Poster Presentation

#01

TITLE: FUNCTIONAL INVESTIGATION OF ANAPC13 GENE IN THE PROGRESSION OF DUCTAL CARCINOMA OF THE BREAST

Authors: Nakahata AM[1], Marin DKK[1], Abuázar CS[1], Ricca TI[1], Costa ET[3], Camargo AA[1], Ferreira JRO[1], Carvalho AF[1], Carraro DM[2]

Authors' Information:

[1] Laboratory of Genomics and Molecular Biology. International Center for Research, A.C. Camargo Cancer Center, São Paulo - SP. Brazil.

[2] National Institute of Science and Technology in Oncogenomics (INCITO), São Paulo - SP. Brazil.

[3] Molecular Oncology Center of Sírio-Libanês Institute.

Corresponding Author:

Nakahata AM. E-mail: adriana_miti@yahoo.com.br

Introduction: Ductal carcinoma *in situ* (DCIS) of the breast is a heterogeneous group of preinvasive tumors which has an uncertain and poorly understood evolution. Previously, our group showed that transcriptional profile alterations in epithelial cells during the progression of ductal carcinoma of the breast mainly occur in the transition from pure DCIS to the *in situ* component of invasive ductal carcinoma (DCIS-IDC) rather than from the *in situ* component of DCIS-IDC to invasive lesions, implying that the molecular program for invasion is already established in the pre-invasive lesion (CASTRO et al., 2008). ANAPC13 was found differentially expressed during cancer progression and was validated by RT-qPCR. Down-regulation of ANAPC13 was observed both at mRNA and protein levels in DCIS-IDC samples when compared to pure DCIS lesions. The presence of the protein in IDC samples was associated with higher rates of overall and disease-free survival in breast cancer patients. Additionally, tumors with low levels of ANAPC13 displayed increased genomic instability observed by copy number alterations, with significant gains in specific regions that display common imbalance in breast cancer, suggesting that down-regulation of ANAPC13 contributes to genomic instability in this disease (Abuazar et al., 2012). **Objectives:** To evaluate the role of ANAPC13 in progression of ductal carcinoma. **Methods:** ANAPC13 overexpressed and knockdown cell lines were obtained by transfection of the MCF-7 cells with expression vectors containing the sense and the anti-sense ANAPC13 gene sequence, respectively. Cell viability and proliferation index of ANAPC13 overexpressed and silenced cell lines were evaluated by MTT, real-time proliferation and glucose consumption assays. In addition, cDNA microarray was performed to identify genes modulated by ANAPC13 expression. Short Time-series Expression Miner (STEM) program was used for identifying co-regulated genes according to ANAPC13 expression. **Results:** Overexpression and silencing of ANAPC13 were associated respectively with the augment and decrease of cell viability and proliferation. This was confirmed by glucose consumption in which ANAPC13 overexpressed cells presented higher consumption of glucoses. In cDNA microarray, ANAPC13 overexpression was related with an enrichment of biological process involved in cellular cycle. **Conclusion:** The modulation in the expression level of the ANAPC13 gene may be involved mainly in cell proliferation process in breast tumor.

#02

TITLE: FUNCTIONAL STUDY OF PROTEINS OF RHO FAMILY GTPASES AND THEIR PROGNOSTIC VALUE IN COLORECTAL CARCINOMAS

Authors: Neto JC[1], Coimbra FJ[2], Aguiar S Jr[3], Begnami MD[1], Macedo MP[1], Soares FA[1], Rocha RM[1]

Authors' Information:

[1] Department of Anatomy Pathology - A.C. Camargo Cancer Center, São Paulo Brazil.

[2] Department of Abdominal Surgery - A.C. Camargo Cancer Center, São Paulo Brazil.

[3] Department of Pelvic Surgery - A.C. Camargo Cancer Center, São Paulo Brazil.

Corresponding Author:

MSc student Julio Cirullo Neto
International Center of Research and Teaching
Department of Anatomy Pathology
Taguá, nº 440, Liberdade. São Paulo - SP. Brazil.
CEP: 01509-900
E-mail: julio_jcn@hotmail.com

Introduction: Colorectal cancer (CRC) is a neoplasia of epithelial origin and covers tumors of the colon and rectum in men and women. It is a treatable and often curable when in the absence of extension to other organs. Approximately 50%-60% of patients diagnosed with colorectal cancer will develop metastases and liver and lungs are the most common sites of metastases. Metastasis is a cascade of molecular and cellular events involving tumor cell intravasation, transport and immune evasion within the circulatory system, arrest at a secondary site,

extravasation and finally colonization and growth. The Rho-like GTPases (which include Rho, Rac and CDC42) are molecular switches that regulate a wide variety of cellular processes. They are crucial for cytoskeletal rearrangements necessary for cell motility, are involved in cell cycle progression and regulate gene transcription. It is becoming increasingly clear that they play an important role in tumorigenesis **Objectives:** Identify differentially expressed proteins of the Rho GTPases family in colorectal tumors and their metastases and evaluate its effect on cell cultures of different tumor groups. **Methods:** Forty cases of CRC who developed metastases and 40 patients who did not develop metastases were retrospectively selected from the files of Department of Anatomy Pathology, A.C. Cancer Center and paraffin material was recovered to perform IHC and IFL. IHC and IFL were performed for markers anti-Rho, anti-Rac, anti-Tiam1, anti-PAK1 and anti-Mrck. The reactions were analyzed on automated equipment MIRAX (3DHistech). Western blot was performed to confirm the specificity of all antibodies and quantify protein expression in cell cultures, the statistical analysis was performed using the Prism 5.2.1 and significance was considered when $p < 0.05$. **Conclusion:** Mrck-β was shown to be important in the formation of metastases in colorectal cancer. Expression is elevated only in metastatic cases shows that the presence of this protein is related to worse prognosis. Tiam1 also shown to be differentially expressed in colorectal tumors and its relationship with Rac1 indicates that when Tiam1 acts on Rac1 leads to metastatic profile. The family of Rho GTPases and molecules involved has a great role in the formation of metastases.

REFERENCES

1. Instituto Nacional de Câncer; Ministério da Saúde. Estimativa de Incidência de Câncer no Brazil para 2010. INCA.
2. Li WH et al. Oncological outcome of unresectable lung metastases without extrapulmonary metastases in colorectal cancer. World J Gastroenterol. v.16, n.26, p.3318-24, 2010.
3. Kaplan RN et al. Bone marrow cells in the 'pre-metastatic niche': within bone and beyond. Cancer Metastasis Rev. v. 25, n. 4, p. 521-9, 2006.
4. Mertens AE et al. Regulation of Tiam1-Rac signalling. FEBS Lett. v.546, n.1, p.11-6, 2003.

#04
TITLE: DCIS PROGRESSION: IDENTIFICATION POTENTIAL PROGNOSTIC MARKERS

Authors: Elias EV[1], Ferreira EN[1], Senz-Abuázar CS[1], Pineda P[1], Castro NP[1], Silva SD[1], Osório CA[2], Carraro DM[1,2]

Authors' Information:

[1] CIPE - International Center of Research and Training - A. C. Camargo Cancer Center, São Paulo - SP. Brazil.

[2] National Institute of Science and Technology in Oncogenomics (INCITO), São Paulo - SP. Brazil.

Corresponding Author:

Elias, EV. E-mail: vani.elias@gmail.com

Invasive ductal carcinoma (IDC) of the breast is one of the most common malignancies and the invasiveness of adjacent tissue is the first step for metastasis establishment, which is responsible for a high death rate among women worldwide. Characterization of the first molecular events crucial for the progression of *in situ* lesion to invasive disease is one of the most challenges of basic research. In our previous study (Castro, 2008), we showed that the most difference in gene expression pattern occurs in pre-invasive lesions, specifically from pure *in situ* ductal carcinoma (DCIS) and the *in situ* component of the lesion that coexists with the invasive ductal carcinoma (DCIS-IDC), and only subtle differences occurs between both components, *in situ* and invasive, of the DCIS-IDC. To assess the subtle differences between epithelial cells from both *in situ* and invasive components of DCIS-IDC lesions, we previously combined RASH (Rapid Subtraction Hybridization) and microarray technologies to identify candidate genes involved in the progression of ductal carcinoma (DC). The aim of the current study was to comprehensively validate the candidate genes, using an independent group of samples, and test them as prognostic biomarkers for DC. Material and Methods: TaqMan assays were performed using a 348-well card with 96 different assays (90 target genes, 5 reference genes and 1 positive control). GeNormplus was applied to identify the most stable reference gene. For data analysis, we used the computer program GraphPad Software®. A fold change of ≥ 2.0 and/or $p < 0.05$ was adopted for the determination of differentially expressed genes. We used the unpaired Student's t test to assess the statistical significance between the mean relative expressions of the analyzed groups. To calculate the fold-change (FC), not logged values were used. For the Student t test, relative expression values were logged in base 2. Protein expression was assessed by immunohistochemistry (IHC) and association with clinicopathological parameters was performed using Tissue Microarray (TMA). **Results:** From 90 candidate genes evaluated, 26% of the genes display concordant expression with the previous cDNA microarray/RASH studies. From them, seven showed more robust differences: CLN-S1A-FC = -2.8, $p = 0.01$; FCGR3A- FC = -3.1, $p = 0.03$, POSTN- FC = -8.5, $p = 0.01$; SAA1-FC = 5.1, $p = 0.01$, SLC37A1--FC = -6.5, $p = 0.02$, TFF1- FC = 4.1, $p = 0.004$, ZEB1- FC = -3.1, $p = 0.032$. TFF1 was assessed by protein expression by IHQ. TMA assays containing 415 primary ductal breast cancer samples, which mRNA was overexpressed in DCIS samples, revealed that TFF1 was positive in 75 cases (19%), showing strong intra-cytoplasmic staining in well--differentiated areas. TFF1 expression was associated with positive estrogen ($p = 0.001$) and progesterone receptor

(p = 0.023). These results revealed novel candidates to be involved in the transition from DCIS to IDC with potential to be used as prognostic molecular markers in breast.

REFERÊNCIAS

1. Castro NP, et al. Evidence that molecular changes in cells occur before morphological alterations during the progression of breast ductal carcinoma. Breast Cancer Res 2008,10:R87.

#05

TITLE: CAN DIFFUSION-WEIGHTED MRI DIFFERENTIATE NON-CYSTIC BENIGN VERSUS MALIGNANT LIVER LESIONS IN CANCER PATIENTS?

Authors: Maria Luiza Testa (Testa ML)[1] Chojniak R[2], Sene LS[3], Damascena AS[4]

Authors' Information:

[1] Mestrado em Ciências - Área de Oncologia - A.C. Camargo Cancer Center, São Paulo - SP. Brazil. Médica Radiologista.
[2] Diretor do Departamento de Imagem do A.C. Camargo Cancer Center, São Paulo - SP. Brazil.
[3] Mestranda em Ciências - Área de Oncologia - A.C. Camargo Cancer Center, São Paulo - SP. Brazil. Médica Radiologista.
[4] Estaticista - CIPE - A.C. Camargo Cancer Center, São Paulo - SP. Brazil.

Corresponding Author:

Maria Luiza Testa (Testa ML). E-mail: mluizatesta@gmail.com
Chojniak R. E-mail: chojniak@accamargo.org.br
Sene LS. E-mail: let_sene@hotmail.com
Damascena AS.E-mail: damascena@cipe.org.br

Background: Diffusion-weighted magnetic resonance imaging is a non-invasive imaging technique that can be quickly performed, has a fast response time, and has recently been applied in studies of the liver. **Objectives:** To compare apparent diffusion coefficient values obtained from diffusion-weighted magnetic resonance imaging of benign and malignant liver lesions. **Materials and Methods:** In 115 adult patients, 367 liver lesions were analyzed. In 57 patients, 105 benign lesions were detected, which included 59 hemangiomas, 42 cysts, 2 adenomas, and 2 focal nodular hyperplasia. In 58 patients, 262 metastatic lesions were detected. Apparent diffusion coefficient values were measured and quantitatively analyzed for each lesion. **Results:** The mean apparent diffusion coefficient values for benign liver lesions, metastatic liver lesions, and cysts were 1.8 x 10^{-3} mm^2/sec, 1.1 x 10^{-3} mm^2/sec, and 2.3 x 10^{-3} mm^2/sec, respectively. The apparent diffusion coefficient value that best classified benign solid lesions from metastases, including pre- and post-chemotherapy treatment lesions, was 1.3 x 10^{-3} mm^2/sec. There was no statistically significant difference between the apparent diffusion coefficient values obtained for metastases and benign solid lesions (p = 0.10), or for metastases treated with systemic chemotherapy or not (p = 0.12). Furthermore, when metastatic lesions were compared before and after chemotherapy with benign solid lesions, the cutoff apparent diffusion coefficient values were 1.3 x 10^{-3} mm^2/sec and 1.2 x 10^{-3} mm^2/sec, respectively. **Conclusions:** In cancer patients, diffusion-weighted magnetic resonance imaging can differentiate benign and malignant liver lesions, and apparent diffusion coefficient values provide an additional diagnostic parameter when evaluated according to strict criteria.

#06

TITLE: ANALYSIS OF SHH AND PTCH1 PROTEIN EXPRESSION IN UTERINE LEIOMYOMA AND LEIOMYOSARCOMA

Authors: Faila Catarina de Souza[1], Natalia Garcia[1], Nilo Bozzini[1], Gustavo Arantes Rosa Maciel[1], Isabela W Cunha[2], Fernando Augusto Soares[2], Edmund Chada Baracat[1], Katia Candido Carvalho[1]

Authors' Information:

[1] Centro Internacional de Pesquisas - A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Departamento de Anatomia Patológica - A.C Camargo Cancer Center, São Paulo, Brazil.

Background: Leiomyoma (LM) and leiomyosarcoma (LMS) are uterine mesenchymal tumors which have variable clinical behavior and may lead to death. Leiomyoma is a benign tumor commonly found in women of reproductive age. Leiomyosarcoma represents approximately 40% of the sarcomas of the uterus. Both are myometrium tumors that show similar cell differentiation, but with different clinical progression. Its etiology and biology are still poorly understood. However, it has been shown that activation of the signaling pathway of Sonic hedgehog (SHH) is related to development of various types of tumor, since this pathway plays an important role in cell proliferation and differentiation. **Objectives:** To evaluate, by immunohistochemistry, the expression of Shh and Patch1 as markers of the Sonic Hedgehog pathway in samples of myometrium, leiomyoma and leiomyosarcoma. **Methods:** We used 80 samples of leiomyoma, 57 leiomyosarcomas and 20 myometrium. Leiomyoma and myometrium samples were obtained from Department of Obstetrics and Gynecology - *Faculdade de Medicina da Universidade de São Paulo*, and samples of leiomyosarcoma from Department of Pathology, A.C. Camargo Cancer Center (both in São Paulo, Brazil). The paraffin-embedded tissues were used in the building of tissue microarray (TMA) blo-

cks for immunohistochemical analysis. **Results:** The immunohistochemical reactions were classified as positive (moderated and stronger staining) or negative (weakly and negative staining). Ptch showed positive reaction in 95% of the myometrium samples, 70% of the leiomyomas with atypia, 99% of the leiomyomas and 84% of the leiomyosarcomas. Shh was found in 30% of myometrium samples, 30% of the leiomyomas with atypia, 22% of the leiomyoma and 39% of the leiomyosarcomas. **Conclusions:** Our results showed Ptch with higher frequency and intensity of expression in leiomyomas, while Shh presented stronger reactivity in 39 percent of leiomyosarcomas. Since Ptch is a Shh receptor, these results need to be further investigated.

#07

TITLE: AN INTEGRATIVE GENOMIC AND TRANSCRIPTOMIC ANALYSIS IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Authors: Yukie Sato-Kuwabara[1], Fabio Albuquerque Marchi[2], Clóvis Klock[3], Cristóvam Scapulatempo[4], Felipe Coimbra[5], Silvia Regina Rogatto[1,6], Fernando Augusto Soares[1,7]

Authors' Information:

[1] Centro Internacional de Pesquisa e Ensino, Hospital A.C. Camargo, São Paulo, Brazil.
[2] Universidade de São Paulo - USP, São Paulo - SP. Brazil.
[3] Hospital Santa Terezinha, Erechim - RS. Brazil.
[4] Hospital do Câncer de Barretos, Barretos, Brazil.
[5] Departamento de Cirurgia Abdominal, A.C. Camargo Cancer Center, São Paulo, Brazil.
[6] Universidade do Estado de São Paulo - UNESP, Botucatu - SP. Brazil.
[7] Faculdade de Odontologia, Universidade de São Paulo, São Paulo, Brazil.

Corresponding Author:

Yukie Sato-Kuwabara. E-mail: yukiesato@gmail.com
Fabio Albuquerque. Marchi E-mail: biomarchi@gmail.com
Clóvis Klock. E-mail: clovisklock@medicinadiagnostica.med.br
Cristóvam Scapulatempo. E-mail: cristovamscapula@uol.com.br
Felipe Coimbra. E-mail: coimbra.felipe@uol.com.br
Silvia Regina Rogatto. E-mail: silvia.rogatto@hcancer.org.br
Fernando Augusto Soares. E-mail: fasoares@hcancer.org.br

Esophageal squamous cell carcinoma (ESCC) is the sixth tumor in frequency in Brazil and despite the improvement of various treatment modalities, the overall survival rates still remain low. In order to identify new possible molecular markers, genomic and transcriptomic data were evaluated using integrative analysis in ESCC samples. Thirty frozen samples of ESCC were carried out in genome-wide expression (GWE) profiling using the Agilent Whole Human Genome Microarray 44K and in Array-CGH (aCGH) using the Agilent Human Genome CGH Microarray 44K following the manufacturer's protocol. Data were extracted and flagged with Feature Extraction and processed using NEXUS 6.0 and TMEV 4.8 Software. A subset of genes identified by the integrated analysis was analyzed for signaling networks using Ingenuity Pathway Analysis (IPA) software. The aCGH analysis revealed 14 significant genomic alterations including 7 gains and 7 losses. A total of 375 genes were mapped to these regions. GWE profile identified 1770 differentially expressed genes in comparison with normal tissue including 573 up regulated and 1197 down-regulated. According to the integrated analysis, 36 genes showed positive correlation (involved in genomic gain and up regulated expression) and 3 genes showed negative correlation (genomic loss and down-regulated expression). According to IPA analysis three significant networks could be defined comprising these genes. The first network comprising 14 of the concordant genes was associated to cellular assembly and organization, cardiovascular system development and function and cell morphology. The second network (11 genes) was related to cancer, immunological disease and cell cycle. The third network (9 genes) was associated to cancer, RNA post-transcriptional modification and organismal development. Eleven genes were involved in genomic gains by aCGH but were down-regulated by GWE profiling, while two genes were involved in losses but their transcripts were up-regulated. The IPA analysis showed that these genes are associated to function as drug metabolism, small molecule biochemistry and nucleic acid metabolism. In this study, it was detected significant genomic alterations and genes differentially expressed involved in important signaling networks that could have an impact on tumor development or progression in ESCC. These genes might be useful as a first step to identify molecular markers for improved diagnostic and therapeutic modalities in this tumor.

