Meeting Abstracts: Papers

Selected for Poster Presentation

XIII Pathology Meeting of Hospital A.C. Camargo

III International Meeting on Investigative Pathology

Abstract 01

Título/Title: A dosagem sérica de Galectina-3 pode auxiliar na identificação de malignidade nos nódulos de tiróide

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Considerando-se que nódulos de tiróide são encontrados em metade da população submetida a ultrassonografia, é imperativo identificar marcadores de malignidade de aplicação populacional. A expressão imunoistoquímica da galectina-3 tem sido utiliizada para caracterizar malignidade e auxiliar no diagnóstico diferencial do nódulo tiroidiano. Esta proteína, ligada a diversos processos vitais para a célula e relacionada a iniciação e progressão tumoral, também pode ser identificada em soro.

Comprovar a possível utilidade clínica da dosagem sérica de Galectina-3 no diagnóstico de malignidade em nódulos tiroidianos, no diagnóstico diferencial e no seguimento do paciente com cancer diferenciado da tiróide (CDT).

Níveis séricos galectina-3 foram dosados através de ELISA em 84 portadores de nódulos submetidos à cirurgia por suspeita de malignidade incluindo 71 pacientes com CDT (65 Carcinomas Papilíferos-CP e 6 Carcinomas Foliculares-CF) e 13 Bócios. Os pacientes foram pareados para sexo, idade e etnia com 91 indivíduos saudáveis. Todos os pacientes com câncer foram conduzidos de acordo com um mesmo protocolo de seguimento por ±1,5anos.

Pacientes com CDT apresentaram dosagens mais elevadas de Galectina-3 (±1,28 ng/mL) do que pacientes com nódulos benignos (±1,16 ng/mL) e controles (±1,15 ng/mL; p=0.0016). Os CP apresentaram expressão mais elevada (±1,28 ng/mL do que controles (±1,15 ng/mL; p=0.0007), mas a expressão de Galectina-3 não diferenciou CDT de nódulos benignos (p=0.2683) nem CP de CF (p=0.7961). Não encontramos relação entre os níveis de Galectina-3 com idade (p=0.8683), sexo (p=0.7889) ou etnia (p=0.8976), com níveis de tiroglobulina sérica (p=0.7597) ou qualquer parâmetro de recidiva/recorrência.

Estes dados preliminares sugerem que a dosagem de Galectina-3 sérica pode vir a ser útil como marcador de diagnóstico para o CDT, mas talvez não seja marcadora de prognóstico ou auxilie no diagnóstico diferencial do nódulo de tireóide.

Abstract 02

Título / Title: A expressão da proteína e de mRNA de MUC1 pode ajudar a diferenciar lesões tireoidianas com padrão folicular

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Os carcinomas bem diferenciados da tiróide (CDT) têm estrutura e função semelhantes as das células foliculares normais, tendo uma boa resposta ao tratamento. Aproximadamente 20 a 30% dos nódulos tireoidianos, mostram após a Punção Aspirativa por Agulha Fina (PAAF), achados citológicos indeterminados, encaminhados para a cirurgia, às vezes desnecessárias, quando se tratava de um nódulo benigno. Portanto, há necessidade de novos marcadores de diagnóstico. MUC1 é uma glicoproteína transmembrana freqüente na superfície das células epiteliais, promovendo uma barreira contra infecções. Nas células epiteliais normais, MUC1 é restrita apenas à superfície apical, mas em células tumorais, sua polarização é perdida, porque MUC1 se torna aberrantemente glicosilada. A proteína MUC1 super expressa no citoplasma das células cancerosas pode ajudar a diferenciar células tumorais de células normais. Analisar a expressão de MUC1 como marcador de diagnóstico em lesões tireoidianas.

Foram construídos dois tissue microarray (TMA) com áreas representativas dos tumores. Utilizamos imunoistoquímica (IHQ) para avaliar expressão da proteína MUC1 em 410 casos com nódulos tireoidianos, incluindo 248 carcinomas papilíferos (CP) e 41 carcinomas foliculares (CF). Dos CP 149 eram clássicos (CPC), 20 variantes de células altas (CPVA) e 79 variantes foliculares (CPVF). Foram estudadas ainda 121 tecidos tiroidianos incluindo 16 tecidos normais (TN) e 105 lesões benignas: 50 hiperplasias e 55 adenomas foliculares (AF). A expressão de RNAm de MUC1 foi analisada por PCR quantitativa (qPCR) em 108 carcinomas, 23 hiperplasias e 19 AF.

A proteína MUC1 foi identificada em 80.2% CP; 48.8% CF; 68,3% CPVF; 70% CPVA; AF 21.8%; 6% (TN), 30% hiperplasias. MUC1 distinguiu tecidos benignos de malignos da tiróide $p<0.0001$ (sensibilidade = 89%, especificidade = 53%). A expressão da proteína MUC1 diferenciou CF de AF com sensibilidade = 62.5 %, especificidade = 67.1 % ($p = 0,0083$); CPVF de AF com sensibilidade = 81.2 %, especificidade = 63.4 % ($p <0,0001$); CPVF de hiperplasia com sensibilidade = 78.2 %, especificidade = 58.3 % ($p <0,0001$); CPC de AF com sensibilidade = 91.6 %, especificidade = 70.4 % ($p <0,0001$). A expressão de RNAm de MUC1 também distinguiu malignos de benignos (Mann-Whitney; $p<0.0001$). Nossos resultados sugerem que expressão de MUC1 pode auxiliar no diagnóstico diferencial das lesões foliculares da tiróide.

Abstract 04

Título / Title: An alternative Brazilian nomogram for predicting breast cancer metastases in non-sentinel lymph node when the sentinel node is positive

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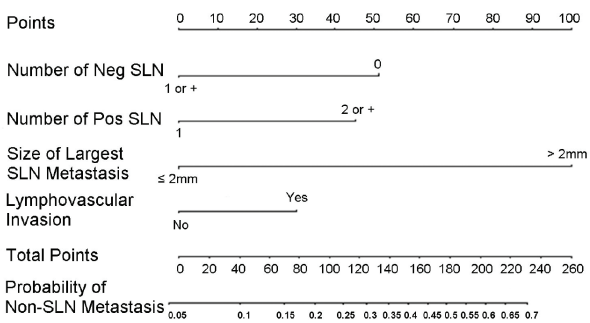
The role of complete axillary lymph node dissection (ALND) after identification of metastasis in sentinel lymph node (SLN) biopsy of women with breast cancer has been questioned. Up to 70% of patients with positive SLN are found to have no other metastasis in non-sentinel lymph node (NSLN) [1]. Several mathematical models (nomograms and scores) have been proposed to predict the likelihood of metastasis in NSLN in order to determine if a complete ALND should be performed or not when the SLN is positive [1,2,3].

The aim of our study was to evaluate risk factors for NSLN metastasis in SLN-positive patients and to propose a mathematical model (nomogram) to predict the likelihood of finding additional positive nodes at ALND.

We reviewed 326 cases of patients with breast cancer and positive-SLN divided into two groups according to the nodal involvement in the ALND: patients with all non-sentinel nodes negative for metastasis, and patients with at least one positive NSLN. Pathological features of the primary tumor (tumor size, histological tumor type and grade, mitotic index, nuclear grade, lymphovascular invasion, estrogen and progesterone receptor status), and SLN (number of SLN, detection method of metastasis and size of SLN metastasis) were assessed. Data were submitted to univariate and multivariate logistic regression, followed by construction of a mathematical model (nomogram) to predict the presence of additional disease in the non-SLN of these patients.

The univariate and multivariate analyses identified the following risk factors for involvement of NSLN with the respective *p* values: size of the largest SLN metastasis ($p<0.001$, $p=0.002$), number of positive SLN ($p=0.006$, $p=0.04$) and negative SLN ($p=0.010$, $p=0.004$), and lymphovascular invasion (LVI), ($p=0.075$, $p=0.085$). The Brazilian Nomogram (Fig. 1) was created using size of largest SLN metastasis, number of positive and negative SLN and LVI. The nomogram was discriminating, with an area under the receiver operating characteristic (ROC) curve of 0.70. Our data showed that the size of largest SLN metastasis, number of positive and negative SLN were predictive risk factors for metastatic involvement of NSLN in patients with positive-SLN. Our nomogram, similar to other models, may represent an additional tool to help physicians and patients who decide whether or not a complete ALND should be performed.



Abstract 04 Figure 1 – Nomogram

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Brazilian Nomogram to predict the likelihood of finding additional positive nodes at ALND. SLN: Sentinel lymph node; Neg: Negative; Pos: Positive.

Abstract 05

Título / Title: Análise da expressão protéica de marcadores de células-tronco tumorais em neoplasias ovarianas epiteliais

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Pelo conceito das células-tronco tumorais (CTT) somente um pequeno subgrupo de células tem capacidade de iniciar e sustentar o crescimento tumoral [1]. Determinadas proteínas têm demonstrado ser marcadores de CTTs. Como os carcinomas epiteliais de ovário são histopatologicamente heterogêneos e a grande maioria das pacientes apresenta recorrência da doença após quimioterapia, a identificação das CTTs poderia contribuir para o conhecimento de seu papel na carcinogênese e desenvolvimento de novas terapias [2]. Analisar a expressão de marcadores de CTTs (CD133, Nestina, AP-2γ, CD31, CD44 e c-Kit) em tumores epiteliais benignos, *borderlines* e malignos e correlacionar com sobrevida global (SG), livre de recorrência (SLR) e com parâmetros patológicos (tipo patológico, local, invasão de cápsula, graduação histológica, por exemplo). Foram selecionadas 129 amostras de neoplasia do epitélio de ovário obtidas do arquivo do Departamento de Anatomia Patológica do Hospital A.C.Camargo-São

Paulo entre 2000 e 2009. Imunoistoquímica foi realizada para avaliar a expressão das proteínas pesquisadas. Para as análises, foram montados Tissue Microarray (TMA). A avaliação das lâminas de TMA foi feita para cada anticorpo utilizando o Sistema Automatizado de Imagem Celular (ACIS). A correlação entre variáveis patológicas e as proteínas estudadas foi realizada pelo teste exato de Fisher/teste do qui-quadrado. Para análises de sobrevida foi usado o Método de Kaplan-Meier e a diferença entre as curvas foi calculada pelo teste de log-rank.

Nestina e CD31 encontraram-se mais expressos em tumores malignos do que nos borderline e benignos ($p<0,05$). c-Kit, assim como CD133 e AP-2γ encontraram-se mais expressos em tumores bem/moderadamente diferenciados em relação aos pouco difenciados ($p <0,05$). Em relação à SG, foram importantes as seguintes proteínas: c-Kit ($p= 0,0004$), CD44 ($p = 0,021$), AP-2γ ($p= 0,0026$) e CD133 ($p= 0,027$). Para a SLR apenas CD44 mostrou-se estatisticamente significativo ($p=0,0021$).

CD133 vem sendo usado na literatura como padrão ouro no diagnóstico de CTTs em neoplasias de ovário. Em nosso estudo, esta mólécula, juntamente com AP-2γ e c-Kit, firmaram sua importância, pois interferiram negativamente na SG e encontraram-se mais expressas em tumores bem/moderadamente diferenciados. Isso mostra que podem estar envolvidas no processo de malignização.

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Abstract 06

Título / Title: Analysis of cell surface carbohydrate expression of in situ ductal carcinoma using lectin histochemistry and immunohistochemistry for p53

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Breast carcinoma is one of the most common neoplasms in women and is a leading cause of cancer related deaths worldwide [1]. The increased number of patients diagnosed with ductal carcinoma *in situ* (DCIS) breast tumors opened up new avenues in research and new dilemmas in clinical practice [2]. Among the various mechanisms of cellular dedifferentiation, glycosylation is frequently amended where changes in carbohydrate profile can be considered biomarkers of the tumorigenic process. Deciphering of the cell surface glycocode, lectins have being used as probes in histochemistry [3].

