information about physiological mechanism of uptake and transport and allows the quantification of the 99mTc-IDA derivatives excretion by hepatobiliary system. Based on these we studied the HEF as an indicator of human LR of patients with hepatic tumors underwent PH.

Material and Methods: 33 patients (13 W and 20 M; 61,3±11,3 years old) with colorectal metastases (n=25), hepatocellular carcinoma (n=4) and other tumors (n=4) were included. Eight patients (24%) were submitted to a major hepatectomy (MAH) and the others (76%) to a minor hepatectomy (MIH). LR was assessed after intravenous bolus injection of 99mTc-N-(3-bromo-2,4,6-trimethylphenylcarbamoilmethyl 1-iminodiacetic acid (Mebrofenin) that was uptaked by the hepatocytes and eventually excreted via billiary pathway without any change to its chemical structure. The HEF is calculated using deconvolution analysis of first pass curve coming from scintigraphic data. We evaluated the pre-operative HEF (T0) and in the 5th day (T5) and one month after PH (T30). We considered the HEF values of 98.8 ± 0.4% (MED ± SD) as normal. For statistical analysis: t-Student test was used.

Results: 1) The mortality and morbility rates were 0% and 15% respectively; 2) the HEF was 98.33±3.36% at T0, 98.7±2.7% at T5 and 97.9±5% at T30 (no significant differences); 3) the HEF values of the patients submitted to a MAH were 98.2 ± 3.1% at T0, 98.7 ± 2.3% at T5 and 97.1 ± 5% at T30, and for those submitted to a MIH were 98.4 ± 3.1% at T0, 98.9 ± 3.3% at T5 and 98.1 ± 5% at T30 (no significant differences).

Conclusion: These results allows to say that the human LR is early enough to normalize the HEF at day 5 after PH, being this evaluation of undoubtedly interest to know the function kinetics and indirectly knowledge about human LR. Additionally, this fast functional liver recovery has high clinical importance, because more aggressive adjuvant chemotherapy can start much early after surgical treatment.

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Estrogen-associated genes expression in uterine leiomyomas

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Uterine leiomyomas are benign smooth muscle tumors and the most common type of gynecological tumor, representing a significant public health problem. It is generally accepted that these tumors are estrogen dependent because they have the ability to enlarge during pregnancy and to shrink during menopause, ovariectomy, and other hypoestrogeneic conditions. There are only a few studies in the literature regarding hormone regulation and steroid hormone receptor status in uterine leiomyomas. Previous studies suggested that the AHR gene, involved in cell proliferation regulation, is a potential marker involved in uterine leiomyomas. AHR gene codifies the dioxin receptor, which forms dimers with another receptor, the ARNT. The complex AHR-ARNT binds DNA sequences to modulate transcription rates of some genes, including the estrogen receptor. The aims of the present study was investigate the ESR1, ESR2, PGR and AHR mRNA expression in 46 uterine leiomyomas and in normal myometrium using quantitative real time PCR to explore the hormonal molecular basis of these tumors. It was detected a down-expression of all genes: 72% of cases for ESR1, 43% for ESR2, 35% for PGR, and 76% for AHR. In addition, in the 46 cases studied, 63% showed an increased ratio of ESR2/ESR1. The expression pattern was compared to clinical-pathological data, including patient age, age at menarche, number of pregnancies, age at first pregnancy, cycle reproductive phase, race, body mass index features, number of myomas, and localization. It was detected that ESR1 and ESR2 expression levels were statistically associated with race (non-white versus white patients) and that PGR gene expression was higher in patients that presented early menarche. These results suggest that ESR1 and ESR2 may play an important role in the development of leiomyoma and that an imbalance in expression of these receptors may contribute to the pathogenesis of the disease. In addition, AHR gene can be assessed as putative marker in the growth and development of uterine leiomyomas.

Both authors (Reis-Rosa, L.A. and Cirillo, P.R.) have contributed equally.

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Proliferation-associated genes correlated to hormonal receptors and Ki-67 status in breast carcinomas

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The determination of estrogen and progesterone receptors expression status is crucial for the decision on therapeutic strategies. However, the routine evaluation of ESR1 and PGR status by immunohitochemistry shows large interlaboratory variability, particularly when these genes are expressed at low levels. The stratification of breast cancer patients based on characteristic patterns of gene expression associated with ESR1/PGR status is important to improve clinical management and useful to overcome these limitations. Recently, our group reported that breast carcinomas with high Ki-67 expression were significantly associated with tumors exhibiting low levels of mRNA and undetectable protein levels of ESR1 and PGR. In the present study, global gene expression analysis was performed in 68 invasive ductal carcinomas (29 cases: training set; 39 cases: validation set) using the CodeLink Human Whole Genome BioArray (GE HealthCare) platform. The samples were grouped according to ESR1 and PGR expression status measured at both the transcript and protein level as well as to their proliferative index (< 25% and > 25% immunopositivity to discriminate Ki-67- and Ki-67+ tumors, respectively) to explore the implications of the Ki-67 status in defining proliferation gene expression signatures. Using signal-to-noise ratio with permutation and leave-one-out cross-validation, 68 sequences differentially expressed ($p<0.001$) were identified between ESR1-/Ki-67+ and ESR1+/Ki-67- tumor samples. A similar analysis comparing PGR-/Ki-67+ versus PGR+/Ki-67- tumor samples showed 83 sequences differentially expressed ($p<0.001$). A set of 17 genes involved in cell proliferation was identified as differentially expressed in both analyses. A subset of these genes was investigated by quantitative real time PCR (qRT-PCR) in an independent group of samples confirming the oligoarray expression data. Moreover, a significant statistical correlation was observed between gene expression and histologic grade. These data point to a set of genes with a role in increasing the proliferative rate of breast tumor cells, revealing novel potential biomarkers involved in breast carcinogenesis.

