CB-P21.
ASSEMBLY OF STRESS GRANULES REQUIRES MICROFILAMENTS AND MICROTUBULES
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Stress granules (SGs) are phase-dense organelles that appear transiently in the perinuclear region of eukaryotic cells upon induction of environmental stress (e.g., heat, oxidative agents, UV, etc). SG assembly is triggered by an abortive translational initiation that leads to the accumulation of stalled polysomal complexes along with RNA-binding proteins (TIA-1/R, HuR, Staufen, etc). SGs are proposed to serve as a triage site that controls the fate of untranslated mRNAs. Our goal is to evaluate the participation of the cytoskeleton and molecular motors in the collapse into SGs of the translational machinery, normally dispersed throughout the cytoplasm. We tested the effect of different microtubule and microfilament-disrupting drugs on SG formation upon exposure of cultured cells to oxidative stress-inducing agents. We found that cytoskeleton-disrupting treatments cause dramatic changes on SG assembly. Disruption of the microtubule network prior to stress induction provokes the formation of SGs of normal size that fail to localize perinervally. Disruption of the actin network induces the accumulation of a larger number of smaller SGs that remain dispersed throughout the cytoplasm. Our results suggest a role for the cytoskeleton for the anchorage and/or for the transport of stress-granule components to the perinuclear region.

CB-P22.
MODIFICATION OF THE C-TERMINUS OF α-TUBULIN BY SITE-DIRECTED MUTAGENESIS: VISUAL ANALYSIS OF THE CYTOSKELETON OF TRANSFECTED CELLS
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The cytoskeleton of eukaryotic cells comprises an interconnected network of microtubules, microfilaments, and intermediate filaments. Microtubules are dynamic structures that play a major role in a wide range of processes, including cell morphogenesis, cell division, intracellular transport and signaling. Microtubules are formed by heterodimers of αβ-tubulin. The α-tubulin gene is highly conserved in eukaryotes. Its C-terminal amino acid is tyrosine which can be removed by tubulin carboxypeptidase and reincorporated by tubulin tyrosine ligase. This post-translational reaction is called the tyrosination/detyrosination cycle, and its physiological role is still unknown. Site-directed mutagenesis was performed on commercial vector pEGFPtrb (Clontech), changing the C-terminal tyrosine codifying codon by another one that codifies for arginine, glycine or aspartic acid. These mutants are not substrate for tubulin carboxypeptidase. Obtained vectors were transfected into NIH 3T3 cells to analyze, by immunofluorescence, the expression of each mutant tubulin, its capacity to assemble into microtubules, and the localization of other cytoskeleton proteins.

CB-P23.
INCORPORATION OF TYROSINE ANALOGUES INTO THE C-TERMINUS OF β-TUBULIN
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α-Tubulin is biosynthesized with a tyrosine at its C-terminus which can be removed by tubulin carboxypeptidase and reincorporated by tubulin tyrosine ligase. We studied the capability of the ligase to incorporate tyrosine analogues into tubulin. Azatyrine induces the reversible phenomenon and can be incorporated into the tubulin's C-terminus as well as into proteins via de novo synthesis. Which of these two mechanisms is responsible of this effect remains unclear. The introduction of a nitro group into the position 3 of the phenolic ring of tyrosine avoids its incorporation into proteins via de novo synthesis, but not into tubulin's C-terminus. Therefore, 3-Nitrotyrosine was synthesized, purified by TLC and characterized by UV-Visible, IR, mass and RMM+H spectroscopy. It was found that 3-nitrotyrosine cannot be incorporated into proteins via de novo synthesis nor into tubulin's C-terminus. No effect was found in cellular proliferation. We also studied if the ligase was able to incorporate other tyrosine analogues. Melphalan could not be incorporated; Thienylalanine and p-naphthylalanine were incorporated with low affinity, and m-fluoro-tyrosine was incorporated very efficiently in vitro. m-F-tyrosine stopped proliferation of C6 cells and changed their morphology. An antibody against the C-terminal m-F-Tyr residue was developed.

