Materials and methods: A phase-contrast microscopy image analysis method to study cell geometry of adherent cells perfused with 10 mg/L cisplatin in culture medium. The method was compared to image analysis and semi-quantitative estimation of scanning electron microscopy images of cells treated the same way.

Results: Phase-contrast light microscopy cell shape changes, as well as the optical halo widening induced by 10 mg/L-cisplatin, correlated well to scanning electron microscopy demonstration of apoptotic morphology with cell membrane blebbing and sprouting.

Discussion: We conclude that image analysis of vital phase-contrast microscopy of adherent single cells is a useful tool to follow early apoptotic changes induced by cancer chemotherapeutics or other agents.

618 POSTER

Urokinase-type plasminogen activator is transcriptionally repressed during12-o-tetradecanoylphorbol-13-acetate-dependent differentiation of hl-60 cells

C. Park[1], J.U. Bang[1], E.J. Yun[1], J.I. Park[1], Y.S. Kim[4], S.G. Paik[4], W.H. Yoon[2,3], S.K. Park[1,4], B.D. Hwang[1,3,4], K. Lim[1,3,4]. [1] *College of Medicine, Chungnam National University, Biochemistry, Daejeon, Korea;* [2] *College of Medicine, Chungnam National University, Surgery, Daejeon, Korea;* [3] *Cancer Research Institute;* [4] *Institute of Biotechnology, Daejeon, Korea*

Background: Urokinase-type plasminogen activator (uPA) is a key regulatory enzyme in a cascade of proteolytic events important for cell migration, tissue restructuring and tumor cell invasiveness. To gain insight on the mechanism of egression of differentiated myeloid cells from bone marrow, transcriptional regulation of uPA gene expression and invasiveness have been investigated during TPA-dependent differentiation of HL-60 cells.

Methods: Human promyelocytic leukemia, HL-60 cell line was obtained from the American Type Culture Collection (CCL 240). Total RNA was prepared by a modification of the method of Karlinsey et al. and Northern blot hybridization was assayed by the method of Virca et al. Nuclear extracts were prepared by the method of Lim et al. with a modification of the method of Gorski et al. The binding activities of nuclear protein factors on DNA sequence elements were determined by DNA mobility shift assay.

Results: uPA mRNA was decreased by TPA and sodium butyrate in HL-60 cells, but vitamin D, retinoic acid and DMSO did not affect. TPA repressed uPA gene expression in time- and dose-dependent manner, whereas PAI-1 was gradually induced. uPA mRNA level of control was almost reduced by pretreatment of actinomycin-D and cycloheximide enhanced uPA mRNA level. In DNA mobility shift assay using oligonuleotide containing GATA-1 binding site or PEA3/AP1 site on the uPA promoter, one specific DNA-protein complex was identified in nuclear extract prepared from control cells, respectively. In nuclear extract prepared from TPA-treated cells, the binding activity of GATA-1 and PEA3/AP1 were vanished. TPA-dependent repression of uPA mRNA was restored by pretreatment of staurosporin and PD98059, whereas SB203580 and tyrphostin were not effect. In DNA mobility shift assay, the binding activity of GATA-1 and PEA3/AP1 were restored by the pretreatment of staurosporin and PD98059. Motility and invasiveness of HL-60 cells were increased to 30 fold and 20 fold after the TPA treatment, respectively.

Conclusion: Reduction of binding activity of GATA-1 and PEA3/AP1 are related to transcriptional repression of uPA gene during TPA-dependent differentiation of HL-60 cells, and uPA activity may be not related to invasion in HL-60 cells. [This work was supported in part by Korea Research Foundation Grant (KRF-005-D00004)].