This work aimed to evaluate the expression profile of cell surface carbohydrates in normal and ductal carcinoma *in situ* (DCIS) of the breast using lectin histochemistry and correlate with p53 immunohistochemistry profile. Biopsies diagnosed with DCIS (n=15) were obtained at the Cancer Hospital of Pernambuco. Tissue slices were treated with trypsin and methanol-H_2O_2 prior to incubation with horseradish peroxidase (HRP) conjugated lectin peanut agglutinin (PNA) at 80μg/mL. Immunohistochemistry for p53 was assayed. Both protocols used DAB-H_2O_2 as HRP substrate. All experiments were realized in duplicate and evaluated in optical microscopy. DCIS was p53 positive in 86.6% of cases with a comedo pattern. In normal tissues (free boards of samples), p53 was negative. PNA, D-galactose specific, was positive in 53.3% of the samples studied. The D-galactose was expressed in neoplastic cells of all types of DCIS. However, PNA staining was mainly in cytoplasm and membrane of neoplastic cells of the transition area DCIS-IDC. In normal tissues, PNA staining was limited to cell membrane presenting a week and punctual staining**.** Results demonstrated a higher expression of D-galactose in samples characterized by cells in transition stage from DCIS to ICD, suggesting PNA as an auxiliary histopathology tool in addition to p53 to help the diagnosis of these lesions.

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Abstract 07

Título / Title: Avaliação da expressão de galectina-3 em carcinoma cervical com ou sem metástase linfonodal associado ao HPV

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A galectina-3 uma proteína da família da lectina induz a angiogênese, permite adesão das células tumorais na membrana basal dos vasos que posteriormente invadirão a corrente sanguínea,e, através de sua propriedade de adesão é capaz de ligar-se as células de órgão distantes facilitando o processo de colonização e desenvolvimento de tumores secundários.Por essas propriedades a galectina-3 está envolvida na progressão tumoral e metástase.Considerando,que o HPV é detectado em 95% dos carcinomas cervicais, este poderia estar envolvido na desregulação da expressão da galectina-3 favorecendo um maior potencial metastático dos carcinomas cervicais invasores.
Avaliar a expressão imunohistoquímica da galectina-3 em lesões malignas de colo uterino, metastáticas e não metastáticas,associadas a infecção pelo HPV a fim de verificar a progressão do câncer cervical e o possível envolvimento do vírus na alteração da expressão de galectina-3.
79 pacientes diagnosticadas com carcinoma de colo uterino, foram subdivididas em dois grupos de acordo com o laudo da biópsia:ausência de metástase(n=52) e presença de metástase linfonodal(n=27).Ainda foram avaliadas as biópsias dos linfonodos pélvicos(79). As amostras foram submetidas ao método de imunohistoquímica, e a detecção de HPV foi realizada através da técnica de PCR.
Das 79 pacientes avaliadas,67(84.8%)apresentaram expressão de galectina-3.Entre os grupos,41(79%) dos carcinomas cervicais invasores sem metástase e 26(96,3%) com metástase expressaram essa molécula.O grupo com ausência de metástase apresentou resultado significante na expressão entre a lesão do colo e seu linfonodo correspondente;entretanto no grupo com presença de metástase linfonodal não foi observado resultado estatisticamente significante.Quanto a análise quantitativa, o teste de Wilcoxon revelou que em ambos os grupos, com ausência de metástase($p< 0,0001$)e com presença de metástase($p<0,0001$) houve diferença estatisticamente significante entre a lesão do colo e seus linfonodos correspondente.Os testes de Mann Whitney e Spearman também revelaram resultados significantes. Em relação à presença ou ausência do HPV e a expressão da galectina-3 não foi observado resultado significante. Segundo nossos achados, a alta expressão de galectina-3 poderia ser considerada um bom marcador biológico para invasão e metástase no carcinoma cervical.

Abstract 09

Título / Title: Avaliação do efeito da inibição da enzima ácido graxo sintase (FASN) pelo orlistate em modelo murino de colonização pulmonar (B16F10/ C57BL6)

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A enzima ácido graxo sintase (FASN) desempenha papel chave na lipogênese de células neoplásicas, sendo considerada um oncogene metabólico em potencial, com papel importante no crescimento e sobrevivência de células tumorais. A alta expressão de FASN é observada vários tumores malignos humanos, incluindo o melanoma, neoplasia maligna que apresenta alto potencial metastático e resistência aos agentes quimioterápicos. Nestes tumores a alta expressão de FASN está associada com pior prognóstico e profundidade de invasão. Diferentes inibidores da atividade de FASN vêm sendo utilizados em diversos estudos, dentre estes a droga orlistate, aprovada pela FDA e utilizada para o tratamento de obesidade, a qual apresentou propriedades anti-tumorais e anti-proliferativas em modelo xenográfico de câncer de próstata e em linhagens tumorais derivadas de câncer de próstata e mama. Os achados recentes de trabalho desenvolvido por nosso

grupo de pesquisa demonstraram uma redução de 50% nas metástases espontâneas para linfonodos em melanomas experimentais pela inibição farmacológica de FASN com a droga orlistate. O objetivo do presente trabalho foi analisar o efeito da inibição da FASN com orlistate em modelo murino de colonização pulmonar e também avaliar seu efeito sobre a atividade gelatinolítica das metaloproteases de matriz (MMPs) 2 e 9 em células B16F10 através de ensaios zimográficos. Foram utilizados 40 camundongos machos C57BL6 com x semanas, os quais receberam implantes de 1x106 células B16F10 na veia caudal lateral. Após x dias do transplante, os animais foram tratados com orlistate (240mg/kg/dia) ou com o veículo etanol pelos períodos de 21 a 28 dias. Observamos redução de 54,3% no número de colonizações metastáticas pulmonares dos animais tratados com a droga em comparação ao grupo controle ($p< 0,005$). Os ensaios zimográficos demonstraram que após o tratamento com orlistate não houve modificação da atividade gelatinolítica das MMPs 2 e 9 secretadas pelas células B16F10. Os resultados deste trabalho monstram que a inibição de FASN com orlistate reduz significativamente o número de metástases pulmonares neste modelo experimental, que faz desta enzima um alvo terapêutico em potencial para estes tumores. Outros estudos são necessários para elucidar os mecanismos pelos quais o processo metastático é inibido após o tratamento com orlistate, nossos resultados sugerem que o tratamento com orlistate parece não estar associado à redução da atividade das MMPs 2 e 9.

Apoio FAPESP.

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Abstract 10

Título / Title: Baixa expressão de bcl-2 em carcinoma de células escamosa de laringe: associação com grau histológico

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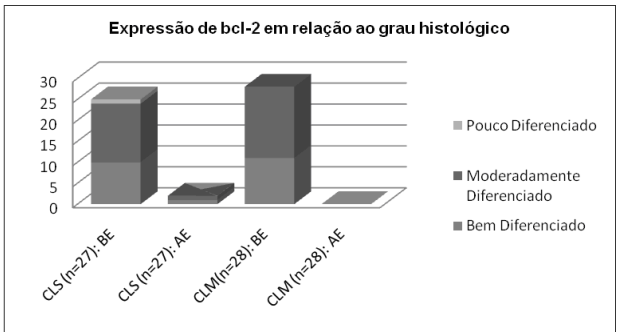
Tumores de laringe representam cerca de 2,0% de todos os cânceres no Brasil, correspondendo a cerca de 8.000 novos casos e 3.000 mortes anualmente. Este tipo de câncer é o mais comum dentre os tumores malignos da cabeça e pescoço. Vários tipos de marcadores moleculares são estudados em câncer de laringe, incluindo proteínas associadas a apoptose como a bcl-2. Essa proteína em condições normais é responsável pela prevenção da morte celular por apoptose em várias situações e alterações na sua expressão pode prolongar a sobrevivência de células defeituosas, facilitando a transformação maligna e consequentemente o câncer. Avaliar a expressão da proteína bcl-2 e sua possível associação com o grau histológico (bem diferenciado, moderadamente diferenciado e pouco diferenciado) em casos de carcinoma de laringe. Neste estudo foi realizada análise imunoistoquímica para bcl-2 em 65 pacientes diagnosticados com câncer de laringe subdivididos em três grupos: 10 carcinomas de laringe *in situ* (CLIS), 27 carcinoma de laringe sem metástase (CLS) e 28 com metástase (CLM). Em todas as biópsias analisadas no presente estudo (n=65) observamos, que a maioria (93,54%), apresentou uma alta incidência de negatividade ou marcação leve quanto a expressão de bcl-2. Quando comparados o padrão de expressão de bcl2 entre os grupos CLIS, CLS e CLM observou-se que não houve diferença significante entre os mesmo tanto na avaliação quantitativa (média de células marcadas) como na qualitativa (intensidade de marcação, porém houve uma tendência a menor expressão de bcl-2 principalmente no grupo mais agressivo (CLM). Ao compararmos a expressão de bcl-2 com grau histológico, observamos que quanto menor o grau de diferenciação celular da lesão, menor foi a expressão de bcl-2 (Figura 1). Semelhante aos resultados encontrados por Chang *et al*(2002) em lesões orais, nossos achados nos leva a concluir que a baixa expressão de bcl-2 nos carcinomas de laringe com metástase (CLM) e nas lesões menos diferenciadas pode ser utilizada como um possível marcador de agressividade nesse tipo de câncer. No entanto estudos com um maior número amostral são necessários para confirmar esse indício.



Abstract 10 Figura 1 - Expressão de bcl-2 e grau histológico
CLS: Carcinoma de laringe sem metástase
CLM: Carcinoma de laringe com metástase

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Abstract 11

Título / Title: BubR1 immunostaining in benign and malignant oral lesions

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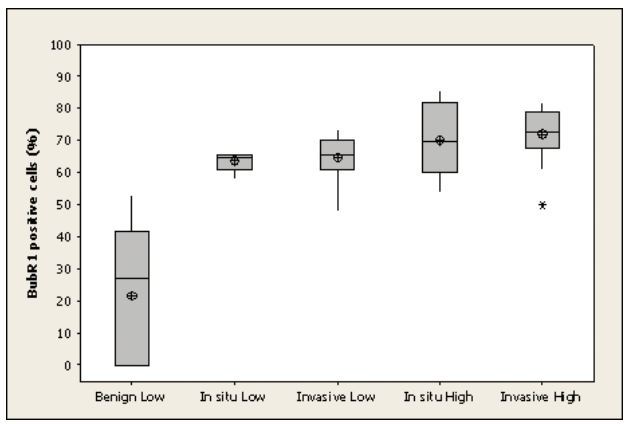
BubR1 is an important protein in the mitotic spindle assembly checkpoint (SAC) that protects the cell from chromosome missegregation and aneuploidy during mitosis 1. The expression of BubR1 has been associated with tumor recurrence, progression and other clinical aspects of cancer 2. Some studies show that karyotypes of malignant oral lesions are near-triploid and present a lot of structural and numerical abnormalities that could influence chromosomal segregation. Our objective was to evaluate the expression profile of BubR1 in different oral lesions and the association of BubR1 expression with patient's cumulative survival. Immunohistochemistry for BubR1 was performed in 63 oral lesions grouped according to their diagnosis (16 non-malignant lesions, 20 in situ carcinomas and 27 invasive carcinomas). Qualitative interpretation of expression was carried out by an experienced pathologist. The low expression was considered when the cells were negative or showed discrete immunostaining and high expression when the cells showed moderate or intense immunolabeling. A mean of ten random microscope fields were selected in order to analyze 1000 cytoplasm. The positive cytoplasms were quantified by a computer-assisted system (Image-Pro Plus - Cybernetics, MD, USA).

According to qualitative evaluation, all benign lesions showed low expression of BubR1, while high expression was observed in 75% of in situ and 70% of invasive carcinomas. The quantitative evaluation for BubR1 was similar to the qualitative one, thus, the mean of positive cells observed in benign lesions was lower than in the carcinomas (Figure 1). After a mean follow-up of 25 months since the date of diagnosis, none of the 10 patients with benign lesion died or presented malignant lesions, while 35% of the in situ and 33% of the invasive carcinoma group have died. Patients with malignant samples and high BubR1 expression showed a shorter cumulative survival (26.8 months) than patients with malignant lesion and low expression (28.69 months), however, the log-rank test was not significant (p=0.129). Our results suggest that BubR1 protein high expression could be a good predictor for malignant oral lesions. On the other hand, we observed no significant association between BubR1 expression and the cumulative survival in our patients.



