Meeting Abstracts:

Papers Selected for Oral Presentation

XIII Pathology Meeting of Hospital A.C. Camargo

III International Meeting on Investigative Pathology

Abstract 44

Título / Title: Viral factors induce hedgehog pathway activation in humans with viral hepatitis, cirrhosis, and hepatocellular carcinoma

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Hedgehog (Hh) pathway activation promotes many processes that occur during fibrogenic liver repair. Whether the Hh pathway modulates the outcomes of virally-mediated liver injury has never been examined. Gene-profiling studies of human hepatocellular carcinomas (HCC) demonstrate Hh pathway activation in HCCs related to chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). Because most HCC develop in cirrhotic livers, we hypothesized that Hh pathway activation occurs during fibrogenic repair of liver damage due to chronic viral hepatitis, and that Hh-responsive cells mediate disease progression and hepatocarcinogenesis in chronic viral hepatitis. Immunohistochemistry and qRT-PCR analysis were used to analyze Hh pathway activation and identify Hh-responsive cell types in archival liver biopsies from 45 patients with chronic HBV or HCV with different stages of fibrosis (F0-4), some of which also presented HCC. Hh signaling was manipulated with cyclopamine in primary human hepatic stellate cells (HSC) and sinusoidal endothelial cells (SEC). Angiogenesis assay was used to investigate if the pathway plays a role in tube formation. SECs were incubated with recombinant Shh, conditioned media from HSC, cyclopamine, short hairpin RNA against smoothened or their respective controls and the length of vascular tubes were measured by morphometry. Hedgehog production was investigated in Huh7 cells infected with JFH-1 and in a rat hepatoma cell line that express the HBV X gene by qRT-PCR. We found increased hepatic expression of Hh ligands (Shh and Ihh) in all patients with chronic viral hepatitis ($p<0.005$), and demonstrated that infection with JFH-1 or ectopic expression of the HBV X gene stimulated cultured hepatocytes to produce Shh ($p<0.005$). The major cell populations that expanded during cirrhosis and HCC (i.e., liver myofibroblasts, activated endothelial cells, and progenitors expressing markers of tumor stem/initiating cells – CD133, K7 and Epcam) were Hh-responsive (Patched and Gli-2 positive) and higher levels of Hh pathway activity associated with cirrhosis and HCC ($p<0.005$). Inhibiting pathway activity in Hh-responsive target cells (HSC and SEC) reduced fibrogenesis ($p<0.005$ for αSMA and $p<0.05$ for col1α1

expression) and angiogenesis ($p<0.05$). HBV/HCV infection increases hepatocyte production of Hh ligands and expands types of Hh-responsive cells that promote liver fibrosis and cancer.
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Abstract 41

Título / Title: The CALM/AF10 interactor CATS is a substrate of KIS, a positive regulator of cell cycle progression in leukemia cells

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CATS is a phosphoprotein initially identified in a Y2H screen as the CALM (PICALM) interacting protein expressed in thymus and spleen. The CATS interaction region of CALM is contained in the leukemogenic fusion protein CALM/AF10, which is found in acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and in malignant lymphoma. CATS sequesters CALM/AF10 in the nucleolus and interferes with the transactivation capacity of CALM/AF10 in a dose-dependent manner. However, the involvement of CATS in malignant transformation seems to go beyond its interaction with CALM/AF10. CATS is highly expressed in leukemia, lymphoma and tumor cell lines but not in non-proliferating T-cells or PBLs. The protein levels of CATS are cell cycle-dependent, induced by mitogens (e.g. PHA) and correlate with the proliferative state of the cell. Thus, CATS can be viewed as a marker for proliferation. To further study CATS function, we searched for CATS interacting proteins. In addition, we analyzed KIS protein expression in primary cells from MDS and leukemia patients. CATS was used as bait in a yeast two-hybrid screen. Protein interaction identified was confirmed by coimmunoprecipitation of overexpressed proteins. *In vitro* kinase assay was performed to investigate whether CATS is a substrate of KIS and to map the residue within CATS, which is phosphorylated. KIS expression was analyzed on bone marrow mononuclear cells (MNCs) of MDS, AML and ALL patients by Western blotting. We studied five healthy donors, 11 MDS patients (seven low-risk [RA/RARS] and four high-risk [RAEB/RAEBt] according to FAB classification), seven AML and one ALL. We identified the kinase interacting with stathmin (KIS or UHMK1) as a CATS interacting partner. KIS is a nuclear serine/threonine kinase that possesses an RNA recognition motif and phosphorylates and regulates the activity of RNA associated factors. Moreover KIS positively regulates cell cycle progression through phosphorylation of $p27^{KIP}$ in leukemic cell lines. We confirmed the CATS-KIS interaction in the yeast system and by co-IP for both CATS isoforms. Using kinase assay we could show that CATS is a substrate for KIS being strongly phosphorylated on its serine 131, which lies within the SGSP consensus sequence for KIS phosphorylation. Finally, Western blotting analysis revealed elevated levels of KIS in MDS, AML and ALL compared to the control samples. Our results show that CATS not only interacts with but is also a substrate for KIS, suggesting that CATS function might be modulated through phosphorylation events. The identification of the CATS-KIS interaction further supports the hypothesis that CATS plays an important role in the control of cell proliferation. Moreover, the elevated levels of KIS in hematological malignances suggest that KIS could regulate CATS activity and/or function in highly proliferating leukemic cells. Thus, our results indicate that CATS function might be important to understand the malignant transformation mediated by CALM/AF10.
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Abstract 03