CB-P24.
COMBINED TREATMENT OF IN VIVO Pancreatic CANCER WITH OLIGODEOLES Se, Zn, Mn PLUS LACHESIS Muta and GEMCITABINE
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We have previously reported the use of oligodeos in Se, Zn, Mn plus Lachesis muta (O-LM) as antitumor drug in pancreatic cancer, both in vivo and in vitro. Besides, gemcitabine (G) have been adopted for several years in recent years in pancreatic cancer. In the present work we have investigated the in vivo use of G O-LM and G+O-LM, in pancreatic cancer xenografts, which were developed in nude mice, by surgical inoculation of Panc-1 cells derived from a human ductal pancreatic adenocarcinoma. When tumor mean diameter reached 0.6 cm, mice were treated with O-LM or both. Relative tumor size at 30 days in O-LM and control (C) groups was significantly greater than in G and combined groups. Histomorphology sections were examined for histopathological changes. The combined treatment showed a significantly minor number of mitosis per field (x10) G O-LM or C. Sections were also examined for apoptosis, proliferating cell nuclear antigens (PCNA) and vascular endothelial cell factor (VEGF) expressing cell. Apoptosis in G and combined groups was greater than O-LM and C groups and the opposite for PCNA. VEGF was expressed almost uniformly in all groups. In conclusion, G and O-LM in vivo combined treatment seemed to be effective in pancreatic carcinoma, presumably due to an increased apoptosis and a decreased proliferation.
CB-P25.
ANTIPROLIFERATIVE ACTIVITY OF HOP EXTRACTS IN TUMOR CELLS
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Previous reports demonstrated multiple biological effects of humulones or α-acids from hop. The aim of the study was to evaluate the antiproliferative and antioxidant activity of hop extracts in human tumor cell lines (MCF-7, T47D, PAJU). Hop extracts used herein are pure, standardized solutions of iso-α-acids: hexahop gold (HG), tetrahop gold (TH) and isohop (IH) (Hasa Hop Products, Inc.). Cell cultures were treated with different extracts (0-100 μg/ml) for 24 h and dose-response curves were obtained. Proliferative activity was evaluated by MTT assay, apoptosis by staining with Hoechst 33258 and antioxidant capacity by determining the oxidation of 2,7 dichlorofluorescein (DCF). Results demonstrated a dose-dependent inhibition of cell growth in all the cell lines treated with HG (p<0.001) and TH (p<0.001). This inhibition was significant as from 25 μg/ml in MCF-7 and PAJU cells and as from 100 μg/ml in T47D. Conversely, no significant growth inhibition was induced by IH in these cell lines. Cells treated with HH, TH or IH did not show significant induction of apoptosis. Regarding DCF oxidation a dose-dependent decrease was observed in all the cell lines treated with TH and HH. In conclusion, TH and HH induced cell growth inhibition, unrelated to induction of apoptosis and possibly associated to the antioxidant capacity of these extracts.

CB-P26.
MOLECULAR MECHANISM OF HUMAN LEUKEMIA JURKAT T-CELL APOTOSIS INDUCED BY PLANT TRYPsin INHIBITORS
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Plants constitute an important source of compounds which can induce apoptosis in a variety of cells. Previously, we reported the isolation of a trypsin inhibitor from Peltophorum dubium seeds (PDTI). This inhibitor, as well as soybean trypsin inhibitor (SBTI), has lecin-like properties and triggers rat lymphoma cell apoptosis. In the present study, we demonstrate that PDTI and SBTI induce human leukemia Jurkat cell death. Induction of apoptosis was confirmed by flow cytometry after propidium iodide labeling, showing a significant increase of the sub G0/G1 fraction. To understand the mechanism of apoptosis, we evaluated caspases involvement and showed caspases-3 and -8 activation by PDTI or SBTI treatment. Consistent with these results, pan caspase inhibitor and caspase-8 inhibitor protected Jurkat cells from apoptosis. However, there was no caspase-9 activation, confirmed by the failure of caspase-9 inhibitor to prevent cell death. We detected a moderate decrease of mitochondrial membrane potential but no significant release of cytochrome c from mitochondria. These results suggest that the intrinsic mitochondrial pathway is not predominant in the apoptotic process. On the other hand, we observed the recruitment of Fas-associated death domain (FADD) to the cell membrane indicating the involvement of this adaptor protein in PDTI- and SBTI-induced apoptosis in Jurkat cells.

CB-P27.
ACTION OF TAMOXIFEN ON THE UTERUS OF RATS BEARING EXPERIMENTAL MAMMARY TUMORS
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Extended tamoxifen (Tam) administration in breast cancer therapy leads to an increased risk for endometrial neoplasia. The objective of this work was to analyze the effect of Tam on the uterus of rats bearing N-nitroso-N-methyleurea-induced mammary tumors: A) Tam (1 mg/kg/day, sc) was administered for 120 days beginning when rats were 40 days old. B) Tam was administered for 30 days. Control groups not receiving Tam treatment or NMU injection were included. Results enclosed in the investigated uteri were: in A) highly significant decrease of uterine weight and absence of steroid receptors expression. Histological studies showed very thin, nearly atrophic endometrial mucosa and also atrophic smooth muscle wall. In B) significant diminution of estrogen receptor expression, increase of progesterone receptor and cystic glandular endometrial hyperplasia. Data were correlated with the expression of: PCNA, c-fos, bax, bcl-2 and insulin growth factor type-I. In A and B, rat estrus cycle was arrested at disestrus (o.m. analysis of vaginal smears). Nuclear aneuploidy and hyperchromatism was observed in the endometrium of NMU-injected rats regardless of Tam treatment. Results show the wide range of biological interrelations established at the end of treatments.

CB-P28.
THE INSULIN GROWTH FACTOR SYSTEM ON MAMMARY TUMORIGENESIS
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Insulin growth factors (IGFs) are important mediators in the growth, development, differentiation and survival of normal and transformed mammary tissue. The aim of this study was to investigate the expression and localization of the insulin growth factor-type 1 receptor (IGF-IR) and insulin receptor (IR) during the mammary carcinogenesis induced in rats by N-nitroso-N-methyleurea (NNU). NNU was injected to 50, 80 and 110 days old rats. Samples of mammary tissue were collected at days 55, 85 and 115, immunohistochemical analysis and Western blot were performed. The antibodies used were a polyclonal rabbit insulin-Rg and IGF-Rg. Expression of both receptors was increased in epithelial and mesothelial cells of mammary gland ducts throughout the observation period. At day 115, a significantly higher expression of both receptors was found when mammary tissue of NNU injected rats was compared to tissue of normal rats. Our results suggest that both IGF-IR and IR, components of the IGF system, have an important role in the promotion/progression phase in the experimental model tested. At present the expression of both receptors is being determined on mammary glands of NNU injected rats under tamoxifen treatment.