Abstract 11 Figure 1 – Profile of BubR1 expression according to the groups of oral lesion.

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Abstract 12

Título / Title: Caspase expression in oral squamous cell carcinoma

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Apoptosis is a genetically programmed form of cell death, of which caspases are the central components. To characterize the expression of caspases 3, 6, 7, 8, 9, and 10 and determine possible correlations between the expression of these proteins and clinicopathologic features of oral squamous cell carcinoma (OSCC). Two-hundred twenty-nine cases of OSCC, arranged in a tissue microarray, were immunohistochemically analyzed. The results were quantitatively analyzed using an automated imaging system (ACIS III) which detects levels of hue, saturation and luminosity, converting this signal into a numerical density measurement that ranges from 0 to 256. All proteins that we examined were expressed in primary OSCC samples. Caspases 8 and 9 were prominently expressed, and caspases 3, 6, 7, and 10 were occasionally expressed. Disease-free survival differed significantly between caspase 7 high- and low-expressing patients, and our multivariate analysis suggested that expression of caspase 7 is an independent prognostic factor for OSCC patients. This study suggests that caspases regulate the tumorigenesis of OSCC and that caspase 7 expression is a predictor of locoregional recurrence of OSCC.
Financial support: FAPESP

Abstract 13

Título / Title: Clinical, ultrasound and scintigraphic parameters of malignancy in thyroid nodule with a first benign cytologic result: a five-year follow-up

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Fine-needle aspiration cytology (FNAC) is the gold standard for evaluating thyroid nodules. However, the false negative (FN) rate of FNAC ranges from 2.3 to 6.2%. The accuracy of a single clinical, US or scintigraphic feature in distinguishing benign from malignant thyroid nodule with initially benign cytologic result is not well established. This study aimed to investigate the value of clinical, US and scintigraphic parameters of malignancy in thyroid nodules with initially benign cytological results. We retrospectively studied 151 patients submitted to thyroid surgery (1998-2008). This study included 194 nodules, 64.4% (125) benign vs. 35.6% (69) malignant, with 46 nodules (23.7% of total) that were diagnosed as benign at first FNAC and underwent a follow-up study that showed a malignant result in 17 (G1). Age, gender, thyroid autoimmunity, family history, growth tumor size, scintigraphic (cold vs. hot nodule) and sonographic features (echogenicity, border regularity, central vascularity, calcifications, nodularity and localization) were compared between the two groups by chi-square and non-parametric test (Mann-Whitney) ($p<0.05$). Of the 17 malignant cases detected, 47.1% was diagnosed by the third FNAC. There were no differences between the two groups in mean age, gender, TSH, free T4, TAb, thyroid dysfunction, compressive symptoms and family history of thyroid cancer. Neither the growth size of the tumor or cold nodule was determinant for malignancy. The laterality and nodularity were not different. Sonographic studies showed a larger mean size in G2 ($p=0.0003$) and positive signs of malignancy in G1: microcalcifications ($p=0.05$), central vascularity ($p=0.031$), irregular border ($p=0.001$), size<2cm ($p=0.001$) with no difference in hypoechogenicity. The border irregularity was the strongest predictor of malignancy followed by microcalcifications, vascularity and diameter <2cm. The mean 5-year follow-up showed stability or reduction size of nodules in 50% of G2 ($p=0.05$). Malignant tumors detected after first benign cytology showed a smaller size and sonographic

suspicious features during a mean five-year follow-up period. The border irregularity was the strongest predictor of malignancy. The scintigraphic study was not useful to the differentiation of two groups. The number and localization of nodules were not predictive of malignancy. No clinical or lab parameter was significant in this study.

Abstract 14

Título / Title: Desferrioxamine, an iron chelator, decreases mortality and parasitemia in *Trypanosoma cruzi*-infected mice through direct action on parasite

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Although DFA is known to reduce the intensity of T. *cruzi* mice infection, the mechanism underlying this effect is still unclear and may involve host and parasite factors. This study aimed to investigate the impact of DFA on mice disease outcome, on *T. cruzi* biology and on host biomarkers. Disease and parasitological studies were performed. The effect of DFA in disease outcome was verified by parasitemia and mortality studies, as well as host iron metabolism, blood cells and lymphocyte subsets analysis. To evaluate the activity of DFA directly on parasites, we tested culture growth inhibition, mobility, membrane integrity and apoptosis. DFA-treated animals presented lower cumulative mortality rate in long term infection and lower parasitemia in both short and long term infection. No effect was observed in iron metabolism markers, erythrogram, leukogram, lymphocyte subsets, except for an increase in lymphocyte counts at 7th d.p.i. DFA-inhibited amastigotes and trypomastigote growth in fibroblast culture decreased parasite mobility, induced minor parasite apoptosis but did not change viability measured by trypan blue staining. DFA action on parasite indicates possible a trypanostatic effect independent of interference on host iron metabolism and with minor effects on lymphocyte subpopulation counts.

Abstract 15

Título / Title: Diagnostic contribution of the detection of the BRAFV600E mutation on fine-needle aspiration specimens of thyroid nodules with indeterminate cytological findings

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Fine-needle aspiration biopsy (FNAB) is considered the method of choice in the preoperative assessment of the thyroid nodules. About 20% of the biopsies show indeterminate findings and the majority of those patients undergo surgery that reveals a benign finding. The BRAFV600E mutation is present in ~50% of papillary thyroid carcinoma (PTC), is highly specific and most clinical studies have associated it with aggressive clinicopathologic behavior and high tumor recurrence. Testing BRAF mutation in FNAB specimens has been shown to refine the diagnostic accuracy of PTC in indeterminate cytology and may provide important value for prognostication. This study aimed to evaluate BRAFV600E mutation test in patients with indeterminate cytology and correlate that with histological findings of matching surgical pathology specimens. Patients subject to thyroid FNAB at A.C. Camargo Hospital from March to October 2009 were prospectively studied with liquid based cytology and BRAF mutation test. Thirty-nine specimens were reported as indeterminate (51% follicular lesion, 31% suspicious for PTC, 13% suspicious for malignancy, 5% inconclusive). PCR and Sanger sequencing method were used for detecting BRAF mutation. Histologically, 25 out of 39 (64%) cases were diagnosed as PTC and 13/39 (33%) were benign lesions. BRAFV600E mutation was detected in 17/25 (68%) of PTCs and not detected in any benign lesion (positive predictive value of 100%). 30/39 (77%) cases were correctly classified by

BRAF status. 62.5% of PTC without BRAF mutation was follicular variant. The BRAF V600E mutation analysis can significantly improve FNAB diagnostic accuracy. It's proposed as a diagnostic adjunctive tool in the evaluation of thyroid nodules with indeterminate cytological findings and can provide useful information for treatment. Positive results can be confidently interpreted as malignant. In our population, 36% of negative results were associated with malignancy, enriched by the follicular variant.

Abstract 16

Título / Title: Differences in the AKT pathway detected by digital quantification of protein expression in different subtypes of breast cancer

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The AKT pathway is involved in breast cancer progression and differential expression among molecular subtypes of breast cancer has been described. Some new modulators of the AKT pathway are under investigation for clinical treatment and quantification at the protein level may be clinically relevant. So far, no standard method to quantify the AKT pathway is available. We evaluated protein expression by immunohistochemistry of AKT, pAKT, mTOR, PTEN, ER, PR and Her2 using tissue microarrays including 56 breast carcinomas, T1/T2 without metastasis, in patients between 40 and 70 years old using tissue microarray. Cases where classified based on the expression of estrogen receptor and Her2. The digital quantification was performed using the ACIS III microscope. Local invasion and proliferation parameters were compared to the molecular profile and AKT, pAKT, mTOR and PTEN levels of expression. The mean age was 53 and the mean tumor size was 2.65 cm (0.4 to 4.5 cm). The mean cytoplasmic expressions were AKT=109.74, pAKT=86.21, mTOR=96.85 and PTEN=121.01. Nuclear staining was observed in 18 (36.0%), 23 (46%), 0 (0%) and 29 (56.7%) cases of AKT, pAKT, mTOR and PTEN, respectively. Triple negative tumors were associated with high mitotic index (p=0.036), low cytoplasmic expressions of AKT and mTOR (p=0.027 and p=0.037, respectively). There was no association between AKT, pAKT, mTOR or PTEN expression and tumor size, number of mitosis or mitotic index. We observed a positive and significant correlation between the expression of PTEN and AKT (r=0.40; p=0.003); PTEN and pAKT (r=0.32; p=0.012); but no correlation between PTEN and mTOR (r=0.06; p=0.76). Triple negative tumors showed lower cytoplasmic expression of AKT and mTOR compared to Luminal A tumors. The high mitotic index observed in triple negative tumors was not associated to AKT pathway overexpression. The digital quantification using immunohistochemistry can detect differences in protein expression of AKT pathway among breast cancer molecular subtypes and may be of clinical use.

Abstract 17

Título / Title: Differential gene expression of *IL1β* and *KLK13* separate desmoid-type fibromatosis (DTF) from benign mesenchymal tumors

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Desmoid-type fibromatosis (DTF) are clonal tumors with fibroblastic proliferation and local aggressiveness, but without metastatic potential. Thus, these tumors present an intermediate behavior, presenting benign and malignant characteristics. Here we show a molecular marker capable of discriminating DTF from benign mesenchymal tumors. Using a cDNA platform representing 4608 genes, we sought genes that discriminate DTF (16 samples) from benign mesenchymal tumors (leiomyoma, neurofibroma and others). Validation of differential expression was done in 28 samples by quantitative Real-Time PCR (QRT-PCR) using TaqMan detection system. cDNA

microarray results pointed to a set of genes that discriminate DTF from the other histological types of STT analyzed. Among differential expressed genes identified in our analysis, the genes *IL1*β (Interleukin-1 beta) and *KLK13* (kallikrein-13) precisely discriminated DTF from the benign samples. QRT-PCR confirmed that DTF have lower *IL1*β (p=0.0027) and KLK13 (p=0.001) transcript levels than all other benign tumors. Our results showed that *KLK13* and *IL1*β could precisely discriminate DTF from benign mesenchymal tumors, representing two important molecular markers. Hence, *IL1*β is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis. *KLK13* expression is regulated by steroid hormones and may be useful as a marker for breast cancer. Thus, the role of these molecules deserves further studies in these tumor groups.

Abstract 18

Título / Title: Epidermal growth factor receptor (EGFR) gene amplification is not the cause of protein overexpression in penile carcinoma

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Squamous cell carcinoma of the penis mainly affects people with poor hygiene habits in undeveloped countries. Epidermal growth factor receptor (EGFR) is a well characterized tyrosine-kinase receptor that has its expression levels increased in diverse tumors, especially in carcinomas. Based on that, this study aimed at evaluating the gene alterations associated with cases with high protein expression levels. Immunohistochemistry (IHC) against EGFR was performed in 195 penile carcinoma samples selected from the medical records of A.C. Camargo Hospital, Brazil. Cases showing strong and complete membrane staining in more than 10% of the tumor cells were considered positive and were submitted to dual-color fluorescence *in situ* hybridization (FISH). Reactions were carried out using fluorescent-labeled probes for EGFR locus and chromosome 7 centromere (Zytovision™) in samples overexpressing EGFR, previously selected by immunohistochemistry. Cases showing two signals of each probe were considered non-altered, those showing more than two signals of each probe were considered polysemic and those showing more EGFR signals compared to centromere signals were considered amplified. In this series, 67 (49.7%) penile carcinoma samples overexpressed EGFR by IHC and were selected for FISH. Protein overexpression was associated with greater risk of recurrence in univariate analysis (p=0,031). Regarding FISH, 31 cases (46%) were uninterpretable, and out of 36 valid cases, 22 (61.1%) were non-altered cases, 12 (33.3%) were polysemic of chromosome 7 and 2 (5.6%) cases presented EGFR amplification. The high number of uninterpretable cases in FISH seems to be related to technical artifacts due to the high quantity of cytokeratin which may block probe penetration in cell cytoplasm and nuclei of these tumor cells. Although EGFR overexpression seems to be associated with worse prognosis, neither gene copy number nor polysemy of chromosome 7 is the main cause of this abnormality in penile tumors. Further studies concerning mutational analysis and clinical data are needed and might be useful for identifying patients who may benefit from EGFR-target therapy.