#08

TITLE: LINEAR MRNA AMPLIFICATION BASED ON *IN VITRO* T7 TRANSCRIPTION AND TEMPLATE-SWITCHING OLIGONUCLEOTIDES FOR THE ASSESSMENT OF THE WHOLE TRANSCRIPTOME BY RNA-SEQ

Authors: Elisa Napolitano Ferreira[1], Gustavo de Campos Molina[1], Renato David Puga[1], Maria Aparecida Nagai[2], Helena Brentani[3], Emmanuel Dias-Neto[1,3], Dirce Maria Carraro[1]

Authors' Information:

[1] International Center of Research and Training/CIPE - A.C. Camargo Cancer Center, Rua Taguá, nº 440 - 1º andar, Liberdade. São Paulo - SP. Brazil. CEP: 01508-010
[2] Radiology and Oncology Department, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil. Avenida Drº Arnaldo, nº 251, Cerqueira Cesar. São Paulo - SP. Brazil. CEP: 01246-000

[3] Department of Psychiatry, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil. Rua Dr Ovidio Pires de Campos, nº 785, Cerqueira Cesar. São Paulo - SP. Brazil. CEP: 05403-010

Corresponding Author:
Elisa Napolitano Ferreira. E-mail: elisa.ferreira@accamargo.org.br
Gustavo de Campos Molina. E-mail: gucmolina@gmail.com
Renato David Puga. E-mail: gucmolina@gmail.com
Maria Aparecida Nagai. E-mail: nagai@usp.br
Helena Brentani. E-mail: helena.brentani@gmail.com
Emmanuel Dias-Neto. E-mail: emmanuel@cipe.accamargo.org.br
Dirce Maria Carraro. E-mail: dirce.carraro@cipe.accamargo.org.br

Background: Whole-transcriptome sequencing analysis has been widely applied for investigating transcriptional alterations in different settings. In many clinical situations, including needle biopsies or laser microdissected cells, limited amounts of RNA are available for whole transcriptome assessment. In these situations, mRNA amplification is required. Here, we describe an mRNA amplification protocol for transcriptome investigation in next-generation sequencing platforms. **Methods:** Total RNA was obtained from two human mammary cell lines expressing different levels of the ERBB2 oncogene (HB4a and C5.2) and submitted to one round of linear amplification based on *in vitro* T7 transcription and template-switch (TS) oligonucleotides. After amplification, double-strand cDNA was obtained using the TS-oligo and the oligo dT for the first and second strand syntheses, respectively. Full-length dscDNA molecules were enzymatically digested with DpnII and ligated to customized linkers, containing a 4-nucleotide barcode for multiplex sequencing by the Genome Sequencer FLX System (454 - Roche) (Carraro et al., 2011). **Results:** To assess the power of this approach, several aspects of the Poly $(A)^+$ transcriptome were evaluated. First, transcript coverage was evaluated and showed full-length coverage, encompassing both the 5' and 3' end of the transcripts, with no decreased representation of the 5' end. Next, relative transcript abundance was compared with non-amplified $PolyA^+$ RNA-Seq (Carraro et al., 2011) and SAGE libraries, and similar expression trends were observed for high- and low-abundance transcripts, suggesting maintenance of the transcript relative abundance. Finally, we assessed transcriptional diversity by comparing the number of different genes represented by amplified and non-amplified Poly $(A)^+$ RNA and a slight decrease in the variability of the genes was detected by the amplified RNA library. This result might be a reflection of the lower sequencing depth of the amplified library, even after the adjustment of the number of reads. **Conclusion:** The mRNA amplification approach presented here is appropriate for assessing the Poly $(A)^+$ transcriptome by RNA-Seq from limited amounts of total RNA revealing a robust preservation of the transcriptome for comparative analysis.

Financial Support: CNPq

REFERENCES

1. Carraro DM, Ferreira EN, de Campos Molina G, Puga RD, Abrantes EF, Trapé AP, Ekhardt BL, Nunes DN, Brentani MM, Arap W, Pasqualini R, Brentani H, Dias-Neto E, Brentani RR. Poly (A)+ transcriptome assessment of ERBB2-induced alterations in breast cell lines. PLoS One 2011,6:e21022.

#09

TITLE: GENOMICS AND TRANSCRIPTIONAL ALTERATIONS MEDIATED BY *BRCA1* MUTATION IN TRIPLE-NEGATIVE EARLY AGE BREAST CANCER

Authors: Elisa N Ferreira[1], Rafael C Brianesi[1], Renan Valieris[1], Jorge E S Souza[1], Marcia C P Figueiredo[1], Felipe C Silva[1], Felipe Fidalgo[1], Andrea Yaguiu[1], Ana C V Krepischi[1], Sandro J de Souza[1], Dirce M Carraro[1]

Authors' Information:
[1] CIPE - International Center of Research and Training - A.C. Camargo Cancer Center, São Paulo, SP, Brazil.

Corresponding Author:
Elisa N Ferreira. E-mail: elisa.ferreira@accamargo.org.br
Rafael C Brianesi. E-mail: rcbrianese@hotmail.com
Renan Valieris. E-mail: rvalieris@gmail.com
Jorge E S Souza. E-mail: jorge@i2bio.org
Marcia C P Figueiredo. E-mail: marcia.figueiredo@hcancer.org.br
Felipe C Silva. E-mail: felipe.silva@accamargo.org.br
Felipe Fidalgo. E-mail: felipe.fidalgo9@gmail.com
Andrea Yaguiu. E-mail: andrea.yaguiu@gmail.com
Ana C V Krepischi. E-mail: ana.krepischi@gmail.com
Sandro J de Souza. E-mail: sandro@i2bio.org
Dirce M Carraro. E-mail: dirce.carraro@cipe.accamargo.org.br

Background: Germline mutations in BRCA1 and BRCA2 genes are associated to hereditary breast cancer leading to early age of tumor onset. BRCA1-carriers preferentially develop hormonal receptor negative tumors. Triple negative breast cancer (TNBC) is characterized by absence of hormonal receptors expression and no over-expression and/or amplification of HER-2. The purpose of this study was to investigate genomic and transcriptional alterations mediated by BRCA1 mutations in TNBC of early-onset patients. **Methods:** Three TNBC early-onset patients (≤ 35 years) were obtained from A.C. Camargo Biobank. One patient was BRCA1-carrier (germline nonsense

mutation - R1751X) and two were BRCA1-non-carriers. Transcriptome alterations were evaluated by RNA-Seq from tumor and normal matched samples and genomic alterations were assessed by exome sequencing using a triplet of DNA samples (blood, normal and tumor breast tissues) and sequenced at SOLiD platform. Somatic copy number alterations were evaluated in tumors by CGH-array. Bioinformatics analyses were performed using Bioscope and CLCBio Workbench. **Results:** Whole transcriptome of BRCA1-associated tumor showed to be more divergent from the BRCA1-negative tumors, as shown by Pearson's correlation based on RNA-Seq data. Normal samples showed similar correlation one to each other. Gene expression comparison between tumor and normal samples of each patient identified 73 transcripts exclusively differentially expressed for the BRCA1-carrier patient. Six of 8 genes selected for validation were confirmed as differently expressed between BRCA1-associated and negative TNBC. Next, from the exome sequencing data, somatic alterations were identified for tumor and normal samples. Preliminary analysis showed that BRCA1-associated tumor presents lower number of genomic alterations compared to BRCA1-negative tumors, whereas, in normal tissues, acquired somatic alterations were more frequent in BRCA1-carrier than BRCA1-negative patients. Additionally, contrary to expected, higher levels of genomic instability were detected in BRCA1-negative tumors compared to BRCA1-associated tumors, based on array-CGH experiments. **Conclusion:** The investigation of genomic and transcriptional alterations mediated by BRCA1 mutations in TNBC contributes to a better comprehension of the tumorigenic process and identification of potential molecular markers for this subtype of breast cancers.

Financial Support: INCITo

REFERENCES

1. Carraro DM, Koike Folgueira MA, Garcia Lisboa BC, Ribeiro Olivieri EH, Vitorino Krepischi AC, de Carvalho AF, de Carvalho Mota LD, Puga RD, do Socorro Maciel M, Michelli RA, de Lyra EC, Grosso SH, Soares FA, de Souza Waddington Achatz MI, Brentani H, Moreira-Filho CA, Brentani MM. Comprehensive analysis of BRCA1, BRCA2 and TP53 germline mutation and tumor characterization: a portrait of early-onset breast cancer in Brazil. PLoS One. 2013;8(3):e57581.

#10

TITLE: CAN PATHOLOGICAL FACTORS PREDICT DISEASE FREE SURVIVAL IN ESTROGEN-POSITIVE, NODE-NEGATIVE BREAST CANCER? MAGEE EQUATIONS INDEPENDENT BIOLOGICAL VALIDATION

Authors: Balacin, Marcelo[1]

Authors' Information:

[1] A.C. Camargo Cancer Center, São Paulo, SP, Brazil.

Corresponding Author:

Marcelo Luiz Balancin. E-mail: mlbalancin@me.com

Background: OncotypeDX® is used to indicate chemotherapy in early stage ER+ (ER+ N0M0) patients, although limited by costs and logistics. Magee equations (ME) are mathematical models based on pathologic data that showed over 98% concordance to OncotypeDX® scores. **Objective:** We tested the ability of ME to predict recurrence in ER+ N0M0 patients using a tissue microarray cohort. **Methods:** On 276 ER+ N0M0 breast cancer patients diagnosed at A.C. Camargo Cancer Center from 1982 to 2005, tumor size and Nottingham score were reviewed. ER and PR expression were evaluated using H-score and Her-2 according to ASCO/CAP guidelines. Ki-67 was categorized in < 10%, 11-50%, 51-90% and > 90%. ME #1 and ME #2 scores were generated [< 18 (low risk-LR), 18-30 (intermediate risk-IR) and > 30 (high risk-HR)]. DFS were compared in survival curves using the log rank test with p -value < 5%. **Results:** Patients aged from 26 to 94 yo (median 60) and median follow-up time was 78 months. Fifty-nine (22.8%) cases recurred at a mean time of 63 months. ME #15y-DFS for LR, IR and HR groups were 92%, 85.7% and 71%, whereas 10y-DFS were 84.4%, 73% and 63%. ME #25y-DFS were 90.2%, 82% and 81%, whereas the 10y-DFS were 79%, 74% and 68% ($p > 0.05$). Hormonal therapy, radiotherapy or chemotherapy was not associated with recurrence. Ki-67 > 10% in patients without chemotherapy ($p = 0.095$) and tumor size > 2cm ($p < 0.0001$) were associated with recurrence. **Conclusion**: High Ki67 and tumor size are the best predictors of recurrence in ER+ N0M0 breast cancer. ME could not be validated to predict recurrence in this cohort.

#11

TITLE: LITERATURE REVIEW WITH CASE REPORT OF CYSTIFIED PARATHYROID CARCINOMA

Authors: Luiz Guilherme Cernaglia Aureliano de Lima[1] MD, Magalhães HLGO[2], Balancin ML[2], Macedo MP[1], Pinto CAL[1]

Authors' Information:

[1] Hospital A.C. Camargo Cancer Center. Departamento de Anatomia Patológica.

[2] Hospital A.C. Camargo Cancer Center.

Corresponding Author:

Magalhães, HLGO. E-mail: hugolhaes@gmail.com
Balancin, ML. E-mail: mlbalancin@me.com
Macedo, MP. E-mail: maripetaccia@hotmail.com
Pinto, CAL. E-mail: coipinto@uol.com.br

Introduction: Cystic parathyroid lesions are rare, usually nonfunctional and misdiagnosed as thyroid cysts. Parathyroid carcinoma is also a rare entity, accounting for < 1% of hyperparathyroidism causes and generally presents as a solid mass. Cystified parathyroid carcinoma is, therefore, an even rarer lesion, usually diagnosed after surgical resection. **Case Report**: A 58 yo, male patient, hypertense, former smoker, presented with a six month history of painless, growing mass, measuring 5.0 cm, associated with primary hyperparathyroidism and dry cough. A thyroidectomy with parathyroidectomy was performed. On gross exam, a cystic, 5.0 cm, right sided lesion, filled with colloid-like material was seen. Paraffin-section revealed a cystic lesion with monotonous small atypical cells, disposed in a solid and cordonal pattern, infiltrating adjacent tissue in a centrifugal fashion. Immunohistochemistry revealed positivity to cyclinD1 and parathormone (PTH) and negativity to thyroglobulin, with a Ki-67 of around 2%. Final diagnosis was cystified parathyroid carcinoma. **Discussion:** Parathyroid cysts are rare lesions, with variable sizes and clinical aspects, ranging from asymptomatic to compressive symptoms due to large size. They are classified by their functional status, as of PTH production, from non--functioning (about 90%) to functioning, each harboring its clinical aspects. Pre-operatory diagnosis, based on fine needle aspiration and ultrasound study, has a high degree of difficulties, by their close resemblance with thyroid lesions and the need of clinical suspicion. Definitive diagnosis was based on invasive and proliferative characteristics of an atypical lesion, differing from an adenoma. **Conclusion:** Differential diagnosis in a cystic cervical lesion should exclude parathyroid origin, especially when located in posterior aspect of the thyroid, including the possibility of malignancy.

#12

TITLE: PREVALENCE OF GERMLINE MUTATIONS AND GENOMIC REARRANGEMENTS IN BRCA1/2 GENES IN HEREDITARY BREAST CANCER UNRELATED BRAZILIAN FAMILIES

Authors: Figueiredo, MCP[1], Silva FC[1], Lisboa BCG[1], Torrezan GT[1], Santos EMM[1], Krepischi AC[1], Achatz MIW[1], Rossi BM[1], Carraro DM[1]

Authors' Information:

[1] International Center of Research and Teaching - CIPE - A.C. Camargo Cancer Center, São Paulo - SP. Brazil.

Corresponding Author:

Figueiredo, MCP. E-mail: marcia.figueiredo@accamargo.org.br

Since the discovery of BRCA1 and BRCA2, germline mutations in these genes have been identified as the cause of hereditary breast and ovarian cancer (HBOC) in up to 30% of these patients. More recently, it has been proposed a novel syndrome called hereditary breast and colon cancer due to the co-occurrence of these tumors in the same individual or in closed relatives. In Brazil, BRCA1/2 mutation frequency has not been thoroughly considered, so comprehensive data was generated in order to determine the frequency of disease-causing mutations in a cohort of 128 Brazilian women, including 108 families fulfilling criteria for hereditary breast and ovarian cancer (HBOC), and 20 for hereditary breast and colon cancer (HBCC). To accomplish this, we use capillary sequencing and multiplex ligation-dependent probes for BRCA1, BRCA2 and the CHEK2 1,100delC variant. Additionally, fourteen cancer susceptibility genes (PTEN, ATM, NBN, RAD50, RAD51, BRIP1, PALB2, MLH1, MSH2, MSH6, TP53,CDKN2A, CDH1 and CTNNB1) were evaluated by array comparative genomic hybridization to identify copy number variations. Overall, the positive detection rate was 22.5% (26% for HBOC and 5% for HBCC patients). BRCA1 gene represented 75% of all pathogenic mutations (21 cases), including two cases with large genomic rearrangements within the BRCA1 gene, whereas BRCA2 mutations were detected in 25% of the cases (7 cases). Additionally, we found a patient with a CHEK2 110delC mutation, which is the first description in a Brazilian patient. Seven (25%) pathogenic mutations were firstly described, including a splice-site BRCA1 mutation, whose pathogenicity was confirmed by the presence of an aberrant transcript showing the loss of the last 62bp of exon 7, due to the creation of a cryptic splice site. The copy number analysis revealed microdeletions of exon-4 and exon-2 in ATM and PTEN, respectively, which were confirmed by duplex quantitative PCR. In summary, our results showed a complex genetic etiology for Brazilian breast cancer families.

Keywords: BRCA 1/2 genes, hereditary breast cancer, mutation.

Financial Support: CAPES and FAPESP

#13

TITLE: THE EXPRESSION OF PRION PROTEIN AND ITS LIGAND HOP ARE ASSOCIATED WITH INVASION IN COLON TUMORS

Authors: Lacerda TCS[1,2], Salles MV[1], Machado C[1], Aguiar Jr.S[3], Cunha IW[4], Martins VR[1,2]

Authors' Information:

[1] National Institute of Science and Technology in Oncogenomics INCITO, A.C. Camargo Cancer Center, São Paulo.
[2] Department of Biochemistry, Chemistry Institute, University of São Paulo.
[3] Department of Pelvic Surgery, A.C. Camargo Cancer Center, São Paulo.
[4] Department of Pathology, A.C. Camargo Cancer Center, São Paulo.

Corresponding Author:

Lacerda, TCS. E-mail: tlacerda@cipe.accamargo.org.br
Salles, MV. E-mail: msalles@cipe.accamargo.org.br
Machado, C. E-mail: cmachado@cipe.accamargo.org.br

Aguiar, Jr.S. E-mail: samuel.aguiar.jr@uol.com.br
Cunha, IW. E-mail: iwcunha@hcancer.org.br
Martins, VR. E-mail: vmartins@cipe.accamargo.org.br

Colon cancer is the fourth most common type of cancer in the world and potential molecular targets for therapy have been studied. One of these targets is the prion protein (PrP^C), a glycosylphosphatidylinositol anchored glycoprotein. PrP^C activity depends on its interaction with proteins, such as HSP70/HSP90 organizing protein (HOP), which besides presenting cytoplasmatic localization, is also secreted in exosome-like microvesicles. PrP^C-HOP complex processes proliferation activity in glioblastomas and melanomas. The aim of this study is to evaluate the expression of PrP^C and HOP in colon tumors and their correlation with tumor processes. A total of 205 cases of colon tumors were organized in tissue microarrays and analyzed by immunohistochemistry using antibodies against PrP^C and HOP. The expression of both PrP^C and HOP were correlated with hematogenic ($p < 0.001$), lymphatic ($p < 0.001$) and perineural ($p < 0.001$) invasion. We also found that the positive correlation with invasion depends upon the conjunct expression of PrP^C and HOP proteins ($p < 0.003$) since tumors showing expression of only one of the proteins did not present correlation with invasion. Furthermore, a direct correlation was found between HOP expression and secretion in colon tumors ($p = 0.0004$). This indicates that the presence of the receptor (PrP^C) and its agonist ligand (HOP) is essential in the different invasion processes. These results point that PrP^C and HOP deserve further investigation as therapeutic targets within these tumors.

Supported by FAPESP.

#14

TITLE: VIDEO ENDOSCOPIC INGUINAL LYMPHADENECTOMY FOR MELANOMA: INITIAL EXPERIENCE OF A SINGLE INSTITUTION

Authors: Eduardo Bertolli[1], Molina AS[1], Macedo MP[1], Pinto CAL[1], Duprat Neto JP[1]

Authors' Information:
[1] A.C. Camargo Cancer Center, São Paulo - SP. Brazil.
Corresponding Author:
Eduardo Bertolli. E-mail: ebertolli@hotmail.com
Molina, AS. E-mail: drandremolina@gmail.com
Macedo, MP. E-mail: maripetaccia@hotmail.com
Pinto, CAL. E-mail: coipinto@uol.com.br
Duprat Neto, JP. E-mail: jduprat@uol.com.br

Introduction: It is known that radical lymphadenectomy can offer durable disease free survival in stage III melanoma patients, but with some early and late complications related to the procedure. Among the radical lymphadenectomies for melanoma treatment, the radical groin dissection (RGD) carries more post-operative morbidity. Patients undergoing RGD may present from 40% to 60% of morbidity due to postoperative wound infection and skin flap necrosis, and may have larger dehiscence sometimes, leading to a long healing process. The video endoscopic inguinal lymphadenectomy (VEIL) is a less invasive procedure than the traditional RGD. **Material and Methods:** Retrospective analysis of five stage III melanoma patients, which underwent video endoscopic inguinal lymphadenectomy in a single institution. **Results:** The median follow up after groin dissection was 8 months (range 6-14). Four patients (80%) underwent sentinel node biopsy (SNB) prior groin dissection and 1 (20%) had clinical groin disease diagnosed by fine needle aspiration. Among patients that underwent SNB, 2 (50%) had one sentinel node, 1 (25%) had 2 and 1 (25%) had 3 sentinel nodes retrieved. The median number of lymph nodes dissected in VEIL was 14 (range 7-23). If we consider the total number of lymph nodes (sentinel nodes and lymph nodes of VEIL), the median was 15 nodes (range 10-24). The median length of drain was 27 days (range 13-36). Regarding postoperative complications, 2 (40%) had infection and 2 (40%) had dehiscence. Only 1 (20%) patient had symptoms and signs of lymphedema. Only 1 (20%) patient had dehiscence and infection. **Conclusion:** VEIL is related to lower rates of postoperative complications. Although in this analysis both infection and dehiscence rate were high, this is a initial experience in a few number of patients. The number of lymph nodes retrieved was higher than the number purposed by quality standards recommendation. VEIL seems to be a safe procedure for the treatment of melanoma inguinal metastasis but learning curve is necessary to achieve less morbidity.