619 POSTER

Depending on the microenvironment, nitric oxide may switch to another way of cell death in cervical carcinoma cell lines treated with staurosporine

Y. Baskin[1], H. Baskin[2], N. Olgun[3], I.H. Bahar[2]. [1] *Regional Institute of Hygiene, Medicine, Izmir, Turkey;* [2] *Dokuz Eylul University, School of Medicine, Microbiology and Clinical Microbiology, Izmir, Turkey;* [3] *Dokuz Eylul University, Oncology Institute, Izmir, Turkey*

Background: Apoptosis is a highly regulated cellular process that can be activated as a result of aberrant proliferation or differentiation, abrogation of cell fates, including proliferation, differentiation, cell survival and apoptosis, contributes to neoplastic transformation. Most chemotherapeutic agents target tumor cell proliferation, leading to the induction of an apoptotic response. Microenvironment of the cancer cell may direct the effect of these agents. Depending on the microenvironment, nitric oxide can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation, nitroxyl anion or peroxynitrite. It has been informed in the literature that the formation of different RNSs cause differences in signal transduction and gene expression. In this case, different RNSs are induced the gain of function or switch to another function for the same protein. In this study, *in vitro* effect of NO on cell death related to microenvironment components was questioned in cervical carcinoma cell which apoptosis was induced by staurosporin.

Material and Methods: *Cells and Experimental Treatments.* The human cervical carcinoma HeLa cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum and 1% penicillin/streptomycin. Cells were cultured in a humidified 5%CO2 atmosphere at 37°C. HeLa cell lines were treated with L-arginine (nitric oxide donor), L-NAME (reversible inducible nitric oxide synthase inhibitor) and, apoptosis inducer staurosporine and hydrogen peroxide (H2O2) (interacts with nitric oxide and generates different metabolites). *MTT assay.* Cell survival and proliferation was studied by a quantitative colorimetric MTT assay. 100 μ l well^{-1} of a 5 mgml^{-1} stock MTT solution was added to cell in a 96well microtiter plate previously seeded at a volume of 100 μ l well^{-1}. This was incubated at 37°C until the purple formazan crystal developed. Finally the MTT-containing medium was removed and 100 μ l of isopropanol with 0.04 N HCl was added to each well. The absorbance on an ELISA plate reader with a test wavelength of 540 nm and reference wavelength of 620 nm. *Hoechst 33342 / PI assay.* The DNA-binding dyes hoechst 33342 and propidium iodide (PI) were used together in a differential dye uptake assay for microscopic identification and quantification of membrane integrity and nuclear morphology.

Results: * Nitric oxide induced a dose and time dependent cell death by apoptosis in cervical carcinoma cell lines.

* Apoptosis inductive effect of NO was more in cells which were pre-induced with staurosporine.

* Hydrogen peroxide, which interacted with and produced different metabolites of NO, changed the effect of NO an apoptosis in staurosporine-induced cervical carcinoma cell line.

Conclusion: Redox homeostasis of microenvironment may designate and change the effect of NO on cell death. Thus, in new treatment protocols the effect of microenvironment should be in consideration.

620 POSTER

Cellular potassium ion deprivation may enhance apoptosis induced by cisplatin

K. Grankvist[1], P. Behnam-Motlagh[1], P.-E. Sandstrom[2], R. Henriksson[3], L. Marklund[1]. [1] *Umea University, Dept. of Medical Biosciences, Clinical Chemistry, UMEA, Sweden;* [2] *Umea University, Dept. of Clinical Sciences, Paediatrics, UMEA, Sweden;* [3] *Umea University, Dept. of Radiation Sciences, Oncology, UMEA, Sweden*

Background: The anticancer drug cisplatin induces cell death by apoptosis. Apoptosis is dependent on cellular loss of potassium ions (K^+). We therefore studied K^+-fluxes and cisplatin-induced apoptosis during K+ ion deprivation of mesothelioma cells with amphotericin B (a K^+ ionophore enhancing K^+ efflux), combined with the Na^+, K^+, $2Cl^-$ cotransport blocker bumetanide (inhibiting K^+ influx).