Abstract 19

Título / Title: Epidermal growth factor receptor (EGFR) high expression rates associates with recurrence in penile cancer

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Penile squamous cell carcinoma is commonly found in undeveloped countries. Epidermal growth factor receptor (EGFR) is a well characterized growth factor receptor that shows increased expression levels in tumors,

mainly in carcinomas. We aimed at evaluating EGFR profile and frequency of expression in penile carcinomas and correlate these findings with clinicopathological features. Immunohistochemistry (IHC) against EGFR was performed in 195 penile carcinoma samples selected from the medical records of A.C. Camargo Hospital, Brazil. Cases showing strong and complete membrane staining in more than 10% of the tumor cells were considered positive. Any other staining pattern was considered negative. Clinicopathological features and survival data were reviewed from hospital records. For statistical analyses, $p<0.05$ was considered significant. Ninety-one cases (49.7%) were considered positive and ninety-two were considered negative, out of which eighty-three (45.4%) showed incomplete membrane staining and nine (4.9%) showed none. Five tumors were uninterpretable due to pre-analytical artifacts. EGFR showed an important heterogeneous expression pattern along the tumor tissue showing variable IHC staining intensity. Positive cases were associated with higher incidence of recurrence in multivariate analyses ($p=0.031$). Our results show that, as in other tumors, EGFR overexpression seems to be associated with poor prognosis and more aggressive penile carcinoma.

Abstract 20

Título / Title: Epithelial-mesenchymal transition protein expression in early stage breast cancer

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Transcriptional repressors have been associated with the epithelial-mesenchymal transition and breast cancer progression in *in vitro* and animal models. Protein expression of TWIST, SLUG and SNAIL in human breast cancer is not well studied. TWIST, SLUG, SNAIL, ER, PR and Her2 protein expression were evaluated using tissue microarray and digital signal quantification in 56 Stage I human breast carcinomas. Tumors were T1/T2 with no metastasis from patients between 40 and 70 years old. Pathological parameters were compared to the molecular profile and TWIST, SLUG and SNAIL expressions. Short-term recurrences were also investigated. The mean age of patients was 53 and the mean tumor size was 2.65 cm (0.4 to 4.5 cm) and the median follow-up 40 months. The number of cases with positive nuclear staining was 1 (1.9%), 23 (45.1%), and 5 (9.8%) for TWIST, SLUG and SNAIL, respectively. Triple negative tumors were associated with high mitotic index ($p=0.036$) but the molecular profile was not associated with tumor grade or size. TWIST showed a moderate direct correlation with SLUG ($r=0.64$) expression and weak direct correlation with SNAIL ($r=0.28$) expression. There was no association between TWIST, SLUG or SNAIL expression and tumor size or grade, number of mitosis or mitotic index. Eight patients develop recurrences (loco-regional or systemic) but no association with TWIST, SLUG and SNAIL expression was observed. TWIST, SLUG and SNAIL showed a coordinated expression but no association with local invasion, cell proliferation or early recurrences was observed in early stage breast cancer. The high mitotic index observed in triple negative tumors was not associated with these tumor repressor expressions.

Abstract 21

Título / Title: Estudo morfológico das células mioepiteliais no desenvolvimento das glândulas salivares humanas: relação entre a maturação das células mioepiteliais e as fases da morfogênese glandular

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A morfogênese das glândulas salivares é um processo dividido em fases: broto, ramificação, canalização e citodiferenciação. Durante essas fases ocorre a diferenciação de componentes glandulares, incluindo as células mioepiteliais. A presença de marcadores

específicos dessas células durante a morfogênese glandular não é totalmente compreendida. O objetivo do nosso estudo foi avaliar a maturação das células mioepiteliais por meio de seus marcadores específicos e correlacionar com as fases da morfogênese das glândulas salivares humanas. Material e Método: Usando a técnica imunoistoquiímica de dupla-marcação, nós avaliamos a expressão de actina músculo liso (AML), calponina, caldesmon, CD10, CD29, proteína S-100, proteína glial fibrilar ácida (GFAP) e p63 nas células mioepiteliais durante a morfogênese da glândula salivar. Para isso espécimes de glândula salivar provenientes da dissecção de 15 fetos humanos entre 8 e 26 semanas de vida intra-uterina (de aborto natural) foram usados. Glândulas salivares adultas foram utilizadas como controles. Na fase canalicular da morfogênese glandular, a proteína p63 foi co-localizada com AML, calponina, CD29 e proteína S-100 nas células mioepiteliais em torno do saco terminal, lóbulos acinar imaturo e ductos intercalares. Na glândula salivar adulta, a expressão dessas proteínas foi observada em células mioepiteliais. GFAP, CD10 e caldesmon não foram detectadas durante a morfogênese e nem em glândulas salivares adulta. A proteína p63 também foi detectada em células basais de todo o sistema ductal. Conclusão: As células mioepiteliais podem ser identificadas na fase canalicular da morfogênese da glândula. Actina músculo liso, calponina, CD29, p63 e proteína S-100 são bons marcadores para células mioepiteliais, embora não sejam específicos para esse tipo celular.

Abstract 22

Title: Evaluation of the prognostic value of peritumoral inflammatory response in patients with penile carcinoma

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In spite of the low incidence in industrialized countries, penile carcinoma (PC) represents 2.1% of all malignancies among Brazilian men. Peritumoral immune response has been described to have influence in prognosis in some tumor types, but its role in PC has not yet been addressed. The purpose of the present study was to evaluate inflammatory infiltrate cell components and to evaluate its relationship with clinicopathological features in order to establish the prognostic value of immune response in patients with PC. One hundred fifty-five patients diagnosed at our hospital, with a minimum five-year-follow-up, were included in the study. Representative paraffin embedded tissue was submitted to immunohistochemical evaluation, using antibodies to CD1a, CD3, CD4, CD8, CD20, CD56, CD68, CD138, granzyme B, S100 protein, HLA-DR and FOX-P3. Elevated plasma cell infiltrate detected by conventional morphology and CD138 correlated with more favorable event-free survival (p=0.04), indicating a protective mechanism of elevated plasma cell counts. However, when the presence of lymph node neoplastic infiltration was evaluated in a multivariate analysis, only this parameter remained as having prognostic value. It is concluded that the role of inflammatory response on prognosis is relevant, especially for CD138, but this importance does not prevail over the value of lymph node status.
Financial support: FAPESP

Abstract 23

Título / Title: Evaluation of the prognostic value of TOP2A and survivin protein expression in sarcomas

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Soft part sarcoma represents a difficult working area, both for the pathologist and the oncologist. Prognostic factors are scarce in these tumors and the search for clinically relevant molecular markers is relatively incipient. We have proposed the present study based on previous results by our group on differential gene expression of soft tissue tumors, which showed significant overexpression of the TOP2a gene in sarcomas. As TOP2a is coded by a gene sited in chromosome 17, we have also included other

genes from the same chromosome in the study, such as HER-2-*neu* and survivin. Our aim was to evaluate immunohistochemical protein expression of the three genes and also assess the gene copy numbers of TOP2a and HER-2-neu by FISH analysis in soft part tumors in order to verify the existence of prognostic value on these parameters. Paraffin embedded tissue from 299 patients were included in the study, of which were 274 sarcomas, 18 desmoid-type fibromatosis, and seven cases of benign tumors. Analysis of multiple parameters showed that combined immunohistochemical expression of TOP2a and survivin was significantly associated with overall survival among sarcomas. This association was also valid when only high grade sarcomas were analyzed. There was no correlation between gene amplification and protein expression of TOP2a. The present results indicate that combined immunohistochemical study of TOP2a and survivin may emerge as a relevant prognostic factor for clinical usage.

Financial support: FAPESP and CNPq

Abstract 24

Título / Title: Evidências da resposta imunológica em Carcinomas Papilíferos da Tiróide: das características moleculares à determinação de perfil clínico patológico.

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O papel da tiroidite linfocítica crônica (TLC) ou ainda de linfócitos infiltrantes de tumores (LIT) no prognóstico do carcinoma papilífero da tiróide (CPT) não é bem conhecido. Não só isso, mas não há conhecimento de possíveis alvos da resposta imunológica anti-tumoral. O objetivo do presente trabalho foi caracterizar o papel da TLC concomitante ao CPT e dos LIT na apresentação inicial e evolução do CPT, correlacionando com o perfil de expressão protéica do tumor. Foi construído um TissueMicroarray com 261 casos de CPT. Os pacientes foram submetidos a um mesmo protocolo de tratamento e de seguimento (tiroidectomia total e subseqüente ablação actínica) e foram seguidos de 12 a 298 meses (53,72±41.01 meses). Dados clínicos, achados intra-operatórios e anátomo-patológicos, assim como de evolução, foram coletados de prontuários. TLC concomitante foi avaliada e todos os casos foram revistos em busca de linfócitos infiltrando o tecido peritumoral e intratumoral. A expressão de MUC1, p53, ATM, NIS e PTEN foi analisada por imunoistoquímica; além disso, 85 tumores foram genotipados para a mutação BRAFV600E. TLC e LIT foram encontrados em 30.59% e 25% dos casos, respectivamente. TLC foi mais freqüente em casos que apresentavam tumores menores que 2 cm ($p=0.0413$), sem invasão extra-tiroideana ($p=0.0158$), sem metástases ao diagnóstico ($p=0.0003$) e aqueles que precisavam de doses acumuladas de raioiodoterapia menores que 250 mCi ($p=0.0016$). A análise de regressão logística multivariada confirmou que a ausência de TLC foi mais freqüente nos pacientes que apresentavam metástases ao diagnóstico e que tinham recorrências ($p=0.0002$). Da mesma forma TLC foi correlacionada com aumento do tempo livre de doença ($p=0.002$). TLC foi mais freqüente em tumores positivos para MUC1 ($p=0.0009$), ATM ($p=0.0039$), p53 ($p<0.0001$) e NIS ($p=0.0189$). LIT foi mais freqüente nos tumores positivos para MUC1 ($p=0.0214$), ATM ($p<0.001$) e p53 ($p<0.0001$). Pacientes sem LIT apresentavam-se mais freqüentemente com invasão extratiroideana (63.27%; $p=0.0042$). A presença de TLC correlacionou-se com a presença de LIT ($p<0.0001$).
Nós sugerimos que tanto TLC quanto LIT podem prever o prognóstico de pacientes com CPT. A expressão de proteínas associadas ao processo tumorigênico pode aumentar a antigenicidade tumoral e facilitar o desenvolvimento resposta imunológica anti-tumoral, representada por TLC e LIT.