#15

TITLE: MERKEL CELL CARCINOMA OF UNKNOWN PRIMARY ORIGIN: CASE REPORT AND REVIEW OF PATHOLOGICAL FEATURES

Authors: Eduardo Bertolli[1], Campagnari M[1], Molina AS[1], Macedo MP[1], Pinto CAL[1], Duprat Neto JP[1]

Authors' Information:
[1] A.C. Camargo Cancer Center, São Paulo - SP. Brazil.
Corresponding Author:
Eduardo Bertolli. E-mail: ebertolli@hotmail.com
Campagnari, M. E-mail: maricampa@hotmail.com
Molina, AS. E-mail: drandremolina@gmail.com
Macedo, MP. E-mail: maripetaccia@hotmail.com
Pinto, CAL. E-mail: coipinto@uol.com.br
Duprat Neto, JP. E-mail: jduprat@uol.com.br

Introduction: Merkel cell carcinoma (MCC) is a rare neuroendocrine tumor of the skin. MCC from an unknown primary origin (MCCUP) can present a diagnostic and the-

rapeutic challenge. When it happens within the lymph nodes in the absence of a primary site, it is even rarer and has only been reported sporadically. **Objectives:** To describe a MCC of unknown origin presented as nodal disease. **Case report:** We present a 68-year-old male patient who developed groin lymphadenopathy. No cutaneous lesions were found. Fine needle aspiration (FNA) biopsy revealed malignant melanoma. No other site of disease was found during staging and we performed groin and iliac lymphadenectomy, in which 5 from 20 lymph nodes had metastatic disease, also reported as melanoma. The patient developed wound-healing problems and during treatment presented with a cervical node. FNA was unable to define the etiology, so we performed excisional biopsy of the node. Immunohistochemistry (IHQ) revealed MCC, which lead to a review of previous diagnosis and they were all considered MCC. Re-staging revealed metastatic disease in retroperitoneum and in right adrenal gland. The patient was referred to chemotherapy and he has been in follow up for one year after the surgery. **Discussion:** There's a controversy in the literature about MCC form a regressed or unknown primary versus lymph nodal MCC. Nevertheless, it represents a very aggressive disease. In our case, it's important to discuss the importance of IHQ in the differential diagnosis between MCC and melanoma.

#16
TITLE: TUMOR NECROSIS FACTOR (TNF) ASSOCIATED TO MELPHALAN IN ISOLATED LIMB PERFUSION (ILP): A ONE-YEAR EXPERIENCE

Authors: Eduardo Bertolli[1], Molina AS[1], Macedo MP[1], Pinto CAL[1], Duprat Neto JP[1]

Authors' Information:
[1] A.C. Camargo Cancer Center, São Paulo - SP. Brazil.

Corresponding Author:
Eduardo Bertolli. E-mail: ebertolli@hotmail.com

Introduction: Isolated limb perfusion (ILP) is a well-established method that allows the regional administration of chemotherapy in patients with advanced melanoma and other malignant neoplasms restricted to the limb. The association of tumor necrosis factor (TNF) with Melphalan as a chemotherapeutic agent has shown good results especially in unresectable lesions or in transit metastasis, that would lead to amputation. In Brazil, TNF was approved for use in January 2012. **Objectives:** To describe and evaluate the patients treated with TNF and Melphalan in ILP at A.C. Camargo Cancer Center since 2012 by the Skin Cancer Department of this institution. **Results:** From January 2012 until February 2013, we have performed seven ILPs with TNF and Melphalan (Table 1). There were 5 melanoma cases, 1 epithelioid hemangioendothelioma (EHE) and 1 myxofibrosarcoma. Four patients (57.1%) were male and median age was 51.14 years (24-83). There were no major complications and all patients were classified as Wieberdink II regarding limb evolution. There were two recurrences (7.42%), and one of them was above the tourniquet line. This patient died seven months after surgery. The other patient started Vemurafenib, and presents with stable disease eleven months after surgery. **Conclusion:** TNF has been recently approved for use in clinical practice in Brazil. Although we have been using it for only one year, we present it as a safe and effective treatment modality for melanoma and other cutaneous neoplasms with multiple in transit metastasis or bulky disease.

Table 1. Clinical features and outcomes of the patients treated with isolated limb perfusion with Melphalan and TNF at the AC Camargo Cancer Center since January, 2012.

Case	MLPS	ADA	NFC	CCFM	DM	PARC	CLR
Gender	F	M	M	M	F	M	F
Age	59	83	66	24	34	48	44
Indication	In transit melanoma metastasis	In transit melanoma metastasis	In transit melanoma metastasis	EHE	Unresectable melanoma metastasis	Sarcoma	In transit melanoma metastasis
Site	RIL	RIL	LIL	RIL	RUL	RIL	LIL
Complications	Shock (Drugs and blood transfusion needed) Wound healing minor complication	Wound infection	Shock (Drugs and blood transfusion needed)	No complications	Extensive tumoral necrosis	Pain	Lymphocele
Outcomes	Recurrence above the tourniquet line. Died 7 months after the surgery	Live without disease, 11 months after surgery	Recurrence and skin metastasis, treated with Vemurafenib. Live with disease, 11 months after surgery	Local recurrence 3 months after surgery, local resection was performed. Live without disease 6 months after surgery	Limb sparing surgery for tumor resection. Live without disease 3 months after surgery	2 months follow up	1 month follow up

EHE: Epithelioid hemangioendothelioma; RIL: Right inferior limb; LIL: Left inferior limb, RUL: Right upper limb.

#17

TITLE: TUMOR NECROSIS FACTOR (TNF) ASSOCIATED TO MELPHALAN IN ISOLATED LIMB PERFUSION (ILP): A ONE-YEAR EXPERIENCE

Authors: Eduardo Bertolli[1], Gibbons IL[1], Campagnari MAC[1], Molina AS[1], Macedo MP[1], Pinto CAL[1], Duprat Neto JP[1]

Authors' Information:
[1] A.C. Camargo Cancer Center, São Paulo - SP. Brazil.

Corresponding Author:
Eduardo Bertolli. E-mail: ebertolli@hotmail.com
Gibbons, I.L. E-mail: ivanalameiras@gmail.com
Campagnari, M. E-mail: maricampa@hotmail.com
Molina, AS. E-mail: drandremolina@gmail.com
Macedo, MP. E-mail: maripetaccia@hotmail.com
Pinto, CAL. E-mail: coipinto@uol.com.br
Duprat Neto, JP. E-mail: jduprat@uol.com.br

Introduction: Metastatic melanoma is very resistant to standard treatment modalities, including chemotherapy and radiotherapy. Diphencyprone (DPCP) is a contact sensitizer with immunomodulator effects, commonly used for alopecia areata and warts. Topical diphencyprone (DPCP) immunotherapy has been shown to cause regression of extensive, rapidly growing recurrent and cutaneous metastatic melanoma when wide local excision is not feasible. It can also be an option for in transit metastasis in patients that cannot manage isolated limb infusion or perfusion. **Objectives:** To describe two cases of cutaneous melanoma treated with DFCP at A.C. Camargo Cancer Center and their clinical outcomes. **Case report:** Our first case is an 84-year-old male patient who presented with extensive disease in face and scalp, which incisional biopsy revealed Breslow 1.1 without any other prognostic factor. Although there was no evidence of metastatic disease, we intended to avoid surgery due to clinical aspects of the patient, so we started topical DPCP. After three months of treatment, the new biopsies revealed no residual disease and re-staging remains without metastatic disease. Our second case is a 64-year-old woman initially treated in 2011 as clinical stage III who attempted wide local excision and groin dissection. She presented with in transit recurrence one year later and we started topical DPCP. We also performed new biopsies after three months, but in one of the lesions there were evidence of residual melanoma. We continued DPCP and after six months there was no evidence neither of local disease nor metastatic disease. They both remain in DPCP treatment. **Conclusion:** There is a lack of evidence in the literature about the use of DPCP in melanoma. We were able to reproduce the good results in primary disease such as in recurrence. DPCP may play a role even in patients with bulky disease or without clinical conditions for surgery.

#19

TITLE: UNCOVERING VULVAR CANCER: INTEGRATED ANALYSIS OF GENOMIC AND TRANSCRIPTOMIC DATA

Authors: Lavorato-Rocha AM[1], Sato-Kuwabara Y[2], Marchi FA[3], Maia BM[1], Rodrigues IS[1], Fernandes GR[4], Baiocchi G[5], Soares FA[1], Rogatto SR[3], Rocha RM[1]

Authors' Information:
[1] Molecular Morphology Laboratory - A.C. Camargo Cancer Center, São Paulo Brazil.
[2] Department of Anatomic Pathology - A.C. Camargo Cancer Center, São Paulo Brazil.
[3] NeoGene Laboratory - A.C. Camargo Cancer Center, São Paulo Brazil.
[4] Catholic University of Brazilia - Brazilia, Brazil.
[5] Department of Gynecology Oncology - A.C. Camargo Cancer Center, São Paulo Brazil.

Corresponding Author:
André Mourão Lavorato-Rocha. E-mail: lavorato.andre@gmail.com
A.C.Camargo Cancer Center - Centro Internacional de Pesquisa e Ensino (CIPE)
Rua Taguá, nº 440, Liberdade. São Paulo - SP. Brazil. CEP: 01508-010
Sato-Kuwabara Y E-mail: yukiesato@gmail.com
Marchi FA E-mail: fmarchibr@hotmail.com
Maia BM E-mail: beatriz.melomaia@gmail.com
Rodrigues IS E-mail: iarabuzzo@hotmail.com
Fernandes GR E-mail: fernandes.gabriel@gmail.com
Baiocchi G E-mail: glbaiocchi@yahoo.com.br
Soares FA E-mail: fasoares@me.com
Rogatto SR E-mail: silvia.rogatto@gmail.com
Rocha RM E-mail: rafael.malagoli@gmail.com

Vulvar squamous cell carcinoma (VSCC) is a rare gynecological cancer, representing approximately 3-5% of genital tumors and 1% of all cancer occurrences in women. However, its incidence has risen considerably and no consistent profile of this disease has been established so far. Also, little is known about the genomic abnormalities of VSCC and how they correlate with gene expression. In order to verify that are the major genomic alterations that lead to gene expression abnormalities that may be linked to tumor progression, we used integrative analysis in VSCC

samples. Seventeen frozen samples of VSCC were used in genome-wide expression (GWE) profiling using the Agilent Whole Human Genome Microarray 60K and in Array-CGH (aCGH) using the Agilent Human Genome CGH Microarray 60K following the manufacturer's protocol. Data were extracted and flagged with Feature Extraction and the aCGH data was processed using NEXUS 6.0. JISTIC was used to classify genes mapped at gains and losses in genomic regions. Differentially expressed genes were identified by SAM statistical test. The CONEXIC algorithm was applied to integrate the data and *in silico* functional analysis were performed using Ingenuity Pathways Analysis (IPA). The aCGH analysis revealed 216 significant genomic alterations including 196 gains and 20 losses. GWE profile identified 3799 differentially expressed genes in comparison with normal tissue including 1352 up-regulated and 2447 down-regulated. The integrated analysis showed that genomic and transcriptomic results were concordant in 47 genes, in which 46 were up regulated and involved in gain of DNA copy number and only 1 gene was down-regulated and associated to genomic loss. According to IPA analysis, three significant networks could be defined comprising these genes. The first network comprised 18 of the concordant genes and was associated to functions as carbohydrate metabolism and cellular maintenance. The second network (18 genes) was related to carbohydrate metabolism, cellular maintenance and drug association. The third network (15 genes) was associated to post-transcriptional modification and hereditary disease. Thereby, important signaling networks were found disrupted by these genes which could influence tumor development or progression in VSCC. Therefore, these genes might be useful as a first step to identify molecular markers to improve diagnosis and therapeutic approaches for this tumor.

#23

TITLE: GENOME-WIDE PROFILE OF EPIGENETIC PATTERNS AND COPY NUMBER ALTERATIONS IN A GROUP OF WILMS TUMORS

Authors: Pramio D[1], Maschietto M[1], Costa SS[2], Silva AG[1], de Camargo B[3], Ferreira EN[1], Rosenberg C[2], Carraro DM[1], Krepischi ACV[1]

Authors' Information:

[1] CIPE - A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Institute of Biosciences, University of São Paulo, São Paulo, Brazil.
[3] INCA - National Institute of Cancer, Rio de Janeiro, Brazil.

Corresponding Author:

Pramio D. E-mail: dimibiomed@hotmail.com
Maschietto M. E-mail: marianamasc@gmail.com
Costa SS. E-mail: silvinha.costa@gmail.com
Silva AG. E-mail: amandagonc@yahoo.com
de Camargo B. E-mail: bdecamar@terra.com.br
Ferreira EN. E-mail: elisa.ferreira@accamargo.org.br
Rosenberg C. E-mail: carlarosenberg@uol.com.br
Carraro DM. E-mail: dirce.carraro@cipe.accamargo.org.br
Krepischi ACV. E-mail: ana.krepischi@gmail.com

Wilms tumor (WT) is the most common kidney malignancy of childhood; nevertheless, its pathogenesis remains largely unknown. Nowadays, WT is a cancer with effective treatment and good prognosis, but tumor relapse occurs at a rate of approximately 15%. Loss of heterozygosity of both 1p and 16q was already associated with an increased risk of relapse and death although detected in a very small subset of patients, making these genetic alterations less sensitive prognostic factors. Therefore, molecular studies are focused in identifying markers to define the minimal therapy while maintaining high survival rates. Somatic DNA copy number alterations (CNAs) are common genetic mutations in cancer, driving huge deviations in normal genic expression, and combined with epigenetic changes, often define key pathogenic events. This study was designed to assess the genome-wide profile of CNAs and DNA methylation in WTs, aiming to identify (epi)genetic markers that could be of clinical and prognostic importance. An array-CGH based survey was performed in a group of 48 WT samples, mainly tumors in stages III and IV, derived from patients with (17) and without (31) relapse in at least three years; CNA data was obtained using an 180K oligoarray platform (Agilent), with an average resolution > 70 Kb, and analysis was performed on the software Nexus 6 (Biodiscovery). Additionally, we delineated the methylomes of two WT using the 450K Infinium platform (Illumina) and the software GenomeStudio, comparing with the epigenomic pattern of healthy adult and embryonic human kidneys. The array-CGH analysis revealed high frequency of arm/whole chromosome alterations at 1q, 7q, and chromosomes 6 and 12 (gains), while losses were recurrent at 7p, 11q, and 16q chromosome regions. WT derived from patients with relapse exhibited a distinctive pattern of genomic alterations, mainly characterized for different frequencies of specific rearrangements. Analysis of small regions affected by recurrent focal alterations disclosed new genes that can be relevant for Wilms tumorigenesis and relapse. Moreover, a preliminary epigenomic landscape of Wilms tumors in relation to different stages of the kidney differentiation was attempted; this analysis will be expanded contributing to elucidate which molecular pathways lead to the impairment of normal kidney differentiation and subsequent oncogenic process.

Financial support: FAPESP/CNPq

#24

TITLE: MUTATIONAL STATUS OF VHL GENE AND CLINICAL SIGNIFICANCE IN RENAL CLEAR CELL CARCINOMA IN BRAZILIAN POPULATION

Authors: Alves MR[1], da Costa WH[2], da Silva FC[3], Carraro DM[3], da Cunha IW[1], Zequi SC[2], Guimarães GC[4], Soares FA[1], Rocha RM[1]

Authors' Information:

[1] Department of Anatomy Pathology - A.C. Camargo Cancer Center, São Paulo Brazil.

[2] Department of Urology - A.C. Camargo Cancer Center, São Paulo Brazil.

[3] Department of Molecular Biology - A.C. Camargo Cancer Center, São Paulo Brazil.

[4] Department of Pelvic Surgery - A.C. Camargo Cancer Center, São Paulo Brazil.

Corresponding Author:

Mariana Rezende Alves. E-mail: mariana.rezendealves@gmail.com

International Center of Research and Teaching. Department of Anatomy Pathology

Taguá, nº 440, Liberdade. São Paulo - SP. Brazil. CEP: 01509-900

Introduction: The most common subtype of renal cell carcinoma (RCC) is the clear cell type (ccRCC) accounting for 75% of cases. In this context, inactivation of VHL gene is thought to be a first event in clear-cell tumorigenic pathway. Thus, we aimed to provide useful predictive or prognostic information in patients with renal clear cell carcinoma cell type (ccRCC) through VHL mutational status. **Methods:** VHL protein expression was analyzed by immunohistochemistry in a TMA containing 148 samples and validated in 62 cases of RNA by qRT-PCR. The mutation profile was assessed in 91 cases by Sanger sequencing. **Results:** VHL was found mutated in 57.1% of cases, being missense mutations present in 28.8%, nonsense in 5.7%, intronic mutations in 15.3%, deletions in 44.2%, indels in 9.6%, duplication in 9.6% and insertion in 1.9% of cases. The prevalence of mutations by exon was: exon 1, 53.8%; exon 2, 30.7%; and exon 3, 15.3%. Concerning VHL protein expression, our data showed high frequency of positivity (78.9%) but no significance between protein expression, clinical data and survival were achieved. Importantly, 86.8% of strong positive cases represented 45 muted cases of the 91 samples evaluated by sequencing. **Conclusion:** Our study presents a panel of 32 novel mutations in VHL gene that can be found in Brazilian population. However, the clinical importance of these mutations remains obscure. Furthermore, the results are not concordant when comparing the presence of mutations and protein and mRNA expression, suggesting that positive pVHL immunostaining would not necessarily indicate a wild-type VHL status. However, by associating downstream proteins/pathways IHC evaluation, such as HIF or PBRM1, it might be possible to provide more insights concerning an overview of the whole biological pathway, leading to more consistent clues on prognosis of the patients.

#25

TITLE: A NOVEL METHOD FOR THE ESTABLISHMENT OF PRIMARY CULTURES OF CANCER STEM CELLS

Authors: Mambelli LI[1,2], Kerkis I[2], Hainaut P[3], Achatz MIW[1]

Authors' Information:

[1] Oncogenetics Department and International Center for Research and Training, A.C. Camargo Hospital.

[2] Laboratory of Genetics, Butantan Institute - São Paulo - SP. Brazil.

[3] International Prevention Research Institute, Lyon, France.

Corresponding Author:

Lisley Inata Mambelli. E-mail: lis_mambelli@yahoo.com.br

Departamento de Oncogenética - Fundação Antonio Prudente/A.C. Camargo Cancer Center; Laboratório de Genética - Instituto Butantan.

Mambelli L.I. E-mail: lis_mambelli@yahoo.com.br

Kerkis I. E-mail: ikerkis@butantan.gov.br

Hainaut P. E-mail: hainautpierre@aol.com

Achatz, M.I.W. E-mail: miachatz@gmail.com

Li-Fraumeni syndrome (LFS), an inherited cancer predisposition syndrome, is associated with germline mutations in TP53. It is characterized by high risk of multiple, early cancers. In Brazil, a variant form of LFS is exceedingly frequent due to a widespread founder TP53 mutation, p.R337H, detected in about 0.3% of the general population in Southern Brazil. This mutation occurs in p53 oligomerization domain and its effect on p53 oligomerization is supposed to be dependent upon pH conditions. Recent studies indicate that p53 plays a critical role in regulating differentiation and asymmetric divisions of Cancer Stem Cells (CSCs). The main objective of this study is to isolate and to characterize CSCs in patients with germline mutations in TP53 gene with Li-Fraumeni syndrome and Li-Fraumeni-like Syndrome. We have isolated and characterized CSCs from tumors of p.R337H mutation carriers. After informed consent, surgical resection fragments were dissociated and brought into culture. Adherent cells and spheroids were derived from different tumor types. Spheroids and stromal cells derived from

a breast cancer (BC) were further analyzed by immunofluorescence and flow cytometry to demonstrate positive immunolabeling for $CD44^+$, $CD24^-$, Oct4, Ki67 and Sox2 antibodies. Time-lapse videomicroscopy showed rapid growth, frequent asymmetric division and absence of senescent phenotypes for least 17 passages. Moreover, we showed by Colony Forming Units assay (CFU) that stromal cells are very clonogenic, once 10^3 cells initially plated were able to form 442 new colonies after 15 days in culture. It was also verified the differentiation potential of stromal cells for the following lineages: adipogenic, osteogenic and muscle-like cells. Adherent stromal cells, as well as oncospheres are able to self-renew and to proliferate *in vitro* for long periods without losing its main features. These properties are similar to stem cell possessing the p53 inactive. However, we observed that treatment of adherent cells with doxorubicin (DNA damaging agent) causes accumulation of p53 and causing induction of cell cycle arrest with low doses of this drug, and cell death/apoptosis in higher doses. Our findings using CSC-p.R337H can provide a very useful model for better understanding the role of specific mutation, as well as the TP53 tumor biology.

#26

TITLE: DOES BACTERIAL INFECTION AFFECT CANCER-SPECIFIC SURVIVAL OF PATIENTS WITH NON-METASTATIC COLORECTAL AND BREAST CANCER?

