Biomedicine & Pharmacotherapy 98 (2018) 357–363

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Synergistic therapeutic effect of diethylstilbestrol and CX-4945 in human acute T-lymphocytic leukemia cells

Jung-Il Jung[a], Kyeong-Yong Park[b], Soon Ae Kim[c], Jiyeon Kim[a,*]

[a] Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon 34824, Republic of Korea
[b] Department of Integrated Material's Development, CHA Meditech Co., Ltd., Daejeon 34025, Republic of Korea
[c] Department of Pharmacology, School of Medicine, Eulji University, Daejeon 34824, Republic of Korea

ARTICLE INFO

Keywords:
DES
T-ALL
Jurkat
IL-2
CK2
NF-κB

ABSTRACT

Human acute T-lymphocytic leukemia (T-ALL) is one of the most commonly diagnosed hematological disorders, and is characterized by poor prognosis and survival rate. Despite the development of new therapeutic approaches, leukemia treatment options remain limited. In this study, we investigated the immunosuppressive and anti-proliferative effects of the synthetic estrogen diethylstilbestrol (DES), both alone and combined with the casein kinase 2 (CK2) inhibitor CX-4945. Our results indicated that DES induced caspase-dependent apoptosis in a human T-ALL cell line (Jurkat cells), while exerting no significant cytotoxicity in normal peripheral blood mononuclear cells (PBMCs). Phytohaemagglutinin and phorbol 12-myristate 13-acetate induced interleukin (IL)-2 production and activation of NF-κB signaling pathways, which were both inhibited by DES. Moreover, DES exerted synergistic effects with CX-4945 on proliferation and IL-2 production in Jurkat cells. Our results demonstrated that DES exerts anti-proliferative and immunosuppressive effects through inhibition of CK2 and the NF-κB signaling pathway in human T-ALL Jurkat cells.

1. Introduction

The most commonly diagnosed hematological disorders are different types of leukemia, including acute myeloid leukemia (AML); chronic myeloid leukemia (CML); chronic lymphocytic leukemia (CLL); and acute lymphocytic leukemia, also called acute lymphoblastic leukemia (ALL) [1]. Among patients with ALL, about 15% have acute T-lymphoblastic leukemia (T-ALL), a subtype that predominantly affects children but can also occur in adults [2]. T-ALL is characterized by low red blood cell counts, abnormal white blood cells in the blood and bone marrow, uncontrolled accumulation of T-cell progenitors, and disruption of immune responses [3]. In particular, CD4+ T lymphocytes contribute to inflammatory responses and autoimmunity through interleukin (IL)-2 expression, which is induced by activation of the T-cell antigen receptor (TCR) and CD3 receptor [4–6]. IL-2 secretion is also mediated by activation of the NF-κB and mitogen-activated protein kinase (MAPK) signaling pathways, which in turn promote T-cell proliferation and inflammatory responses [7–10]. Despite extensive research and evaluation of newly developed drugs, patients with T-ALL continue to suffer poor outcomes and inflammation-related side effects. Prognosis is especially poor when T-ALL occurs in adults over 40 years of age.

Diethylstilbestrol (DES) is a synthetic form of estrogen that has endocrine-disrupting effects during fetal development, with physiological concentrations potentially causing malformations during pregnancy [11,12]. DES is also associated with increased risks of cervical and breast cancer. Data suggest that DES acts as an antagonist against androgens, progesterone, and mineralocorticoid receptors, and thereby suppresses prostate cancer cell survival [13,14]. Preclinical studies in a human prostate cancer xenograft model demonstrate that combined treatment with DES and docetaxel produces an improved therapeutic response by inducing cell cycle arrest in the G2–M phase and by modulating androgen steroidogenesis [15,16]. Reports further show that pharmacological doses of sex steroids and analogs—including estradiol, DES, progesterone, and testosterone—exert cytostatic and cytotoxic activity in human leukemia cells [17,18]. Based on these previous findings, we hypothesized that DES would have potent inhibitory activity against proliferation and inflammatory responses in human T-ALL cells, as well as in prostate cancer and other leukemia cells.

CX-4945 (Silmitasertib) is an orally available ATP-competitive inhibitor of two subunits of casein kinase 2 (CK2): CK2α and CK2α′. CX-4945 exerts anti-proliferative activity through inhibition of CK2-

* Corresponding author at: Department of Biomedical Laboratory Science, School of Medicine, Eulji University, 77, Gyeryong-ro 771beon-gil, Jung-gu, Daejeon 34824, Republic of Korea.
E-mail addresses: 96k620@gmail.com (J.-I. Jung), pky@chamc.co.kr (K.-Y. Park), sakim@eulji.ac.kr (S.A. Kim), yeon@eulji.ac.kr (J. Kim).

https://doi.org/10.1016/j.biopha.2017.12.078
Received 19 September 2017; Received in revised form 15 December 2017; Accepted 15 December 2017

mediated signaling pathways, including MAPK and PI3K/Akt signaling, in various types of solid tumors and hematological malignancies [19–21]. Notably, combined treatment with CX-4945 and other existing drugs shows synergistic cytotoxic effect on proliferation of lymphocytic leukemia cells through the suppression of NF-κB signaling [22–24].

In the present study, we investigated the NF-κB signaling pathway, inflammatory responses (*e.g.*, cytokine production), and cell survival in T-ALL cells overexpressing mRNA for CK2α and CK2α′. We additionally examined the inhibitory effects of DES combined with CX-4945. Our results indicated that DES combined with CX-4945 exerted synergistic inhibitory effects on T-ALL cell proliferation. We also found that phorbol 12-myristate 13-acetate (PMA)/phytohaemagglutinin (PHA) induced activation of the NF-κB signaling pathway and IL-2 production. These findings provide new evidence that the combination of DES and CX-4945 synergistically exerts therapeutic activity against T-ALL through suppression of CK2 expression and the NF-κB signaling pathway.

2. Materials and methods

2.1. Materials

We purchased Ficoll-Histopaque solution, phytohaemagglutinin (PHA), and phorbol 12-myristate 13-acetate (PMA) from Sigma Aldrich (USA). Fetal bovine serum (FBS), phosphate-buffered saline (PBS), Dulbecco's modified eagle medium (DMEM), RPMI 1640 medium, 100 U/mL penicillin, and 100 μg/mL streptomycin were purchased from Corning Life Science (USA). We obtained diethylstilbestrol (DES) and CX-4945 from Selleck Chemicals (USA). The MUSE® Annexin V and Dead Cell Assay Kit was purchased from Merck Millipore (Germany), and the IL-2 ELISA kit from KOMABIOTECH (Korea). The Cell Counting Kit-8 was purchased from Dojindo Molecular Technologies (USA), and the Caspase-Glo® 3/7 assay system from Promega (USA). We purchased primary antibodies specific for poly [ADP-ribose] polymerase-1 (PARP-1), phosphorylated (p)-p65, p65, Lamin B1, and actin from Santa Cruz Biotechnology, Inc. (USA), and we purchased antibodies raised against caspase-3 and cleaved caspase-3 from Cell Signaling Technology, Inc. (USA). NE-PER nuclear and cytoplasmic extraction reagent was purchased from Thermo Fisher Scientific (USA).

