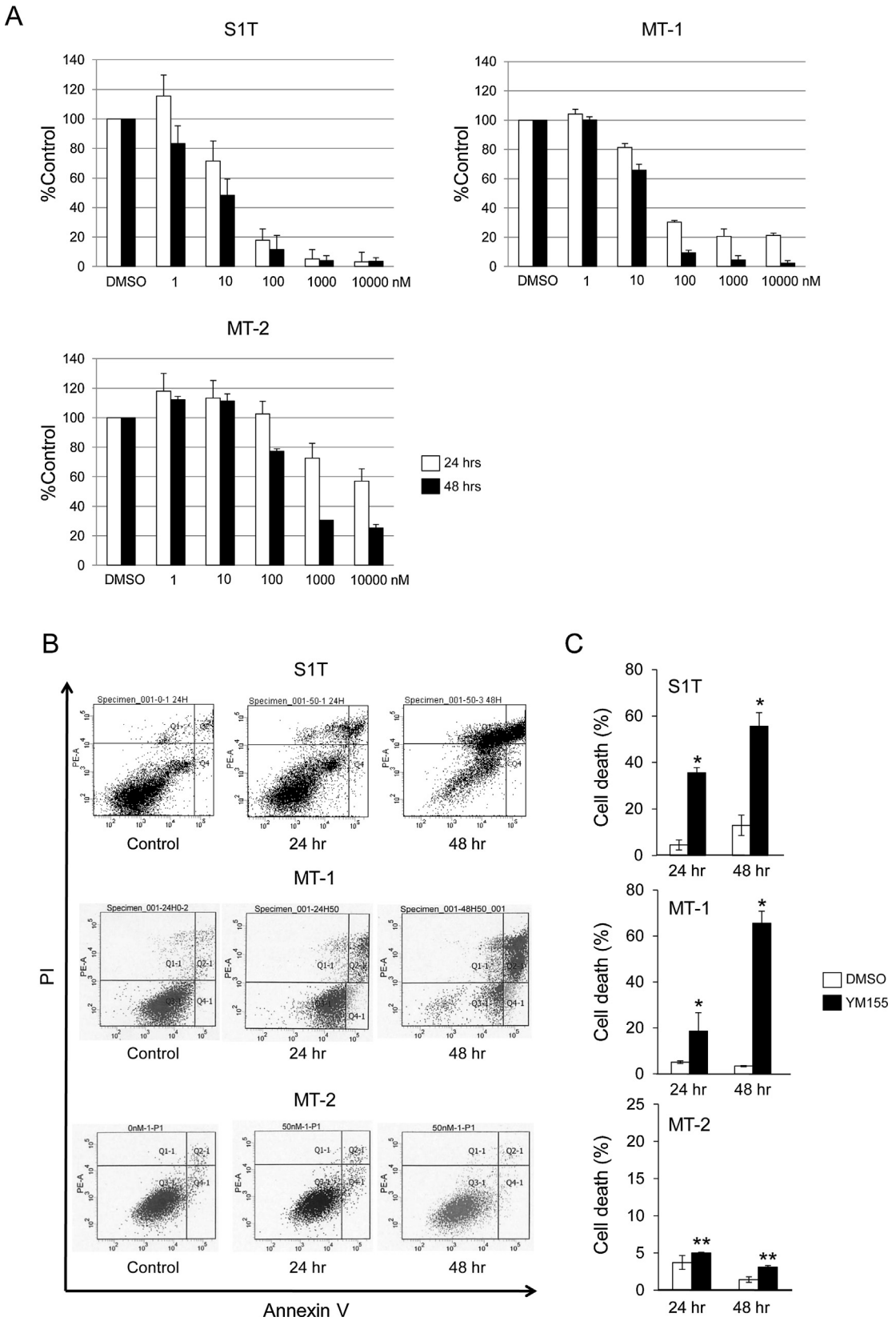

Fig. 1. Growth inhibition and cell death of S1T, MT-1, and MT-2 cells by YM155. (A) S1T, MT-1 and MT-2 cells were cultured in a 96-well tissue culture plate in the absence or presence of YM155 at the indicated concentration for 24 or 48 h. Cell viability was measured using the MTT assay. Data are expressed as the mean percentage of the control cells, representing the average of triplicate sample testing. Error bars represent 1 standard deviation (SD) from the mean for triplicate wells. Results are representative of three independent experiments. (B) S1T, MT-1, and MT-2 cells were cultured in a 6-well tissue culture plate in the absence or presence of YM155 (50 nM) for 24 h or 48 h without medium exchange. The frequency of cell death was measured using flow cytometric analysis with annexin-V/PI staining. Representative dot-plots of S1T, MT-1 and MT-2 cells are shown. (C) Data represent the average of triplicate samples, and error bars represent one SD from the mean for triplicate wells. Results are representative of three independent experiments. *$P < 0.05$ (control versus YM155), **no significant difference.