Authors: Attiê R[1], Chinen LTD[2], De Lima VCC[2]

Authors' Information:

[1] Department of Pharmacy - A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] Department of Medical Oncology - A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:

Attiê R. E-mail: regina.attie@hcancer.org.br
Chinen LTD. E-mail: ltdchinen@gmail.com
De Lima VCC. E-mail: vladcclima@gmail.com

Background: Inflammation has been linked to cancer since the nineteenth century and its study can help to develop better strategies for cancer prevention and treatment. Some infectious agents are recognized as major components of chronic inflammation associated with cancer. Some data suggest that infection may have a protective effect against cancer, and some infection agents can even be used in therapy, such as BCG (*Bacillus Calmette-Guérin*) in bladder cancer. **Objective:** This study aimed to verify the role of bacterial infections on cancer-specific survival of patients undergoing treatment or treated for colorectal cancer and breast cancer. **Methods:** Two retrospective cohorts were analyzed, encompassing non-metastatic colorectal and breast cancer patients hospitalized between January 2006, and April, 2010, due to suspected or confirmed bacterial infection, identified from the medical records and from the Hospital Infection Control Service (SCIH) registry. Survival curves were calculated using Kaplan-Meier method and the impact of clinical variables was estimated using log-rank test. Variables statistically associated with cancer-specific survival in univeriate analysis, were included in a Cox multivariate model. **Results:** From a total of 2,595 SCIH records, 214 elegible patients were identified and divided into two groups: Infected and control (non-infected). For patients with colorectal cancer, the higher the number of infections, the worse the cancer-specific survival. Neutrophil count was also associated with worse cancer-specific survival. Infection remained as an independent predictor of worse cancer-specific survival, along with clinical staging ($p = 0.01$). Regarding breast cancer, the only independent predictor of cancer-specific survival was surgery as an isolated tretament. **Conclusions:** The development of infection during or after cancer-specific treatment is associated with decreased cancer-specific survival in colorectal cancer patients.

REFERENCE:

1. Arnold IC, Hitzler I, Müller A. The immunomodulatory properties of helicobacter pylori confer protection against allergic and chronic inflammatory disorders. Front Cell Infect Microbiol 2012; 2:10.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001; 357: 539-45.
3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008; 454:436-44.

#27

TITLE: DENDRITIC CELL PHENOTYPE AND FUNCTION BEFORE AND AFTER ONCOLOGIC SURGERY: A CASE REPORT

Authors: Clavijo Salomón MA[1], Pizzo CRP[1], Barbuto JAM[1]

Authors' Information:

[1] Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

Corresponding Author:

Attiê R. E-mail: regina.attie@hcancer.org.br
Chinen LTD. E-mail: ltdchinen@gmail.com
De Lima VCC. E-mail: vladcclima@gmail.com

Introduction: Dendritic cells (DCs) are responsible to recognize cancer cells and orchestrate an immune response against them by priming lymphocytes, which will maintain that response throughout life. However, if a tumor is established, dendritic cells and lymphocytes are victims of scape mechanisms that allow tumor growth and dissemination. Although these scape mechanisms are well documented in the literature, dendritic cell and lymphocyte status after an oncologic surgery remain less studied. Purpose: Here, we report the phenotypic and functional changes of monocyte-derived dendritic cells (Mo-DCs) of a 34-year-old male before and after partial nephrectomy due to chromophobe renal cell carcinoma. **Methods:** Monocytes from the patient's peripheral blood, before and after partial nephrectomy, were differentiated into DCs by culture, in the presence of GM-CSF and IL-4 for seven days. To obtain mature Mo-DCs, TNF-α was added at day 5 and 48 hours later Mo-DCs were harvested, phenotyped and co-cultured with CFSE-labeled allogeneic T lymphocytes for an additional 5 days. Mo-DCs phenotype, lymphoproliferation and regulatory T cell induction was determined by flow cytometry (BD™). **Results:** After surgery, immature and mature Mo-Ds decreased frequency and expression of PDL-1 (from 63.2% to 38.2% and from 89.7% to 79.2%, respectively). In mature Mo-DCs, the expression of CD86, CD80, HLA-DR and CD205 was downregulated and the expression of CCR7, CD11c and CD209 was upregulated. On the other hand, after surgery, mature Mo-DCs capacity to stimulate T cell proliferation increased (from 16.6% to 36.6%) along with the expression of IL-2R (from 57% to 96%) and with the improvement of the CD4: CD8 ratio (from 54%: 45% to 81%: 19%). Finally, Mo-DCs capacity to induce regulatory T cells decreased after surgery (from 16.2% to 3.8%). One-year postoperative follow-up shows no tumor recurrence. **Conclusion:** Together, this data shows that the presence of a tumor affects Mo-DCs but after tumor removal there is a reestablishment of its phenotype and function. These findings are clinically relevant since functional dendritic cells could play an important role in preventing recurrence or metastasis, once the tumor mass is removed.

Supported by: FAPESP #2009/54599-5, 2011/05331-0 and 2012/23478-0.

REFERENCES

1. Steinman R et al. Taking dendritic cells into medicine. Nature 2007;27;449(7161):419-426.
2. Banchereau J et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767-811.
3. Pinzon-Charry A et al. Dendritic cell dysfunction in cancer: A mechanism for immunosuppression. Immunol Cell Biol 2005;83:451-461.

#28

TITLE: ANALYSIS OF PTEN/AKT IN THE DEVELOPMENT OF HUMAN ORAL TISSUE AND ORAL SQUAMOUS CELL CARCINOMA: ROLE IN MALIGNANT TRANSFORMATION

Authors: Buim MEC[1], Lourenço SV[1,2], Soares FA[1,2]

Authors' Information:
[1] Department of Anatomy Pathology, Hospital A.C. Camargo, São Paulo.
[2] Department of General Pathology, Dental School, University of São Paulo, Brazil.

Corresponding Author:
Buim MEC. E-mail: mebuim@hotmail.com
Lourenço SV. E-mail: silvialourenco.70@terra.com.br
Soares FA. E-mail: soaresfernando@terra.com.br

Oral squamous cell carcinoma (OSCC) is a prevalent public health problem and is characterized by high degree of local aggression and lymph node metastasis. Signaling through the PTEN/AKT pathway is responsible for balancing survival and apoptosis cell. The goal of this study was to analyze the association of the PTEN and AKT expression with clinicopathological feature of the OSCC patients. **Methods:** Expression of PTEN and AKT gene/protein was investigated in 68 cases of OSCC, obtained from the files of Anatomic Pathology Department from A.C. Camargo Cancer Center (Brazil), by qRT-PCR and IHC. **Results:** Low PTEN gene and protein expression occurred in 66% and 63%, respectively, OSCC cases. However, no association was observed between this molecule and clinicopathological parameters. High phosphorylate AKT (pAKT) protein expression was associated with poor/moderated differentiated histological grade (p = 0.029); no association was observed between pAkt protein expression and other clinicopathological parameters. AKT gene expression, by qRT-PCR, was associated with clinical stage (p = 0.034), perineural infiltration (p = 0.014) and tumor size (p = 0.037). Conclusion: Our results suggest that downregulation of PTEN can be important to OSCC tumorigenesis and can contribute to oncogene activation, and high pAKT expression might be an unfavorable prognostic marker in OSCC.

#29

TITLE: ROLE OF REACTIVE OXYGEN SPECIES IN CARCINOGENESIS-RELATED EVENTS IN BREAST CANCER CELL LINES

Authors: Hecht F[1,2], Leitão AAC[2], Carvalho DP[1], Fortunato RS[2]

Authors' Information:
[1] Laboratório de Fisiologia Endócrina Doris Rosenthal, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
[2] Laboratório de Radiobiologia Molecular, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

Corresponding Author:
Hecht, Fabio. E-mail: hecht@ufrj.br
Leitão, Álvaro Augusto Costa. E-mail: acleitao@biof.ufrj.br
Carvalho, Denise Pires. E-mail: dencarv@biof.ufrj.br
Fortunato, Rodrigo Soares. E-mail: rsfortunato@yahoo.com.br

Context: Breast cancer is the second most common type of cancer worldwide and the most common among women. Several factors are associated with an increased susceptibility to the development of this pathology. Several phenomena may explain the relationship between these risk factors and the process of carcinogenesis. The most remarkable being oxidative stress, which is characterized by a disturbance in redox balance of the cell, leading to oxidation of cell components such as proteins, lipids and DNA. Reactive oxygen species (ROS) are derived from several sources, such as the electron transport chain, cytochrome P450 enzymes and enzymes whose only function is the production of ROS, the NAPDH Oxidases (NOX). The relationship between oxidative stress and cancer has begun to be elucidated in the past decade and its influence on cellular events related to the success of tumor progression, such as proliferation, migration, apoptosis, angiogenesis and cell invasion, have been experimentally demonstrated. **Objectives:** Since a relationship between oxidative stress and carcinogenesis has been already suggested in several other tissues, the aim of the study was to demonstrate a possible role of NAPDH Oxidase-derived ROS on cellular events related to mammary carcinogenesis through an *in vitro* model. **Methodology:** Breast tumor cell lines (MCF-7 and MDA-MB-231) and a non-tumor cell line (MCF-10A) were treated with a specific inhibitor of NAPDH Oxidases (VAS2870) and parameters related to tumor initiation and progression will be evaluated, such as intracellular and extracellular ROS production, cell proliferation, cell viability and cell migration. **Results:** In regard to ROS production, increased extracellular enzymatic activity was found in tumor cells MCF7 and MDA-MB-231, when compared to the non-tumoral cell line MCF10A. The intracellular ROS production was significantly higher in the cell line MDA-MB-231 compared to MCF10, however, was decreased in the MCF7 cells. Inhibition of NOXs showed a dose-dependent inhibitory effect on cell viability and proliferation. The qualitative migration assay (wound-healing) revealed that the inhibition of NOXs decreases MDA-MB-231's cell migration. **Conclusion:** Based on the preliminary results, we suggest that ROS exert an important role in both the initiation and tumor progression by modulating cellular mechanisms known to be related to the carcinogenesis process, such as ROS production, proliferation and migration.

#30

TITLE: THE OVER-EXPRESSION OF A SINGLE ONCOGENE ALTERS THE PROTEOMIC LANDSCAPE OF MICROPARTICLES

Authors: Amorim MG[1,2], Fernandes GR[1], Martins-de-Souza D[3], Nunes DN[1], Dias-Neto E[1]

Authors' Information:
[1] Laboratory of Medical Genomics, A.C. Camargo Cancer Center - São Paulo - SP, Brazil.
[2] Curso de Pós-graduação em Oncologia, Fundação Antônio Prudente - São Paulo - SP, Brazil.
[3] Max Planck Inst. & Ludwig-Maximilians-Universität - Munich, Germany.

Introduction: Content differences may confer distinct biological functions to heterogeneous microparticle (MP) populations. The over-expression of one oncogene dramatically alters cell phenotype, but it is unknown whether it affects MP-content. **Materials and Methods:** MPs were isolated from conditioned medium of HB4a, a human mammary luminal epithelial cell line, and C5.2, a transformed HB4a clone over-expressing HER2, by differential sedimentation at 20 Kg (20k) and 100 Kg (100k). MPs were characterized by transmission electron microscopy and proteomic analysis. SDS-PAGE was used for protein prefractionation (cells only) prior to nanoflow liquid chromatography mass spectrometry (nLC-MS/MS) and label-free (MPs) or isotope-coded protein labeling (cells) methods were used for proteome analysis. Experiments were performed in duplicates and only proteins identified by at least two peptides with 95% confidence were considered. **Results:** Proteomic data revealed high HER2 levels in C5.2 cells (> 400x) and in both C5.2-derived MPs, and allowed the quantitation of 589 (20k), 421 (100k), and 1468 (cells) proteins. 122 proteins were exclusively in 20k MPs, and 60 proteins were exclusively in 100k MPs. HER2 over-expression resulted in the upregulation of 17 and 13% and the downregulation of 13 and 7% of the proteins (20k and 100k MPs respectively). A high level of protein heterogeneity was seen between the MP subsets. Only 2/102 and 14/138 proteins increased in HB4- and C5.2-MPs respectively were seen in both MP subsets. Proteins capable of inducing malignant transformation were found in both MP subsets

including two proteins involved in cell motility and invasion, cofilin and CD44, both increased > 90x. **Conclusions:** MP subsets differentially shuttle many proteins, suggesting that their content is not arbitrarily defined and probably reflects distinct functions. HER2-upregulated proteins in MPs may drive cellular malignancy and are potential biomarkers for HER2+ cancer patients.

Financial Support: FAPESP.

#31

TITLE: STEM-CELL MARKERS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA AND THE INVOLVEMENT OF MIRNAS IN THEIR REGULATION

Authors: Bárbara de Lima Brito[1], Prudente FP[1], Lourenço SV[2], Ianez RF[1], de Sousa EA[1], Silva MMC[1], Kowalski LP[3], Soares FA[1,2], Coutinho-Camillo CM[1]

Authors' Information:

[1] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.

[3] Department of Otorhinolaryngology and Head and Neck Surgery, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:

Bárbara de Lima Brito (apresentador). E-mail: barbaralj@ig.com.br
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil
Prudente, FP. E-mail: fpprudente@gmail.com
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
Lourenço, SV. E-mail: silvialourenco@usp.br
Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.
Ianez, RF. E-mail: rere_fraga@hotmail.com
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
de Sousa, EA. E-mail: elenpkn@yahoo.com.br
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
Silva, M M C. E-mail: marlonfilme@hotmail.com
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
Kowalski, LP. E-mail: lp_kowalski@uol.com.br
Department of Otorhinolaryngology and Head and Neck Surgery, A.C. Camargo Cancer Center, São Paulo, Brazil.
Soares, FA. E-mail: soaresfernando@terra.com.br
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo and Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.
Coutinho-Camillo, CM. E-mail: claumcc@terra.com.br
Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Background: Recent studies have shown that the ability to initiate tumors may be due to the presence of tumor stem-cells (CSCs) which exhibit characteristics of both stem-cells and cancer cells. Head and neck squamous cell carcinoma (HNSCC) is the fifth most common tumor worldwide and is histologically heterogeneous, consisting of different types of cells, including tumor cells, stromal cells, inflammatory cells and some studies have demonstrated the presence of stem-cell markers in these tumors. MicroRNAs (miRNAs) are approximately 22 nucleotide non-coding RNA molecules that regulate gene expression post-transcriptionally and many miRNAs have been described as regulators of stem-cells in different types of cancer, controlling their self-renewal and differentiation. **Objectives:** To investigate the presence of stem-cell markers in head and neck squamous cell carcinoma (HNSCC) and associate the results with the expression of stem-cell regulator miRNAs. **Material and Methods:** The immunoexpression of integrin-β1, CD24, CD44, ALDH1 and CD133 proteins was analyzed in 35 HNSCC patients and the results were semi-quantitatively analyzed using a conventional optical microscope, considering the patterns and intensity of staining. The expression of let-7a, miR-34, miR-125b, miR-138, miR-145, miR-183, miR-200b, miR-203, miR-205, miR-302a was evaluated by Real Time RT-PCR. **Results:** Five out of 35 cases of HNSCC (14.3%) showed positive cytoplasmic staining of ALDH1; integrin-β1 was expressed in 32 out of 33 cases (97.0%); 24 out of 35 cases (68.6%) showed positive membranous staining of CD44; CD24 was expressed in 30 out of 34 cases (88.2%) and 2 out of 35 cases (5.7%) showed positive membranous staining of CD133. The expression of stem-cell regulator miRNAs is being performed by using TaqMan microRNA Assays. The expression of RNU44 and RNU48 was used as an endogenous control. **Conclusions:** Our study provides the evidence of the expression of putative stem-cell markers in head and neck squamous cell carcinoma and the involvement of miRNAs in the regulation of these markers is under investigation.

Financial Support: CNPq 475449/2011-8 and CAPES.

#32

TITLE: SPLICING VARIANT OF TRIM37 AS A POTENTIAL MOLECULAR MARKER FOR BREAST CANCER

Authors: Tatiana Iervolino Ricca[1], Maria Cristina Rangel[1], Elisa Napolitano e Ferreira[1], Elen Pereira Bastos[1], Laura Tojeiro Campos[1], Luiz Paulo Camargo[1], Adriane Mary Pimentel[1], Maria do Socorro Maciel[1], João Gonçalvez Filho[1], Helena Paula Brentani[1], Sandro José de Souza[2], Dirce Maria Carraro[1]

Authors' Information:
[1] Laboratory of Genomics and Molecular Biology - CIPE/A.C. Camargo Cancer Center, São Paulo, SP, Brazil.
[2] Ludwig Institute for Cancer Research, São Paulo - SP. Brazil.

There has been evidence showing that alternative splicing occurs in at least 95% of human genes increasing the proteomic diversity, since 80% of these events occur within the coding region. Some splicing variants are preferentially expressed in human tumors and can be considered as potential molecular markers for more accurate diagnostic and prognostic factors, as well as therapeutic targets. The aim of this study was to identify overexpressed splicing variants in breast cancer and test them as molecular markers. For that, 270 exons previously identified as overexpressed in tumor tissues by *in silico* analysis were selected, immobilized onto nylon membranes and interrogated by 31 tumor and 11 normal breast samples. This analysis showed 14 exons over expressed in breast cancer and 3 of them (exon 23, 5 and 5 in TRIM37, MK-STYX and BRRN1 genes, respectively) fulfilled stringent criteria through quantitative RT-PCR validation, using the initial set of 31 breast cancer samples and an independent set of 40 additional samples. Correlation of transcripts expression with clinical and histopathological data reported positive associations only between the expression of the TRIM37 splice variant containing exon 23 and the presence of estrogen and progesterone receptors, as well as the absence of p53 mutation. These associations were not observed when the expression of TRIM37 variant skipping the same exon was analyzed, indicating that they are related to the presence of the exon 23. Additionally, when the total protein content of tumor and normal breast cell lines were assessed using anti-TRIM37 antibody, a remarkable imbalance was observed in expression level of the protein product from TRIM37 exon 23 inclusion variant. Altogether, these results suggest that this particular isoform may be functionally important in the context of breast cancer. In this regard, functional analyses is going to be performed using breast cancer cell lines overexpressing either TRIM37 gene containing or skipping exon 23, and cell lines silenced either for exon 23 and a constitutive exon of the TRIM37 gene.

#33
TITLE: CIRCULATING TUMOR CELLS AS AN INDICATIVE OF EARLY RECURRENCE IN OVARIAN CANCER: A CASE REPORT

Authors: Rocha BMM[1],Sanches SM[1], Baiocchi G[2], Campanha D[1], Aguiar CM[1], Fanelli MF[1], Soares FA[3], Chinen LTD[1]

Authors' Information:
[1] Department of Clinical Oncology, A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Department of Gynecology, A.C. Camargo Cancer Center, São Paulo, Brazil.
[3] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:
Rocha, BMM. E-mail: bruna.malagoli@hotmail.com
Sanches, SM. E-mail: solange.sanches797@gmail.com
Baiocchi, G. E-mail: glbaiocchi@yahoo.com.br
Campanha, D. E-mail: dancanbr@yahoo.com.br
Aguiar, CM. E-mail: carol_motta_aguiar@hotmail.com
Fanelli, MF. E-mail: mffanelli@gmail.com
Soares, FA. E-mail: fasoares@me.com
Chinen, LTD. E-mail: ludmillatdchinen@hotmail.com

Background: Ovarian cancer is the tenth leading cause of cancer death worldwide. The epithelial ovarian cancer is usually diagnosed at advanced stages of disease, and despite its primary responsiveness to therapy, it has a high recurrence rate due to selection/acquisition of chemoresistance of residual cells. It is believed that the spread of cancer requires circulating tumor cells (CTCs) and that these can be tumor stem cells (CSC). **Objective:** Correlate CTCs and their molecules expression with clinical outcomes. **Methods:** CTC counting and characterization were performed using cytokeratin-dependent immunomagnetic separation (Miltenyi) and ISET (isolation by size of tumor cells; Rarecells). **Results:** This is a case report of a 60-year-old Brazilian woman, diagnosed in 2006, clinical stage III and submitted to optimal cytoreduction. She made 8 cycles of adjuvant chemotherapy (carboplatin/paclitaxel), but had recurrence diagnosed in June 2007, with peritoneal and capsular liver implants and retroperitoneal lymphadenopathy. She was submitted to secondary cytoreduction with intraperitoneal hyperthermic chemotherapy with cisplatin/mitomycin and remained well until April 2011, when an exophytic lesion in spleen was detected. A splenectomy was performed (March 2012) and showed a recurrence from primary ovarian cancer with no evidence of other sites of recurrence. In April 2012, the first blood sample collected showed two CTCs. The patient was maintained at follow-up with no clinical evidence of recurrence on imaging scans. In June 2012, she developed abdominal pain and distension, suggesting intestinal obstruction which was resolved with clinical support. Considering the hypothesis of cancer recurrence, a new blood sample was collected,

in order to search CTCs and to characterize them. More than 50 CTCs were found, confirmed in another sample collected some weeks later. By immunocytochemistry of her ISET membrane, a strong double-staining for CD44/c-Kit, marker of ovarian CSC (Foster R et al., 2012) was observed. There was no expression of MRP-5 (multidrug resistance associated protein 5) and CK7. **Conclusion:** The patient's clinical history strongly suggests that current symptoms are due to a recurrence of ovarian cancer, but until now, there is no image documentation that can confirm this. She remains in follow-up with imaging and CTCs counting being performed until confirmation of a relationship between CTCs counts and early diagnosis of recurrence.