2.2. PBMC isolation and cell culture

Our use of human primary peripheral blood mononuclear cells (PBMCs) and bone marrow cells from ALL patients was approved by the International Review Board of Eulji University (EU 17-03). We also collected heparinized venous peripheral blood from healthy adults for PBMC isolation. PBMCs were harvested by gradient centrifugation using Ficoll-Histopaque solution, and then washed with PBS and re-suspended in DMEM supplemented with 10% heat-inactivated FBS and 1% antibiotics. We purchased human acute T-lymphoblastic leukemia Jurkat cells (No. 40152) from the Korean Cell Line Bank (KCLB), and cultured these cells in RPMI 1640 medium containing 5% FBS and 1% antibiotics. All cell cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. Cell viability assay

PBMCs or Jurkat cells were seeded at a density of 1.0×10^4 cells/well in a 96-well plate and cultured for 24 h. After incubation, cells were treated for 24–72 h with DES and/or CX-4945 in complete media containing 5% FBS. Cell viability was measured using the Cell Counting Kit-8 following the manufacturer's instructions. Absorbance was measured using a Multiscan FC microplate photometer (Thermo Fisher Scientific, USA). All experiments were performed in triplicate.

2.4. Flow cytometry

Jurkat cells were seeded at a density of 1.0×10^5 cells/mL in 24-well plates, and incubated for 24 h. Next, the cells were exposed for 24 h to DES (0–10 μM) in complete media containing 5% FBS. The cells were then harvested and incubated with the MUSE® Annexin V and Dead Cell Assay Kit. To measure the fraction of apoptotic versus dead cells, we used the MUSE® Cell Analyzer (Merck Millipore, Germany), and data analysis was performed using the MUSE® Annexin V and Dead Cell software module (Merck Millipore, Germany).

2.5. Western blot analyses

Cytoplasmic or nuclear fractions of Jurkat cell lysates were prepared using NE-PER nuclear and cytoplasmic extraction reagent. After protein quantification, these cytoplasmic or nuclear extracts (40 μg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were labeled with primary antibody for specific protein detection, and then incubated with HRP-conjugated secondary antibodies. Antibody binding was visualized using Luminata™ Forte Western HRP Substrate (Merck Millipore, Germany). To determine relative protein expression, the band intensities were measured using X-ray films and development solution (Fujifilm, Tokyo, Japan).

2.6. Quantitative real-time PCR (qRT-PCR) analyses

Jurkat cells were treated for 24 h with a combination of PHA (1 μg/mL) plus PMA (50 ng/mL), with or without DES, in culture media containing 5% FBS. Next, total RNA was isolated using the AccuPrep® RNA Extraction Kit (Bioneer Corp., Daejeon, Korea). From 1 μg of total RNA, cDNA was synthesized using oligo (dT) primers (Bioneer Corp., Daejeon, Korea) and the RocketScript™ Reverse Transcriptase Kit (Bioneer Corp., Daejeon, Korea). We performed quantitative real-time RT-PCR using ExcelTaq 2X Q-PCR Master Mix (SMOBiO, Hsinchu, Taiwan) and the CFX96™ Real-Time PCR System (Bio-Rad, Sacramento, CA, USA) with the following cycling conditions: 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The IL-2 mRNA level was normalized to GAPDH as an internal standard. Statistical significance was assessed using the Student's *t*-test with GAPDH-normalized $2_T^{-\Delta\Delta C}$ values. The following primers were used: IL-2 forward, 5′-ACTTTCACTTAAGACCCAGGGA-3′; IL-2 reverse, 5′-AGTGTTGAGATGATGCTTTGACA-3′; GAPDH forward, 5′-GAGTCAACGGATTTGGTCGT-3′; GAPDH reverse, 5′-GATCTCGCTCCTGGAAGATG-3′. All reactions were performed in triplicate, and data were analyzed using the $2_T^{-\Delta\Delta C}$ method [25].

2.7. Enzyme-linked immunosorbent assay (ELISA)

PBMCs (1.0×10^5 cells/mL) or Jurkat cells (1.0×10^4 cells/well) were seeded in 96-well plates, and incubated for 24 h. The cells were then treated for 24 h with PHA (1 μg/mL) plus PMA (50 ng/mL), with or without DES, in culture media containing 5% FBS. After incubation, the amount of IL-2 in the supernatant was measured using the human IL-2 ELISA kits according to the manufacturer's instructions.

2.8. Analysis of combined drug effects

We analyzed the effects of the drug combination using the CalcuSyn software program (Biosoft, Cambridge, UK). To determine whether the result of treatment with the two compounds was additive or synergistic, we applied combination index (CI) methods derived from the median effect principle of Chou and Talalay [26]. The CI was calculated by the formula published by Zhao et al. [27]. A CI of 1 indicated an additive effect between the two compounds, a CI $>$ 1 indicated antagonism, and a CI $<$ 1 indicated synergism.