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Identification of a gene expression signature correlated to breast cancer prognosis

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Breast carcinoma is a heterogeneous disease with different molecular subtypes being characterized by distinct morphological appearances, genetic alterations, and clinical presentation. This heterogeneity poses a major challenge in its diagnosis and treatment. Breast cancer can also display notorious distinct clinical characteristics in different patient and ethnic populations, having divergent clinical courses despite having similar histopathologic histopathology appearances. Brazilians form one of the most heterogeneous populations in the world, the result of five centuries of interethnic crosses between peoples from three continents: the European colonizers and immigrants; African slaves; and the autochthonous Amerindians. For this reason, molecular signatures of breast carcinomas from Brazilian patients can contribute to identify new molecular markers common at several ethnicities. To this end, global gene expression profiles of a a set of 43 primary breast tumors samples were evaluated using high-density oligoarrays (Platform CodeLink Human Whole Genome BioArray, GE HealthCare). Multiple statistical methods (signal-to-noise ratio with permutation, leave-one-out, t-test) were applied, combining the prognostic information and clinical outcome. Based on clinical criteria and traditional markers used in clinical practice two subgroups of patients were defined: a group of 25 patients were evaluated as “good prognosis”, of which 5 of them presented metastasis; and a group of 18 patients considered of “poor prognosis”, from which 4 of them showed metastasis. The comparison between all patients with metastasis (9 cases) with the group of good and poor prognosis identified an expression signature comprising 52 genes (leave-one-out, $p<0.001$) able to distinguish good prognosis patients without metastasis from patients who had metastasis independent of the molecular prognosis. We also identified an expression signature comprising 134 genes (t-test, $p< 0.02$) able to differentiate patients with poor prognosis that showed no metastasis (for more than five years after the surgical procedure), from patients who developed metastasis. This gene expression signature correctly stratified patients into good prognosis group or poor prognosis group. Differential expression of a subset of these genes was independently confirmed in a larger set of tumor samples using quantitative RT-PCR. Cross-reference of these signature with available breast cancer prognosis signatures such as Oncotype DXtradeTM (Genomic Health Inc.) and MamaPrintTM (Agendia Inc.) showed a limited overlap. These results point to a novel gene expression signature of breast

carcinomas capable of distinguishing samples according to clinical course and progression of the disease.

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Effects of mycotoxins on apoptosis of human immune system

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Apoptosis is an important process in a wide variety of different biological systems and also in chemical-induced cell death.

The immune system is now recognized as a target organ for many xenobiotics such as drugs and chemicals, which are able to trigger unwanted apoptosis or to alter the regulation of programmed cell death. Reducing the number of immune-competent cells after xenobiotic treatment can lead to immunosuppressive effects, resulting in an increased susceptibility to tumors or infections diseases.

Mycotoxins are secondary metabolites produced by microfungi that are capable of causing diseases and death in humans and animals at low concentrations. The immunotoxic effects can be mediated by direct toxin interactions with lymphocytes and other blood cells, provoking, among others effects, a rapid and strong apoptosis of human lymphoid cells.

Our objective was to explore the effects of deoxynivalenol (DON) and ochratoxin A (OTA) on apoptosis of immune-competent cells using cell lines as model.

The effects of both mycotoxins on apoptosis of lymphocytes at the cellular and molecular level were studied using flow cytometric analysis, western blotting or ELISA system. We found that toxin effects was origin-dependent and dose dependent and both mycotoxins caused inhibition of cell proliferation, mediated by activation of apoptosis pathway. In conclusion DON and OTA by apoptosis-induced in vitro on cells lines model may have some negative effects on human immunosystem that support further investigations

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Lipoxygenase expression and intracellular localization in different types of cancer