REFERENCES

1. Foster R et al. Ovarian cancer stem cells: working towards the root of stemness. **C**ancer Lett 2012.

#35
TITLE: FUNCTIONAL ANALYSIS OF NOVEL MISSENSE VARIANTS FOUND IN LYNCH SYNDROME PATIENTS

Authors: Ferreira JRO[1], Silva FCC[1], Nakahata AM[1], Elias EV[1], Santos EMM[1], Carvalho AF[1], Carraro DM[1]

Authors' Information:
[1]International Center of Research and Teaching - CIPE - A.C. Camargo Cancer Center, São Paulo - SP. Brazil.

Corresponding Author:
Dirce Maria Carraro. E-mail: direce.carraro@accamargo.org.br

Background: Lynch syndrome (LS) is the most common form of hereditary colorectal cancer and is associated with germline mutations in one of several mismatch repair (MMR) genes, mainly MLH1 and MSH2. Most MMR truncated mutations are known to cause LS, however, a significant proportion of mutations are single amino acid substitutions that do not necessarily result in a dysfunctional protein. Functional testing of MMR protein variants is of great importance to distinguish pathogenic relevant mutations from non--pathogenic polymorphisms. The aim of this study is to evaluate functionally three novel variants in MLH1 and MSH2 genes found in previous work by our group (Valentin et al., 2011) **Methods:** The full-length cDNA for both genes were cloned in pcDNA3.1 His A and the alterations were constructed by site-directed mutagenesis. In addition, we used two computational algorithms (SIFT and POLYPHEN-2) for pathogenicity prediction. For functional assays, the colon cancer cell lines SW-480 (MLH1 (+/+), MSH2 (+/+)) was used as positive control, and the HCT-116 (MLH1 (-/-)) and LoVo (MSH2 (-/-)) were used for transfection. **Results:** To investigate the functional consequences of the amino acid substitutions, we first mapped the alterations in the protein domains of each correspondent gene. In MLH1, the variant c.2027 (T > C) was located in the interaction domain with PMS2/MLH3/PMS1 and in the MSH2 variants are located in ATPase (c.2187 (G > T)) and DNA binding (c.23 (C > A) domains. The computational algorithms classified the MLH1 variant c.2027 (T > C) and the MSH2 variant c.2187 (G > T) as pathogenic, while c.23(C > T) variant was classified as non-pathogenic. In order to assess the presence of these variants in a control population, we screened 96 DNA samples from healthy subjects with no family history of cancer. The MLH1 variant T8M (c.23C > T) was found in two healthy individuals, revealing that this probably a neutral variant and has no effect on protein function as predicted by in silico analysis. We expressed the wild type protein hMLH1 (pcDNA3.1 MLH1-wt) and hMSH2 (pc DNA3.1 MSH2-wt) along with the site-directed mutants for MLH1 (pc DNA3.1 MLH1-L676P) and MSH2 (pc DNA3.1 MSH2-M729I) in a transient transfection system. The expression levels for recombinant proteins were examined by western blot analysis with anti-MLH1 and anti-MSH2 antibodies. We will perform assays to investigate the protein-protein interaction, cellular localization and mismatch repair activity.

Financial Support: CNPq and FAPESP

REFERENCES

1. Valentin MD, da Silva FC, dos Santos EM, Lisboa BG, de Oliveira LP, Ferreira Fde O, Gomy I, Nakagawa WT, Aguiar Junior S, Redal M, Vaccaro C, Valle AD, Sarroca C, Carraro DM, Rossi BM. Characterization of germline mutations of MLH1 and MSH2 in unrelated south American suspected Lynch syndrome individuals. Fam Cancer. 2011;10:641-7.60:12.

#36
TITLE: BREAST CANCER DNA COPY NUMBER ALTERATIONS PROFILING ACCORDING TO HISTOLOGIC GRADE AND VASCULAR INVASION STATUS

Authors: Felipe Fidalgo[1], Tatiane Rodrigues[2], Érica Araújo[1], Dirce M Carraro[1], Carla Rosenberg[2], Victor Piana Andrade[3], Ana C V Krepischi[1]

Authors' Information:
[1] CIPE, A.C. Camargo Cancer Hospital, Brazil.
[2] Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil.
[3] Department of Surgical and Investigative Pathology, A.C. Camargo Cancer Hospital, Brazil.

Background: Breast cancer is an important cause of cancer-related death in women, and invasive ductal carcinomas of non-special type (IDC-NST) correspond to 80% of all invasive breast tumors; in spite of significant advances in diagnostic and treatment, several major unresolved clinical and scientific problems remain. The vascular invasion status and the histologic grade of breast tumors are clinical parameters of high prognostic value; both are features indicating the level of tumor aggressiveness by morphological criteria. To the best of our knowledge, there are only a few studies that delineated the profile of genomic coy number alterations (CNAs) associated with specific breast cancer grades; none of them investigated the molecular profile related to the presence or not of vascular invasion. **Material and methods:** A group of 57 breast IDC-NST was studied evaluating the CNA pattern according to different histologic grades and presence of vascular invasion. The comparative genomic hybridization based on microarrays (array-CGH) was performed in a whole-genome 60K platform (Agilent Technologies). Selected CNAs were validated using quantitative RT-PCR. The Nexus Copy Number 6.0 software (BioDiscovery) was used for CNA calling. Minimum common regions of recurrent aberrations were obtained by implementing the global frequency statistical approach of the STAC method; the Mann-Whitney test was used to compare two unpaired groups, and the Kruskall-Wallis to compare three unpaired groups through the software GraphPad PRISMA5. **Results:** Using the STAC analysis, histologic grade III breast tumors exhibited mainly gains at 7p22, 8q22, 8q24, and losses at 9p21, and 5q11-q23; the most significant CNA event associated with grades I and II tumors was the 16p gain. The same approach identified 16p gain, and losses at 9p, 13, 16q in tumors negative for vascular invasion. In the group with vascular invasion, losses at 11q, 17q12, 18q and 20q were detected; more importantly, a peak of 11q24 loss, harboring only two genes, was strongly associated with the presence of vascular invasion. We also inspected the frequency of six well-characterized breast cancer amplicons (19q12 (CCNE1), 8q24 (MYC), 11q13 (CCND1), 8p12 (FGFR1), 8p12 (ZNF703), and 20q13.2 (ZNF217) in the different tumor groups; we found a higher percentage of some of these driver rearrangements among high-grade tumors 19q12, 8q24, and 8p12; in the group positive for vascular invasion 20q13.2, 19q12, and 8q24. Additionally, we validated by qRT-PCR three new focal high amplitude rearrangements that were detected in breast tumors grade III with vascular invasion: 4q13.3 (ADAMTS3 gene), 12p12.3 (RERGL gene), and 11p11.2 (HSD17B12 gene) **Conclusion:** The recurrence of rearrangements in the cancer genome can provide clues about relevant genes influencing aggressiveness and progression. In this study, we investigated the genome of 57 breast IDC-NST and specific genomic regions were associated with the histologic grade III group and with tumors positive for vascular invasion, indicating genes possibly related to these features. In addition, breast cancer driver genes were found at higher frequencies in both high-grade and positive for vascular invasion tumors, suggesting that a panel of bonafide cancer rearrangements could indicate the tumor aggressiveness level, and be of therapeutic and prognostic value. Furthermore, the analysis disclosed focal rearrangements harboring genes potentially associated with tumor aggressiveness and/or vascular invasion, such as ADAMTS3, RERGL, and HSD17B12.

Financial support: FAPESP and CNPq.

#37

TITLE: IDENTIFICATION AND FUNCTIONAL VALIDATION OF NOVEL WILMS TUMOR POSSIBLY ASSOCIATED MUTATIONS

Authors: Bruna D Barros[1], Giovana T Torrezan[1], Elisa N Ferreira[1], Mariana Maschietto[2], Ana C V Krepischi[1], Dirce M Carraro[1]

Authors' Information:

[1] CIPE - International Center of Research and Training - A. C. Camargo Cancer Center, São Paulo - SP. Brazil.

[2] MHCB Unit, UCL Institute of Child Health.

Corresponding author:

Dirce Maria Carraro - dirce.carraro@accamargo.org.br

Background: Identification of molecular alterations that triggers Wilms Tumor (WT) onset is crucial to improve diagnosis and prognosis. It is estimated that WTX, WT1 and CTNNB1 alterations accounts for 30% of WTs, however, most of the cases remain without an identified driver mutation. Results from a previous study of our group pointed APC and PLCG2 as candidate genes possibly altered in WTs1. Thus, the aim of the project was to identify genomic alterations in the complete sequence of APC, CTNNB1, WT1, WTX and PLCG2 genes by target massive parallel sequencing, to define the mutational spectrum of the genes and evaluate the intronic nucleotide substitution pattern. **Material and Methods:** Complete genomic regions of the genes (430Kb) were amplified by long range PCR in 15 WTs and 3 controls, giving a total of 60 amplicons per sample that were mixed, barcoded and sequenced on an Ion PGM™ Sequencer. Point mutation and indels not present in controls were validated by capillary sequencing. Validated alterations were

screened in an independent group of 39 WTs and 96 controls. Missense mutations identified in the study will be assessed for their functional impact by *in vitro* assays carried on tumor cell lines (HEK293) transfected with the mutated plasmids obtained by site-directed mutagenesis. **Results:** For the 15 tumors and 3 normal controls we obtained 400,000 sequences per sample in average. At exonic region, 4 out of the 4 identified missense alterations were validated. Three of them were not identified in the 99 controls being classified as possibly associated with WTs: 2 at APC (Ile2541Val, Met1413Val) and one at PLCG2 (Asn946Ser). At intronic region, tumor's substitutions were classified in two groups: SNPs (alterations present in controls or SNPs databases) and somatic substitutions (remaining alterations). Tumors presented an over-representation of G:C > A:T changes ($p > 0.001$) and a reduction of A:T > G:C changes ($p > 0.001$) when compared to the SNPs variants. Mutated plasmids for the three APC and PLCG2 variants were already produced and functional assays are ongoing **Conclusions:** This study provides insights into the spectrum of WT substitution mutations occurring in exonic and intronic regions of the five associated genes, revealing a low frequency of point mutations in the coding sequencing of these genes and an over-representation of somatic G:C > A:T transitions. The impact of the identified mutations is going to be further investigated by *in vitro* assays.

REFERENCES

1. Maschietto M et al. Molecular profiling of isolated histological components of Wilms tumor implicates a common role for Wnt signaling pathways in the kidney development and in the tumor. Oncology 2008,75:81-91.

#38
TITLE: A METAGENOMIC SURVEY OF BACTERIAL POPULATIONS IN HUMAN ORAL CAVITY CANCER

Authors: Thomas AM[1], Gomez LFB[1,2], Puga RD[1], Lima LA[1], Rossi L[4], Amorim MG[1], Francisco ALN[5], Mendes G[3], Carvalho MB[4], Kowalski LP[5], Nunes DN[1], Dias-Neto E[1,6]

Authors' Information:

[1] Laboratory of Medical Genomics, International Research Center, A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Universidad de Los Andes, Bogota, Colombia.
[3] Life Technologies, São Paulo, Brazil.
[4] Hospital Heliópolis, São Paulo, Brazil.
[5] Head and Neck and Ortohinolaryngology Department, A.C. Camargo Hospital, São Paulo, Brazil.
[6] Laboratory of Neurosciences (LIM-27), Inst. Psychiatry, HC-FMUSP, São Paulo, Brazil.

Corresponding author:
Thomas AM. E-mail: andrewmaltezthomas@hotmail.com

Background: Alcohol and tobacco are the two most important external agents that cause oral squamous cell carcinomas (OSCC). Another exogenous element that may have an important role in this scenario is the oral cavity's microbiota, which can generate high amounts of carcinogenic agents, such as acetaldehyde. In this study, the oral cavity's bacterial microbiota composition was evaluated using metagenomic large-scale sequencing, a method that does not rely on *in vivo* growth or cultivation of bacteria (a relevant aspect since most of the human microbiota is not cultivable) and allows their identification and quantification after *in silico* analysis. **Methods:** In order to evaluate the bacterial populations present in the biofilms of patients with OSCC, three groups were studied: 1) 10 individuals without OSCC that do not smoke nor drink; 2) 5 individuals without OSCC that smoke and drink on a daily basis; 3) 11 individuals that have OSCC and use these two drugs. To do so, the polymorphic region V1 of the 16S-rDNA gene was amplified by PCR, the resulting amplicons were barcoded with specific sequences for each individual and sequenced on the Ion Torrent™ platform (Life Technologies). The resulting sequences were then compared to those present in public databases, such as the Ribosomal Database Project. **Results:** A total of 2,332,475 sequences were obtained with an average amplicon size of 113 nucleotides and suggest a higher bacterial diversity in patients with OSCC when compared to the group without cancer, and the group without cancer that smoke and drank. The higher bacterial diversity observed in patients with oral cancer was further explored by separating the samples into small tumors (T1-T2) and large tumors (T3-T4), which indicated the later had a more diverse microbiome. The most abundant phyla found were *Firmicutes* (35.7%), *Actinobacteria* (31.2%), *Proteobacteria* (29.4%), *Bacteroidetes* (3.3%) and *Fusobacteria* (0.1%). **Conclusions:** The results obtained in this study should help understand the bacterial composition of patients with oral cancer, a first step in evaluating the role that common carcinogens play on the microbiota and oral cavity cancer.

Keywords: metagenomics, next generation sequencing, oral cancer

Financial support: CAPES, CNPq

 # 39

TITLE: AN INTEGRATED APPROACH TO IDENTIFY CANDIDATE DRIVER REGIONS OF HEPATOBLASTOMA

Authors: TC Rodrigues[1], F Fidalgo[2], EF Napolitano[2], I Werneck[3], CML Costa[3], DM Carraro[2,3], ACV Krepischi[2,3], C Rosenberg[1]

Authors' Information:

[1] Institute of Biosciences, University of São Paulo, Brazil.
[2] CIPE, A.C. Camargo Hospital, São Paulo, Brazil.
[3] National Institute of Science and Technology, São Paulo, Brazil.

Corresponding author:

Tatiane Cristina Rodrigues. E-mail: tatiane.cr@hotmail.com

Hepatoblastoma (HB) is the most common liver cancer in children and adolescents. Molecular data about HB are still scarce and remain clinically inconclusive. In the present study, we describe the patterns of somatic copy number alteration (SCNA) associated with DNA methylation and gene expression data in HB investigated by high resolution approach. We performed array-CGH in a whole-genome 180K platform, applying Genomic Workbench 6.9 software (Agilent) for calling genomic imbalances. Cytogenetic characterization of HB revealed a hallmark pattern: the occurrence of whole chromosome or large chromosome aneuploid segments. The global profile of SCNAs showed only a few alterations in each sample, which indicates that HBs have less genetic instability than most solid tumors. Trisomy for chromosomes 2 and 20, recurrent aberrations in HB, are simultaneously present in one sample. Another tumor has a small high gain region at 2q adjacent to a small deletion, narrowing an area known to be genetically relevant in HB. This amplicon includes less than 60 genes that are found as gained in 3 other tumors of our casuistic. Gains in mosaic are found at 1q, 3p and X, and losses at 22q, including a known cancer susceptibility gene not yet related to HB. Integration of the methylation and gene expression data for each gene located in the 2q amplicon allowed narrowing the list of relevant genes for HB. For the evaluation of the DNA methylation patterns we have performed experiments at the 450K BeadArray platform (Illumina) according to the manufacture's protocol and used Genome Studio software and IMA computational package for analysis. Additionally, we selected 90 genes for expression analysis, combining genes affected by recurrent copy number changes in our study with others already related to HB by previous studies, using the SYBR-green based customized array RT^2 qPCR Primer Customized Assay (Qiagen Technologies) following manufacturer's instructions. This integrated approach will point to the strongest candidates genes that to participate as drivers in the oncogenic process in HB.

Financial support: FAPESP.

#40

TITLE: APOPTOSIS IN HUMAN SALIVARY GLAND: EXPRESSION OF SURVIVIN AND CASPASE-3 IN PLEOMORPHIC ADENOMA AND MUCOEPIDERMOID CARCINOMA

Authors: Victoria Saavedra[1], Lourenço SV[2], Ianez RF[1], Nagai MA[3], Kowalski LP[4], Soares FA[1,2], Coutinho-Camillo CM[1]

Authors' Information:

[1] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.
[3] Discipline of Oncology, Department of Radiology and Oncology, Medical School, University of São Paulo - SP, Brazil.
[4] Department of Otorhinolaryngology and Head and Neck Surgery, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding author:

Victoria Saavedra (apresentador). E-mail: vick-saave@hotmail.com
Lourenço, SV. E-mail: silvialourenco@usp.br
Ianez, RF. E-mail: rere_fraga@hotmail.com
Nagai, MA. E-mail: nagai@usp.br
Kowalski, LP. E-mail: lp_kowalski@uol.com.br
Soares, FA. E-mail: soaresfernando@terra.com.br
Coutinho-Camillo CM. E-mail: claumcc@terra.com.br

Background: Pleomorphic adenoma is the most common benign tumor of major salivary glands. It shows a marked histological diversity with epithelial, myoepithelial, and mesenchymal components in a variety of patterns. Mucoepidermoid carcinoma is the most common malignant, locally invasive tumor of the salivary glands, especially of the parotid gland. Apoptosis is a genetically programmed form of cell death and aberrations of the apoptotic mechanisms that cause excessive or deficient programmed cell death have been linked to a wide array of pathologic conditions. **Objectives:** To characterize the expression of survivin and caspase-3 proteins and determine possible associations between the expression of these proteins and clinicopathologic features of the tumors. **Material and Methods:** Fifty-five cases of mucoepidermoid carcinoma and 50 cases of pleomorphic adenoma were analyzed by immunohistochemistry

and the results were semi-quantitatively analyzed, considering the patterns and intensity of staining. **Results:** Thirty-six out of 50 cases of pleomorphic adenoma (72.0%) showed predominantly positive nuclear staining of survivin and 14 cases (28.0%) showed negative staining. Positivity was observed predominantly in ductal structures, myoepithelial cells and tumors presenting condroid metaplasia. Tumors with myxoid metaplasia were negative with rare positive epithelial cells. Thirty-seven out of 44 cases of mucoepidermoid carcinoma (84.1%) showed positive cytoplasmic and/or nuclear staining of surviving and 7 cases (15.9%) showed negative staining. Nuclear positivity was observed predominantly in intermediate cells and ductal structures. Low grade tumors with mucous cells presented predominantly cytoplasmic staining. Twenty-four out of 41 cases of pleomorphic adenoma (58.5%) showed positive cytoplasmic staining of caspase-3 and 17 cases (41.5%) showed negative staining. Positivity was observed predominantly in luminal structures and was focally distributed. Twenty-six out of 46 cases of mucoepidermoid carcinoma (56.5%) showed positive cytoplasmic staining of caspase-3 and 20 cases (43.5%) showed negative staining. Positivity was observed predominantly in intermediate cells and was focally distributed. **Conclusions:** Our study provides evidence of mechanisms of anti-apoptotic signaling in salivary gland neoplasms, mainly shown by high expression of survivin in cases of mucoepidermoid carcinoma and in solid areas of pleomorphic adenoma.