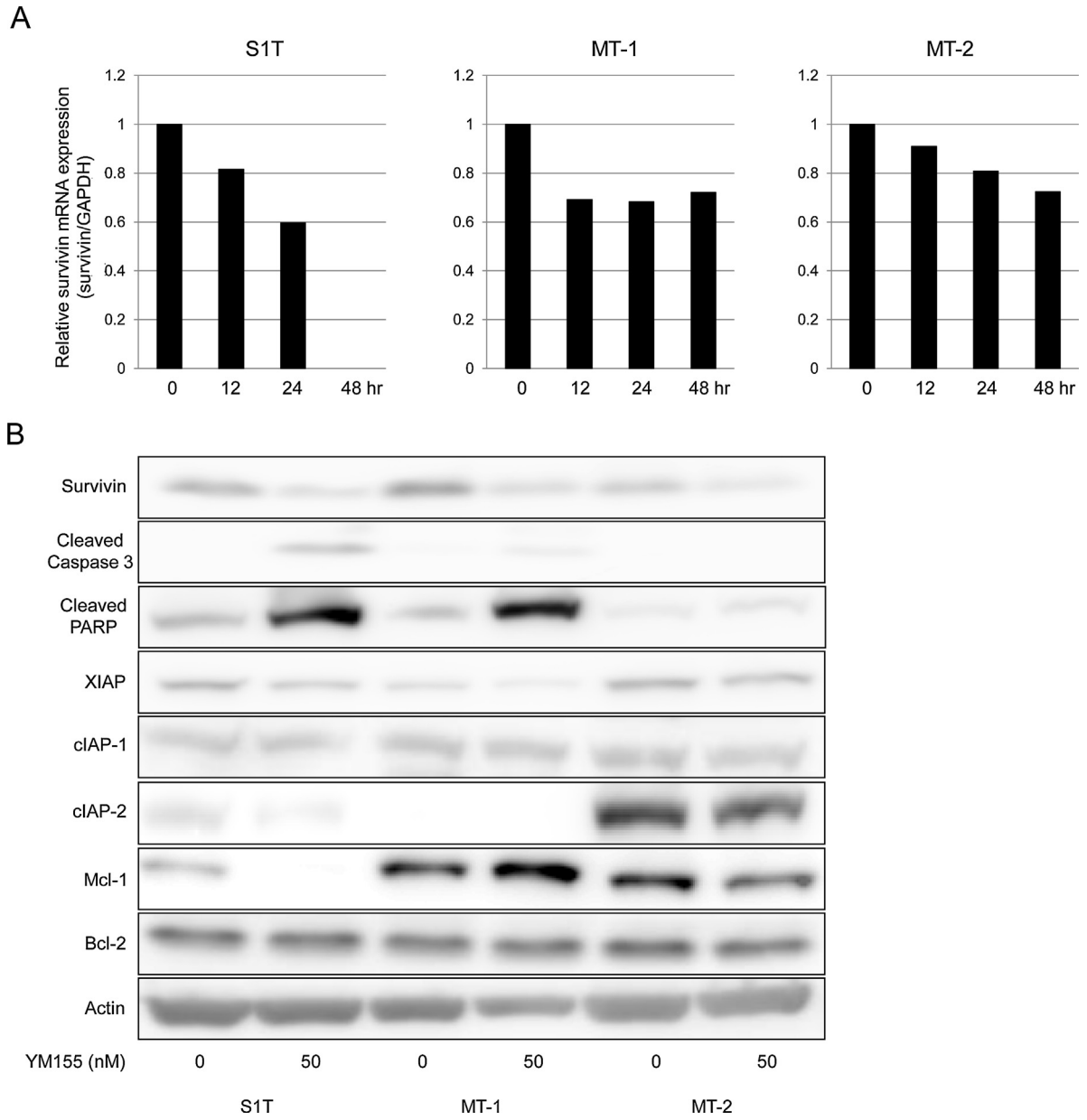

Fig. 2. YM155 suppresses survivin mRNA and protein expression levels in ATL cells. (A) YM155 inhibits survivin mRNA levels in ATL cells. S1T, MT-1, and MT-2 cells were treated with 50 nM YM155 for the indicated time periods. After drug treatment, total RNA was prepared. The relative expression of survivin mRNA was quantified by real-time RT-PCR analysis. Columns, mean value of two independent experiments. (B) YM155 suppresses survivin expression levels in ATL cells. S1T, MT-1, and MT-2 cells were treated with 50 nM YM155 for 24 h. Immunoblotting was performed with specific antibodies for survivin, cleaved caspase-3, cleaved PARP, XIAP, Mcl-1, cIAP-1, cIAP-2, Bcl-2, and β-actin. The immunoblot shown is representative of two independent experiments.

mRNA levels in these cells after 12 h, 24 h and 48 h. We next examined the expression of anti-apoptotic proteins, including survivin, in these cells treated with YM155. As shown in Fig. 2B, survivin protein expression was suppressed 24 h after the treatment with YM155 of these cells. YM155 also suppressed Mcl-1 expression in S1T cells. However, YM155 treatment did not affect the other anti-apoptotic proteins such as XIAP, cIAP-1/2 and Bcl-2. These