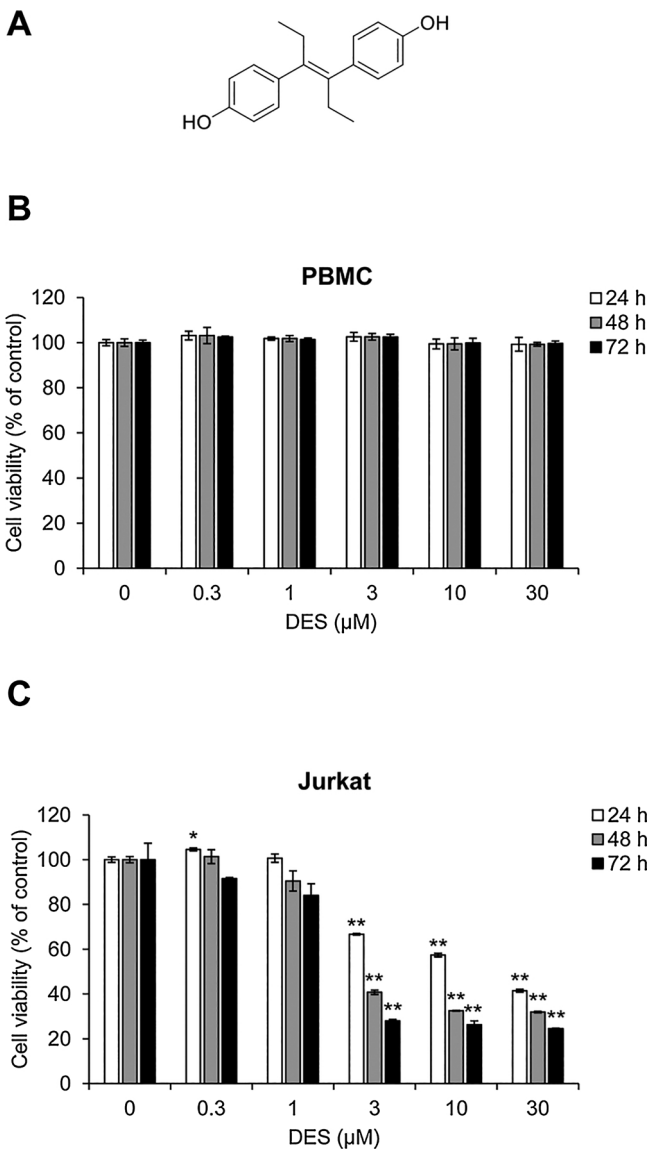

Fig. 1. Anti-proliferative effects of diethylstilbestrol (DES) in T-ALL Jurkat cells. (A) Chemical structure of DES. (B and C) PBMCs and Jurkat cells were treated with DES (0–30 μM) for 24–72 h, and then the cell viability was measured by CCK-8 assay. Experiments were performed in triplicate. Data represent mean ± SD. *p < .01; **p < .001 (vs. DES-free controls).

2.9. Statistical analyses

Data are presented as mean ± SD. Statistical significance was determined using Student's *t*-test or two-way ANOVA. Differences from controls were considered significant when p < .01.

3. Results

3.1. DES induces caspase-dependent apoptosis of T-ALL cells

To determine the optimal diethylstilbestrol (DES) concentration for investigating anti-proliferative effects, we initially assessed cytotoxicity using normal PBMCs (Fig. 1A). DES had no appreciable effects on PBMC viability (Fig. 1B), but dose-dependently inhibited the viability of Jurkat cells (Fig. 1C). To elucidate the mechanism of DES-induced cell death, we next used flow cytometry to further examine the inhibitory effect of DES on cell viability. DES treatment led to a dose-dependent increase in the population of Annexin V- and 7-Aminoactinomycin D (AAD)-positive apoptotic cells (Fig. 2A), as well as increased caspase-3/7 activity (Fig. 2B). Western blot analysis revealed that DES treatment provoked increased expression of cleaved caspase-3 and of PARP-1, which is the cleaved product of a caspase target in the nucleus (Fig. 2C). These results demonstrated that DES induced caspase-dependent apoptosis in Jurkat T-ALL cells, with no cytotoxic effects in PBMCs.

3.2. DES inhibits the PMA/PHA-mediated NF-κB signaling pathway and IL-2 production

NF-κB signaling is involved in various cellular mechanisms, including immune processes, inflammation, and stress responses [28], and specifically plays crucial roles in proliferation, cytokine production, and the pro-inflammatory response in activated T lymphocytes [9]. Notably, p65 phosphorylation in NF-κB signaling regulates IL-2 gene expression in activated T lymphocytes [8]. To investigate whether DES could modulate T-lymphocyte responses, we next examined the immunosuppressive effect of DES on gene expression and paracrine IL-2 in the culture medium upon stimulation with PMA and PHA (PMA/PHA). PMA/PHA treatment gradually increased the levels of phosphorylated p65 from 20 to 60 min (Fig. S1A). To further investigate the relationship between NF-κB signaling activation and IL-2 expression, we used qRT-PCR and ELISA to measure IL-2 mRNA expression and IL-2 production in stimulated Jurkat cells. PMA/PHA treatment time-dependently increased IL-2 mRNA expression and IL-2 secretion in the cell culture medium (Fig. S2B and C).

Based on these results, we next evaluated how DES treatment affected the activation of NF-κB signaling by PMA/PHA, and the inhibition of IL-2 mRNA expression and IL-2 secretion. DES suppressed p65 phosphorylation in the cytosol and nuclear fractions (Fig. 3A), and dose-dependently inhibited the PMA/PHA-induced IL-2 mRNA expression and IL-2 secretion in Jurkat cells (Fig. 3B and C). To further elucidate the immunosuppressive effects of DES, we assessed PMA/PHA-induced IL-2 production in human PBMCs (Fig. 3D). Similar to our findings in Jurkat cells, DES also inhibited PMA/PHA-induced IL-2 secretion in human PBMCs. These results suggested that the PMA/PHA-induced activation of NF-κB signaling stimulated IL-2 mRNA expression and IL-2 production in T-ALL Jurkat cells and PBMCs, and that this process could be inhibited by DES treatment.

3.3. CK2α mRNA was overexpressed in bone marrow cells of T-ALL patients

CK2 overexpression has been observed in hematological cancers, including AML, CLL, MM, and ALL [29–32]. Moreover, suppression of CK2 expression (using siRNA transfection or specific inhibitors) reportedly inhibits the downstream PI3K/Akt signaling pathway, thereby inducing apoptosis of various types of cancer cells [19,33]. Here we performed qRT-PCR analysis to investigate the transcriptional activity of the *CSNK2A1*, *CSNK2A2*, and *CSNK2B* genes (which encode the CK2 subunits, CK2α, CK2α′, and CK2β) in the bone marrow cells of patients with ALL.

Although there were slight differences among samples, the *CSNK2A1* mRNA expression levels were generally higher in the bone marrow cells of ALL patients compared to in normal PBMCs (Fig. 4A). Notably, some samples from ALL patients showed *CSNK2A1* mRNA expression levels of over 30–50 times higher than in normal PBMCs. Additionally, half of the bone marrow cell samples from ALL patients showed higher *CSNK2A2* transcriptional activity compared to normal PBMCs (Fig. 4B). However, *CSNK2B* mRNA expression levels were lower in cells from ALL patients than in normal PBMCs (Fig. S2).