Abstract 25

Título / Title: Experiência Clínica do Tratamento de Mieloma Múltiplo em uma Única Instituição Brasileira

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O mieloma múltiplo (MM) é caracterizado pela proliferação de um único clone de células plasmocitárias, produtor de imunoglobulina. Devido à heterogeneidade no curso da doença, a identificação de fatores preditivos torna-se importante no manejo do tratamento. Analisar a sobrevida global (SG) e sobrevida livre de progressão (SLP) dos pacientes com diagnóstico de MM admitidos no Hospital A.C.Camargo e suas correlações com as características clínicas e laboratoriais, na busca de fatores preditivos (FP). Foram analisados retrospectivamente prontuários de 96 pacientes com MM, entre 2000 e 2007, tratados com diversos esquemas terapêuticos. Para análise de SG e SLP foi usado o método Kaplan-Meier e para verificar diferença entre as curvas para as variáveis observadas, o teste de log-rank. Em relação ao sexo, a população estudada foi homogênea. A idade mediana foi de 60 anos (28-83 anos). Proteína M foi detectável em 89,6%. A proporção de IgG, IgA e MM biclonal foi de 49%, 22% e 1%. Dentre os MM secretores de cadeia leve, a proporção foi de: 52,1% kappa, 25% lambda e 2,1%, ambos. 66,7% dos MM eram estágio clínico IIIA e 25,0%, 18,8%, 25,0% eram ISS 1, ISS 2 e ISS 3. 68,7% dos pacientes tinham KPS > 70% e 44,8% apresentavam ECOG < 2. 25% dos pacientes apresentaram-se ao diagnóstico com insuficiência renal, 74,0% com dor óssea e 31,3% com mais de 3 lesões ósseas detectadas ao radiograma. 82,3% dos pacientes fizeram uso de bisfosfonato. O cariótipo foi normal em 14,6% dos casos. 39,6% dos pacientes foram submetidos a TMO e 8,3% realizaram transplante em tandem. Os principais esquemas de quimioterapia foram: 37% VAD, 24% MP e 16,7% TaloDex. A SG mediana dos pacientes foi de 57, 9 meses (1,84-144,7 meses) e a SLP de 70,42 meses (0,3–118,35 meses). Na avaliação de FPs foi observado que ter realizado ou não TMO, ao contrário do que está estabelecido na literatura, não interferiu nas SLP e SG. Porém, níveis de β2-microglobulina > 3,5 (mediana de 113,9 meses x 25 meses; p= 0,004) interferiram de forma positiva na SLP. De forma inversa, pacientes com ISS 3 (média de 78,64 meses, mediana não atingida x média de 40,9 meses; mediana de 12,62 meses; p= 0,013) apresentaram melhor SLP. Por último, níveis de neutrófilos ≥ a 1500 (média de 68,3 meses, mediana não atingida x média de 11,7 meses, mediana de 5 meses; p < 0,001) levaram a maior SLP.Em nossa amostra, β2-microglobulina > 3,5, ISS igual a 3 e níveis de neutrófilos ≥ a 1500 demonstraram ser FPs de SLP em pacientes com MM, corroborando dados da literatura.

Abstract 26

Título / Title: Development of nonalcoholic steatohepatitis and adenoma in inbred mice submitted to a high-fat diet

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Nonalcoholic steatohepatitis (NASH) is a chronic liver disease associated with obesity, type-2 diabetes, and hyperlipidemia. Here we describe an experimental model of non-alcoholic fatty liver disease (NAFLD) induced by a high fat diet (HFD). Male C57Bl/6 mice were fed for 12 months with a HFD (58 cal% fat) or a low fat diet. Animals were examined at different time points for weight gain, serum transaminases (AST and ALT), serum cholesterol and triglycerides, fasting blood glucose, and histological liver alterations. Mice fed with HFD became obese, developed glucose intolerance and hyperglycemia, had high aminotransferase levels, and increased concentration of serum cholesterol and triglycerides. Histological analysis of the liver showed steatosis, ballooning hepatocytes, Mallory bodies, inflammatory infiltration, and perisinusoidal fibrosis. The presence of adenoma was detected in livers of mice fed over 10 months with HFD. Our results indicate that the HFD model reproduces many of the aspects of NAFLD, being a model similar to human NAFLD, reproducing many of its laboratory and histopathological features, and therefore can be useful to the understanding of NASH pathogenesis and its treatment. Furthermore, this model may be useful to the understanding of liver oncogenesis in the context of metabolic syndrome.

Abstract 27

Título / Title: Expression of cyclooxigenase-2 and p53 in neighboring invasive and *in situ* components of breast tumors

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The aim of this study was to assess the relationship between the expression of COX-2 and p53, estrogen (ER) and progesterone (PR) hormone receptors, and HER-2 in the *in situ* (DCIS) and invasive components of ductal carcinomas (IDC) of the same breast, and to compare these expressions between the two histological components.

The expressions of COX-2, p53, and hormone receptors were assessed with immunohistochemistry (IHC) in 87 patients with IDC with areas of DCIS. IHC and fluorescent *in situ* hybridization (FISH) were used to assess HER-2 status. The intraclass correlation coefficient and chi-square were calculated to assess the cross-tabulation of COX-2 expression in the *in situ versus* invasive components. Logistic regression models fit specifically for the comparison of marker expression in the DCIS versus IDC and the COX-2 positive and negative groups. COX-2 was expressed in 61% of the *in situ* components and in 58% of the invasive components. Forty-four percent of the cases expressed COX-2 in both components. Of the tumors that expressed COX-2 in the invasive component, 17% were negative for the enzyme in the *in situ* component. By contrast, of the tumors that expressed COX-2 in the *in situ* component, 17% were negative for the enzyme in the invasive component (p= 0.02). There was no difference in COX-2 expression comparing DCIS and IDC (p= 0.80). In the *in situ* component, there was a statistical borderline increase in p53 expression in tumors that also expressed COX-2 (p= 0.07). The proportions of tumors that expressed HER-2 (p= 0.73), ER (p= 0.25) and PR (p= 0.57) did not differ according to the COX-2 status. There was a marginally increased proportion of ER-positive specimens in the group of tumors that expressed COX-2 (p= 0.07) in the IDC. The expression of COX-2 was similar in the *in situ* and invasive components of the breast carcinomas. COX-2 positivity relates marginally with the expression of p53 in the *in situ* component, and with the ER expression in the invasive components.

Abstract 27 Table 1 - Expression of tumor markers in the in situ and invasive regions of the breast tumors

	In situ (%)	Invasive (%)	p	p adjusted
COX2				
Neg	29 (39)	36 (42)		
Pos	44 (61)	49 (58)	0.86	0.80
Unknown	14	2		
P53				
Neg	40 (48)	58 (67)		
Pos	43 (52)	28 (33)	0.01	<0.01
Unknown	4	1		
HER2				
Neg	68 (79)	61 (72)		
Pos	17 (21)	23 (28)	0.35	0.49
Unknown	2	3		
ER				
Neg	27 (31)	21 (25)		
Pos <50%	11 (12)	14 (15)		
Pos =>50%	49 (57)	52 (60)	0.55	0.36
Unknown	0	0		
PR				
Neg	35 (41)	46 (54)		
Pos<50%	29 (32)	21 (24)		
Pos=>50%	23 (27)	20 (22)	0.23	0.08
Unknown	0	0		

Neg - negative; Pos - Positive

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Abstract 29

Título / Title: Frutalin,α-D-galactose-binding lectin from *Artocarpus incisa: a* new prostate carcinoma marker.

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Lectins have been used as tools in malignant cell research. Frutalin (FT) discriminates benign from malignant thyroid tissues as an immunohistochemical (IHC) marker, both in the native biotinylated and rabbit antibody forms [1]. Recently, a few studies have revealed the ability of native FT and recombinant antibody forms to distinguish prostate hyperplasia, atrophy and carcinoma as tumor marker [2,3], while Gal3 decrease in cancer transformation [4]. The study aimed to test these findings in core biopsy summated to basal cell evaluation with p63 and high molecular weight keratins. FT was obtained by affinity chromatography on *Adenanthera pavonina* galactomannan. Mouse monoclonal antibodies from NOVOCASTRA (Gal3) and DAKO (p63, keratins) were used. Formalin-fixed, paraffin-embedded prostate tissues were cut at 4 μm and stained using biotinylated frutalin as probe (300μg/ml) and heat pH 6 retrieval solutions for the monoclonal markers (usual dilution) using the Advance™ IHC method. Pilot cases (20 hyperplastic and 20 carcinoma) and 20 prostate core biopsy with normal, atrophic, hyperplastic, suspicious and carcinoma were evaluated. All the samples with carcinoma were negative for Gal3 and showed moderate to strong FT expression including 96 focal areas from 16 cases with none or few basal cells. FT biotinylated is a good marker for usual prostate carcinoma disclosing suspicious small areas and dismiss atrophy. It is useful alone and when associated with traditional tests supporting diagnosis. In the future, other groups in addition to Minho and Porto University may receive FT for testing.

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Abstract 30

Título / Title: Geração e identificação de anticorpos monoclonais scFv contra células tumorais de tireóide

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Fragmentos de anticorpos recombinantes têm se tornado ferramentas importantes em diversas áreas, tais como: Biologia Molecular, Farmacêutica e pesquisa Médica. Avanços recentes estão relacionados à aplicação desses anticorpos na oncologia, com estratégias diagnósticas e terapêuticas para diferentes carcinomas (1). Neste estudo, uma biblioteca de fragmentos de anticorpos monoclonais scFv foi construída utilizando RNA total de sangue periférico de 25 pacientes com Carcinoma Diferenciado da Tireóide. Essa biblioteca scFv foi selecionada utilizando os métodos Bioppaning and Rapid Analysis of Selective Interactive Ligands (BRASIL) e Phage display contra células tumorais de tireóide, com o objetivo de encontrar ligantes específicos a superfície celular tumoral. Os clones selecionados foram identificados por Dot blotting, e a reatividade contra proteínas de tumor, adenoma e bócio foi analisada por Elisa. O clone scFv-C1 apresentou melhor reatividade pelas proteínas tumotais e foi escolhido para a imunoistoquímica. Esta foi realizada com lâmina de Micro-arranjo de tecido (TMA) com duzentos e vinte nove casos de tireóide, sendo 110 Carcinomas, 52 Adenomas Foliculares, 49 Bócios e 18 tecidos normais de tireóide. O anticorpo scFv-C1 reagiu especificamente aos tecidos de câncer, com reatividade ao citoplasma das células tumorais, foi capaz de distinguir o Grupo Câncer do Controle (Bócio, Adenoma e tireóide normal) com significância estatística ($p<0,0001$) e entre os carcinomas reagiu melhor com os tumores pequenos (TNM 1 e 2) e com pouca agressividade ($p=0,050$). O fragmento de anticorpo scFv-C1 pode ser um potencial candidato a

biomarcador para o diagnóstico do Câncer de tireóide.
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Abstract 31

Título / Title: The role of p40 in the course of the infection of c57bl/6 mice with *Leishmania braziliensis*

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L. braziliensis (Lb) is the main agent of tegumentary leishmaniasis in Brazil. Mechanisms involved in resistance and susceptibility to the infection are not clear. We evaluated the role of p40 in the course of the infection of C57BL/6 mice with Lb. Knock-out (KO) mice for p40 (C57BL/6p40-/-), which do not produce IL-12 or IL-23 were used. Mice were infected in the dermis of the ear with Lb (10^5 parasites). Lesions size, parasitic load and dissemination to the spleen were monitored. Histological analysis of inflammatory response in the skin lesions, draining lymph nodes (DLN), spleen and liver were performed at 1, 5 and 10 weeks after infection. Early skin inflammatory response was evaluated 6, 12 and 24 hours after infection. Both groups of mice developed skin lesions and parasites were found in the DLN. After 10 weeks of infection, wild mice controlled parasites in the site of inoculation but not in the DLN. They did not show dissemination of infection to the spleen or liver. KO mice were unable to control infection and had progressive lesions with dissemination of parasites. Inflammation in the skin was distinct in wild as compared to KO mice. In the early phase of the infection, KO mice had milder inflammatory response with fewer neutrophils. At 1 week, KO mice showed milder infiltrate, mainly macrophagic, whereas wild mice had a mixed infiltrate constituted by macrophages, lymphocytes and neutrophils. At 5 weeks, both group of mice had a dense, diffuse, and mixed inflammatory infiltrate with parasitized macrophages. Parasitism was more intense in KO mice. At the 10^{th} week, inflammation persisted in wild mice but parasites were not detected. KO mice showed dense mixed inflammatory infiltrate with huge amounts of parasites within macrophages. The DLN in wild mice showed persistent parasitism associated with a granulomatous inflammatory response. Increasing amounts of parasites were present in LN of KO mice with progressive alteration of LN architecture and no granulomas were seen. After the 5^{th} week, parasites were found in the spleen and liver of KO mice, associated with inflammatory infiltrate of macrophages. Our results clearly confirm the importance of p40 in the control of *L. braziliensis* infection and suggest that its absence determine alterations in the dynamics and composition of the inflammatory infiltrate that are important to the containment of the parasites in C57BL/6 mice.