results indicated that YM155 inhibited survivin protein expression in part via transcriptional repression. In addition, caspase-3 and PARP were activated in S1T and MT-1 cells but not in MT-2 cells 24 h after treatment with YM155. To further confirm the type of cell death, a caspase-3 inhibitor Z-DEVD-FMK was added to the cells in combination with YM155. When cells were treated with YM155 (10 nM) alone or in combination with Z-DEVD-FMK (100 μM) for 48 h in S1T, MT-1, and MT-2 cells, Z-DEVD-FMK prevented YM155 mediated cell growth arrest and cell death in S1T and MT-1 cells (Fig. 3A and B).

3.3. Targeting STAT3 as a strategy to overcome drug tolerance of MT-2 cells to YM155

To investigate the mechanism of drug tolerance of MT-2 cells to YM155, we examined the phosphorylation status of representative signaling molecules in S1T, MT-1, and MT-2 cells. As shown in Fig. 4A, STAT3, STAT5 and AKT were constitutively phosphorylated in MT-2 but not in S1T and MT-1 cells. We also found the viral transactivator protein Tax expression in MT-2 but not S1T and MT-1 cells. Because activated STAT3 has been shown to be associated with drug resistance in cancer cells [23], we hypothesized that STAT3 phosphorylation may contribute to drug tolerance of MT-