Based on these results, we next investigated the relationship between CK2 expression and ALL cell survival. To this end, we evaluated the anti-proliferative and immunosuppressive effects of DES plus CX-4945—an ATP-competitive inhibitor of both the CK2α and CK2α′ catalytic subunits. To evaluate the inhibitory effect of DES, we detected the protein expressions of CK2α and CK2α′ by western blot analysis. DES inhibited the mRNA and protein expressions of both CK2α and CK2α′

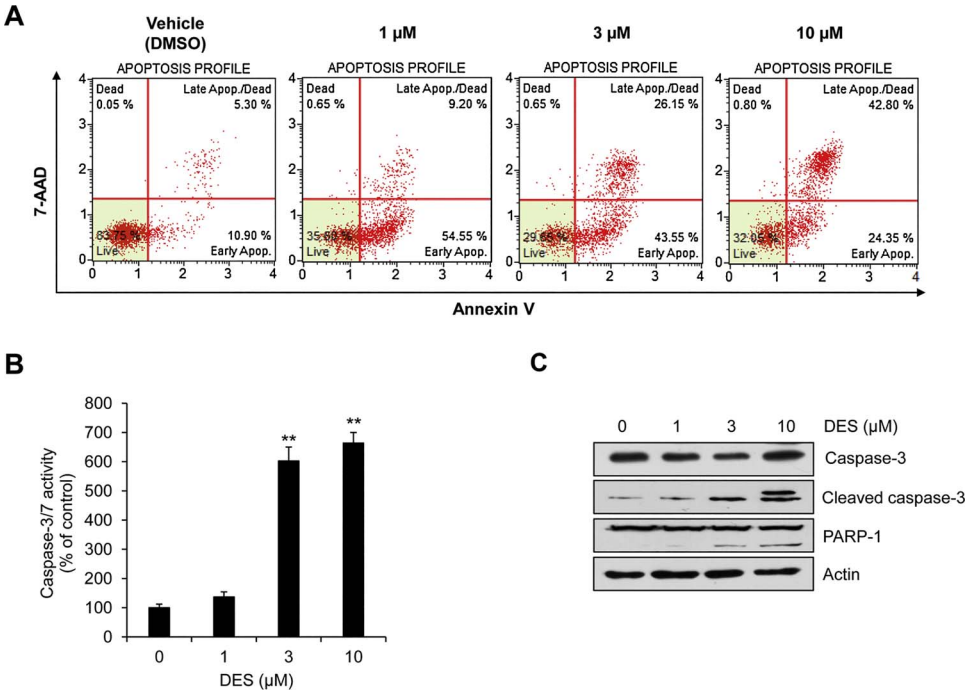

Fig. 2. DES induces caspase-dependent apoptosis. (A) Jurkat cells were incubated with DES for 48 h, and then the populations of apoptotic and dead cells were analyzed using the MUSE Cell Analyzer. (B) Caspase-3/7 activity was measured using the Caspase-Glo 3/7 Assay System. Experiments were performed in triplicate. Data represent mean ± SD. **p < .001 (vs. DES-free controls). (C) Expressions of the cleaved formed of PARP-1 and caspase-3 were evaluated by western blot, using actin as a loading control.

(Fig. 5A and B). These results suggested that the cells of T-ALL patients exhibited overexpression of the catalytic subunits of CK2, and that DES has inhibitory effects on mRNA or protein expression in T-ALL cells.

3.4. DES/CX-4945 synergistically inhibits proliferation and IL-2 production in stimulated T-ALL cells

Next, we assessed cell viability to determine whether CK2 inhibition by DES and CX-4945 caused synergistic effects in Jurkat cells. Compared to the results of single treatment with DES or CX-4945, combined treatment had an increased inhibitory effect on cell

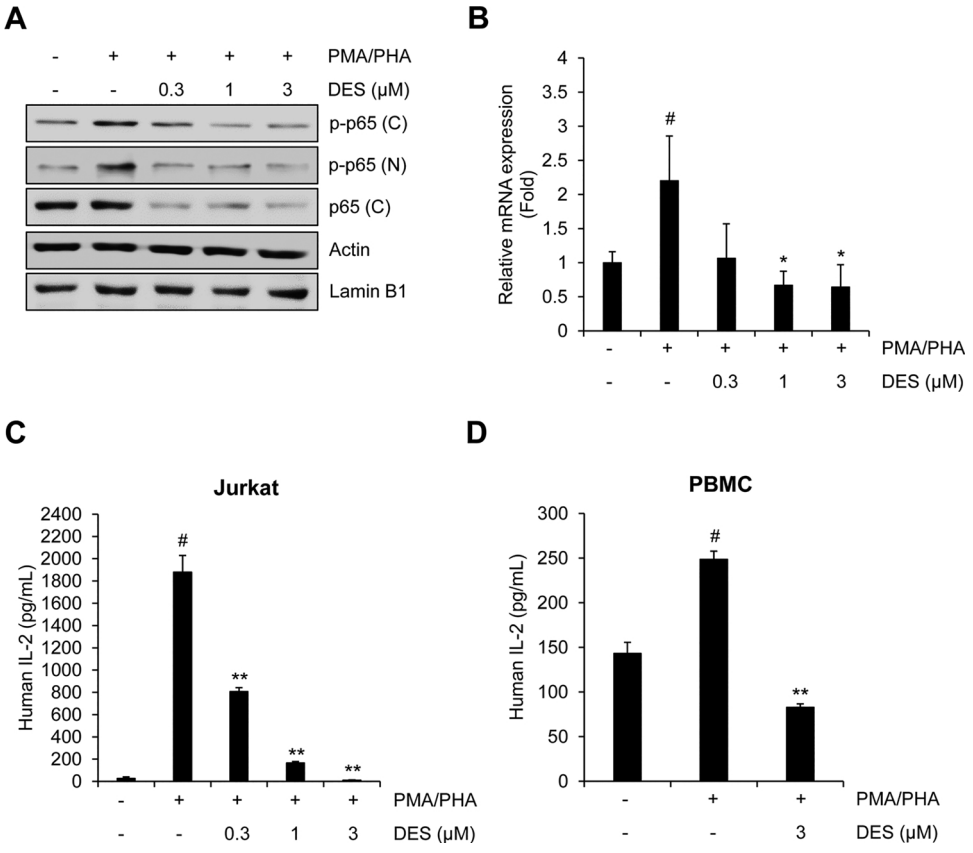

Fig. 3. DES inhibits NF-κB signaling and IL-2 secretion in stimulated T-ALL cells. (A) Jurkat cells were pretreated with DES at the indicated concentrations, and then treated with PHA (1 μg/mL) plus PMA (50 ng/mL) for 1 h. Western blot analysis was performed to measure expression of cytosolic or nuclear p65 and its phosphorylated form. Actin and Lamin B1 were used as loading controls for the cytosolic and nuclear fractions, respectively. (B) Jurkat cells were incubated for 6 h with PHA (1 μg/mL) plus PMA (50 ng/mL) and/or DES (3 μM) in RPMI1640 media containing 0.1% FBS. After RNA extraction and cDNA synthesis, we performed qRT-PCR to measure mRNA expression of the indicated genes, using GAPDH as an internal control. Differences from controls were considered significant when #p < .01 (vs. the PMA/PHA and DES untreated cell population); *p < .01; **p < .001 (vs. the cell population treated with only PMA/PHA).