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Lipoxygenases (LOX), particularly 5-LOX and 12-LOX, have been implicated in carcinogenesis and several LOX-inhibitory drugs and natural products have been tested in preclinical studies and early drug trials for anti-carcinogenic activity and clinical effects. Knowledge is still lacking on LOX and their products with respect to biological activities, particularly intracellularly. The expression and localization of 5- and 12-LOX was studied by immunohistochemistry in 10 samples from cancers and normal tissue of pancreas, breast, colon, stomach, prostate and lung. Staining intensity, localization and proportion of positive cells were scored. Furthermore, malignant cell lines from pancreas (PANC-1) and breast (T-47D) were synchronized by serum starvation before adding 10% FCS. Cells were stained for 5- and 12-LOX by immunoperoxidase or immunofluorescence after 0, 2 and 6 hours and analysed by light and confocal microscopy. The expression of 5-LOX was more marked in cancer compared with the normal counterpart for most tissues except colon (very little expression) and stomach (marked expression in normal and malignant tissue). The nuclear envelope was the most prominent localization. For 12-LOX the expression was generally more marked than that of 5-LOX; little or no difference was seen between cancer and normal tissue in breast, colon and stomach, but cancer of pancreas, prostate and lung showed increased expression. The nucleus and nuclear envelope stained strongly for 12-LOX. The in vitro experiments showed appearance of 5-LOX in the nuclear envelope following stimulation but 12-LOX was always seen at the nuclear envelope. Nuclear expression of 12-LOX changed following stimulation and showed different behaviour in the two cell lines; a transient increase in PANC-1 but a decrease in T-47D. In conclusion, changes in LOX expression in cancer are variable depending on tissue. Increased expression was seen for 5-LOX in cancer of breast and pancreas and for 12-LOX in cancer of pancreas, prostate and lung. As the activating protein of 5-LOX (FLAP) is located in the nuclear envelope, the shift in intracellular localization in the in vitro experiments might indicate a link to the cell cycle. The considerable differences between cancers of different origin, reflected also in the different behaviour of 12-LOX seen in the two cell lines, will have to be kept in mind when designing strategies for chemoprevention or treatment of cancer based on LOX inhibition.

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Effect of stimuli treatment on proliferation and apoptosis of tumor cells

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The main obstacle against the success of therapy in many cancers seems to be the impossibility of eradication of all tumor cells. Increase of replicative capacity, loss of cell adhesion and angiogenesis process represent aggravating factors of clinical evolution for cancer patients. Breast and ovarian cancers represent some malignancies with high incidence and mortality throughout women, their ethiology involving many genetic, immunological and biochemical factors. Malignant evolution depends on the genetic profile of tumor, which dictates its reaction to citotoxic action exerted by chemotherapeutical agents or contributes to a resistant phenotype. Structural or gene expression alterations are responsible not only for the appearance of cancer, but also for the clinical responses of patients to chemotherapy. The present study focused on the potential influence of stimuli treatment (doxorubicin, cytokines, curcumin) on proliferation by cell cycle phases and apoptosis of breast and ovarian tumor cells. Experiments were performed on human breast and ovarian adenocarcinoma cell lines, levels of resistance being tested by measuring the expression of P-glycoprotein during cultivation of MCF-7, MDA-MB-231 and SK-OV-3 cells with stimuli. Sensibitility of tumor cells to stimuli treatment was evaluated by measuring the cytotoxicity induced by treatment using MTT or XTT colorimetric methods. We have also analyzed by flow-cytometry the influence of stimuli treatment on antigene expression of bcl-2, p53, Ki-67, Fas and P-glycoprotein, correlated with the modifications of apoptosis and cell cycle phases. Progression through cell cycle phases was evaluated by PI technique and flow-cytometry analysis, while percentages of apoptotic cells were detected by using Annexin V - FITC/PI coloration, followed by flow-cytometry. In addition, gene expression of molecules under study was analyzed by RT real-time PCR, and the results correlated with antigen expression detected by flow-cytometry. Data obtained could lead to a selection of patients who might benefit the most of antitumor immunotherapeutical strategies focused on diminishing the primary tumor and controlling/eliminating the metastases. Knowing the modifications induced by chemotherapeutical agents in human tumor cell lines will make possible the identification of new gene alterations associated with the resistant phenotype, which might be taken into account for future gene therapy of breast and ovarian cancers.

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Experimental/Molecular therapeutics, pharmacogenomics 3

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TSA regulates P-glycoprotein and multidrug resistance associated protein expression in cancer cells

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Multidrug resistance (MDR) constitutes a major obstacle for success of cancer treatment. Although several mechanisms could be involved in the acquisition of this phenotype, the role of two different membrane proteins, P-glycoprotein (Pgp) and multidrug resistance associated protein (MRP) has been well established. Both proteins are members of the same ATP-binding cassette superfamily of transport proteins.

We have studied the effects of histone deacetylase inhibitors, such as TSA and SAHA on Pgp and MRP expression in different cancer cell models, including HT-29 and HCT-15 human colon carcinoma cell lines; IMIM-PC-1, RWP-1 and IMIM-PC-2 human pancreatic adenocarcinoma cell lines; MCF-7 and MCF-7/Adr human breast carcinoma; HL-60, HL-60R, K-562 and K562/Adr human leukaemia cell lines. In all these cell lines we