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AN ANTISENSE OLIGOMER COMPLEMENTARY TO C-MYB ENTREP BY APC LIPOSOME INHIBITS PROLIFERATION AND INDUCES DIFFERENTIATION OF HL-60 ACUTE MYELOID LEUKEMIA CELLS. Yu Zhi Wang, Li Wu. Institute of Radiation Medicine Beijing, R.P.China.

To study the role of c-myb gene in regulation of AML cells, 18-mer antisense oligonucleotides anti-myb were synthesized and tetradecyl-modified phosphorothioate oligomer to enhance the penetration into cells. We determined the number of cell formation of hematopoietic colonies in the semisolid medium and the expression of c-myb-mRNA by PCR. The data indicate that the leukemia cell incubated with Ason for 20-h and the culture was continued for 5-d at various concentrations (20-80 µg/ml) in medium, the proliferation and of the expression of myb-mRNA in HL-60 were inhibited. APC (Alkylphosphocholines) are phosphocholine esters with antitumor activity by inhibiting cell proliferation. APC liposomes were tested against HL-60. The results showed that the APC liposomes can entrap the antisense-oligomers and enhance the antineoplastic activity significantly. The activity of inhibition was depended upon concentrations of Ason. The effects of inhibition of liposome-Asons on the proliferation of leukemia cells were more strong than the effects of free Ason. NBT tests indicated that HL-60 cells were differentiated into the terminal stage

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INDUCTION OF APOPTOSIS IN HUMAN MYELOID LEUKEMIA CELLS BY ANTIMETABOLITES AND ANTHRACYCLINES. V.D. Kravtsov, M.L. Kozlov, (Intro. by S.Kranitz), Vanderbilt Univ. and VA Medical Center, Nashville, TN, USA. The induction of apoptosis by 2',2'-difluorodeoxyuridine (dfU, gemastabine), cytosine arabinoside (ara-C), daunomycin and idarubicin was compared in human HL-60 promyelocytic leukemia cells. At logarithmic phase of cell growth, cell cycle analysis by flow cytometry revealed 38% of G₀/G₁, 36% of S- and 16% of G₂/M-phase cells. Cells were cultured and exposed to a wide concentration range of the drugs in a 96-well micro plate. Apoptosis was monitored for 40 hours using an automatic microculture kinetic (MICK) assay (Lab Invest, 74, 557, 1996). Morphological changes in cells were analyzed in Giemsa stained cytospin preparations. We have found marked differences in the apoptosis-inducing potency of the drugs. In order to induce a similar extent of apoptosis in HL-60 cells, 0.025 µM dfU or 0.5 µM ara-C was required. At these concentrations both drugs induced maximal apoptosis (about 50% apoptotic cells) after 30h incubation. However, at 0.05 µM dfU and 2.5 µM ara-C a portion of the cells underwent apoptosis by 6h. Further increases to 5, 10, 20, 40, 80, and 160 µM of the drugs resulted in a gradual dose-dependent increase in the portion of cells dying by apoptosis by 6h with a subsequent decrease in the extent of apoptosis by 30h. These findings confirm at least two mechanisms by which cell cycle specific antimetabolites can induce apoptosis in tumor cells. Apoptosis induced by low or moderate drug concentrations evolves from the incorporation of the active drug metabolites into DNA of cycling cells and thus requires a relatively prolonged time to develop. High dosages of the same drugs kill both quiescent and cycling cells within a few hours due to a toxic inhibition of cytoplasmic enzymes followed by activation of a common pathway of apoptosis. The latter mechanism may account for the rapid decrease in the blast counts which is often observed in AML patients following high-dose therapy with ara-C. Anthracyclines kill cells via inhibition of topoisomerases and/or generation of free radicals and thus can affect tumor cells at any stage of the cell cycle. We observed maximal apoptotic responses after 28h exposures to 0.5 µM daunomycin or after a 13h exposure to 0.1 µM idarubicin. With 1, 2.5, 5 or 10 µM daunomycin, the time to maximal apoptosis was found to be 19h, 15h, 17h and 8.0h, respectively. Similarly, with 1, 10 and 20 µM idarubicin the time to maximal apoptosis was shortened to 8, 5.5, and 3.6h. Increases in the drug concentrations not only shortened the time to maximal apoptosis, but resulted also in a decreased extent of apoptosis and increased proportion of necrotic cells. We hypothesize that at very high doses, antimetabolites and anthracyclines may trigger apoptosis through a general toxic injury of susceptible cells. This mechanism is distinct from the specific antimetabolic and direct DNA damaging effects that cause apoptosis at lower doses of the drugs.