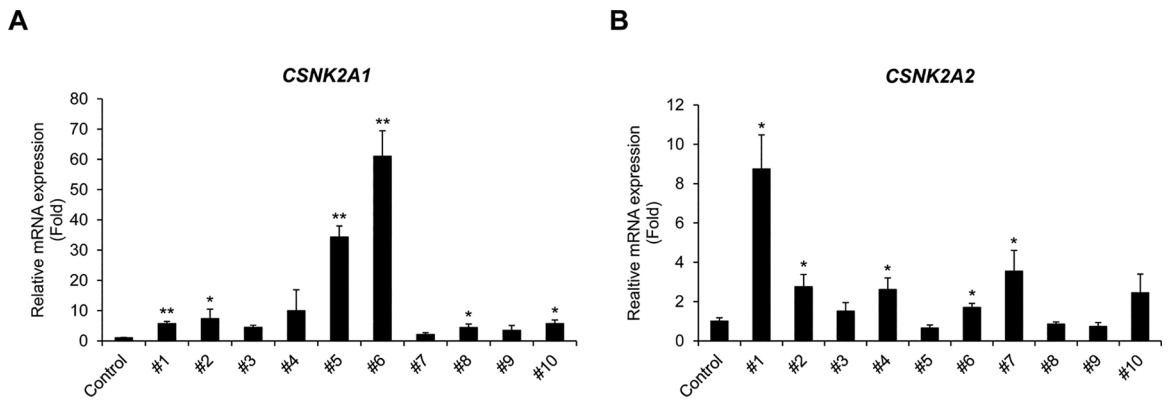

Fig. 4. The mRNA expression of CK2α and CK2α′ in cells from patients with ALL. (A and B) The mRNA expression levels of CK2α and CK2α′ were measured by qRT-PCR analysis in normal PBMCs (n = 3) and in bone marrow cells from ALL patients. The control group represents the average of the qRT-PCR results obtained from analyzing PBMCs from three healthy individuals. *p < .01; **p < .001 (vs. control).

proliferation (Fig. 6A). We further calculated the combination index (CI) using the raw data shown in Fig. 5A, which confirmed a synergistic effect at concentrations above 3 μM (Table 1).

We also confirmed the effects of DES and CX-4945 on PMA/PHA-induced mRNA expression and paracrine IL-2. Our preliminary results demonstrated that PMA/PHA increased both CK2α and CK2α′ expression after 20 min of incubation, while CK2β was not affected (Fig. S3). To confirm that the CK2 inhibition induced by combined treatment had an immunosuppressive effect, we next assessed IL-2 mRNA expression and IL-2 production in activated Jurkat cells. IL-2 mRNA expression and IL-2 production were inhibited by single treatment with DES (3 μM) or CX-4945 (10 μM) (Fig. 6B and C). Compared to the single treatment, combined treatment exerted synergistic effect on the IL-2 mRNA expression and IL-2 production. These results suggested that the DES-induced inhibition of CK2 expression can show synergistic activity with CX-4945 (and likely other small molecules with CK2-inhibiting effects) to impact the proliferation and IL-2 production in T-ALL cells.

4. Discussion

Despite the development of many drugs for leukemia treatment and an overall improved prognosis, T-ALL remains an aggressive and refractory hematologic cancer. Therapeutic strategies for T-ALL have primarily focused on multi-agent chemotherapy or hematopoietic stem cell transplantation. Importantly, various immune responses that occur during leukemia progression can result in deterioration of the treatment effect and/or increasing severity of symptoms. Thus, there remains a need for leukemia treatments that can suppress the immune response and induce cancer cell death.

As research and development costs and investments have increased over the past several decades, the pharmaceutical industry has experienced substantial growth. However, although the number of new drug pipelines has increased, this has not led to a substantial increase in the number of approved new drugs. Pharmaceutical companies are currently looking for new methods to reduce research and development costs. One such cost-saving strategy is the concept of drug repositioning, in which existing drugs are applied to other medical indications. For example, we examined the efficacy of the receptor tyrosine kinase inhibitor dovitinib in regulating multiple myeloma proliferation and BMP-2-induced osteoblast differentiation [34,35]. Drug repositioning has the advantages of expediting drug development and processing, and reducing investment risk [36,37].

DES was originally synthesized as an antagonist of three estrogen receptor isotypes, but later studies demonstrated that DES could also be useful for cancer treatment [38,39]. In one previous report, DES showed cytotoxic activity against human leukemia cell lines, including HL-60, K562, U937, U266, and Jurkat cells [18]. In our present study, we applied the practice of drug repositioning, evaluating the efficacy of DES for inhibiting T-ALL cell proliferation and PMA/PHA-induced pro-inflammatory responses. Our results showed that DES induced apoptosis in Jurkat cells in a manner involving caspase-3/7 activation, without exerting significant cytotoxicity in normal PBMCs. DES also inhibited PMA/PHA-induced mRNA expression and secretion of IL-2—a potent T cell growth factor—through inhibition of the NF-κB signaling

