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[Lab. of Pharmaceutics]

Decreased Thymosin β_4 in Apoptosis Induced by a Variety of Antitumor Drugs.

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As many antitumor drugs can kill tumors through the induction of apoptosis, the effect of these drugs would presumably be enhanced if they were used in combination with other drugs which interact with the apoptotic processes. To clarify the biological events involved in the induction of apoptosis, we examined changes in the proteins associated with induction of apoptosis by antitumor drugs. When Molt-4 cells were exposed to antitumor drugs such as etoposide, ICRF-193 and neocarzinostatin, they exhibited apoptotic cell death as determined by flow cytometry using fluorescein isothiocyanate (FITC) labeled annexin V staining of phosphatidylserine on membranes and detection of hypo-diploid cells. Following the induction of apoptosis, a low molecular weight of protein was commonly decreased, which was identified to be thymosin β_4 by HPLC analysis, and the morphology of actin filaments changed into clumps formations. These results suggest that decreased thymosin β_4 is involved in the induction of apoptosis by the anti-tumor drugs.

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Purification of a Growth-Suppressing Factor for Bovine Artery Endothelial Cells from Mouse Lymphoma P388D1 Cells.

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A growth-suppressing factor for bovine artery endothelial cells (BAEGSF) was purified from the conditioned medium of a mouse lymphoma P388D1 cell culture in the presence of carboxymethylated curdlan. The purified BAEGSF showed two bands with silver staining on a SDS-polyacrylamide gel under reducing condition and their molecular weights were estimated as approximately 55 and 63 kDa, while the molecular weight of the purified BAEGSF was estimated as about 65 kDa by gel filtration using Superdex 200HR. BAEGSF was shown not to have a lethal effect on endothelial cells, but had an inhibitory action on the proliferation of these cells. Furthermore, the growth-suppressing activity of BAEGSF for bovine artery endothelial cells (BAE) was not inhibited by anti-transforming growth factor- β (TGF- β), anti-tumor necrosis factor- α (TNF- α), and anti-interleukin-1 (IL-1) antibodies. These results suggest that BAEGSF is different from TGF- β , TNF- α , and IL-1 which have been reported to inhibit BAE growth.

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Growth Suppressing Factor for Endothelial Cells Exhibits Tumor Regressing Activity.

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Endothelium growth suppressing and tumor-regressing activities were copurified from the conditioned medium of P388D1 culture in the presence of carboxymethylated curdlan by a procedure including ammonium sulfate fractionation and six column chromatographies. The intravenous administration of the purified growth suppressing factor for endothelial cells to sarcoma 180-bearing mouse caused a rapid decrease in the number of viable tumor cells in tumor lumps within 16h. Immunohistochemical study showed that the intravenous injection of the purified factor to sarcoma 180-bearing mouse resulted in hemorrhagic disorder all over the tissue in the tumor lump. The purified factor significantly inhibited *in vitro* tubulogenesis of bovine artery, human umbilical vein, and adult human dermal microvascular endothelial cells on collagen gel. These findings demonstrate that endothelium growth suppressing factor may bring about the regression of a solid tumor by the inhibiting angiogenesis.

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Activation of Macrophages and Neutrophils by an Endothelium Growth Suppressing Factor.

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An endothelial cell growth-suppressing factor (EGSF) was purified from the serum-free conditioned medium of the mouse P388D1 culture in the presence of carboxymethylated curdlan. The purified EGSF showed two bands corresponding to the molecular masses of 55 and 63 kDa by silver staining on a SDS-polyacrylamide gel under reducing conditions. This factor strongly suppressed the proliferation of endothelial cells from bovine artery, human umbilical vein, and human dermal vas capillare and this suppression was observed to be reversible. We found that EGSF was a potent chemoattractant for macrophages and neutrophils. EGSF mediated the adhesion of neutrophils to BAEs and transendothelial migration of neutrophils. Macrophages stimulated by EGSF produced nitrite in a dose-dependent manner. These findings suggest that EGSF acts not only as a potent inhibitor for the growth of endothelial cells but also an activator for macrophages and neutrophils.