ASSOCIATED WITH ENDOMETRIOSIS PROGRESSION

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Endometriosis is a chronic illness hormone-dependent which is defined by the presence of endometrial tissue outside the uterus. The endocrine disrupting chemicals may be involved in the development and progression of disease. Hexachlorobenzene (HCB) is a pesticide that acts as an endocrine disruptor modulating the hormonal signaling. Aberrant expression of estrogen and progesterone receptors (ER, PR) has been associated with progression of endometriosis, which is referred to as estrogen-dependent and progesterone-resistant. Aromatase is the key enzyme in the estrogen biosynthesis and it is essential for establishment and growth of endometriosis lesions. Previous results showed that HCB induces cell migration and invasion of endometrial cells, increases ERalfa; and reduces PR levels in lesion and eutopic endometrium in rat model. Our aim was to evaluate the dependence of ER on migration (scratch motility assay) and invasion (transwell assay) in endometrial stromal cells (T-HESCs), and to investigate the hormone receptor profile (WB) in T-HESCs and primary cultures of endometrial stromal cells from eutopic endometrium of control (CESC) women. Cells were exposed to HCB (0.005, 0.05, 0.5 and 5 µM) for 24h. Results showed that the pesticide enhanced cell migration (HCB 5 µM, $p<0.001$) and invasion (HCB 0.5 µM, $p<0.001$) in an ER dependent manner in T-HESCs. Moreover, HCB increased expression of Aromatase (0.005, 0.05 and 0.5 µM, $p<0.01$) and ERalfa; (0.5, $p<0.05$), while it reduced PR protein levels (5 µM, $p<0.05$). Instead, the ERß levels were not modified. In CESC, HCB enhanced ERalfa (0.5 µM, $p<0.05$), ERß (0.05, µM $p<0.05$) and Aromatase (0.5 µM, $p<0.05$) protein levels. Also we compared the HCB exposure effects with 17-ß-estradiol, observing that both showed a similar action on REalfa protein expression. In conclusion, our results demonstrated that HCB would act as a xenoestrogen inducing an invasive profile contributing to the development and progression of the disease.

0303 - NEUTRALIZING CAPACITY OF ANTISERA OBTAINED BY IMMUNIZATION WITH BOTHROPS ALTERNATUS VENOM BLOCKED IN THEIR METALLOPROTEINASES

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Specific treatment for snake bite accidents with antivenoms, obtained from immunized animals, is the only recommended therapy. Bothropic intoxication is characterized by proteolytic, coagulant and hemorrhagic effects that induce local tissue damage and systemic alterations that could lead to death. Snake venom metalloproteinases, zinc- dependent, play a relevant role in the pathogenesis of intoxication. They provoke a disruption of the hemostatic system, restricting their use as immunogen in high doses. In previous work, it was demonstrated that is it possible to immunize mice with high doses of Bothrops alternatus venom (BaV) neutralized with disodium salt of ethylenediaminetetraacetic acid (Na2EDTA) avoid of hemorrhagic lesions and then animals reported higher antibodies titer. The present study was performed to test the neutralizing ability of sera obtained from animals treated whit venom-Na2EDTA, and evaluate the effect of this immunogen on pulmonary parenchyma. Groups of 5 BALB/c mice were immunized subcutaneously with BaV, or BaV/Na_2EDTA emulsified with Freund's adjuvant (complete first and incomplete-booster). On day 50 serums were collected for neutralization assays: proteolytic activity on azocasein, phospholipase activity on erythrocytes and thrombine-like activity on citrated plasma. Animals were sacrificed and lungs removed for histological analysis (hematoxylin-eosin). The results showed that the capacity of neutralize each of one of the three enzyme activities assayed was significantly higher ($p<0.05$) by the serum obtained from animals immunized with BaV/Na_2EDTA. Histological analysis showed that the pulmonary parenchyma from immunized mice with BaV was significantly affected (pneumonitis, $p<0.05$), in respect to those were BaV/Na_2EDTA treated. Results demonstrated that the BaV/Na_2EDTA immunogen has a lower organic impact with respect to BaV and, at the same time, providing a serum with high neutralizing capacity.