Abstract 32

Título / Title: Avaliação da expressão das proteínas p53 e bcl-2 em carcinomas de laringe

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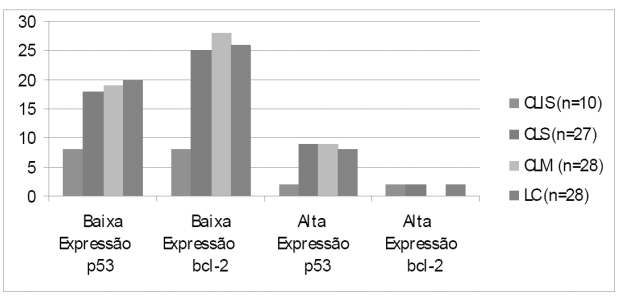
Anualmente 644.000 novos casos de câncer de cabeça e pescoço são diagnosticados, sendo que dois terços desses casos ocorrem em países em desenvolvimento. Carcinoma de células escamosas de laringe é o câncer mais comum da região de cabeça e pescoço representando cerca de 2.8% de novos casos no mundo. No Brasil tumores de laringe representam cerca de 2.0% de todos os cânceres. Já está bem estabelecido que o consumo de álcool e

tabaco são considerados os principais fatores etiológicos. No entanto, os eventos moleculares que induzem as transformações da mucosa normal ao carcinoma invasivo da laringe ainda são desconhecidos. O mecanismo de oncogênese na laringe pode ser controlado por vários fatores, entre eles proteínas envolvidas na regulação do ciclo celular como a p53 e na apoptose como a bcl-2. Esse estudo teve como objetivo determinar a expressão de p53 e bcl-2 nos carcinomas de laringe assim como verificar se a ausência ou presença dessas proteínas estaria associada à invasão e metástase neste tipo de carcinoma. Foi realizada imunoistoquímica com avaliação quantitativa e qualitativa para p53 e bcl-2 em 65 pacientes diagnosticados com câncer de laringe, sendo 10 carcinomas *in situ* (CLIS), 27 carcinomas sem metástase (CLS) e 28 com metástase (CLM), e seus respectivos linfonodos cervicais (LC). A expressão qualitativa das proteínas p53 e bcl2 foi semelhante no grupo CLIS. No entanto, nos grupos CLS, CLM e LC foi observada uma diferença estatisticamente significante entre as marcações [OR= 6,3 (IC 95% 1,2- 32,5); OR= 27,8 (IC 95% 1,5- 505,8); OR= 5,2 (IC 95% 1- 27,2)] respectivamente. Da mesma forma a análise quantitativa mostrou que a média de células marcadas com p53 e bcl-2 não diferiu no grupo CLIS. Já nos grupos CLM, CLS e LC houve maior número de células expressando p53 do que bcl-2. Apesar da estreita relação existente entre as proteínas p53 e bcl-2 na regulação do ciclo celular, nossos achados levam a crer que esta interação esteja possivelmente prejudicada nos tumores de laringe à medida que estes se tornam invasivos.



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Abstract 33

Título / Title: Hipoexpressão de PTEN em neoplasias da mama com alterações polissômicas do cromossomo dez

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A Fosfatase Homóloga a Tensina (PTEN) é um gene supressor de tumor que pode estar mutado ou deletado em uma grande variedade de tumores humanos. O gene PTEN, localizado no cromossomo 10, age como uma fosfatase lipídica, opondo-se à via da PI3-K/Akt. A supressão do PTEN resulta em aumento da ativação desta via, que se correlaciona com pior prognóstico em pacientes com câncer de mama. A expressão transcriptômica e proteômica de genes localizados em cromossomos polissômicos de células neoplásicas ainda não é bem elucidada. 58 casos de câncer de mama invasivo foram selecionados. Tecido parafinado foi utilizado para a confecção de lâminas coradas em H&E utilizadas na reclassificação histológica, confirmação diagnóstica, construção do tissue microarray (TMA), estudo imunoistoquímico (IHQ) e Hibridação in situ Fluorescente (FISH). Para análise de positividade de PTEN pela IHQ, um sistema de escore combinatório entre a intensidade e a proporção de células marcados no tumor foi utilizado. As lâminas de FISH foram analisadas em microscópio de fluorescência. RT-PCR para a quantificação de mRNA de PTEN foi realizado em amostras de tumor a fresco dos mesmos casos. Na análise IHQ para PTEN houve 5 casos negativos (0), 18 casos 1+, 22 casos 2+, 8 casos 3+, e 5 casos 4+. A análise dos resultados do qRT-PCR mostrou hipoexpressão em 76,3% dos casos e correspondência com os resultados de IHQ. No FISH, houve 45 casos sem alteração gênico-cromossômica, 5 com deleção hemizigótica de PTEN, 2 com deleção homozigótica e 6 com polissomia do cromossomo 10. A expressão de PTEN avaliada por

RT-PCR foi significativamente maior nos casos em que não houve alteração gênico-cromossômica (p = 0.023) e menor naqueles com deleção homozigótica do gene PTEN (p<0.001). Tumores com polissomia do cromossomo 10 apresentaram expressão significativamente menor em comparação com os tumores sem alteração (p = 0.0056) e com deleção hemizigotica (p = 0,0086) e semelhante aos tumores com deleção homozigótica. A expressão protéica e de mRNA do gene PTEN parece estar diminuída em tumores que apresentam alterações polissômicas de seu cromossomo, mesmo quando alterações no gene, em si, não é observada pelo FISH. Estudos futuros de hipermetilação em regiões promotoras destes genes em tumores polissômicos podem contribuir para o entendimento destas alterações.

Abstract 34

Título / Title: Impacto da associação de temozolamida à radioterapia na sobrevida de pacientes com gliomas malignos

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Impacto da associação de temozolamida à radioterapia na sobrevida de pacientes com gliomas malignos. Apesar de relativamente incomuns, os gliomas malignos estão associados à morbidade e mortalidade elevadas. Mesmo com tratamento otimizado, a sobrevida mediana para pacientes com glioblastomas (GBM) é de 12 a 15 meses e 2 a 5 anos para pacientes com gliomas anaplásicos. Em publicação recente de estudo fase III, foi demonstrado ganho de sobrevida em pacientes com GBM após terapia adjuvante com temozolamida concomitante à radioterapia. Analisar o impacto em sobrevida da introdução da temozolamida no tratamento adjuvante de pacientes com gliomas de alto grau tratados no Hospital AC Camargo. Foram avaliados retrospectivamente 79 pacientes com gliomas de alto grau tratados no Hospital AC Camargo entre Julho de 1995 e Fevereiro de 2009. Os pacientes foram analisados quanto ao uso de temozolamida em associação a radioterapia ou radioterapia isolada no contexto adjuvante. A terapia combinada consistia de temozolamida 75 mg/m^2 diariamente concomitante à radioterapia seguido de mais 6 ciclos de temozolamida 150 a 200 mg/m^2 do D1 ao D5 a cada 28 dias após o término da radioterapia. Para o cálculo da sobrevida global (SG) foi empregado o método de Kaplan-Meier e a diferença entre as curvas foi analisada pelo teste de log-rank. O diagnóstico histopatológico foi GBM em 60 pacientes (76,7%), astrocitomas anaplásicos (AA) em 16 pacientes (19,5%), e glioma maligno SOE em 3 pacientes (3,8%). A idade mediana na população foi de 52,9 anos (19,45 – 81,49 anos).Cerca de 43% dos pacientes foram submetidos a ressecção completa do tumor, enquanto 38% tiveram ressecção subtotal e 11% apenas biópsia. Em relação ao tratamento, 39 pacientes (49,4%) receberam temozolamida concomitante à radioterapia como terapia adjuvante. Destes, 32 pacientes receberam temozolamida após o término da radioterapia. A SG mediana de toda a amostra foi de 20,3 meses (0,23 a 161 meses). O grupo que recebeu temozolamida teve uma SG de 28,7 meses em comparação a 12,7 meses do grupo que recebeu radioterapia isolada (p = 0,08). O tratamento com temozolamida associado à radioterapia em pacientes com gliomas malignos de alto grau no Hospital AC Camargo proporcionou melhor SG em relação à radioterapia isolada. A ausência de significância estatística deve-se, provavelmente, ao tamanho da amostra. Os dados de sobrevida aqui demonstrados corroboram com os encontrados na literatura.

Abstract 35

Título / Title: Implications of type and density of tumor inflammatory infiltrate in gastric carcinomas submitted to neoadjuvant therapy

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Tumors often contain infiltrates of immune cells. These infiltrates represent the result of interplay between the host immune system and tumors during their development and growth. Intratumoral T cells with an effector (memory) phenotype showed favorable influence on the prognosis of patients with malignant neoplasms. However, tumors have elaborated methods to circumvent such a response to T regulatory cells. The aim of the study was to examine the main intratumoral lymphocyte subpopulation correlated with pathological response in gastric carcinomas (GC) submitted to neoadjuvant chemotherapy. The authors evaluated the inflammatory infiltrate by immunohistochemistry for CD3, CD4, CD8, CD20, CD138, CD68, CD1a, and S100 protein in 19 patients who had received neoadjuvant chemotherapy based on a cisplatin scheme followed by surgery. The pathological response was defined by the ratio of viable cells and fibrosis. The intratumoral inflammatory infiltrate was predominantly characterized by three types of lymphocytes (CD3+, CD4+, and CD8+). The number of T cells was the same in the two groups of GC. The plasmocytic infiltrated was scarce in almost all tumors examined but the increased number of CD138+ cells was correlated with major response in the GC, characterized by presence of viable tumor cells less than 50% of the tumor bed with increased fibrosis (>50%). Positivity for dendritic cells was not found in any case. The intratumoral inflammatory infiltrate in GC submitted to neoadjuvant chemotherapy consisted of T CD3+, CD4+ and CD8+ cells; depletion of CD138+ cells may reveal susceptibility to response to neoadjuvant chemotherapy and could be a reason for the scope of tumor cells from the mechanisms of gastric immune control.

Abstract 36

Título / Title: MGMT as a potential prognostic marker in breast cancer

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MGMT repairs DNA damages, via alkylation, by removing a methyl group from the O6 position of guanine. It acts as a tumor suppressor gene in normal cells and prevents DNA mutation. We evaluated the MGMT expression in breast tumors correlating it with other prognostic factors. Sixty-four cases of invasive breast carcinomas were randomly selected for a TMA construction. Immunohistochemistry (IHC) was performed for MGMT and also for ER, PR, HER2, Ki67, p53, p63, e-cadherin, CK5 and CK14 for luminal and basal phenotype classification. IHC was evaluated following the guidelines for each marker most recommended in the literature. Fluorescent *in situ* Hybridization (FISH) was performed in those cases considered 2+ in order to assess *HER2* gene amplification status. qRT-PCR was performed in frozen tissue from our tumor bank for all cases in order to evaluate mRNA expression of MGMT. Fourteen cases were triple-negative (21.8%) and, among those, seven cases were basal-like carcinomas (10.9%). 25 cases (39%) were luminal-like type A, four cases were (6.25%) luminal-like type B, and one case (1.5%) was HER2-like type. MGMT showed significant lower expression in the basal-like tumors when compared to the luminal-like ones (p=0,007). Basal-like phenotype tumors presented higher positivity for p53 and Ki67 than the luminal types (p=0.025 and p=0.003 respectively). Positive p53 and high Ki67 tumors showed significant lower expression of MGMT (p=0.0184 and p=0.0081 respectively). MGMT assessment by IHC or molecular biology techniques may represent an important prognostic factor in breast cancer.

