(Fig.1). The purpose of this study is to verify the effects of MCS-C2 on the cell cycle progression, and to clarify the action of mechanism on MCS-C2-inducing cell cycle arrest and apoptosis in prostate cancer cells.

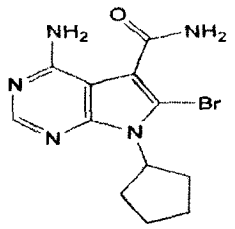

Fig. 1. Structure of MCS-C2.

Methods: LNCaP, DU145, and PC3 cells treated with MCS-C2 were evaluated for antiproliferative effect using cell viability test, flow cytometric analysis (Fig.2), TUNEL assay (Fig.3), and microscopic examination. To clarify the action of mechanism of MCS-C2, we also performed immunoblot assay for the proteins involved in cell cycle progression and apoptosis (Fig.4).

Results: PC3 cells treated with MCS-C2 resulted in the elevated protein level of E2F1 and rapid degradation of cyclinB in the absence of the modulation of mRNA levels; this is accompanied by the G1 phase arrest and subsequent apoptosis. The elevated level of E2F1 was due to the enhanced stability of E2F1 demonstrating a prolonged half-life. MCS-C2 modulates

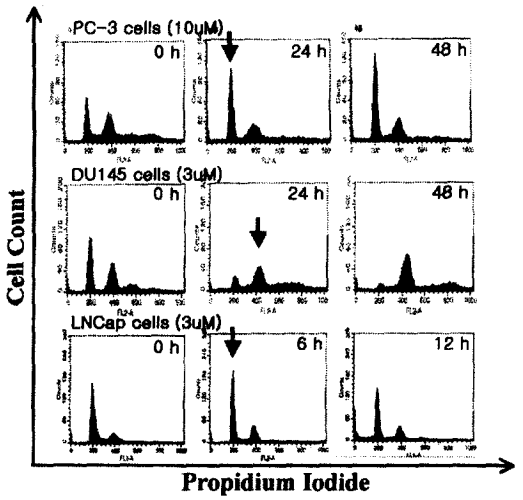

Fig. 2. Analysis of cell cycle regulation.

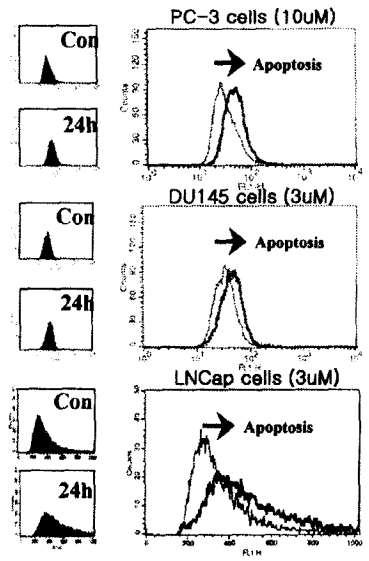

Fig. 3. TUNEL assay.

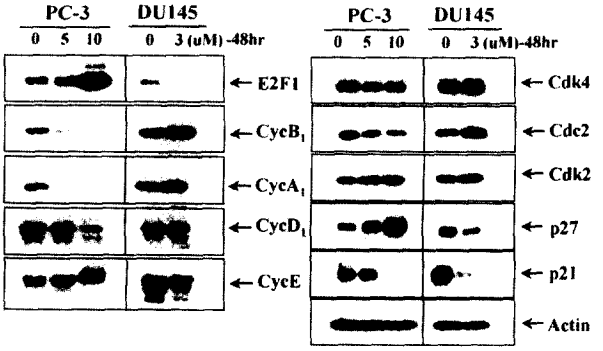

Fig. 4. Western blot analysis.

Conclusion: MCS-C2 induces cell cycle arrest and apoptosis via regulation of protein ubiquitiantion pathway in prostate cancer cells. Accordingly, MCS-C2 might be a novel candidate with a therapeutic potential against prostate cancer cells.

622 POSTER

Cyclosporin-A enhances docetaxel-induced apoptosis through inhibition of NF-kappaB activation in human gastric carcinoma cells.

C. Nakahara[1], T. Morisaki[1], H. Matsunaga[2], K. Nakamura[1], N. Yamanaka[1], H. Kuga[1], H. Kuroki[1], E. Baba[1], M. Tanaka[3], M. Katano[1].
[1] *Kyushu University, Cancer Therapy and Research, Fukuoka-City, Japan;*
[2] *Saga Medical School, Faculty of Hospital Pharmacy, Saga-City, Japan;*
[3] *Kyushu University, Surgery and Oncology, Fukuoka-City, Japan*

Background: Our preliminary study revealed that cyclosporin-A (CsA), which is an immunosuppressive drug, can suppress constitutive activation of nuclear factor-κ B (NF-κ B) in human gastric carcinoma cells. On the other hand, tubulin inhibitor, docetaxel (TXT), has been indicated to induce NF-κ B activation in several malignant cells. We hypothesized that CsA can enhance TXT-inducing apoptosis in human carcinoma cells through inhibition of NF-κ B activation.

Materials and Methods: Two human gastric carcinoma cell lines (GCTM-1 and MK-1), a colon carcinoma cell line (DLD-1), a pancreas carcinoma cell line (NOR-P1), a human embryonic pulmonary fibroblast cell line, and human umbilical vein endotherial cells were used as targets. Apoptotic cell death was verified morphologically by nuclear fragmentation assay with Hoechst staining. Nuclear translocation of NF-κ B was determined by immunostaining and electrophoretic mobility shift assay (EMSA). The therapeutic effects of a combination of TXT and CsA were assessed in a mouse peritoneal dissemination model.

Results: A combination of CsA (5 μ M) and TXT (10 nM) significantly enhanced apoptotic cell death in all carcinoma cell lines but not in non-malignant cell lines in comparison with the single agent alone. These effects were also observed in seven fresh carcinoma cells isolated from 8 patients with malignant ascites or pleural effusions. TXT had no expressions of MDR-1 gene in GCTM-1 cells and CsA had little influence upon TXT uptake and efflux in these carcinoma cell lines. With immunostaining and EMSA, TXT induced NF-κ B activation in the carcinoma cell lines, and combination of CsA with TXT markedly suppressed NF-κ B activation in the carcinoma cells. In addition, combination of NF-κ B decoy instead of CsA with TXT also induced apoptosis in the carcinoma cells. A combination of CsA and TXT significantly suppressed peritoneal dissemination in a murine peritoneal dissemination model.

Conclusions: Our data indicate that TXT induces both apoptosis pathway and anti-apoptosis pathway (NF-κ B activation) in gastric carcinoma cells. CsA inhibits the anti-apoptotic pathway. As a result, CsA enhances TXT-induced apoptosis mainly through the inhibition of TXT-induced NF-κ B activation. Treatment with a combination of CsA and TXT will prove to be a useful therapeutic strategy for cancer patients, especially for patients with multiple drug resistance.