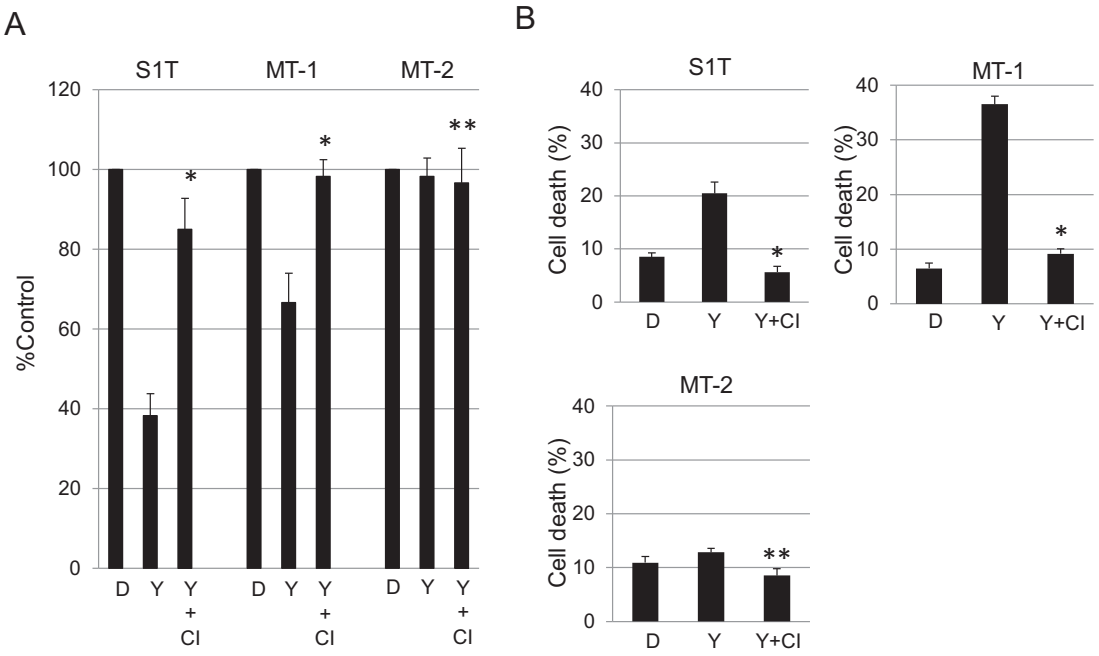

Fig. 3. YM155 induces cell death at least in part via the caspase-dependent manner. (A) S1T, MT-1 and MT-2 cells were cultured in a 96-well tissue culture plate in the presence of DMSO, YM155 (10 nM), or YM155 plus caspase-3 inihibitor Z-DEVD-FMK (100 μM) for 48 h. Cell viability was measured using the MTT assay. Data are expressed as the mean percentage of the control cells, representing the average of triplicate sample testing. Error bars represent one SD from the mean for triplicate wells. Results are representative of two independent experiments. (B) S1T, MT-1 and MT-2 cells were cultured with YM155 (10 nM) alone or in combination with caspase-3 inihibitor Z-DEVD-FMK (100 μM) for 48 h. The frequency of cell death was measured using flow cytometric analysis with annexin-V/PI staining. Data represent the average of triplicate samples, and error bars represent one SD from the mean for triplicate wells. Results are representative of two independent experiments. D, DMSO; Y, YM155; CI, caspase-3 inhibitor. *$P < 0.05$ YM155 plus caspase-3 inhibitor versus YM155 alone. **no significant difference.