Materials and methods: Apoptosis was detected by nucleosome formation and caspase-3 activity. To study K^+ fluxes we quantified the K^+ analogue $^{86}Rb^+$ in cisplatin-induced apoptosis of mesothelioma cells.

Results: Amphotericin B, combined with bumetanide, markedly augmented cisplatin-induced nucleosome formation and caspase-3 activity. It is suggested that amphotericin B augmented cisplatin-induced apoptosis by increasing K^+ efflux, and that amphotericin B combined with bumetanide enhanced cisplatin-induced apoptosis by reduction of K^+ influx combined with stimulation of K^+ efflux.

Discussion: K^+ flux modulation could possibly be used to enhance the antitumour efficacy of cisplatin treatment.

621 POSTER

Cell cycle arrest and induction of apoptosis by novel Cdk inhibitor MCS-C2 is associated with deregulated ubiquitination pathway in prostate cancer cells

C. Lee, M. Kim, Y. Cho. *College of Medicine, Hanyang University, Medical Genetics, Seoul, Korea*

Background: To search for a specific inhibitor of cell cycle regulation in human cancer cells, we synthesized an analogue of toyocamycin, MCS-C2

(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.

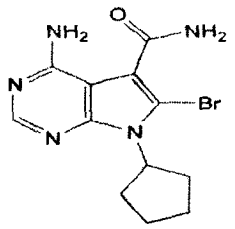

Fig. 1. Structure of MCS-C2.

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

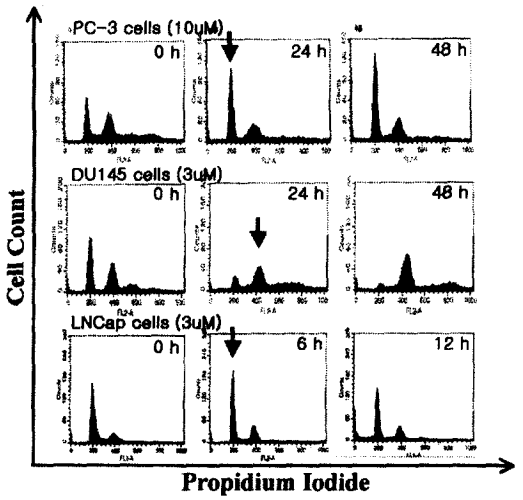

Fig. 2. Analysis of cell cycle regulation.

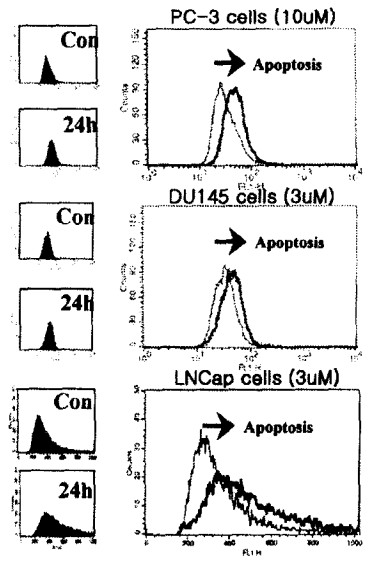

Fig. 3. TUNEL assay.

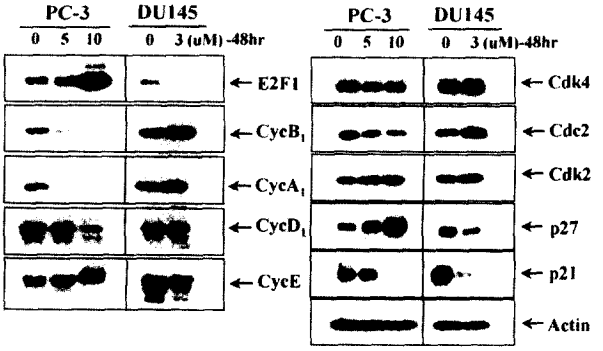

Fig. 4. Western blot analysis.

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.