Título/ Title: A genomic and transcriptomic integrated analysis in penile carcinoma

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Penile carcinoma (PC) is a rare neoplasm frequently associated with high-risk human papillomavirus (HPV) types. In this study, the most relevant copy number variations and gene expression alterations in PC were evaluated by performing an integrative analysis of the data.

DNA and RNA were extracted from 13 PC samples. Genomic copy number variations and gene expression were assessed by 4x44K platforms (Agilent). CGH-array data were extracted with Feature Extraction 10.1.1.1.1 software and analyzed by Genomic Workbench Standard 5.0.14, statistical algorithm ADM-2 and sensitivity threshold of 6.0. Gene expression data were analyzed using TMeV 4.5 software (*www.tm4.org)* with SAM algorithm, 1000 permutations and no false discovery rate (FDR). The integrated analysis was performed with covariance > 30% and exclusion of outliers and noise. HPV was genotyped in all samples using *LINEAR ARRAY HPV Genotyping Test* (Roche).

One hundred seventy-one copy number alterations were verified, corresponding to an average of 13.15±6.85 CNVs per individual. The most common copy number gains were detected at 2q21.3, 8q24.12, 13q32.1, and 8q; losses were found in 8p and Y chromosome. Three hundred genes differentially expressed were found mainly associated with cell metabolism and transcription regulation. The integrative genomic and transcriptomic analysis revealed positive correlation for 44 out of 102 significant genes, including *TNFRSF11B* and the tumor suppressor gene *CDKN2A*. Three out of thirteen samples of PC were positive for HPV subtype 16. Unsupervised gene expression analysis revealed no differences between HPV positives and negatives cases.

A significant positive correlation was verified between copy number alterations and gene expression analysis independent of HPV genotype. To the best of our knowledge, this is the first study showing an integrative genomic and transcriptomic analysis in penile carcinoma revealing putative markers for this disease frequently detected in developing countries.

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Abstract 28

Título / Title: FGFR2 gene expression discriminate desmoid-type fibromatosis from fibrosarcomas

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Fibroblastic tumors represent a group of tumors that include benign tumors to high grade sarcomas. Between these two extremes, desmoid-type fibromatosis (DTF) are clonal tumors with fibroblastic proliferation and local aggressiveness but without metastatic potential. The aim of this study was to determine differential gene expression profile and define molecules able of discriminating among Desmoid-type fibromatosis (DTF) and fibrosarcoma (FS). Using a cDNA platform representing 4608 genes, we sought a gene that could discriminate desmoid-type fibromatosis (19 samples) from fibrosarcomas (04 samples). Validation was done in 124 samples (112 DTF and 12 FS) by immunohistochemistry and 25 samples by Quantitative Real Time-PCR (QRT-PCR). cDNA microarray results pointed to a set of genes that discriminate DTF from fibrosarcomas. Among differential expressed genes selected for validation, FGFR2 (Fibroblast Growth Factor Receptor 2) was able to separate precisely DTF from fibrosarcomas. QRT-PCR and immunohistochemical results corroborate with cDNA data with DTF showing higher expression levels of FGFR2 than fibrosarcomas. Mathematical analysis showed significant statistical values of differential expression between these two groups (p=0.0081). Our results pointed to FGFR2 transcript and protein levels as a potential discriminator between these fibroblastic tumors. The roles of these molecules deserve further studies in these tumor groups.