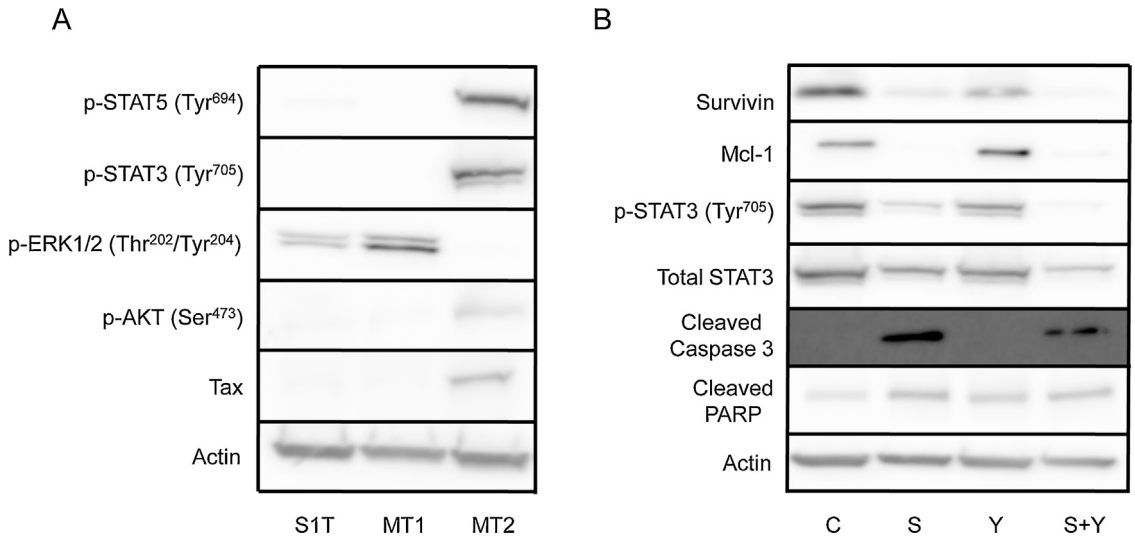

Fig. 4. The combination of STAT3 inhibitor and YM155 may overcome tolerance to YM155. (A) STAT3, STAT5, and AKT are constitutively phosphorylated in MT-2 but not in S1T and MT-1 cells. Tax protein is also expressed in MT-2 cells but not in S1T and MT-1 cells. Immunoblotting was performed with specific antibodies for p-STAT5 (Tyr694), p-STAT3 (Tyr705), p-ERK1/2 (Thr202/Tyr204), p-AKT (Ser473), Tax and β-actin. The immunoblot shown is representative of two independent experiments. (B) MT-2 cells were cultured with STAT3 inhibitor S3I-201 or YM155 alone or in combination for 24 h. Immunoblotting was performed with specific antibodies for survivin, Mcl-1, p-STAT3 (Tyr705), STAT3, cleaved caspase-3, cleaved PARP and β-actin. The immunoblot shown is representative of two independent experiments. (C) MT-2 cells were cultured with S3I-201 or YM155 alone or in combination for 24 h. Cell viability was measured using the MTT assay. Data are expressed as the mean percentage of the control cells, representing the average of triplicate samples. Error bars represent one SD from the mean for triplicate wells. (D) MT-2 cells were cultured with S3I-201 or YM155 alone or in combination for 24 h or 48 h. The frequency of cell death was measured using flow cytometric analysis with annexin-V/PI staining. Data represent the average of triplicate samples, and error bars represent one SD from the mean for triplicate wells. Results are representative of two independent experiments. C, Control; S, STAT3 inhibitor S3I-201; Y, YM155. *$P < 0.05$ YM155 plus S3I-201 versus YM155 alone.

2 cells to YM155. The STAT3 inhibitor S3I-201 suppressed STAT3 phosphorylation and survivin expression (Fig. 4B). Interestingly, S3I-201 also suppressed Mcl-1 expression. In addition, S3I-201 or in combination with YM155 induced caspase 3 and PARP activation. In contrast, treatment with YM155 alone for 24 h did not induce the cleavage of caspase 3 and PARP while it suppressed survivin expression in MT-2 cells. We next investigated the antiproliferative effect of S3I-201 in combination with YM155 on YM155-tolerant MT-2 cells. The concomitant treatment of MT-2 with S3I-201 and YM155 overcame tolerance, which was shown by a decrease of prolifera-

tion of approximately 55% when cells were treated with YM155 and S3I-201 versus YM155 alone ($P < 0.05$; Fig. 4C). To evaluate potential synergy of combination of these two compounds on inhibition of cell proliferation, combination index analysis was performed. As combination index of YM155 and S3I-201 were <0.9, synergy effect was indicated. We next examined the effects of co-treatment with S3I-201 and YM155 on cell survival (Fig. 4D). The concomitant treatment for 48 h showed a significant increase of annexin V-positive and/or PI-positive cells versus either S3I-201 or YM155 alone ($P < 0.05$).