Financial support: FAPESP research grant 11/02051-6

#41

TITLE: EXPRESSION OF CD9 TETRASPANIN IN VULVAR CARCINOMAS

Authors: Leonardo T Costa[1], Rafael M Rocha[2], Isabela W Cunha[3], Fernando A Soares[3], Gustavo A R Maciel[1], Edmund C Baracat[1], Katia C Carvalho[1]

Authors' Information:

[1] Laboratório de Ginecologia Estrutural e Molecular - LIM58, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

[2] Centro Internacional de Pesquisas - Hospital A.C. Camargo, São Paulo, Brazil.

[3] Departamento de Anatomia Patológica - Hospital A.C. Camargo, São Paulo, Brazil.

Background: Vulvar carcinomas represent approximately 95% of all vulvar tumors. When diagnosed in early stages, it usually presents a good prognosis, and survival rates depend upon inguinal lymph node status. However, about 30% of the patients present lymph node metastasis at the time of the diagnosis. Young women, in general, have vulvar carcinoma associated with HPV infection. The mechanisms of biological behavior of vulvar cancer remain unclear. Tetraspanins are proteins that are involved in several biological processes including cell proliferation, adhesion, invasion and migration. The reduction or lack of expression is frequently reported in metastatic tumors with poor prognosis. Moreover, tetraspanins are thought to be involved in viral infections, such as HPV and might have a role in its pathophysiology. **Objectives:** To analyze CD9 tetraspanin expression profile in vulvar carcinoma and to compare to normal tissue. **Methods:** All samples were obtained from the frozen sample bank of the Pathology Department of A. C. Camargo Cancer Center, São Paulo, Brazil. Quantitative Real Time PCR (QRT-PCR) was performed using TaqMan probe detection system for CD9 mRNA using 30 samples. Mann-Whitney test was used for statistical analysis. Immunohistochemistry validation was performed in a TMA containing 150 paraffin-embedded samples using specific antibody against CD9 protein. **Results:** QRT-PCR showed lower CD9 mRNA amount in tumor than normal tissue (fold of -3 times). Although fifth (50%) percent of the samples have expressed this protein, the majority then showed weakly staining. Additionally, all positive samples presented reactivity on cellular membrane or cytoplasm. **Conclusion:** Low or lack expression of CD9 seems to be involved in malignant transformation of vulvar neoplasm, since normal tissue presents higher protein and transcript expression.

#42

TITLE: COMPREHENSIVE GENETIC SCREENING OF FIVE MISMATCH REPAIR GENES AND CLINICAL FEATURES OF BRAZILIAN PATIENTS SUSPECTED FOR LYNCH SYNDROME

Authors: Felipe Cavalcanti Carneiro da Silva[1], Giovana Tardin Torrezan[1], Márcia Cristina Pena Figueiredo[1], Érika Maria Monteiro Santos[1], Maria Isabel Waddington Achatz[1], Wilson Toshihiko Nakagawa[1], Benedito Mauro Rossi[1], Fábio de Oliveira Ferreira[1], Dirce Maria Carraro[1]

Authors' Information:

[1] Laboratory of Genomics and Molecular Biology, A.C.Camargo Cancer Center, São Paulo - Brazil.

Corresponding author:

Felipe Cavalcanti Carneiro da Silva. E-mail: felipe.silva@accamargo.org.br

Lynch syndrome (LS) accounts for 3-5% of all colorectal cancers (CRC) and is inherited in an autosomal dominant fashion due to germline mutations in the mismatch repair genes MLH1, MSH2, MSH6, PMS1 and PMS2. LS is characterized by early CRC onset, multiple tumors (synchronous and metachronous) and extracolonic cancers such as endometrial, small bowel, ureter/renal pelvis, ovary, hepatobiliary tract, gastric, brain and sebaceous. Since the genetic screening became available, the molecular diagnosis of LS has been successfully increasing; however, it is still limited to developed countries and to the most frequently mutated genes MLH1 and MSH2. Hence, the aims of the study was to determine the frequency of pathogenic mutations in the five MMR genes by capillary sequencing and MLPA analysis, as well as to identify the most frequent extracolonic manifestations in 116 Brazilian families suspected for LS, in which 50 (43%) fulfilled the Amsterdam criteria (AC) and 66 (57%) fulfilled the Bethesda guideline (BG). Forty-six out of 116 (40%) were found to harbor pathogenic mutations. MSH2 was the most frequent mutated gene with 26 mutations, including 5 large genomic rearrangements, followed by MLH1 (16 mutations), MSH6 (4 mutations) and PMS2 (1 mutation). The positive detection rate for patients meeting the AC was 64% (32/50), whereas for BG the detection rate was 21% (14/66). Regarding tumor localization, 31 mutation carriers (67.5%) had proximal colon cancer, while 55 non-carriers (78.5%) had distal colon cancer. Synchronous/metachronous tumors were found in 25% of carriers, in contrast with only 2.9% in non-carriers. From the 116 selected families, we have collected information regarding CRC and extracolonic manifestations in a total of 2359 individuals. CRC was found in 325 family members, and the most frequent extracolonic tumors identified were gastric (39 cases), followed by breast (36 cases) and endometrial (19 cases). Interestingly, breast cancer was inversely correlated with Lynch syndrome, in which the majority was found in non-mutation carriers. It is well know that establishing the diagnosis is challenging and requires knowledge and surveillance. Thus, recognition of individuals and families with hereditary predisposition to cancer according to clinical and molecular features can contribute substantially to improve results related to diagnosis and characterization of LS.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Instituto Nacional de Ciência e Tecnologia em Oncogenômica (INCITO)

#43

TITLE: EXPRESSION OF PI3K/AKT PATHWAY IN ADENOID CYSTIC CARCINOMA AND PLEOMORPHIC ADENOMA OF SALIVARY GLANDS

Authors: Ianez, RCF[1], Coutinho-Camillo CM[1], Pinto CAL[1], Soares FA[1], Lourenço SV[2]

Authors' Information:

[1] Department of Surgical Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] General Pathology Department, Dental School, University of São Paulo, Brazil.

Corresponding

Ianez, RCF. E-mail: rere_fraga@hotmail.com, Department of Surgical Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Coutinho-Camillo, CM. E-mail: claumcc@terra.com.br - Department of Surgical Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Pinto, CAL. E-mail: coipinto@uol.com.br, Department of Surgical Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Soares, FA. E-mail: soaresfernado@terra.com.br, Department of Surgical Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Lourenço, SV. E-mail: silvialourenco@usp.br, General Pathology Department, Dental School, University of São Paulo, Brazil.

Introduction: Pleomorphic adenoma (PA) and adenoid cystic carcinoma (ACC) are the most common benign and malignant salivary gland neoplasms, respectively. They are originated from the intercalated duct region and are composed by luminal structures and myoepithelial cells. In a previous study we detected that protein c-kit is involved in the process of salivary gland morphogenesis and PA; this protein appeared to be related to pluripotent cells. Additionally, recent reports have shown that alterations in KIT gene are present in ACC. Based on this evidence, we further investigated the expression of PI3K/Akt pathway involved in the Kit signaling cascade in ACC and PA. **Material and Methods:** The PI3K/Akt pathway was investigated in 50 cases of PA and 50 cases of ACC using immunohistochemistry. **Results:** In PA c-kit was positive in isolated luminal cells, and myoepithelial cells were positive for alpha and beta PI3k, phospo-Akt and phosphor-mTor. In ACC, neoplastic luminal structures were positive for c-Kit and the other proteins showed positivity in myoepithelial cells especially in cribriform areas. **Conclusion:** Expression of the downstream proteins of the c-kit cascade in myoepithelial cells in both PA and ACC may be indicative that these neoplastic cells share common proliferative pathways, despite being benign or malignant.

#44

TITLE: POTENTIAL ANTI-TUMORIGENIC EFFECTS OF AMP-KINASE (AMPK) ON PAPILLARY THYROID TUMOR CELLS LINEAGES

Authors: Juliana Cazarin de Menezes[1], Andrade BM[1], Carvalho DP[1]

Authors' Information:

[1] Laboratório de Fisiologia Endócrina, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.

Corresponding

Primeiro Autor: Juliana Cazarin de Menezes. E-mail: julianacazarin@gmail.com
Andrade, BM. E-mail. brunoma@biof.ufrj.br
Carvalho, DP. E-mail: dencarv@biof.ufrj.br

Introduction: Although poorly understood, the role of AMPK in carcinogenesis seems to be related to two opposing functions: (1) promotion of tumor cells survival in unfavorable metabolic situations; (2) decrease in cell proliferation. We recently described that AMPK activation decreases iodine uptake and stimulates glucose uptake in rat thyrocytes (Andrade et al., 2011, Andrade et al., 2012), two changes that are also observed in thyroid tumor progression. However, the role of AMPK in thyroid cancer is not known. **Objective:** Evaluate, *in vitro*, the role of AMPK on cellular processes involved in thyroid carcinogenesis. **Methods:** Normal human thyrocyte lineage (NTHY-ORI) and two papillary carcinoma lineages BCPAP (BRAF v600e mutation) and TPC-1 (RET/PTC translocation) were treated with the pharmacological activator of AMPK, AICAR (1mM) for 24h. AMPK expression, cell proliferation, cell adhesion, cell migration and cell cycle were evaluated. **Results:** Total and phosphorylated AMPK are expressed in the 3 different lineages. However, the basal expression of phosphorylated AMPK is higher in BCPAP. We observed a reduction in cell number quite similar between the 3 lineages after Aicar treatment for 24h (NTHY-ORI C: 1.00 ± 0.041; A: 0.754 ± 0.032, $p < 0.01$) (BCPAP C: 1.00 ± 0.023; A: 0.592 ± 0.037, $p < 0.001$) (TPC-1 C: 1.00 ± 0.034; A: 0.688 ± 0.085, $p < 0.05$). When the cell cycle was evaluated under the same experimental conditions we observed a reduction of cell proliferation with a G0/G1 phase arrest in NTHY--ORI and G0/G1 and S phase arrest in BCPAP. In TPC-1 cells, we could not observe reduction of cell proliferation. Aicar also produced an increase in cell adhesion (NTHY--ORI C: 1.000 ± 0.027; A: 1.117 ± 0.03, $p < 0.05$) (BCPAP C: 1.000 ± 0.01; A: 1.267 ± 0.028, $p < 0.001$) (TPC-1 C: 1.00 ± 0.029; A: 1.155 ± 0.029, $p < 0.05$) and a reduced cell migration ability in all the three lineages. **Conclusion:** AMPK activation reduces cell proliferation in the normal thyrocyte cell lineage NTHY and in the papillary carcinoma cell lineage BCPAP. In TPC-1 cells we could not observe a reduction of cell proliferation. However, we observed a reduction in TPC-1 cell number after AMPK activation. We also demonstrated an increase in cell adhesion and reduction of cell migration ability, which may be related to a reduced metastatic ability and, consequently, a less aggressive phenotype. These data strongly suggests a potential anti-tumorigenic effect of AMPK activation on papillary thyroid tumor cells lineages, *in vitro*.

REFERENCES

1. Andrade BM et al. A novel role for AMP-kinase in the regulation of the Na+/I--symporter and iodide uptake in the rat thyroid gland. Am J Physiol Cell Physiol, 2011.
2. Andrade BM et al. AMP-Activated Protein Kinase Upregulates Glucose Uptake in Thyroid PCCL3 Cells Independent of Thyrotropin. Thyroid, 2012.

#45

TITLE: CIRCULATING TUMOR CELL ASSESSMENT IN A METASTATIC BREAST CANCER CASE

Authors: Carvalho FM[1], Chinen LTD[1,2], Andrade WP[3], Soares FA[1], Rocha RM[1]

Authors' Information:

[1] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Department of Clinical Oncology, A.C. Camargo Cancer Center, São Paulo, Brazil.
[3] Department of Mastology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding

Fernanda Machado de Carvalho. E-mail: femcarvalho@yahoo.com.br
Chinen, LTD. E-mail: ltdchinen@gmail.com
Andrade, WP. E-mail: wesley.andrade@hotmail.com
Soares, FA. E-mail: fasoares@gmail.com
Rocha, RM. E-mail: rafael.malagoli@gmail.com

Metastasis is the most common cause of mortality in cancer patients. In the context of breast cancer, survival time for metastatic patients varies from less than 9 months to over 3 years. Currently, it has been hypothesized that the metastatic process is initiated by a subpopulation of circulating tumor cells (CTCs) found in patient blood and that CTCs could be an important biomarker of this process. Despite advances in detection and progression of breast cancer, CTCs were found in patients many years after mastectomy without evidence of disease. For metastatic breast cancer patients, using CellSearch™ platform, ≥ 5 CTCs/7.5 mL of peripheral blood are associate with worse prognosis[1]. However, there are few studies focused on molecular characterization of CTCs that would provide a useful target for treatment development. Thus, we aimed to present

the case report focused on isolation and characterization of CTCs from peripheral blood sample of a 45-year-old woman diagnosed with HER2-overexpressing metastatic breast cancer with *sites* of metastasis in bone, lung, lymph node, liver, and peritoneum 9 years after detection of primary tumor. Primary tumor was an Estrogen and Progesterone Receptor-positive, HER2-negative breast cancer. To accomplish this, we used a non-automated technique, ISET™ (Isolation by Size of Epithelial Tumor Cells (RareCell Diagnostics, Paris, France). The present method is based on blood filtration through a 10 spot-polycarbonate membrane with 8-µm-diameter cylindrical pore. ISET membrane was used for dual-color immunocytochemistry for CTC identification and characterization according to the manufacturer's instructions. To evaluate and distinguish CTCs from White Blood Cells (WBCs) contaminants, besides morphology analysis, dual-color immunocytochemistry (DAB+/Permanent Red; Dako™) were carried out using the following antibodies: anti-pan cytokeratin (Clones AE1/AE3, Dako™) and anti-CD45 (clone 2B11+ PD7/26, Dako™), a WBC surface marker. Isolated cells were considered CTC when negative for CD45, hyperchromatic and irregularly shaped nuclei, nuclear size ≥ 12 µm, nuclear to cytoplasmic ratio more than 50%, and/or in presence of CD45-negative clusters of cells containing three or more nuclei [Circulating Tumor Microemboli (CTM)][2]. Four spots of ISET membrane was used for CTC enumeration and the result was extrapolated to 10 mL of peripheral blood 2.Two other spots were used to perform dual-color immunocytochemistry: anti-CD45 together with antibodies against cellular metabolism proteins [anti PGC1 alpha (Clone 4C1.3, Calbiochem™) and anti COXIV (Cell Signaling Technology™)]. PGC1 alpha is a key transcriptional regulator of energy metabolism involved in tumor growth by regulation of coordinately regulates mitochondrial and fatty acid metabolism supporting lipogenesis[3]. Cytochrome C oxidase subunit IV (COXIV) is considered a mitochondrial marker and one of the major regulation sites for oxidative phosphorylation. Our results showed a total of 40 CTCs/10 mL of patient peripheral blood and 1 CTM, which presents highly metastatic potential and survival advantages in comparison with isolated CTCs[2]. All cells that fulfilled the morphological criteria for CTCs were negative for immunostaining with anti-CD45; furthermore these cells presented heterogeneity in immunostaining for pan-cytokeratin. CTCs in CTM were negative while some isolated CTCs presented positivity for pan-cytokeratin.

CTM was not found in the evaluated spots for cellular metabolism markers, on the other hand, some isolated CTCs showed immunopositivity for PGC1 alpha and COX IV antibodies showing a metabolically active profile, which might contribute for their survival, resistance, survival and adaptation in novel microenvironments as bloodstream and other organs leading to metastatic process. This case report emphasizes that the detection and characterization of CTCs as strategies to lead to personalized treatment and development of new therapeutic agents focusing, for example, in cellular metabolism. In this context, our next steps will be evaluation of the same markers in a larger cohort of breast cancer patients, establishing a comparison between CTCs profile with breast cancer cells lineage and immunohistochemistry results of the tissue samples. Furthermore, verifying if there will be correlation among these results, clinical data and disease progression of patients with breast cancer.

REFERENCES

1. Cristofanilli M et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:8
2. Krebs MG et al. Analysis of circulating tumor cells in patients with non-small cell lung cancer using epithelial marker-dependent and -independent approaches. J Thorac Oncol 2012;7
3. Bhalla K et al. PGC1α promotes tumor growth by inducing gene expression programs supporting lipogenesis. Cancer Res. 2011;1:71(21).

#46
TITLE: P90 RIBOSOMAL S6 KINASES (RSKS) ARE DIFFERENTIALLY EXPRESSED IN GLIOBLASTOMA-DERIVED CELL LINES AND CONTROL CELLULAR PROLIFERATION

Authors: Roffé M[1], Lupinacci FCS[1], Hajj GNM[1], Martins VR[1]

Authors' Information:

[1] A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding

Roffé, M. E-mail: mroffe@cipe.accamargo.org.br
Lupinacci, FCS. E-mail: lupi.bio@gmail.com
Hajj, GNM. E-mail: ghajj@cipe.accamargo.org.br
Martins, VR. E-mail: vmartins@cipe.accamargo.org.br

The RAS/ERK signaling pathway is essential for the control of normal cell proliferation, survival, growth and differentiation. Dysregulation of this pathway is involved in the pathology of a large number of human cancers. The Extracellular signal-Regulated Kinase-1 and 2 (ERK1/2) play a pivotal role in the RAS/ERK signaling pathway by phosphorylating, and thus regulating, several downstream targets. The 90 kDa ribosomal S6 kinases (RSKs) are a group of ERK1/2 substrates. RSKs phosphorylate several cytosolic and nuclear proteins, and they have been implicated in the regulation of several processes, including cell survival, proliferation, cell growth and motility. Four RSK isoforms (RSK1-4) are expressed in humans and they share a high degree of sequence homology. Increasing evidence links RSK1 and RSK2 to several aspects of human cancer pathology. Despite the structural and functional

similarity among RSK1 and RSK2, some types of tumors are only affected by RSK1 and not by RSK2, and *vice versa*. It has been shown, for example, that RSK2 is overexpressed and activated in highly invasive head and neck squamous cell carcinoma (HNSCC) cell lines and promotes metastasis, whereas RSK1 does not. RSK2 expression also correlated with metastatic progression in patients with HNSCC. In lung adenocarcinoma patients, RSK1 expression decreases during metastasis. Accordingly, the silencing of RSK1, but not of RSK2, increases cell motility and invasiveness of A549 cells. The aim of this work was to explore the relationship between RSKs and glioblastomas (GBMs), the most common and aggressive primary brain tumor in humans. Using immunoprecipitation followed by silver staining of SDS-PAGE we determined that the ratio of RSK1 to RSK2 is of about 3.1 in the GBM-derived cell line LN-18. We then studied by immunoblotting the expression of RSK1 and RSK2 in other GBM cells and found that in PTEN-positive cells (LN-18 and LN-229) the RSK1/RSK2 ratio is > 1 and in PTEN-negative cells (A172, U-87MG and U-118MG) is < 1. The RSK inhibitor SL0101-1 inhibited proliferation of LN-18, LN-229 and U-87MG cells more efficiently than Rapamycin, both in basal conditions and in the presence of EGF. These results point to a differential regulation of the expression of RSK 1 and 2 in GBMs cell lines that might result in distinct pathological phenotypes. The effect of the inhibition of RSK on proliferation of GBMs underscores the importance of the RAS/ERK signaling pathway for this type of cancer cells.

Financial Support: FAPESP, National Institute of Translational Oncogenomics (INCiTO) and National Institute of Translational Neurosciences

#47

TITLE: IDENTIFICATION OF NOVEL CANCER SUSCEPTIBILITY GENES THROUGH EXOME SEQUENCING OF COLORECTAL POLYPOSIS PATIENTS WITHOUT *APC* OR *MUTYH* GERMLINE MUTATIONS

Authors: Torrezan GT[1], Silva FCC[1], Ferreira EN[1], Krepischi ACV[2,3], Santos EMM[4], Rossi BM[5], Carraro DM[1,2]

Authors' Information:

[1] Laboratory of Genomics and Molecular Biology - A. C. Camargo Cancer Center, São Paulo - SP, Brazil.

[2] National Institute of Science and Technology in Oncogenomics (INCITO), São Paulo - SP, Brazil.

[3] Laboratory of Structural Genomics - A. C. Camargo Cancer Center, São Paulo - SP, Brazil.

[4] Hereditary Colorectal Cancer Registry Biology - A. C. Camargo Cancer Center, São Paulo - SP. Brazil.

[5] Barretos Cancer Hospital - Pio XII Foundation, Barretos - SP. Brazil.