Abstract 40

Título / Title: Raman spectroscopy applied to the diagnosis of breast cancer: biochemical classification, suitability for immunohistochemical studies and *in vivo* application

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Breast cancer is the second most common type of cancer among the world population, with a survival rate of 61% after 5 years. Raman spectroscopy is an optical technique that can provide information about the molecular composition of an investigated sample. Recently, it has emerged as a non-destructive analytical tool for the biochemical characterization of breast tissues due to several advantages such as sensitivity to small structural changes, non-invasive sample capability, and for non-requirement sample preparation. However, Raman experiments are scarce due to the great difficulty to differentiate the subtypes of breast cancers, and also, only a few experiments have used animal models for the study of clinical applicability of Raman spectroscopy diagnosis. This work was divided into two stages. In the first stage, we evaluated several human breast tissue biopsies to obtain a differentiation between normal tissues and 7 subtypes of breast pathologies by FT-Raman *ex vivo*. In the second stage, we evaluated and discriminated benign and malignant mammary lesion in animal model through *in vivo* FT-Raman spectroscopy associated to immunohistochemistry exam (MMP-19). Raman *ex vivo* - 194 Raman spectra from breast tissues were classified into 8 groups: normal breast tissue, fibrocystic condition, duct carcinoma in situ, duct carcinoma in situ with necrosis, infiltrating duct carcinoma not otherwise specified, colloid infiltrating duct carcinoma, and invasive lobular carcinomas. Raman *in vivo* - 32 Sprague-Dawley rats were mammary gland tumors induced with a single dose of 75 mg/kg p.o. of DMBA. Histological analysis indicated that mammary hyperplasia, cribriform, papillary and solid adenocarcinomas were found. PCA and LDA with cross-validation were applied as spectral classification algorithm. For the human breast tissue experiment, we were able to establish the biochemical basis for each spectrum that plays a special role in the carcinogenesis process. For the animal study, it was possible to show that normal and adenocarcinoma tissues discrimination was possible using transcutaneous *in vivo* Raman collection mode. This study has demonstrated the utility of Raman spectroscopy as a potential tool for the screening of breast cancer, enabling a quick and simplistic breast cancer diagnosis, minimizing the patient trauma, time delay, and high medical costs of biopsies.

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References

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Abstract 08

Título / Title: Avaliação da infecção de camundongos CBA por *Leishmania amazonensis* e *Leishmania major* na derme da orelha

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Camundongos CBA são resistentes à *L. major* e susceptíveis à *L. amazonensis*. A maior parte dos estudos tem sido feito com inoculação de parasitos no subcutâneo e não na derme como ocorre na infecção natural. Decidimos comparar o curso da infecção na derme da orelha e avaliar diversos parâmetros. Os CBA/J formam inoculados com $5x10^5$ *L. amazonensis* ou *L. major* na derme da orelha. Os camundongos inoculados com *L. amazonensis* desenvolveram lesões progressivas enquanto nos inoculados com *L. major* as lesões foram discretas. Na primeira semana o infiltrado inflamatório caracterizou-se pela predominância de neutrófilos até 12 horas e de macrófagos após 24 horas, nos dois grupos. Após 45 dias, nos animais inoculados com *L. amazonensis* o infiltrado inflamatório foi intenso, composto por macrófagos com vacúolos grandes repletos de amastigotas e foi moderado com predominância de macrófagos naqueles inoculados com *L. major*. A carga parasitária (45 dias) de *L. amazonensis* foi 40 vezes maior do que a de *L. major* nas orelhas, mas 100 vezes menor nos linfonodos; e a produção de IFN-α foi maior tanto nas orelhas (3X) quanto nos linfonodos (10X) dos animais inoculados com *L. major*. Não foi detectada diferença na produção de IL-4 entre os grupos. A produção de ROS, uma hora

após a inoculação dos parasitos, foi significativamente maior ($p < 0,0001$) nas orelhas inoculadas com *L. major*. A inibição da produção de superóxido com difeniliodonio (100μM/Kg) aumentou 3 vezes a carga parasitária de *L. major* em neutrófilos peritoniais. Em ensaios de migração de células inflamatórias a partir de folhetos das orelhas colados em cultura observou-se que as células macrofágicas infectadas com *L. amazonensis* migram menos que as infectadas com *L. major*. Os resultados mostram que camundongos CBA, inoculados na derme da orelha, são resistentes à *L. major* e susceptíveis à *L. amazonensis* no local da infecção, mas são mais permissivos à *L. major* do que à *L. amazonensis* no linfonodo de drenagem. A menor capacidade migratória dos macrófagos infectados com L. amazonensis pode estar relacionado com a menor carga parasitária no linfonodo. A diferença na produção de superóxido nas fases inicias da infecção pode ser crítica na determinação do curso da infecção.