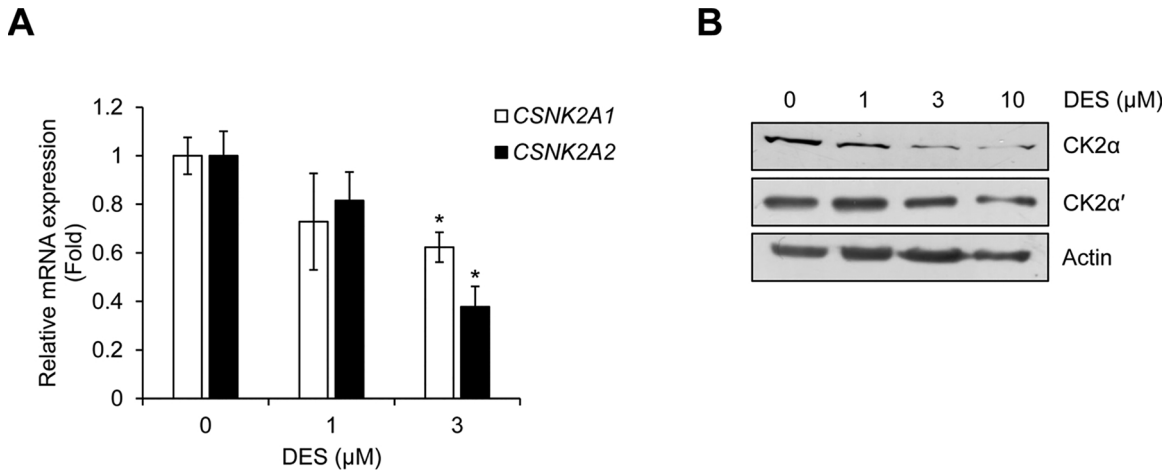

Fig. 5. DES inhibits mRNA and protein expressions of CK2 subunits. Jurkat cells were treated with DES for 6 and 24 h. (A) CK2α and CK2α′ mRNA expressions were measured by qRT-PCR. (B) CK2α and CK2α′ protein expressions were measured by western blot analysis. Actin was used as a loading control. *p < .01 (vs. DES-free control).

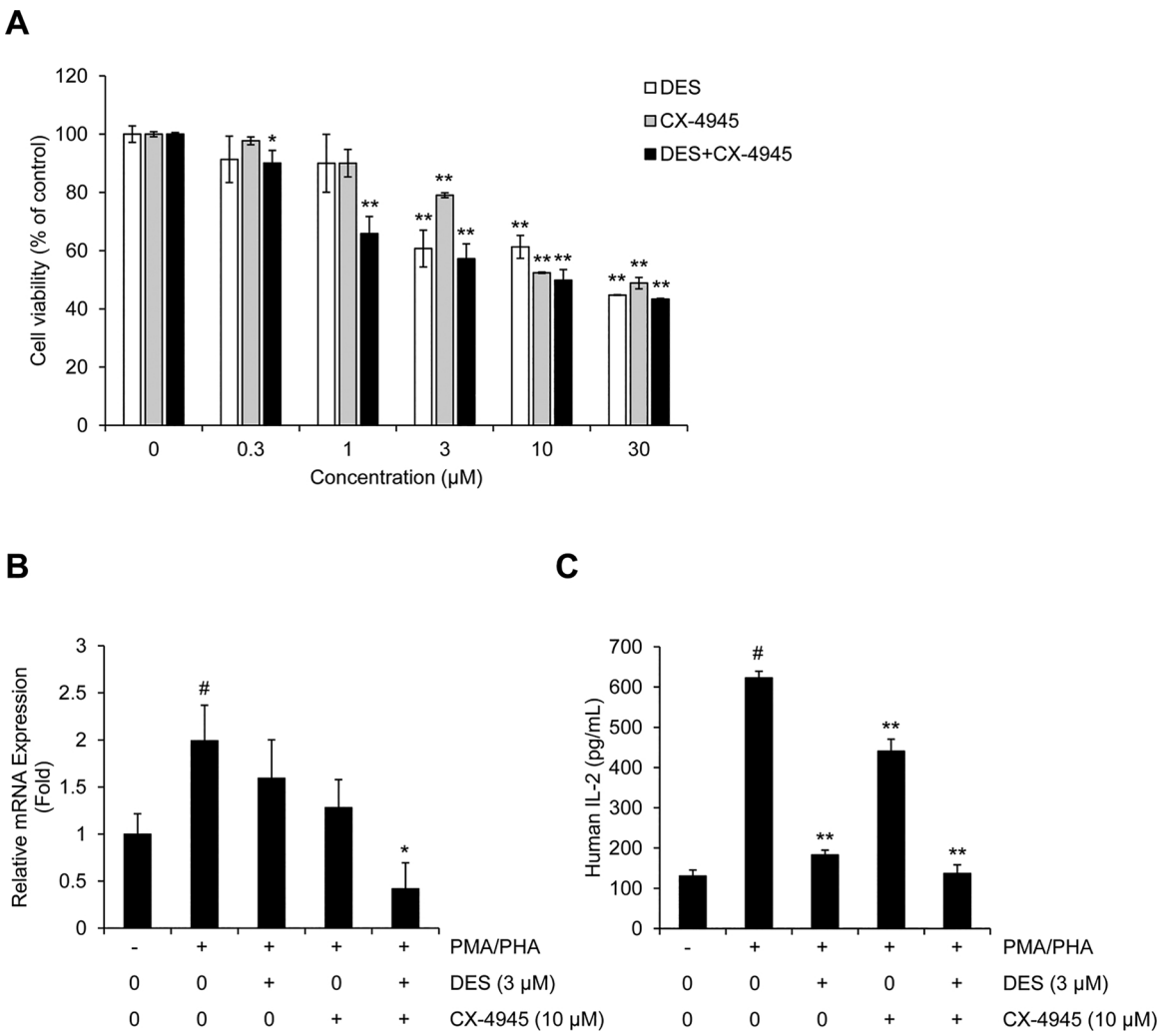

Fig. 6. Treatment with a combination of DES and CX-4945 exerted a synergistic inhibitory effect in T-ALL cells. (A) Jurkat cells were treated with DES, CX-4945, or a combination of DES plus CX-4945 for 24 h and then cell viability was measured. Data represent mean ± SD. *p < .01; **p < .001 (vs. DES-free controls). (B and C) Jurkat cells were pretreated for 2 h with DES (3 μM) and CX-4945 (10 μM) individually or in combination, and were then treated with PMA (50 ng/mL) plus PHA (1 μg/mL) for 6 and 24 h. IL-2 mRNA expression was detected by qRT-PCR analysis (B) and the amounts of secreted IL-2 in the culture medium were measured by ELISA (C). Experiments were performed in triplicate. #p < .01 (vs. the PMA/PHA, DES, and CX-4945 untreated cell population); *p < .01; **p < .001 (vs. the cell population treated with only PMA/PHA).

Table 1
Combination index (CI) values for the two-drug combination against Jurkat cell viability.

DES (μM)	CX-4945 (μM)	CI value
0.3	0.3	0.97077
1	1	1.17815
3	3	0.59826
10	10	0.42268
30	30	0.71071

pathway.

Moreover, we demonstrated that this inhibitory effect of DES was improved when used in combination with the CK2 inhibitor CX-4945. Prior reports show that CX-4945 has anti-proliferative effects, and it has previously been administered as combinatorial therapy with existing anti-cancer drugs for hematological cancer treatment [23,40]. In our present study, compared with the single-treatment group, combined treatment with DES and CX-4945 synergistically inhibited Jurkat cell proliferation as well as IL-2 mRNA expression and secretion. These results indicated that DES may have a better therapeutic effect when combined with a drug that exerts a CK2-inhibitory effect on ALL cells showing high CK2 expression.