0322 - PESTICIDES EXPOSURE ENHANCE HIF-1ALPHA, VEGF AND NOS-2 EXPRESSION IN MDA-MB-231 HUMAN BREAST CANCER CELLS

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Organochlorine pesticides such as Hexachlorobenzene (HCB) and organophosphate Chlorpyrifos (CPF) are synthetic compounds used worldwide as insecticides, herbicides and fungicides. Recently, HCB and CPF have become important as a potential risk factor in breast cancer. We demonstrated that these compounds induce proliferation, migration and invasion in human breast cancer cells. Angiogenesis plays a role in local tumor growth and metastasis. It has been observed a correlation between elevated levels of hypoxia inducible factor-1 alpha (HIF-1alpha), tumor metastasis and angiogenesis. HIF-1alpha induces genes like vascular endothelial growth factor (VEGF), nitric oxide synthase-2 (NOS-2) and cyclooxygenase-2 (COX-2). VEGF acts on tumor endothelial cells to increase their proliferation, survival and migration. COX-2 and NOS-2 promote tumor angiogenesis. The aim of our work was to examine the HCB or CPF action on breast cancer angiogenesis. We studied the effects of HCB (0.005, 0.05, 0.5 and 5 µM) or CPF (0.05, 0.5, 5 and 50 µM) exposure in MDA-MB-231 breast cancer cells in dose-response curves on: a) HIF-1alpha expression, b) VEGF secretion, c) COX-2 and d) NOS-2 protein levels (Western blot). Our results showed that MDA-MB-231 exposed for 6 h to CPF increases HIF-1alpha ($p<0.05$) and NOS-2 expression ($p<0.05$) at all assayed doses, as well as VEGF secretion at 0.05, 0.5 and 5 µM ($p<0.05$). Besides, CPF (0.05, 0.5 and 5 µM) stimulates COX-2 levels at 24 h ($p<0.05$). On the other hand, HCB for 6 h enhances HIF-1alpha levels at 0.05, 0.5 and 5 µM ($p<0.05$); NOS-2 expression and VEGF secretion at all assayed doses ($p<0.05$); meanwhile, at 24 h HCB (0.05 and 5 µM) increases COX-2 levels ($p<0.05$). In conclusion, our results demonstrate that HCB and CPF stimulate proangiogenic factors in MDA-MB-231 cells. Altogether, these data highlight that the pesticide exposure could promote the angiogenic processes contributing to mammary carcinogenesis.

0323 - HEXACHLOROBENZENE EXPOSURE INDUCES PRO-ANGIOGENIC MICROENVIRONMENT IN AN IN VITRO MODEL OF ENDOMETRIOSIS

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Endometriosis, characterized by ectopic growth of endometrial tissue, is a common gynecological disease. The symptoms are pelvic pain and infertility, affecting women quality of life. Angiogenesis is critical in the development and maintenance of endometriotic lesions. It is a multistep process of new blood vessel formation that involves secretion of growth factors and extracellular matrix degradation, as well as migration, proliferation, and tube formation by endothelial cells. Vascular endothelial growth factor (VEGF) is an angiogenic factor that plays an important role in endometriosis progression. Exposure to endocrine-disrupting environmental pollutants is associated with the disease etiology. Hexachlorobenzene (HCB) is a pesticide that induces toxic reproductive effects in laboratory animals. Previous results showed that HCB increases the volume of endometriotic like-lesions, microvessel density and VEGF levels in a rat endometriosis model. The present study examined the effect of HCB on endometriosis angiogenesis in vitro. Human endometrial stromal cells (T-HESCs) were exposed to HCB (0.005, 0.05, 0.5 and 5 µM) or vehicle for 48 h, and the conditioned media were then used to stimulate EA.hy926 endothelial cells to evaluate cell proliferation (MTT assay and PCNA expression), migration (scratch motility assay) and tube-like structure formation in a Matrigel assay. The results showed that HCB (0.005-0.05 µM) induced VEGF secretion ($p<0.05$) in T-HESCs cells. Moreover, the conditioned media treatment enhanced cell proliferation (0.005-5 µM, $p<0.05$), migration (0.005-0.5 µM, $p<0.05$), and tube formation increasing total tube length (0.005 µM, $p<0.05$; 0.5 µM $p<0.01$) and branching points (0.5 µM, $p<0.05$) in endothelial cells. Our results demonstrated that HCB exposure induces angiogenic factors secretion in human endometrial cells T-HESC, triggering an increase in the ability of endothelial cells to form new vessels, a critical event for the endometriosis progression.