Corresponding

Torrezan, GT. E-mail: giovana.torrezan@accamargo.org.br
Silva, FCC. E-mail: felipe.silva@accamargo.org.br
Krepischi, ACV. E-mail: ana.krepischi@accamargo.org.br
Santos, EMM. E-mail: emonteiro@accamargo.org.br
Rossi, BM. E-mail: rossibm@gmail.com
Carraro, DM. E-mail: dirce.carraro@cipe.accamargo.org.br

Background: Patients with multiple colorectal adenomas are currently screened for germline mutations in two genes, APC and MUTYH. APC-mutated patients present classic or attenuated familial adenomatous polyposis (FAP/AFAP), while patients carrying biallelic MUTYH mutations exhibit MUTYH-associated polyposis (MAP). However, about 10-15% of polyposis patients do not harbor mutations in these genes, suggesting that other yet unknown polyposis-predisposing genes could exist. In previous studies of our group (Torrezan et al. 2011, Torrezan et al. 2012 and Torrezan et al. 2013), 23 unrelated FAP and MAP patients were screened for APC and MUTYH mutations through DNA sequencing, array-CGH, MLPA and duplex qPCR analysis. In total, 21 mutated patients were identified in this cohort (91%) - 6 patients carried MUTYH mutations, 14 carried APC pathogenic mutations and one carried a novel APC missense variant of unknown clinical significance (p.Val1789Leu). The aim of the present study is to screen the two polyposis patients that were negative for APC and MUTYH mutations for novel susceptibility genes by next generation exome sequencing. In addition, we will evaluate the pathogenicity of the novel APC missense variant p.Val1789Leu with *in vitro* assays. **Methods:** Genomic DNA of patients ID20 and ID22 was extracted from lymphocytes and the coding regions were capture using the TargetSeq™ Exome Enrichment Kit. Library construction was performed according to manufacturer instructions and submitted to paired-end (75 bp x 50 bp) sequencing at SOLiD 5500 xl platform. For the novel missense variant, immunoprecipitation and cellular localization assays will be performed on colon tumor cell lines transfected with plasmids expressing the wild type APC gene, an APC pathogenic mutation and the p.Val1789Leu variant produced by site-directed mutagenesis. **Results:** A total of 55,014.151 reads were sequenced for patient ID20 and 58,820.164 for patient ID22, leading to an expected coverage of more than 100x. Currently, we are performing the mapping of the resulting sequences and bioinformatics analysis to identify novel or rare mutations, which are predicted to impact gene function. **Conclusions:** Exome sequencing and functional studies can allow identification of causative mutations in individuals with polyposis phenotypes, having a direct impact on patient treatment, as well as surveillance and prevention for at-risk family members.

Keywords: APC, exome sequencing, FAP, MAP, MUTYH

Financial Support: CNPq and FAPESP.

REFERENCES

1. Torrezan GT et al. Mutational spectrum of the APC and MUTYH genes and genotype-phenotype correlations in Brazilian FAP, AFAP, and MAP patients. Orphanet J Rare Dis. 2013;8:54.
2. Torrezan GT et al. A novel SYBR-based duplex qPCR for the detection of gene dosage: detection of an APC large deletion in a familial adenomatous polyposis patient with an unusual phenotype. BMC Med Genet. 2012;13:55.
3. Torrezan GT et al. Breakpoint characterization of a novel large intragenic deletion of MUTYH detected in a MAP patient: case report. BMC Med Genet. 2011;12:128.

#48
TITLE: MOLECULAR ALTERATIONS IN GLIOMAS EVALUATED USING LARGE SCALE SEQUENCING OF MRNAS ASSOCIATED WITH POLYRIBOSOMES

Authors: Fernanda Cristina Sulla Lupinacci[1]. Roffé M[1], Ferreira EN[1], Martins VR[1], Carraro D[1], Hajj GN[1]

Authors' Information:
[1] A.C. Camargo Cancer Center, Instituto Nacional de Neurociência Translacional, Instituto Nacional de Oncogenômica. São Paulo, Brazil.

Corresponding
Fernanda Cristina Sulla Lupinacci. E-mail: flupinacci@cipe.accamargo.org.br
Roffé, M. E-mail: mroffe@cipe.accamargo.org.br
Ferreira, EN. E-mail: elisa.ferreira@accamargo.org.br
Martins, VR. E-mail: vmartins@cipe.accamargo.org.br
Carraro, D. E-mail: dirce.carraro@cipe.accamargo.org.br
Hajj, GN. E-mail: ghajj@cipe.accamargo.org.br

Context: Glioblastoma is among the most aggressive types of tumors and less responsive to chemotherapeutic agents, thus a better understanding of the behavior of these tumors may help to develop new treatments for this disease. Currently, many genome-wide projects attempt to define general patterns of gene expression based on deep sequencing or microarray data from total mRNA populations. However, this approach provides little information about the molecular mediators of tumor biology, because the expression levels of mRNAs do not necessarily reflect the levels of proteins. On the other hand, the identification of mRNAs target of translational alterations in tumors can show gene expression profiles that better reflect the population of proteins. **Objectives:** In this project we intend to identify mRNAs differentially translated in glioblastomas by deep sequencing of mRNAs associated with polysomes (actively engaged in translation). **Methods:** Polysomes were isolated from lysates of the GBM cell line LN18 by continuous gradient of sucrose 5-47% ultracentrifugation. Total RNA was extracted with TRIzol® and the ribosomal RNA was depleted with Ribominus™ (Life technologies). cDNA libraries were made with Ion Torrent™ kit. Libraries were deep sequenced in Ion PGM (Life technologies). **Results:** LN18 cells were treated or not with BI-D1870, a selective inhibitor of RSK, a kinase involved in the translational control. We were able to isolate polysomes in both conditions with 1µg of RNA of total extracts and 0.5 µg of RNA from polysome samples. Depletion of rRNA had an efficiency of 15% for total extracts and 1% for polysome samples. Sequencing was performed by Ion PGM successfully with 84% of beads loading and 36% of sequence reads with a total of 3,163.212 readings. Differentially translated mRNAs were obtained in response to treatment with the RSK inhibitor. **Conclusion:** We were able to successfully establish the technique for large-scale sequencing of polysome mRNAs and observed mRNAs differentially translated in two different conditions. We intend to apply this technique to define differentially translated mRNAs in glioblastoma samples paired with normal tissue from the biobank of the A.C. Camargo Cancer Center.

Financial Support: FAPESP

#49
TITLE: HER2 STATUS IN GASTRIC CARCINOMAS: IMMUNOHISTOCHEMISTRY TESTING USING THREE DIFFERENT ANTIBODIES

Authors: José Ivanildo Neves[1,2], Maria Dirlei FS Begnami[1]

Authors' Information:
[1] Departamento de Anatomia Patológica do A.C. Camargo Cancer Center.
[2] Doutorando do A.C. Camargo Cancer Center.

Gastric cancer (GC) is the second leading cause of cancer-related death worldwide. Amplification of HER2 is found in about 20% of breast carcinomas and the treatment with trastuzumab, a monoclonal antibody has been quite effective. In GCs, the amplification of HER2 has been reported in a frequency of 7 to 27%. This variability is mainly due to the different methodologies used for research and interpretation of gene and protein expression. Therefore, there is an unquestionable need for standardization of protocols and interpretation of achievement tests for determination of HER2 status in CGs to properly select patients who will benefit from the use of trastuzumab. **Objectives:** Evaluate the HER2 protein expression in GC using three different clones of anti-HER2 primary antibodies (Clone SP3, HercepTest and 4B5). **Methodology:** 762 GCs from total

or partial gastrectomy performed in the period 1980-2006 at A.C. Camargo Cancer Center were selected. The cases were arranged in 7 TMA blocks containing two cylinders with 1mm diameter representing different areas of tumors. The immunohistochemical reactions were performed on 2 slides of each TMA block with different levels of depth. The analyses were done in four representative areas of the tumors. **Results:** Of 762 cases, 664 cases were evaluated for SP3 (13% of the cases were excluded), 673 and 684 were evaluated for 4B5 and Herceptest (12% and 13% were excluded cases, respectively). Scores 0 and 1 using the SP3 antibody, Herceptest and 4B5 were detected in 88%, 94% and 92% of the cases respectively. 55 (8%) cases stained with SP3 were classified with a score of 2, 40 (4%) and 36 (5%) cases showed this score using the Herceptest and 4B5, respectively. This group of patients is classified as undetermined or doubtful for the status of HER2 and is mandatorily being tested by *in situ* hybridization. Different from that for the other scores, we note that the frequency of score 3 cases were very similar using the SP3 and Herceptest (4% and 5%) and somewhat lower (2%) with the use of 4B5. **Conclusions:** This study shows that the immunohistochemical expression of HER2 is dependent on the clone of primary antibody used. Scores 0 and 1 were more frequent using 4B5 and Herceptest antibodies. The antibody SP3 detected more frequently the CG score 2 and 4B5 antibody showed lower frequency of cases score 3. To confirm the best antibody for HER2 status by immunohistochemistry in GC will be necessary to perform the *in situ* hybridization for gene amplification.

REFERENCES

1. Kononen, J., L. Bubendorf, et al. "Tissue microarrays for high-throughput molecular profiling of tumor specimens." Nat Med 1998;4(7):844-847.
2. Hofmann, M., O. Stoss, et al. "Assessment of a HER2 scoring system for gastric cancer: results from a validation study." Histopathology 2008;52(7):797-805
3. Bang, Y. J., E. Van Cutsem, et al. "Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial." Lancet 2010;376(9742):687-697.7-805.

#50

TITLE: PROLIFERATIVE VERRUCOUS LEUKOPLAKIA

Authors: Vitor Bonetti Valente[1], Lima PS[1], Onofre MA[1], Massucato EMS[1], Navarro CM[1]

Authors' Information:

[1] Faculdade de Odontologia de Araraquara - UNESP. Araraquara, Brazil.

Corresponding

Vitor Bonetti Valente. E-mail: vitorbvalente@foar.unesp.br
Lima, P. S. E-mail: patriciaslima@foar.unesp.br
Onofre, MA. E-mail: maonofrefoar@uol.com.br
Massucato, EM. S. E-mail: emaria@foar.unesp.br
Navarro, CM. E-mail: cnavarro@foar.unesp.br

Background: Proliferative verrucous leukoplakia (PVL) is an aggressive form of oral leukoplakia with high morbidity and mortality rates. PVL is a persistent and progressive condition that requires very close follow-up, along with early and aggressive treatment to increase the probability of a favorable outcome. **Objectives:** The aim of this study was to compare epidemiological aspects of PVL with malignant transformation (MT) and PVL non-transformed (NT). **Methodology:** It was a retrospective study. The medical records of patients attended to at the Oral Medicine Service, Department of Diagnosis and Surgery, during the period of 1995 to 2011 were evaluated. Based on this previous selection, the medical records of PVL patients according to diagnosis criteria proposed by Hansen et al. (1985) were considered for this study. All data were entered into a database with the Epi Info version 3.3.2 (available for download at: http://www.cdc.gov/epiinfo/epiinfo.htm), to use od Epi Info the results were encoded in alphanumeric system. **Results:** From 10,385 clinical files, we found 13 (0.12%) PVL patients; 6 MT and 7 NT. The mean age for the groups MT and NT was 71.2 and 59 years respectively. Women were 66.6% and 85.7% while the men were 33.3% and 14.3% for MT and NT groups. Tobacco and alcohol consumption was not associated to the malignant transformation. The main anatomical site for malignant transformation of PVL was the buccal mucosa and alveolar ridge, while benign PVL predominate on tongue. The majority of the patients presented multifocal PVL. Conclusions: PVL is more common in female, over 71 years old, non-smokers and nonalcoholic. PVL generally was multifocal and the period for malignant transformation was 3.2 years in average. Based on this data, we conclude that early diagnosis is very important to improve the prognosis, but this is a difficult point considering the innocent microscopical aspect of PVL along all the follow-up until malignant transformation.

REFERENCES

1. Hansen et al. Proliferative verrucous leukoplakia. A long-term study of thirty patients. Oral Surg Oral Med Oral Pathol 1985; 285:98.
2. Cabay et al. Proliferative verrucous leukoplakia and its progression to oral carcinoma: a review of the literature. J Oral Pathol Med 2007; 255:61.
3. Bagan et al. Malignant transformation of proliferative verrucous leukoplakia to oral squamous cell carcinoma: a series of 55 cases. Oral Oncology 2011; 732:35.

#51

TITLE: CLINICOPATHOLOGICAL ASPECTS OF PATIENTS WITH ORAL DYSPLASIA AND SQUAMOUS CELL CARCINOMA ON LOWER LIP: A CASE SERIES

Authors: Vitor Bonetti Valente[1], Andrade CR[1], Pereira-Filho VA[1], Onofre MA[1], Massucato EMS[1], Navarro CM[1]

Authors' Information:

[1] Faculdade de Odontologia de Araraquara - UNESP. Araraquara, Brazil.

Corresponding

Vitor Bonetti Valente. E-mail: vitorbvalente@foar.unesp.br
Andrade, CR. E-mail: clevertonroberto@foar.unesp.br
Pereira-Filho, VA. E-mail: dinho@foar.unesp.br
Onofre, MA. E-mail: maonofrefoar@uol.com.br
Massucato, EMS. E-mail: emaria@foar.unesp.br
Navarro, CM. E-mail: cnavarro@foar.unesp.br

Context: The control of oral cancer is based on at least two points: prevention and early diagnosis. Educative campaigns are important to inform the population about risk factors and early signs and symptoms of oral cancer. The treatment at the initial stages improves the prognosis and increases the survival. **Objectives:** The aim of this study was to present a case series of 4 lip changes, consisting of 2 diagnosis of severe dysplasia and microinvasive squamous cell carcinoma, and the other two in reference to invasive squamous cell carcinoma. **Results:** Case 1: 53-year-old White woman. Systemic Health: Arterial Hypertension and Depression. Risk Factors: alcohol consumption. Clinical Aspects: diffuse erythematous spots. Histopathologic Diagnosis: severe dysplasia. Treatment: vermilionectomy. Case 2: 67-year-old White man. Systemic Health: Depression. Risk Factors: chronic exposure to ultraviolet radiation. Clinical Aspects: erythematous and whitish spots. Histopathologic Diagnosis: microinvasive squamous cell carcinoma. Treatment: vermilionectomy. Case 3: 55-year-old White man. Systemic Health: Arterial Hypertension. Risk Factors: chronic exposure to ultraviolet radiation. Clinical Aspects: ulceration with indurated edges. Histopathologic Diagnosis: well differentiated squamous cell carcinoma (T1N0M0). Treatment: surgical resection followed to vermilionectomy. Case 4: 61-year-old White man. Systemic Health: Arterial Hypertension and Diabetes. Risk Factors: chronic exposure to ultraviolet radiation, tobacco and alcohol consumption. Clinical Aspects: exophitic nodule with crust, fissures and indurated edges. Histopathologic Diagnosis: squamous cell carcinoma ($T_2N_0M_1$) infiltrating striated muscle. Treatment: surgical resection followed to neck dissection. **Conclusions:** This case series illustrates the importance of the early diagnosis of labial squamous cell carcinoma to reduce treatment sequelae, to increases the survival and to improve quality of life.

REFERENCES

1. Abreu L et al. Lip cancer in Western Australia, 1982-2006: a 25-year retrospective epidemiological study. Aust Dent J 2009;130:5.
2. Luna-Ortiz K et al. A Lip cancer experience in Mexico. An 11-year retrospective study. Oral Oncol 2004;992:9.
3. Tilakaratne et al. Grading oral epithelial dysplasia: analysis of individual features. J Oral Pathol Med 2011;533:40.

#52

TITLE: NO SOMATIC MUTATIONS IN THE GENE *P16^{INK4}* IN MALIGNANT NON-PALPABLE BREAST LESIONS

Authors: Delmonico L[1,2], Menezes L[1], Franco MF[3], Moreira AS[4], Azevedo CM[3], Alves G[1]

Authors' Information:

[1] Instituto Nacional do Câncer INCA, Rio de Janeiro (RJ), Brazil.
[2] Universidade do Estado do Rio de Janeiro, Rio de Janeiro (RJ), Brazil.
[3] Hospital Universitário Gafrée-Guinle, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro (RJ), Brazil.
[4] Plataforma de Sequenciamento PTDIS - Instituto Oswaldo Cruz, Rio de Janeiro (RJ), Brazil.

Corresponding

Delmonico L. E-mail: lucasdelmonico@gmail.com
Menezes L. E-mail: liviademenezes@yahoo.com
Franco MF. E-mail: marcofelipe20@hotmail.com
Moreira AS. E-mail: smoreiraaline@gmail.com
Azevedo CM. E-mail: azevedocma@bol.com
Alves G. E-mail: gbrown@inca.gov.br

Introduction: Breast cancer is the second most common type of cancer worldwide and the most common among women. The number of new cases expected for Brazil in 2013 is 52 per 100 thousand women (MS, 2013). During carcinogenesis there are several changes in the genes that control the cell cycle, however, only a few studies describe the role of somatic mutations in p16^{INK4} in the evolution for initial breast cancer.
Objectives: Search for mutations in p16^{INK4} tumor suppressor gene (exons 1-3) in non-palpable breast lesions.
Methodology: The fragments of breast lesions were obtained from female patients who underwent excisional biopsy examination, core biopsy or mammotomy according to the routine of the University Hospital Gaffrée Guinle - RJ. The extraction of DNA from the samples was performed according to Sambrook et al., 1989. Approximately 100 ng DNA was applied in polymerase chain reaction (PCR) using the primers described by Robertson et al., 2010. To sequence the amplified fragments, the PCR products were purified with the GFX kit® PCR DNA and Gel Band Purification (GE).

The purified products were sequenced in sequencer 3130 Genetic Analyzer from Applied Biosystems. To analyze the samples, a comparison was made with a standard sequence of the gene $p16^{INK4}$ (NG_007485.1 GenBank) with the sequences generated by sequencing. This comparison was made using the programs BioEdit Sequence Alignment and Sequencer Demo Version Gene Codes. **Results:** A total of 20 DNA samples from non palpable lesions were obtained, that were subsequently classified as invasive ductal breast carcinoma, variants histological grade II and III. No mutation in the gene $p16^{INK4}$ was detected by automated sequencing. **Conclusion:** To determine which tumor suppressor genes can reveal the trend of the evolution of a breast lesion is still a matter of discussion. Besides the genes undergo fluctuations regarding mutations in tumor tissue, there is little information about their influence on prognosis. This work was carried out to search for mutations in the gene $p16^{INK4}$, but this is not the end. Other possible genetic changes should be investigated (Loss Heterozygosity and methylation), to insure the functionality of the gene in the initial injury.

Financial Support: Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ APQ 1 (E-26/110.319/2008 e E-26/110.803/2009)

REFERENCES

1. MS - Ministério da Saúde - INCA (Instituto Nacional do Câncer - http://www2.inca.gov.br/wps/wcm/connect/tiposdecancer/site/home/mama/cancer_mama - acesso em 22/04/2013 - 16hs:14min.
2. Robertson L et al. Survey of familial glioma and role of germline *p16INK4A/p14ARF* and *p53* mutation. Fam Cancer 2010, 9(3); 413:21.
3. Sambrook J et al. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press 1989.

#53
TITLE: COMPLICATIONS OF CANCER TREATMENT IN HEAD AND NECK

Authors: Ferrisse TM[1], Navarro CM[1], Onofre MA[1], Bufalino A[1], Massucato EMS[1]

Authors' Information:
[1] Universidade Estadual Paulista "Júlio de Mesquita Filho" Câmpus de Araraquara - Faculdade de Odontologia. Brazil.