4. Discussion

Survivin has shown to be overexpressed in ATL leukemia cells but not in normal tissues [5,24,25]. A recent study showed that a high expression level of survivin mRNA was a predictor for ATL prognosis in the clinical setting [26]. In addition, down-regulation of survivin expression with shRNA decreased survivin expression and cell viability in ATL cells [5]. These results strongly suggest that survivin is an attractive therapeutic target in ATL cells. YM155 is a survivin suppressant identified by a high-throughput screening of compounds that selectively inhibited survivin promoter activity [13]. YM155 inhibits the growth of various tumor cells, including those of hematological malignancies [13,27].

To clarify the effects of YM155 on ATL cells, we examined whether YM155 could induce cell growth arrest and cell death in ATL cells. We observed that YM155 suppressed cell proliferation in a dose-dependent manner in S1T, MT-1, and MT-2 cells. However, treatment with 10 μM of YM155 for 48 h did not completely inhibit proliferation of MT-2 cells, whereas YM155 with a lower concentration (1 μM) completely inhibited proliferation of S1T and MT-1 cells, suggesting a lower sensitivity of MT-2 cells to YM155 in terms of antiproliferative effect. In addition, treatment with 50 nM of YM155 for 48 h induced cell death in S1T and MT-1 but not in MT-2 cells. These findings suggest that YM155 has a potent antitumor activity for S1T and MT-1 cells, while MT-2 cells are tolerant to YM155.

To investigate the cytotoxic mechanism of YM155 in ATL cells, we examined if YM155 suppresses survivin expression in ATL cells. Quantitative PCR and immunoblot analyses showed that YM155 treatment reduced survivin mRNA and protein levels in S1T, MT-1, and MT-2 cells. In addition, immunoblot analysis showed that YM155 induced the activation of caspase-3 and PARP in S1T and MT-1 but not in MT-2 cells. In addition, caspase-3 inhibitor Z-DEVD-FMK prevented YM155 mediated cell growth arrest and cell death in S1T and MT-1 cells. These results indicate that YM155 induced caspase-dependent apoptosis in part via the transcriptional repression of survivin in S1T and MT-1 cells.