0483 - SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT: A NEW MARKER FOR ESTROGENICITY OF ENDOCRINE DISRUPTOR COMPOUNDS

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Endocrine disruptor compounds (EDCs) comprise naturally occurring and synthetic substances widely spread in the environment that adversely affect human and wildlife. Screening methods and ideal biomarkers to determine EDC potency need to be exhaustively improved. Soluble guanylyl cyclase alpha1 subunit (sGC alpha1) is an abundant cytosolic protein ubiquitously expressed in most tissues. We previously showed that sGC alpha1 is specifically and highly up-regulated by estrogen (E2) in vivo and in vitro, although it lacks estrogen responsive elements. The aim of this work was to evaluate sGC alpha1 protein expression as a potential marker for xenoestrogenic EDC exposure. First, the effect of E2 on sGC alpha1 expression was tested in several E2-dependent cell lines (MCF-7, ECC-1 and GH3). Following experiments were performed using GH3 cells since they are commonly included in in vitro EDCs screening tests. Cells were incubated for 48 h with a wide variety of xenoestrogenic EDCs: Cd, Pb, Cr, Ni, As, ethynylestradiol, diethylstilbestrol, bisphenol A, hexachlorobenzene, and chlorpyrifos at a range of doses from nM to pM. sGC alpha1 protein levels were determined by Western blot. E2 increased sGC alpha1 expression in all cell lines tested: MCF-7 (% of control (C), 130.53 ± 8.06*), ECC-1 (232.26 ± 6.94***), and GH3 (208.7 ±10***), (*$p<0.05$, ***$p<0.001$ vs. respective C). E2 augmented sGC alpha1 through estrogen receptor (ER) activation (sGC alpha1 protein levels, % of C; E2: 208.7 ± 10***, ICI 182,780: 113 ± 9, ICI 182,780+E2: 86 ± 5###, $p<0.001$ vs. respective C). sGC alpha1 expression was strongly up-regulated by all the EDCs tested even by those exhibiting low or null ER binding capacity ($p<0.05$). Natural hormones not binding ER (progesterone, prolactin, and insulin) were unable to modify sGC alpha1 levels. Here we provide evidence that in vitro sGC alpha1 protein assay may be a very sensitive and powerful tool to identify compounds with estrogenic activity, which could improve current mammalian-based screening methods.

0489 - CYTOTOXICITY OF ZINC NANOPARTICLES BIOSYNTHESIZED BY MICROORGANISMS ON HUMAN KERATINOCYTE CELL LINE

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Metal nanoparticles –NPs– (10-100 nm) have an important antimicrobial activity which suggests possible biomedical applications. Zinc NPs (ZnNPs) are widely used for different products. The aim of this work was to investigate the toxicity of ZnNPs biosynthesized by microorganisms, in a human keratinocyte cell line (HaCaT). ZnNPs were synthesized using Pseudomonas aeruginosa (ATCC 27853) and were characterized by UV-Vis spectroscopy and by transmission electron microscopy. HaCaT cells were incubated for 4 h and 24 h at different ZnNPs dilutions (1/2, 1/5 and 1/10). RPMI 1640 culture medium with 5% FBS, a metal precursor salt solution of $ZnSO_4$ (0.1 and 0.25 mM), and a bacterial growth control of biosynthesis (BGC), were used as controls. Cell viability was evaluated by MTT assay, crystal violet and neutral red tests; reactive oxygen species (ROS) were studied by DCF-DA; superoxide dismutase (SOD) activity was determined by riboflavin-NBT method; and reduced glutathione (GSH) by Ellman reactive. ZnNPs cell capture assays were performed by fluorescence microscopy and changes in cell migration were evaluated by wound healing assay. As determined by fluorescence microscopy ZnNPs were able to enter HaCaT cells. The toxicity assays indicated that cell viability was significantly altered by ZnNPs 1/2 and BGC conditions after 4 h and 24 h incubation. ROS levels increased after 4 h incubation with ZnNPs 1/2, 1/5, and BGC, while a 24 h incubation, 1/10 dilution also augmented ROS. SOD activity increases at all ZnNPs dilutions tested, and with BGC. GSH was not modified by any treatment. Finally, the presence of ZnNPs and BGC in the culture media affected cell migration. Altogether these results suggest that ZnNPs are able not only to enter into skin cells but also to modify human keratinocyte viability, oxidant/antioxidant cell balance and cell migration. More studies are needed to unravel the mechanisms underlying these alterations.

Bioinformática, genoma, proteoma y nuevas tecnologías / Bioinformatic III

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0481 - RESPONSE OF MACROPHAGES IN CONTACT WITH SYNTHETIC BIOMATERIALS BASED ON POLY-N-ISOPROPYLACRYLAMIDE AND COPOLYMER HYDROGELS

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