There remains some controversy regarding the effects of DES on sex steroid-related diseases, such as breast cancer, ovarian cancer, and prostate cancer. However, it can be expected that the therapeutic effects of commonly used anticancer agents may be enhanced by concomitant administration of appropriate concentrations of DES [41]. DES exerts anti-cancer activity by inducing cell cycle arrest at G2–M phase, which results in docetaxel-induced apoptosis in human androgen-independent prostate cancer [41]. Our present findings suggest that DES may improve the therapeutic efficacy of anti-cancer agents, and this observed synergism when combined with existing drugs could lead to improved cancer treatment. We are presently conducting further *in vitro* studies in other leukemia cell lines, with the aim of elucidating the molecular mechanisms of DES's anti-cancer activity in greater detail. We hope that this study will contribute to increasing the efficacy of chemotherapeutic agents for ALL treatment.

5. Conclusion

In summary, our present study provided evidence regarding the therapeutic mechanism behind DES's anti-proliferative activity in T-ALL. We additionally demonstrated that T-ALL cells express high levels of CK2 subunits, and that CK2 inhibition by CX-4945 showed synergistic effects promoting the inhibition of survival and IL-2 production in T-ALL cells.

Conflict of interest

The authors have declared that no competing interests exist.

Acknowledgements

The biospecimens for this study were provided by the Keimyung University Dongsan Hospital Korea Regional Biobank, a member of the National Biobank of Korea, which is supported by the Ministry of Health and Welfare. All samples derived from the National Biobank of Korea were obtained with informed consent under institutional review board-approved protocols. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2017R1D1A1B03027968).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2017.12.078