Treatment with 50 nM of YM155 did not induce cell death in MT-2 cells. To elucidate the mechanism of drug tolerance in MT-2, we examined the intracellular signaling in S1T, MT-1 and MT-2 cells. We found that STAT3, STAT5 and AKT were constitutively phosphorylated in MT-2 but not in S1T and MT-1 cells; both of which were sensitive to YM155. We previously showed that resveratrol, a well-known polyphenolic compound, induced cell death accompanied by inhibition of constitutive phosphorylation of STAT3 but not STAT5 in MT-2 cells [28]. In addition, resveratrol inhibited cIAP-2 and Mcl-1 expression and activated caspase-3 and PARP in MT-2 cells. In the present study, YM155 suppressed survivin expression but not Mcl-1 and cIAP-2 protein expression in MT-2 cells. STAT3 activation results in upregulation of various genes involved in cell survival and proliferation, such as those encoding Bcl-2, Bcl-X_L, Mcl-1, cyclin D1, and c-Myc [29–31]. Epling-Burnette et al. showed that inhibition of STAT3 signaling leads to apoptosis in lymphocytes through decreased Mcl-1 expression [32]. Bhattacharya et al. showed that inhibition of STAT3 by dominant negative-STAT3 decreased mRNA and protein levels for Bcl-2, Mcl-1, and cIAP-2 in polyamine-depleted cells [33]. In addition, our previous study showed that STAT3 inhibitor S3I-201 suppressed Mcl-1 and cIAP-2 expression in the HTLV-1-infected T cell line, HUT-102 [28]. These observations suggest that Mcl-1 and cIAP-2 are downstream targets of STAT3. In the present study, we found that S3I-201 suppressed Mcl-1 expression and activated caspase-3 and PARP in MT-2 cells. In contrast, YM155 did not suppress Mcl-1 expression. In addition, it did not cleave caspase-3 and PARP. These findings suggest that downregulation of survivin is not sufficient to lead to cell death in MT-2 cells and that STAT3-Mcl-1 axis may play a role in survival of MT-2 cells. To clarify this, further experiments such as specific inhibition of Mcl-1 by small interfering RNA approach should be performed. In the present study, we showed the viral transactivator protein Tax expression in MT-2 but not S1T and MT-1 cells. Tax is known to disrupt transcriptional control of cytokines, cytokine receptors and immuno-modulatory proteins in T cells. Horiuchi et al. demonstrated that Tax enhanced interleukin-6 receptor (IL-6R) expression in JPX-9 cell line, which is derived from Jurkat cell line expressing Tax cDNA. The authors also demonstrated that IL-6/soluble IL-6R enhanced proliferation of HTLV-1-infected cells in association with activation of STAT3 [34]. Thus, constitutive phosphorylation of STAT3 in MT-2 cells might be related to Tax protein expression. In addition, activation of STAT3 and STAT5 in MT-2 cells might be also attributed to activating mutations affecting JAK kinases. If that were the case, therapeutic approach base on JAK inhibitors would be an alternative treatment. However, we have not analyzed the mutation status of JAK kinases.

Our data showed that STAT3 inhibitor S3I-201 enhanced the inhibitory effect of YM155 on proliferation of MT-2 cells. It is quite likely that the enhanced antiproliferative effect by YM155 in combination with S3I-201 is due to the inhibition of anti-apoptotic proteins including survivin, Mcl-1, and cIAP-2 in MT-2 cells. Thus, targeting STAT3 may overcome drug tolerance of MT-2 cells to YM155.

Phase I and II trials with YM155 have reported on its safety and tolerability in patients with melanoma, prostate cancer, non-small cell lung cancer, and lymphomas [14–17]. Unfortunately, the trial exploring the effectiveness of YM155 as a single agent in lymphoma patients has been terminated early due to the modest activity [15]. Whereas the 2.4% response rate in the trial is lower than anticipated, there was evidence of biologic activity in the series. The experience with a number of small molecules suggests that modest single agent activity should not be a deterrent to further drug development. In vivo ATL model, YM155 in combination with anti-CD52 monoclonal antibody alemtuzumab has recently been shown to have therapeutic efficacy [18]. Thus, YM155 more likely has a role in potentiating the efficacy of the other agents. Recently, a worldwide meta-analysis showed that the combination of zidovudine (AZT) and interferon-α (IFN-α) is highly effective in the leukemic subtypes of ATL and should be considered as standard first-line therapy in that setting [35]. This combination has also improved long-term survival in patients with smoldering and chronic ATL as well as a subset of patients with acute ATL. In addition, ATL lymphoma patients benefit from chemotherapy induction with concurrent or sequential antiretroviral therapy with AZT/IFN-α. To prevent relapse, clinical trials assessing consolidative targeted therapies such as antiretroviral agents in combination with monoclonal antibodies or novel agents are needed [35]. Because survivin is an attractive target in ATL cells, YM155 should be also included in these clinical trials in the future.

In conclusion, we demonstrated a markedly enhanced antiproliferative effect in YM155-tolerant ATL cells with the combination of the survivin suppressant YM155 and STAT3 inhibitor S3I-201.

Our data suggest that a clinical trial of such a combination is warranted in patients with ATL.

Acknowledgments

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