Abstract 37

Título / Title: O6-methyguanine-DNA methyltransferase evaluated by immunohistochemistry: best practice for clinical and research assessment

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MGMT repairs DNA damage and acts as a tumor suppressor gene in normal cells preventing DNA mutation. Several different methods for MGMT immunohistochemical (IHC) testing have been used resulting in no universally accepted standard. We evaluated the IHC expression of MGMT of five different primary antibodies in 59 invasive breast carcinomas. Fifty-nine breast carcinomas were randomly selected for a TMA construction. Five different primary antibodies against MGMT were used for the IHC study: clone MT3.1 (NeoMarkers, GeneTex and Santa Cruz), SPM287 (Santa Cruz) and MT23.2 (Zymed). Heat-induced antigen retrieval in citrate and Advance™ IHC detection system were used. IHC was visually analyzed by the microscope and automated analyzed by software applied to digital slides. qRT-PCR was performed in all tumors for transcript expression quantification. The antibody SPM287, Santa Cruz, showed the highest sensitivity ($p<0.001$) and the antibody MT3.1, Santa Cruz, showed the least sensitivity ($p<0.001$). The antibody MT23.2, Zymed, showed higher levels of cytoplasm staining, which was not observed in the other antibodies tested ($p<0.001$). Fifty-nine samples, 94.9%, showed hypoexpression of MGMT when compared to normal breast evaluated by qRT-PCR ($p<0.001$). SPM287, Santa Cruz, was the only antibody which showed a positive and significant correlation with the results obtained from qRT-PCR ($p=0.027$). The antibody SPM 287, Santa Cruz, presented as the most sensitive and specific antibody for the IHC evaluation of MGMT. This antibody seems to be of reliable and effective use for research and clinical practice in breast cancer.

Abstract 38

Título / Title: Pancreatic adenosquamous carcinoma of the pancreas: clinicopathologic review in three patients

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Pancreatic adenosquamous carcinoma (PAC) is a rare morphological variant of adenocarcinoma, accounting for 1 - 4% of all pancreatic carcinomas. These tumors are characterized by the presence of both glandular and squamous components and at least 30% of the tumor should be comprised of the squamous component. This tumor has not been associated with any specific clinical syndromes or serum data. The prognosis is very poor, less favorable than the invasive ductal tumor even after curative resection. A previous report found that squamous cells carcinomas grow at twice the speed of adenocarcinoma. Therefore, once it has a squamous component, it may exhibit a higher degree of malignancy. Despite the rarity, PAC occurs worldwide and more data are required to better understanding of this lesion. The purpose of this study was to identify clinicopathologic features of this rare morphological tumor. Three patients with pain, anorexia, jaundice, median age of 66 years, presented with tumors; two tumors were in the head of the pancreas and one was caudal. The median tumor size was 4.5 cm, all were node positive and median overall survive was 6 months. The origin of adenosquamous tumor is not well defined. Squamous metaplasia of pre-existing adenocarcinoma has been suggested as a mechanism underlying the histogenesis of pancreatic adenosquamous carcinomas. Other authors report that neoplasia occurs again. Most metastases (in this study and literature) were from the adenocarcinoma component and not from the squamous component. The major sites of metastases are liver and peritoneal dissemination. In summary, we report a well-characterized series of adenosquamous carcinomas of the pancreas with clinicopathologic features.

Abstract 39

Título / Title: Psoriasin expression pattern and its correlation to clinical and pathological features in penile cancer

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Psoriasin (S100A7) has been shown to be overexpressed in early stages of carcinogenesis of breast, bladder and some epithelial skin tumors. Penile carcinoma is an important issue for developing countries, as it is significantly more common there than in developed nations. Therefore, this study has focused on evaluating S100A7 protein levels and correlating them to clinical and pathological data. Immunohistochemistry analyses were performed in a retrospective series of 366 penile carcinomas using tissue microarray method. Clinicopathological features and survival data were reviewed from hospital records. For statistical analyses, $p<0.05$ was considered significant. In our series, 242 cases (77.6%) were negative for the staining, whereas 70 (22,4%) were positive. Fifty-four cases could not be assessed due to material loss in the array. Regarding the clinicopathological features, negative S100A4 staining showed association with invasion of corpus cavernosum. No association with survival was observed. Although high expression of psoriasin in tumors has been already described in literature, no association with disease prognosis has ever been made. The association observed in the present study corroborates with the idea that this protein is overexpressed in the early events of tumor progression but not in advanced tumors.

Abstract 42

Título / Title: The expression of cyclooxigenase-2 (COX-2) is higher in serous compared to mucinous borderline ovarian tumors and correlates with cell proliferation activity

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Borderline ovarian tumors generally share a relatively indolent behavior. Several factors have been found to correlate with a poorer prognosis and/or further development of frankly malignant disease, such as: mucinous type, cell proliferation rate and detection of peritoneal implants during surgical staging. Studies suggest that overexpression of COX-2 is related to carcinogenesis by increasing the cell proliferation activity, promoting neoangiogenesis and reducing apoptosis. A lower expression of COX-2 may be related to the development of borderline ovarian tumors. The study included 86 borderline epithelial tumors of the ovary; 36 serous, 46 mucinous and 4 seromucinous, obtained from women who underwent surgery between January 1998 and December 2004. Expression of COX-2 and Ki67 proteins were evaluated with immunohistochemistry, in paraffin-embedded sections of the ovarian tumors. Most women had stage I disease, although a higher proportion of advanced disease was found in women with serous (14.7%) or seromucinous (50%) neoplasia ($p=0.001$). Thirteen women were found to harbor peritoneal implants. The proportion of cells with positive nuclei for Ki67 was significantly higher in mucinous tumors ($p=0.014$). Serous tumors presented a higher expression of COX-2 compared to mucinous ones (68.5% versus 52.5%; $p<0.01$). Tumors with >=15% of nuclei positive for Ki67 also had a higher expression of COX-2. The higher expression of COX-2 did not correlate with peritoneal implants and disease stage. These results

Abstract 42 Table 1 - Multivariate analysis of the expression of COX-2 in ovarian bordeline

	Mean percentage of cels expressing COX-2	p value	COX-2 integrated score	p value
Histological type				
Mucinous	52.5 (25.7)	Ref.	1.2 (0.8)	Ref.
Seromucinous	66.5 (33.7)	0.13	1.6 (0.9)	0.12
Serous	68.5 (22.5)	<0.01	1.7 (0.8)	<0.01
Ki67				
<15%	58.7 (26.8)	Ref.	1.4 (0.8)	Ref.
=>15%	61.1 (26.8)	0.04	1.5 (0.9)	0.03
Peritoneal implants				
No	58.4 (25.6)	Ref.	1.4 (0.8)	Ref.
Yes	68.4 (25.3)	0.53	1.7 (0.9)	0.33
Stage				
I	58.4 (25.5)	Ref.	1.4 (0.8)	Ref.
II	35 (12.7)	0.61	0.6 (0.4)	0.79
III	75.7 (22.7)	0.29	1.9 (0.7)	0.19

Ref = referential

indicate that the expression of COX-2 is positively related to the cell proliferation rate and was higher in serous than in mucinous borderline ovarian tumors. However, the study design prevents the assessment of whether COX-2 is itself a modulator of the tumor's behavior or a surrogate marker of the biological events that lead to increased aggressiveness.

Financial support: FAPESP and CNPq

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Abstract 43

Título / Title: Trypsin x neuraminidase in lectin histochemistry: a biochemistry study of Lewy Bodies in Parkinson disease

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Parkinson´s Disease (PD) is a progressive, neurodegenerative and idiopathic disorder characterized by a progressive loss of dopaminergic neurons of the *substantia nigra* (locus niger) of the midbrain. Histopathology tests show the *substantia nigra* eosinophilic hyaline intracytoplasmic inclusions known as Lewy Bodies (LB), which is indicative of PD diagnosis. Despite its relevance, LB biochemical composition is still unclear. Lectin histochemistry is a technique used to investigate the profile of carbohydrate expression in cell surface glycoconjugates. Enzymatic digestion is an important step in the protocol, in which enzymes perform functions of induction and/or modulation of lectin-carbohydrate recognition event. An enzyme commonly used in lectin histochemistry is trypsin, a protease that hydrolyzes bonds of lysine and arginine, arginyl or lysyl derivatives, and amide groups of lysine and arginine. Neuraminidase is an enzyme that acts on residues of terminal monomeric sialic acid of glyco moieties in membrane glycoconjugates. This study aimed to compare the action of trypsin and neuraminidase on lectin histochemistry protocol in brain tissues (n=11) *post-mortem* diagnosed as PD obtained from the Death Verification Service of Pernambuco at UFPE (SVO-UFPE). Slices (4 μm) were treated separately with trypsin solution 0.1% (w/v) and neuraminidase (1mg/mL), followed by incubation with methanol-H_2O_2 solution and incubation with horseradish peroxidase labeled lectins (Con A - specific for glucose/mannose, PNA - galactose/N-acetyl-galactosamine specific, LTA and UEA-I - L-fucose specific). Tissues treated with trypsin showed no staining for Lewy Bodies. Neuraminidase treatment led to the staining of Lewy Bodies in all cases when LTA (40μg/mL) and UEA-I (20μg/mL) were used. Results indicate that Lewy Bodies express L-fucose residues recognized by lectin histochemistry after neuraminidase treatment. The absence of lectin-carbohydrate recognition does not imply an absence of corresponding lectin specific carbohydrate but that it may be inaccessible in a glycoconjugate structure. Studies regarding carbohydrate profile of LB are under progression.

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Abstract 45

Título / Title: The human epidermal growth factor receptors in gastric carcinomas: a study using fluorescence *in situ* hybridization and immunohistochemistry

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The human epidermal growth factor receptors (EGFR) family consists of four members: ErbB-1 (HER1), ErbB-2 (HER2), ErbB-3 (HER3) and ErbB-4 (HER4). These receptors activate numerous downstream pathways in response of extracellular ligands, regulating diverse processes including differentiation, migration, proliferation, and survival. Alterations in EGFR family members play a role in the development and progression of many human cancers. In gastric carcinomas (GC), HER1 and HER2 overexpressions are thought to be prognostic factors and targets of novel biological agents. The effect of HER3 or HER4 expression in GC has not been sufficiently studied. HER3 expression is observed frequently in advanced GC with poor prognosis and HER4 gene expression seems to be higher in GC tissue in comparison with adjacent gastric mucosa. In this study, we explored gene and protein expression of the EGFR family in GC in order to establish new potentially prognostic factors. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) were carried out in 221 GC using tissue microarray. IHC positivity for EGFR was defined by score 2+ or 3+ according with intensity and frequency of membranous staining. In addition, the intensity of the cytoplasm staining was also determined for HER3 and HER4 overexpression. HER1, HER2, HER3, and HER4 overexpression was found in 3/197 (1.5%), 24/207 (12%), 121/193 (64%) and 41/183 (22%) cases respectively. FISH assay was performed according to the protocol previous described. In each case, 100 tumor nuclei were evaluated. Cases showing a gene/CEN fluorescence ratio ^{3} 2 were considered positive for gene amplification. FISH detected HER1 and HER2 amplification in 1.3% and 8% of the cases. Amplifications for HER3 and HER4 were not observed. Overall, alterations of the four members of EGFR were significantly associated with parameters involved with tumor progression, including the depth of tumor invasion, involved lymph nodes, and tumor stage. We herein showed a strong correlation between HER2, HER3 and HER4 overexpression and low-grade tumors, corresponding to the intestinal type GC according to Lauren's classification. In addition, HER2 amplification was significantly related with worse survival. These results reveal that all the members of the EGFR family are activated in GC. The exact mechanisms involved in the HER3 or HER4 alterations in gastric cancer, especially in the intestinal type, remains unclear since amplifications were not found. Gaining further understanding into the oncogenic mechanism of EGFR family may not only help in the development of targeted therapy in gastric patients but might accelerate the acceptance of a novel taxonomy of cancer which is based on the genomic perturbations in cancer genes and cancer gene families and their response to targeted agents.