References

[1] S.M. Noor, R. Bell, A.C. Ward, Shooting the messenger: targeting signal transduction pathways in leukemia and related disorders, Crit. Rev. Oncol. Hematol. 78 (2011) 33–44.
[2] C.H. Pui, W.E. Evans, Treatment of acute lymphoblastic leukemia, N. Engl. J. Med. 354 (2006) 166–178.
[3] M. Belmonte, C. Hoofd, A.P. Weng, V. Giambra, Targeting leukemia stem cells: which pathways drive self-renewal activity in T-cell acute lymphoblastic leukemia? Curr. Oncol. 23 (2016) 34–41.
[4] S.M. Steward-Tharp, Y.J. Song, R.M. Siegel, J.J. O'Shea, New insights into T cell biology and T cell-directed therapy for autoimmunity, inflammation, and immunosuppression, Ann. N. Y. Acad. Sci. 1183 (2010) 123–148.
[5] J.E. Smith-Garvin, G.A. Koretzky, M.S. Jordan, T cell activation, Annu. Rev. Immunol. 27 (2009) 591–619.
[6] T.R. Malek, I. Castro, Interleukin-2 receptor signaling: at the interface between tolerance and immunity, Immunity 33 (2010) 153–165.
[7] C. Dong, R.J. Davis, R.A. Flavell, MAP kinases in the immune response, Annu. Rev. Immunol. 20 (2002) 55–72.
[8] G.R. Crabtree, N.A. Clipstone, Signal transmission between the plasma membrane and nucleus of T lymphocytes, Annu. Rev. Biochem. 63 (1994) 1045–1083.
[9] F. Mercurio, A.M. Manning, Multiple signals converging on NF-kappaB, Curr. Opin. Cell Biol. 11 (1999) 226–232.
[10] N. Mori, M. Fujii, S. Ikeda, Y. Yamada, M. Tomonaga, D.W. Ballard, N. Yamamoto, Constitutive activation of NF-kappaB in primary adult T-cell leukemia cells, Blood 93 (1999) 2360–2368.
[11] R. Troisi, E.E. Hatch, L. Titus-Ernstoff, M. Hyer, J.R. Palmer, S.J. Robboy, W.C. Strohsnitter, R. Kaufman, A.L. Herbst, R.N. Hoover, Cancer risk in women prenatally exposed to diethylstilbestrol, Int. J. Cancer 121 (2007) 356–360.
[12] J.R. Palmer, L.A. Wise, E.E. Hatch, R. Troisi, L. Titus-Ernstoff, W. Strohsnitter, R. Kaufman, A.L. Herbst, K.L. Noller, M. Hyer, R.N. Hoover, Prenatal diethylstilbestrol exposure and risk of breast cancer, Cancer Epidemiol. Biomark. Prev. 15 (2006) 1509–1514.
[13] C.C. Coss, A. Jones, D.N. Parke, R. Narayanan, C.M. Barrett, J.D. Kearbey, K.A. Veverka, D.D. Miller, R.A. Morton, M.S. Steiner, J.T. Dalton, Preclinical characterization of a novel diphenyl benzamide selective ERα agonist for hormone therapy in prostate cancer, Endocrinology 153 (2012) 1070–1081.
[14] J. Shamash, S.J. Sarker, Comment on 'anti-tumour activity of abiraterone and diethylstilboestrol when administered sequentially to men with castration-resistant prostate cancer', Br. J. Cancer 110 (2014) 266–267.
[15] C.N. Robertson, K.M. Roberson, G.M. Padilla, E.T. O'Brien, J.M. Cook, C.S. Kim, R.L. Fine, Induction of apoptosis by diethylstilbestrol in hormone-insensitive prostate cancer cells, J. Natl. Cancer Inst. 88 (1996) 908–917.
[16] R.B. Montgomery, M. Bonham, P.S. Nelson, J. Grim, E. Makary, R. Vessella, W.L. Stahl, Estrogen effects on tubulin expression and taxane mediated cytotoxicity in prostate cancer cells, Prostate 65 (2005) 141–150.
[17] D.S. Salomon, D.T. Vistica, Steroid receptors and steroid response in cultured L1210 murine leukemia cells, Mol. Cell Endocrinol. 13 (1979) 55–71.
[18] M.V. Blagosklonny, L.M. Neckers, Cytostatic and cytotoxic activity of sex steroids against human leukemia cell lines, Cancer Lett. 76 (1994) 81–86.
[19] H.J. Chon, K.J. Bae, Y. Lee, J. Kim, The casein kinase 2 inhibitor, CX-4945, as an anti-cancer drug in treatment of human hematological malignancies, Front. Pharmacol. 6 (2015) 70.
[20] A. Siddiqui-Jain, D. Drygin, N. Streiner, P. Chua, F. Pierre, S.E. O'Brien, J. Bliesath, M. Omori, N. Huser, C. Ho, C. Proffitt, M.K. Schwaebe, D.M. Ryckman, W.G. Rice, K. Anderes, CX-4945, an orally bioavailable selective inhibitor of protein kinase CK2, inhibits prosurvival and angiogenic signaling and exhibits antitumor efficacy, Cancer Res. 70 (2010) 10288–10298.
[21] J. Kim, S.H. Kim, Druggability of the CK2 inhibitor CX-4945 as an anticancer drug and beyond, Arch. Pharm. Res. 35 (2012) 1293–1296.
[22] R.C. Prins, R.T. Burke, J.W. Tyner, B.J. Druker, M.M. Loriaux, S.E. Spurgeon, CX-4945, a selective inhibitor of casein kinase-2 (CK2), exhibits anti-tumor activity in hematologic malignancies including enhanced activity in chronic lymphocytic leukemia when combined with fludarabine and inhibitors of the B-cell receptor pathway, Leukemia 27 (2013) 2094–2096.
[23] F. Buontempo, E. Orsini, A. Lonetti, A. Cappellini, F. Chiarini, C. Evangelisti, C. Evangelisti, F. Melchionda, A. Pession, A. Bertaina, F. Locatelli, J. Bertacchini, L.M. Neri, J.A. McCubrey, A.M. Martelli, Synergistic cytotoxic effects of bortezomib and CK2 inhibitor CX-4945 in acute lymphoblastic leukemia: turning off the pro-survival ER chaperone BIP/Grp78 and turning on the pro-apoptotic NF-κB, Oncotarget 7 (2016) 1323–1340.
[24] H. Lian, D. Li, Y. Zhou, E. Landesman-Bollag, G. Zhang, N.M. Anderson, K.C. Tang, J.E. Roderick, M.A. Kelliher, D.C. Seldin, H. Fu, H. Feng, CK2 inhibitor CX-4945 destabilizes NOTCH1 and synergizes with JQ1 against human T-acute lymphoblastic leukemic cells, Haematologica 102 (2017) e17–e21.
[25] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method, Methods 25 (2001) 402–408.
[26] T.C. Chou, P. Talalay, Quantitative analysis of dose-effect relatioships: the combined effects of multiple drugs or enzyme inhibitors, Adv. Enzyme Regul. 22 (1984) 27–55.
[27] Zhao L, M.G. Wientjes, J.L. Au, Evaluation of combination chemotherapy: integration of nonlinear regression, curve shift, isobologram, and combination index analyses, Clin. Cancer Res. 10 (2004) 7994–8004.
[28] M.J. May, S. Ghosh, Rel/NF-kappa B and I kappa B proteins: an overview, Semin. Cancer Biol. 8 (1997) 63–73.
[29] J.S. Kim, J.I. Eom, J.W. Cheong, A.J. Choi, J.K. Lee, W.I. Yang, Y.H. Min, Protein kinase CK2alpha as an unfavorable prognostic marker and novel therapeutic target in acute myeloid leukemia, Clin. Cancer Res. 13 (2007) 1019–1028.
[30] F.A. Piazza, M. Ruzzene, C. Gurrieri, B. Montini, L. Bonanni, G. Chioetto, G. Di Maira, F. Barbon, A. Cabrelle, R. Zambello, F. Adami, L. Trentin, L.A. Pinna, G. Semenzato, Multiple myeloma cell survival relies on high activity of protein kinase CK2, Blood 108 (2006) 1698–1707.
[31] F. Piazza, S. Manni, M. Ruzzenem, L.A. Pinna, C. Gurrieri, G. Semenzato, Protein kinase CK2 in hematologic malignancies: reliance on a pivotal cell survival regulator by oncogenic signaling pathways, Leukemia 26 (2012) 1174–1179.
[32] L.R. Martins, P. Lúcio, M.C. Silva, K.L. Anderes, P. Gameiro, M.G. Silva, J.T. Barata, Targeting CK2 overexpression and hyperactivation as a novel therapeutic tool in chronic lymphocytic leukemia, Blood 15 (2010) 2724–2731.
[33] R.A. Faust, S. Tawfic, A.T. Davis, L.A. Bubash, K. Ahmed, Antisense oligonucleotides against protein kinase CK2-alpha inhibit growth of squamous cell carcinoma of the head and neck in vitro, Head Neck 22 (2000) 341–346.
[34] H.J. Chon, Y. Lee, K.J. Bae, B.J. Byun, S.A. Kim, J. Kim, Traf2- and Nck-interacting kinase (TNIK) is involved in the anti-cancer mechanism of dovitinib in human multiple myeloma IM-9 cells, Amino Acids 48 (2016) 1591–1599.
[35] Y.H. Son, S.H. Moon, J. Kim, The protein kinase 2 inhibitor CX-4945 regulates osteoclast and osteoblast differentiation in vitro, Mol. Cells 36 (2013) 417–423.
[36] T.T. Ashburn, K.B. Thor, Drug repositioning: identifying and developing new uses for existing drugs, Nat. Rev. Drug Discov. 3 (2004) 673–683.
[37] C.R. Chong, D.J. Sullivan Jr., New uses for old drugs, Nature 448 (2007) 645–646.
[38] T. Grenader, Y. Plotkin, M. Gips, N. Cherny, A. Gabizon, Diethylstilbestrol for the treatment of patients with castration-resistant prostate cancer: retrospective analysis of a single institution experience, Oncol. Rep. 31 (2014) 428–434.
[39] A. Omlin, C.J. Pezaro, S. Zaidi, D. Lorente, D. Mukherji, D. Bianchini, R. Ferraldeschi, S. Sandhu, D. Dearnaley, C. Parker, N. Van As, J.S. de Bono, G. Attard, Antitumour activity of abiraterone and diethylstilboestrol when administered sequentially to men with castration-resistant prostate cancer, Br. J. Cancer 109 (2013) 1079–1084.
[40] F. Buontempo, E. Orsini, L.R. Martins, I. Antunes, A. Lonetti, F. Chiarini, G. Tabellini, C. Evangelisti, C. Evangelisti, F. Melchionda, A. Pession, A. Bertaina, F. Locatelli, J.A. McCubrey, A. Cappellini, J.T. Barata, A.M. Martelli, Cytotoxic activity of the casein kinase 2 inhibitor CX-4945 against T-cell acute lymphoblastic leukemia: targeting the unfolded protein response signaling, Leukemia 28 (2014) 543–553.
[41] R.B. Montgomery, P.S. Nelson, D. Lin, C.W. Ryan, M. Garzotto, T.M. Beer, Diethylstilbestrol and docetaxel: a phase II study of tubulin active agents in patients with metastatic, androgen-independent prostate cancer, Cancer 110 (2007) 996–1002.