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HUMAN MONOCYTOID LEUKEMIA CELL LINES ARE HIGHLY SENSITIVE TO APOPTOSIS INDUCED BY 2-DEOXYCOFORMYICIN AND DEOXYADENOSINE. N. Nilsu¹, M. Umada¹, Y. Honma². ¹First Dept. of Internal Medicine, Toho Univ. School of Medicine, Tokyo, ²Dept. of Chemotherapy, Saitama Cancer Center Research Institute, Saitama, Japan. (Intro. by U. Sawada)

Adenosine deaminase inhibitor, 2-deoxycoformycin (DCF), has demonstrated significant anti-proliferative effects on leukemia and lymphoma cell lines. When DCF was exposed in the presence of deoxyadenosine (dAd), the concentration required to induce apoptosis of monocytoid leukemia cells was much lower than that for myeloid, erythroid or lymphoma cell lines. Among the cell lines tested, U937 cells were the most sensitive to the treatment. Expression of the Fas antigen, as measured by flow cytometry, showed a peak 24 hrs after DCF treatment. Analysis of expression of bcl-2, bax, bcl-XL, and c-myc mRNAs by RT-PCR showed significant decrease on the expression of bcl-2, bcl-XL, and c-myc in the DCF-treated U937 cells, while bax expression remained unchanged. Induction of ICE and CPP32 activity was accompanied with the apoptosis, and treatment with their inhibitors significantly suppressed the apoptosis induced by DCF. The effective concentration of DCF inducing apoptosis of U937 cells was 1/1000 of that for lymphoma cell lines, compared as molar concentrations. The combination therapy with DCF and dAd may be of clinical value for the treatment of acute monocytic leukemia.

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ATYPICAL VARIANT OF ACUTE MONOBLASTIC LEUKEMIA (AML, M5a). D. Gluzman, I. Abramenko, N. Elous, L. Sklyarenko, Simonet N. I., O. Vasilenko, R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology National Academy of Science, Kyiv, Ukraine; French-Ukrainian Center "Children of Chernobyl", Kyiv, Ukraine.

Phenotype of blast cells of 24 patients with AML, M5a have been studied by immunoperoxidase staining and flow cytometry. The used MoAbs were CD34, CD33, CD13, CD14, CD15, CD16, CD64, CD71, CD42, CD61, CD62, CD38, HLA-DR, CD10, CD19, CD20, CD37, CD3, CD4, CD8, CD45RA, CD45RO. Also cytochemical reactions (activity of MPO, AcP, ANAE, CAE, PAS-reaction) were performed. Heterogeneity of AML, M5a was revealed. Blast cells were presented as relatively differentiated cells (CD34-CD33+CD13+CD14+CD15+CD64+ ANAE+) and monoblasts of moderate stage of maturation (CD34-CD33+/-CD13+/-CD14+CD15+CD64+ ANAE low). In all cases the minor myeloblastic component was detected. Separate group of AML, M5a was revealed. It was presented by 4 patients. Blast cells had L2 morphology and minimal evidence of lineage-specific differentiation. In one patient blast cells may be considered as pluripotent cells: CD34+CD36-cyHLA-DR+CD15+cyCD7+CD33-CD13-CD64-. In other patients they were distinguished by absence of CD34 only. Low activity of ANAE, 3-9% of MPO+ blasts, simultaneous expression of HLA-DR and CD7 in cytoplasm indicated on monocytic direction of its differentiation. Cytogenetic abnormalities were nonspecific (hypodiploidy 32-42 in one patient; i(17q) and del(12)(p12) in second and normal karyotype;