Abstract 46

Título / Title: Residual cancer burden of breast cancer after neoadjuvant chemotherapy: a study of 20 cases in A.C. Camargo Hospital using M.D. Anderson's protocol

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Neoadjuvant chemotherapy (NeoCT) has been widely used for treating locally advanced breast cancer (BC) and large tumors with resection possibility. Some studies correlates CT tumor response with survival. NeoCT-treated women with tumor extinction at mastectomy showed improvement of survival rates and considered as pathological complete response (pCR). Most authors consider pCR as the absence of residual invasive tumor or metastasis to lymph nodes. pCR can be used as a marker for evaluating treatment efficacy. There is no standardized method for grading BC pathological response to CT and different methods have been proposed. *Symmans* et. al recently created an index to calculate residual cancer burden (RCB). We report our experience on evaluating tumor response to CT using this index and correlate the findings with pre treatment molecular immunophenotype. Twenty patients with BC and NeoCT with posterior mastectomy and axillary lymph node dissection were studied. Mastectomy specimens were processed using Symman's protocol, sorting patients as having pCR, minimal (RCB-I), moderate (RCB-II), or extensive (RCB-III) residual disease. pCR was seen in 3 of the 20 cases studied (15%), and the distribution between immunosubtypes

was: one triple negative non basal subtype, one luminal A and one luminal B. RCB-1 was seen in five cases (25%), three luminal A, one luminal B and one triple negative non basal subtype. RCB-II could be seen in nine cases (45%), six luminal A and three luminal B. Three cases (15%) were classified as RCB-III, two basal types and one luminal B. Pathology response in patients with NeoCT evaluated in our hospital by Symman's protocol showed a trend towards worse response in triple negative basal type tumors.

Abstract 47

Título / Title: Molecular markers for predicting progression of pure ductal carcinoma *in situ* of the breast

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The identification of molecular factors necessary for the progression of pure ductal carcinoma *in situ* of the breast (pure DCIS) is one of the main concerns of the scientific community. Our research group previously identified a group of genes differentially expressed between pure DCIS and *in situ* component of lesions with co-existing invasive ductal carcinoma (DCIS-IDC), using the microarray technique in a cell-based resolution experiment (Castro *et al*., 2008). The aim of the study was to discover novel molecular markers able to predict invasiveness potential in a DCIS group of breast carcinomas by qRT-PCR and Immunohistochemistry (IHC). For qRT-PCR, 9 genes were selected, for which there were available antibodies. The qRT-PCR reactions were performed in ABIPrism 7900 Sequence Detection System and the criterion of gene expression difference was ≥ 2-fold change. IHC was performed in a tissue microarray (TMA), using a polymeric system, ADVANCE (DAKO™). The categorical variables were analyzed using the chi-square or Fisher´s exact test. Overall survival and disease-free survival probabilities were calculated by the Kaplan-Meier method, and the log-rank test was used for the comparison of survival curves. The Cox regression model was used to estimate the relative risks. All results were considered statistically significant when $p \leq 0.05$. The expression of four genes (*ADFP*, *ARHGAP19*, *ANAPC13* and *CLTCL1*) was confirmed by qRT-PCR, in the initial sample set used in the microarray experiment. IHC was conducted to evaluate the corresponding proteins, using an independent group of 44 pure DCIS and 36 DCIS-IDC samples arrayed in TMA. Two genes, *ANAPC13* ($p=0.041$) and *CLCTL1* ($p=0.049$), confirmed the expression at protein level, showing significant higher cytoplasm staining in the pure DCIS group. In addition, their prognostic potential was also investigated in a TMA composed of 187 invasive breast cancer samples. Strong cytoplasm staining of ANAPC13 was associated with the absence of recurrence ($p=0.01$) and presented a marginal significance with three or less compromised lymph nodes ($p=0.09$), and also a significant association with a longer period of overall and disease free survival (log-rank test, $p=0.0025$ and $p=0.026$, respectively). *ANAPC13* is likely to be a good breast cancer marker, being a potential candidate to be introduced into the clinical practice.

Financial support: CEPID and FAPESP.

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Castro NP, Osorio CA, Torres C, Basto EP, Mourão-Neto M, Soares FA, Brentani HP, Carraro DM. Evidence that molecular changes in cells occur before morphological alterations during the progression of breast ductal carcinoma. Breast Cancer Res 2008;10:R87.

Abstract 48

Título/ Title: A Sociologia Celular reflete a influência da expressão de proteínas neoplásicas na arquitetura do carcinoma papilífero da tireóide

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Introdução: Entre carcinomas papilíferos da tireóide (CPT) há grande variação da expressão de proteínas, tais como MUC e NIS, conforme o grau de diferenciação do clone neoplásico. A diferenciação pode refletir-se na arquitetura histológica e ser extremamente sutil e imperceptível ao observador humano. A análise sintática da estrutura é uma ferramenta computacional que permite quantificar a "sociologia celular" e descrever de maneira exata as relações topográficas entre as células de um tecido. A distribuição espacial das células pode ser caracterizada e quantificada usando-se modelos geométricos que, aplicados ao tecido, fornecem informações úteis sobre a distribuição espacial e as relações entre as células. Objetivo: Analisar a correlação entre marcadores moleculares (proteínas MUC1 e NIS; RNAm de MUC1) e mudanças da arquitetura neoplásica com o intuito de estudar mais detalhadamente a fisiopatologia do CPT. Material e Métodos: Imagens digitalizadas de 50 lâminas de amostras de CPT, coradas com HE, tiveram seus núcleos marcados com o uso de software desenvolvido pelo nosso grupo de estudo. Após a marcação dos núcleos da imagem tumoral, obtivemos dados geométricos sobre a topologia das células tumorais. Características histopatológicas do tecido tumoral e dados clínicos dos pacientes foram coletados. A expressão protéica de MUC1 a NIS foi demonstrada por imunoistoquímica e o RNAm de MUC1 obtido por PCR em Tempo Real. Resultados: A porcentagem de células neoplásicas que expressam MUC1 é maior em carcinomas com baixa densidade de células neoplásicas (p=0,01), que também revelam núcleos com maior densidade óptica (p=0,002), porém com maior variabilidade desta característica (p=0,005). O RNAm de MUC1 está mais expresso em células com grande variação da densidade óptica da cromatina (p=0,0005). Carcinomas com maior número de células positivas para NIS mostram uma arquitetura tecidual mais regular (p=0,03), pois menores são os valores de desvio padrão do perímetro e da área dos polígonos de Voronoi. **Conclusão:** A análise revela modificações sutis da arquitetura do tecido, não detectáveis pelo olho humano. Da mesma forma, as diferenças de expressão de proteínas como MUC1 e NIS são acompanhadas por re-arranjo geométrico das células neoplásicas, sugerindo que alterações da expressão de marcadores moleculares podem indicar mudanças da fisiopatologia da neoplasia.

Abstract 49

Título/ Title: MMP9 immunohistochemical expression by Hodgkin-Reed-Sternberg cells as a prognostic marker in young patients with classical Hodgkin lymphoma

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To identify differences in gene regulation according to the status of Epstein Barr virus (EBV) infection in classical Hodgkin lymphoma (cHL), we compared the expression profiles of three EBV-negative (L-428, L-1236, KM-H2) and one EBV-positive (L-591) Hodgkin Lymphoma (HL) cell lines. We observed that 756 genes are significantly up- or downregulated in the EBV-negative cell lines, as compared to the EBV-positive cell line. For four of the differentially expressed genes (Caspase-1, Caveolin-1, CCL20, and MMP9), immunohistochemical validation was performed in a tissue microarray (TMA) containing 148 cHL cases and the results compared to EBV infection status and patient outcome. Only CCL20 expression by Hodgkin-Reed-Sternberg (H-RS) cells was associated with EBV infection ($p<0.0001$). On the other hand, Caspase-1 and MMP9 expression by H-RS cells significantly associated with lower disease-specific survival rates in patients between 15 and 45 years old, and the expression of MMP9 by neoplastic cells emerged as an independent factor of unfavorable prognosis. These results suggest the ability of H-RS cells to explore different signaling pathways, regulating different genes according to EBV infection status. Of these, CCL20 protein expression was shown to be specifically associated with EBV infection in cHL cases. We also observed the expression of other proteins by H-RS cells, of which MMP9 might be promising as an independent prognostic factor for this group of patients.

Financial support: FAPESP and CNPq

Abstract 50

Título/ Title: A influência da expressão dos microRNAs miR15-a e miR16-1 na expressão de Bcl-2 em ceratocistos odontogênicos

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Contexto O ceratocisto odontogênico é uma neoplasia de natureza odontogênica que apresenta uma alta taxa de recorrência. O tratamento é cirúrgico e a marsupialização é utilizada como etapa inicial para involução da lesão. Os microRNAs miR15a e miR16-1 são RNAs pequenos que não codificam proteínas e funcionam como reguladores negativos do gene antiapoptótico Bcl2 em nível pós-transcricional. Objetivos Investigar a expressão do mRNA de Bcl2 e da proteína Bcl2 e dos microRNAs miR15-a e mirR16-1 em ceratocistos odontogênicos e investigar a alteração da expressão destes após a marsupialização. Materiais e Métodos Foram incluídos no estudo um total de 28 amostras de ceratocisto, incluindo lesões primárias e marsupializadas, esporádicas e sindrômicas. O mRNA e os microRNAs foram quantificados por meio de PCR em tempo real, utilizando um *pool* de folículos dentários. A proteína Bcl2 foi investigada por imuno-histoquímica. Resultados Todas as amostras de ceratocisto (exceto uma) demonstraram expressão aumentada de mRNA Bcl2 em comparação com os folículos, além de forte expressão imuno-histoquímica desta proteína. 85% das amostras apresentaram baixos níveis de expressão de miR15-a e/ou miR-16-1 comparados com os folículos pericoronários. Além disto, os casos que apresentavam amostras coletadas antes e após a marsupialização demonstraram aumento dos níveis de miR15-a e 16-1 e diminuição de expressão protéica após este procedimento. Conclusões Os resultados sugerem um perfil antiapoptótico das células neoplásicas , sendo que a expressão reduzida de miR15a e miR16-1 parece contribuir para este perfil. Por ultimo, a marsupialização leva a uma redução da expressão de Bcl2 e os microRNAs podem estar envolvidos nesta regulação. [Apoio CNPq e FAPEMIG]

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Abstract 51

Título/ Title: Characterization of the mechanisms of PLCG2 down-regulation in Wilms

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Wilms Tumor (WT), an embryonic kidney tumor, arises from metanephric blastemal cells which were unable to complete the differentiation process. WT presents histological and molecular characteristics that resemble normal nephrogenesis. WT resemble embryonic kidneys concerning down-regulation of PLCG2 at mRNA and protein level, which was observed in WT and early embryonic kidneys, suggesting it to be associated with WT onset (Maschietto et al., 2008). The aim of this study was to investigate PLCG2 promoter methylation status in WT samples. CpG islands in PLCG2 were predicted using Meth Primer v1.1B software. HEK293 was treated with 5μM of 5-AzadC and DNA and total RNA were purified. DNA was bisulfite converted with EZ DNA Methylation Gold (Zymo) and primers flanking the sequence regions with higher concentrations of CpG sites were used for PCR and sequencing by Sanger method. For confirming methylation status, pyrosequencing of the fragments was applied. First, 4,000 bp (-2,000 to +2,000 from first exon) were analyzed and predicted two CpG islands: from -469 to -323 and from -227 to +777. For investigating if PLGC2 expression is under DNA methylation control, quantitative RT-PCR from both non-treated and treated cells was performed and revealed huge activation of PLCG2 in HEK293 after 5-AzadC treatment, suggesting methylation as the

mechanism of PLCG2 expression control. A region from -634 to +298, that contemplates the first and a portion of the second island, was amplified from bisulfite converted DNA of HEK293 cells. After sequencing, the C to T conversion efficiency was assessed by absence, in the chromatogram, of C peaks in the C original positions in non CpG sites. In accordance to Sanger method, pyrosequencing did not reveal difference in the methylation status of this region between treated and non-treated cells. On the other hand, in the region from +642 to 1,045, that contains the final region of the second island, nine out of 19 CpG sites were found to be hypermethylated in non-treated cells.PLCG2 expression is under methylation control. Nine CpG sites may be, at least partially, involved in its expression control. Higher coverage of CpG islands by sequencing analysis is required for determining the CpG involved in PLCG2 regulation. Analysis of methylated CpGs will be extended to WT, fetal and differentiated kidney samples and the result correlated to expression level and clinical information.

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Reference

Maschietto M et al., Molecular Profiling of Isolated Histological Components of Wilms Tumor Implicates a Common Role for the Wnt Signaling Pathway in Kidney and Tumor Development. Oncology 2008; 75:81-91.