Corresponding Author:
Ferrisse T.M. E-mail: tuliomferrisse@gmail.com
Navarro, C.M. E-mail: cnavarro@foar.unesp.br
Onofre, M.A. E-mail: maonofrefoar@uol.com.br
Bufalino, A. E-mail: andreiabufalino@bol.com.br
Massucato, E.M.S. E-mail: emaria@foar.unesp.br

Context: Radiotherapy is a primary or adjunctive treatment modality, often used in the treatment of malignant neoplasia, including those affecting the head and neck region. The normal tissue injuries caused by radiation are consequences of cell damage and can lead to functional impairment. Among the complications stand hyposalivation, radiation caries, dysphagia, taste changes, osteoradionecrosis and mucositis. The most common clinical presentations of oral mucositis are erythema and ulceration, and their classification according to the World Health Organization, is related to feeding difficulties. Among the forms of treatment and prevention, the use of low-power laser has been recommended as well as topical and systemic treatments. The objective of this work is to forward the importance of monitoring patients receiving radiotherapy for malignancies in the head and neck region in the oral point of view. **Case report:** N.V., 68-year-old female, attended the Oral Medicine Service forwarded to an otolaryngologist and presenting chief complaint "sore throat". During the interview, the patient reported that the pain appeared two months ago, especially when drinking cold drinks and the pain has increased. She is smoker for 51 years and former alcoholic for 9 years. The intraoral examination was noted an ulcer with elevated borders and hardened, bed with necrotic, approximately 5 cm, located in the retromolar region and expanding towards the oropharynx. Incisional biopsy was performed and the histopathological diagnosis was Squamous Cell Carcinoma. The patient was referred to an oncologist and treatment instituted was surgical removal of the lesion, with a safety margin and lymph node dissection, supplemented by radiotherapy. **Results:** The patient was re-referred to the Oral Medicine Service for treatment and monitoring of complications in the oral cavity, facing the radiotherapy and the main pathology observed in this period was oral mucositis. The recommended treatment protocol followed the Service. We also observed other complications such as radiation burns, hyposalivation and pseudomembranosa candidiasis. **Conclusion:** Oral mucositis is the main adverse effect of radiotherapy, his study, on the pathogenesis, clinical presentation and treatment is very important for improving the quality of life of these patients.

REFERÊNCIAS

1. Rodrigues-Caballero et al. Cancer treatment-induced oral mucositis: a critical review. Int. J. Oral Maxiffofac. Surg. 2012; 41:225-238.
2. Brito, C.A et al. Effect of chlorhexidine and low power laser in the prevention and treatment of oral mucositis. Rev.Odontol. UNESP.2012; 41(4):236-241.

#54

TITLE: CLINIC-PATHOLOGICAL CORRELATION OF PROTEIN EXPRESSION OF COMPLEX CD44/ERM (EZRIN/ RADIXIN/MOESIN) IN PENILE CARCINOMA

Authors: Andrade JB[1], Lopes A[2], Soares FA[3]

Authors' Information:
[1] Department of Anatomy Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.
[2] Department of Pelvic Surgery, A.C. Camargo Cancer Center, São Paulo, Brazil.
[3] Department of Anatomy Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:
Juliana Bartholo de Andrade. E-mail: jujubandrade@yahoo.com
Ademar Lopes. E-mail: ademar-lopes@uol.com.br
Fernando Augusto Soares. E-mail: fasoares@hcancer.org.br

Introduction: Lymph node metastasis is the most important prognostic factor in patients with penile carcinoma. Understanding the molecular processes that may be involved in metastatic dissemination would improve the prognostic tools to better evaluate the patients, thus allowing an appropriate treatment planning, avoiding the morbidity of unnecessary surgery. Some studies have emphasized the influence of cellular adhesion in the development of tumorigenesis and metastasis as the proteins of complex CD44/ERM (Ezrin/Radixin/Moesin). **Objectives:** The present study aimed to evaluate the expressions of CD44 protein family and ERM (Ezrin/Radixin/Moesin) in penile carcinoma and correlate them to the clinical-pathological factors and clinical behavior of disease. Material and **Methods:** Three hundred and eighty-four patients were selected, retrospectively, undergoing surgery for squamous cell penile carcinoma, with or without inguinal-iliac lymphadenectomy at A.C. Camargo Cancer Center (São Paulo, Brazil) during the period of January 1953 to December 2000. The evaluation of the expressions of CD44 and ERM family was performed by immunohistochemistry in blocks of TMA (tissue microarray), that subsequently were analyzed by digital microscopy system ACIS III (Dako®). The immunoreactivity for CD44, Ezrin and Moesin were categorized as strong, weak and negative. The immunoreactivity for Radixin was categorized into positive and negative. **Results:** CD44, Ezrin and Moesin showed strong expression in 35.2%, 12.5% and 52.6% of the cases, respectively. Radixin showed positive expression in 44.0% of the cases. Decreased expression of CD44 was significantly correlated with perineural invasion and with infiltration of the cavernous body. The positive expression of Ezrin and Moesin was significantly correlated with circumcision. Furthermore, the positive expression of Ezrin was significantly correlated with the histological differentiation degree and influenced significantly disease-free survival. There was no correlation with the expression of Radixin. **Conclusion:** Ezrin protein expression in penile carcinoma seems to be an independent prognostic factor in disease-free survival, being able to become an important molecular marker in penile carcinoma. However, the results suggest that further investigations are needed in order to define the expression pattern of the complex CD44/ERM.

#55

TITLE: CIRCULATING TUMOR CELLS IN PATIENTS WITH COLORECTAL CANCER TREATED WITH BEVACIZUMAB: WHICH CELLS ARE WE REALLY COUNTING?

Authors: Abdallah EA[1], Sanches SM[1], Fanelli MF[1], Rocha BMM[1], Ocea LMM[1], Buim MEC[1], Dettino ALA[1], Mingues NB[1], Chinen LTD[1]

Authors' Information:
[1] Department of Clinical Oncology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:
Abdallah, EA. E-mail: emne22@gmail.com
Sanches SM. E-mail: solange.sanches797@gmail.com
Fanelli, MF. E-mail: mffanelli@gmail.com
Rocha, BMM. E-mail: bruna.malagoli@hotmail.com
Ocea, LMM. E-mail: enf_lumend@yahoo.com.br
Buim, MEC. E-mail: mebuim@hotmail.com
Dettino ALA. E-mail: aldolad@hotmail.com
Mingues, NB. E-mail: nattalia.breve@hotmail.com
Chinen, LTD. E-mail: ltdchinen@gmail.com

Background: Colorectal cancer (CRC) was the third most commonly diagnosed cancer in men in the world, and the second in women in 2008 (Jemal et al., 2011). The first strategy for treatment is complete resection of the lesion. However, some patients experience recurrence, believed to be due to residual micrometastases. Traditional diagnostic methods are unable to detect CTCs present in these sites and released into the circulation. **Objective:** To count and correlate CTC levels with progression free survival (PFS). **Methods:** Prospective study made by blood collection of patients with metastatic or advanced CRC. Blood was collected before the beginning of chemotherapy and after 60 days, in accordance with image exams. The enrichment of CTCs was made by direct immunomagnetic labeling of positive cytokeratin (CK) cells. These cells were permeabilized and labeled with antibody against pan-CK

conjugated to phycoerythrin to identify epithelial cells. Leucocytes were identified by anti-CD45 antibody. CTCs were analyzed by immunofluorescence and by light microscope and quantified by 8 mL of blood. PFS curves were made by Kaplan Meier method and the differences between curves were analyzed by log-rank. **Results:** There were included 16 patients treated with FOLFOX or FOLFORI and bevacizumab. The median age was 63.5 years (30-81). The majority of patients was men (62.5%) and included at stage IV (68.7%). The PFS after the treatment was observed by image exams and showed a media of 6.14 months (0.79-8.55 months). The median CTCs numbers detected in these patients were 23.5 CTCs/8 mL at baseline. Patients with lowest levels of CTCs (above the median) showed worse PFS (4.15 months) in relation to those with higher levels of CTCs (7.78 months, $p = 0.037$). The same was true for the CTCs counts in the first follow-up (4.20 x 7.73 months, $p = 0.047$, respectively). **Conclusion:** Although our CTCs counts seems conflicting, the lowest counts found in patients with worst PFS can be explained by the inhibition of tumor angiogenesis by bevacizumab, which may lead to hypoxia, invasive cell behavior and epithelial mesenchymal transition (EMT), as postulated by Gazzaniga et al. (2011). As the method used was based on epithelial markers, it is possible that these patients with poorest PFS were under EMT. The expression of EMT and endothelial cells markers in CTCs filtered on ISET are under investigation in our lab.

REFERENCES

1. Jemal A et al. Global cancer statistics. CA Cancer J Clin 2011;61:134.
2. P. Gazzaniga et al. Circulating tumor cells, colon cancer and bevacizumab: the meaning of zero. Annals Oncol 2011;22:1930-31.

#57
TITLE: BIAS OF CYTOKERATIN-BASED CIRCULATING TUMOR CELLS COUNTING: A CASE REPORT

Authors: Chinen LTD[1], Rocha BMM[1], Carvalho FM[2], Aguiar CM[1], Abdallah EA[1], Campanha D[1], Dettino LAL[1], Mingues NB[1], Oliveira TB[1], Soares FA[2], Fanelli MF[1]

Authors' Information:

[1] Department of Clinical Oncology, A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:

Chinen, LTD. E-mail: ltdchinen@gmail.com
Rocha, BMM. E-mail: bruna.malagoli@hotmail.com
Carvalho, FM. E-mail: femcarvalho@yahoo.com.br
Aguiar, CM. E-mail: carol_motta_aguiar@hotmail.com
Abdallah, EA. E-mail: emne22@gmail.com
Campanha, D. E-mail: dancanbr@yahoo.com.br
Dettino, LAL. E-mail: aldolad@hotmail.com
Mingues, NB. E-mail: nattalia.breve@hotmail.com
Oliveira, TB. E-mail: thiagobueno32@yahoo.com.br
Soares, FA. E-mail: fasoares@me.com
Fanelli, MF. E-mail: mffanelli@gmail.com

Background: Circulating tumor cells (CTCs) have been reported to be an important prognostic biomarker in metastatic patients. However, their clinical use and impact is still under debate. **Objective:** Comparatively assess two CTC detection methods according to the patient's clinical follow up. **Methods:** CTC counting and characterization were performed during follow up in a patient with metastatic undifferentiated non-small cell lung cancer by using cytokeratin-dependent immunomagnetic separation (Miltenyi) and ISET (isolation by size of tumor cells; Rarecells). **Results:** Blood samples were collected before chemotherapy. Miltenyi kit showed a total of 214 CTCs/8 ml with high expression of CK; the ISET showed 588 CTCs/8 mL. Carboplatin and paclitaxel treatment were initiated. A CT scan performed after the third cycle of chemotherapy revealed progression of disease to liver (multiple nodules) and retroperitoneal lymph nodes. Miltenyi kit showed 5 CTCs/8 ml with heterogeneous expression of CK while the ISET approach showed 1024 CTC in 8 ml, consistent with the radiologic progression. The pattern of CKs expression observed could be explained by a partial or incomplete epithelial-mesenchymal transition (EMT) process. Immunocytochemistry for N-Cadherin was performed and a weak staining was observed in the majority of CTCs isolated by ISET pointing out the presence of a CTC population with mesenchymal features. We decided to look for Vimentin in the first and second blood collection in double-staining with pan CK. Vimentin was highly expressed in the majority of CTCs from the first collection and moderately expressed in all CTCs isolated from the second time. Pan CK expression, an epithelial marker, was negative in CTC isolated by ISET (first and second blood samples). After three cycles of ineffective chemotherapy, the patient´s disease gradually progressed and she was initiated on palliative second-line chemotherapy. This evolution was in agreement with CTC results obtained by ISET (increased number and EMT), but not with CTC results obtained by cytokeratin-based CTC isolation. **Conclusion:** These findings strongly suggest that a relevant number of CTC were in EMT and detected by ISET but not by the method based on CK expression. The method which only identifies epithelial cells does not allow to assess the effect of the therapy and brings bias.

#58

TITLE: ANALYSIS OF CANCER STEM CELL MARKERS IN CIRCULATING TUMOR CELLS OF PATIENTS WITH OVARIAN CANCER

Authors: Rocha BMM[1], Sanches SM[1], Campanha D[1], Abdallah EA[1], Ocea LMM[1], Mingues NB[1], Guimarães APG[1], Baiocchi G[3], Soares FA[2], Fanelli MF[1], Chinen LTD[1]

Authors' Information:

[1] Department of Clinical Oncology, A.C. Camargo Cancer Center, São Paulo, Brazil

[2] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

[3] Department of Gynecology, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:

Rocha, BMM. E-mail: bruna.malagoli@hotmail.com
Sanches, SM. E-mail: solange.sanches797@gmail.com
Campanha, D. E-mail: dancanbr@yahoo.com.br
Abdallah, EA. E-mail: emne22@gmail.com
Ocea, LMM. E-mail: enf_lumend@yahoo.com.br
Mingues, NB. E-mail: nattalia.breve@hotmail.com
Guimarães, APG. E-mail: andreapgg@gmail.com
Baiocchi, G. E-mail: glbaiocchi@yahoo.com.br
Soares, FA. E-mail: fasoares@me.comi
Fanelli, MF. E-mail: mffanelli@gmail.com
Chinen, LTD. E-mail: ludmillatdchinen@hotmail.com

Background: Ovarian cancer is the sixth most common tumor in women and the tenth leading cause of cancer death worldwide. Despite the primary responsiveness to therapy, this disease has a high recurrence rate due to selection or acquisition of chemoresistance by residual cells (Visvader JE et al., 2008). In this context, circulating tumor cells (CTCs) have been considered an important biomarker for dissemination and progression of cancer disease. Trying to explain the process of initiation and progression of cancer, there is one model that establishes the existence of more undifferentiated cells, capable of self-propagating, differentiating itself from other cells and the ability to recapitulate the original tumor *in vivo* and *in vitro*. They are called tumor stem cells (CSCs) (Burgos-Ojeda D et al., 2012). **Objectives:** Search for markers of CSCs in CTCs isolated from patient's blood sample with recurrent ovarian cancer. **Methods:** We included 12 patients at the time of disease recurrence. Blood was collected before the beginning of chemotherapy and after 60 days. Isolation and characterization of CTCs from a patient blood sample were performed by ISET™ (Isolation by Size of Epithelial Tumor Cells; RareCell Diagnostics, Paris, France). We performed double staining immunohistochemistry (CD44/C-kit; CD44/ALDH1) in isolated CTTs searching for CSCs's markers. **Results:** All patients were treated with carboplatin before the recurrence. Of these, 41.7% had CTCs expressing CD44/c-Kit or CD44/ALDH1, markers of CSCs, which are described in the literature as a cell type responsible for chemoresistance (Foster R et al., 2012). By each marker analysis, 91.7% of patients showed expression of CD44, 16.7% of c-Kit and 33.3% of ALDH1. No CK7 expression was observed in any CTC isolated from any patient. The median of CTCs in the first blood sample was 23 cells (maximum 300, minimum 2), while this value was 8.5 cells in the second collection (maximum 252, minimum 0). All patients are being followed up: 75% are in treatment, 16.7% are without treatment and 8.3% progressed to death. **Conclusion:** Isolation and analysis of CTCs, responsible for tumor dissemination, and CSCs, necessary for the maintenance of tumor growth, gives higher emphasis to metastatic cascade. CSC properties may be related to subsequent recurrence and chemoresistance presented by all patients in this study. It seems that CTTs and CTCs are not necessarily separate populations of cancer cells.

REFERENCES

1. Burgos-Ojeda D et al. Ovarian cancer stem cell markers: prognostic and therapeutic implications. Cancer Lett 2012;322:1-7.
2. Foster R et al. Ovarian cancer stem cells: working towards the root of stemness. Cancer Lett 2012.
3. Visvader JE et al. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nature Reviews Cancer 2008;8:755-68.

#59

TITLE: EXPRESSION OF HER2 AND TOPOISOMERASE IIα (TOP2A) IN PENILE SQUAMOUS CELL CARCINOMA

Authors: Alice Muglia Thomaz da Silva[1], Isabela W. Cunha[2], Gustavo C. Guimarães[3], Fernando A. Soares[1,2]

Authors' Information:

[1] International Center for Research and Education - CIPE, Hospital A.C. Camargo, São Paulo, SP, Brazil.

[2] Departments of Anatomic Pathology.

[3] Pelvic Surgery, Hospital A.C. Camargo, São Paulo, SP, Brazil.

Corresponding Author:

Alice Muglia Thomaz da Silva E-mail:alicemuglia@hotmail.com

Background: Penile carcinoma (PC) is a rare tumor; however, in developing regions its incidence is alarming. Presence of lymph node metastasis (LNM) is the most important prognostic factor, and molecular markers to

predict it have been pursued. HER2, member of EGFR family, has its overexpression associated with poor prognosis and presence of LNM in breast tumors, and it is generally co-amplified with a neighboring gene on chromosome 17, topoisomerase IIα (TOP2A), a target for anthracyclines. **Material And Methods:** Immunohistochemistry was performed in 143 slides from patients with PC. HER2 was classified as positive or negative according to the presence of cytoplasmic staining. TOP2a expression was automatedly evaluated by ScanScope XT, Aperio. Percentage of stained nuclei was quantified and considered positive cases showing nuclear staining in more than 25% of tumor cells. Comparison between categorical variables was performed by the Pearson chi-square or Fisher's exact test. Survival rates were calculated using the Kaplan-Meier method and the curves were compared by log-rank test. In all statistical tests, the alpha error was set at 5%. **Results:** Nuclear positivity for TOP2a was heterogeneous and seen in 74.2% (106) cases, whereas increased expression (> 25% of positive nuclei) represented 51.8% (58) of cases. Cytoplasmic (and no membrane) expression of HER2 was observed in 20.3% (29) of cases. Expression of both markers was associated with high histological grade (p = 0.047 and p = 0.004, respectively). HPV infection was present in 20.8% of the sample, among which 51.3% were infected by HPV 16 only, 21.6% by HPV 18 only, and 2.7% by both types concomitantly. HPV and TOP2a expression had no association with survival (p = 0.723 and p = 0.119, respectively). On the other hand, HER2 expression had a negative impact (p = 0.009). A marginal statistical association was observed between the expression of TOP2a and HER2 (p = 0.055), but not of these two markers with HPV infection (p = 0.535 and p = 0.546, respectively). **Conclusions:** Expression TOP2a and HER2 are associated with higher histological grade, a known bad prognostic indicator in PC. Marginal association of the expression of these two markers might suggest that they are coamplified and, therefore, investigations regarding chromosome 17 status might be useful. Cytoplasmic expression of HER2 seems to have an impact on patients' prognosis, probably dimmerizing with its partner EGFR.

Financial Support: Capes and FAPESP.

#60

TITLE: EPITHELIAL-MESENCHYMAL TRANSITION IN CERVIX CARCINOMA: DOWNREGULATION OF E-CADHERIN IN INVASION FRONT

Authors: Priscila Samara Saran BSc[1], Silvia Vanessa Lourenço PhD[2], Cláudia Malheiros Coutinho-Camillo PhD[1], Fernando Augusto Soares MD PhD[1,2]

Authors' Information:

[1] Department of Anatomic Pathology, Hospital A.C. Camargo, São Paulo - SP. Brazil.

[2] Department of General Pathology, Dental School, University of São Paulo, São Paulo - SP. Brazil.

Corresponding Author:

Priscila Samara Saran. Hospital A.C. Camargo. Centro Internacional de Pesquisa e Ensino. Rua Taguá, nº 440 São Paulo - SP. Brazil. CEP: 01508-010. E-mail: prisamara1@yahoo.com.br

Background: Squamous cell carcinoma of the cervix is a common gynecological malignancy, being the second most common neoplasm in Brazilian women. Its pathogenesis is partially regulated by adhesion molecules and metalloproteinases that participate in the process of tumor cell motility towards invasion in adjacent tissues. This process also involves a phenotypic cell conversion known as epithelial mesenchymal transition (EMT), which is characterized by loss of E-cadherin and expression of other molecules that help the mechanisms of tumor secondary growth. **Objectives:** To evaluate the occurrence of EMT in the invasion front of the squamous cell carcinoma of the cervix. **Methods:** The expression of E-cadherin and Ki-67 was evaluated by double labeling immunohistochemistry in 80 cases. The results were qualitatively analyzed using a conventional optical microscope, considering the positivity patterns and intensity of staining. **Results:** The expression of E-cadherin was decreased at the front of invasion. In these areas only a small number of cells expressed Ki67; in the center of tumor islands most cells were positive for Ki-67 and expressed E-cadherin in a strong membrane pattern. **Conclusion:** E-cadherin loss seems to be an important event in areas of invasion front of squamous cell carcinoma of the cervix. The Institutional Ethics Committee approved this study (Protocol number 1468/10) and it was supported by FAPESP grants 11/03670-1 and 11/18483-2.

#61

TITLE: RADICAL PROSTATECTOMY AND POSITIVE SURGICAL MARGINS: TUMOR VOLUME AND GLEASON SCORE PREDICTS CANCER OUTCOME.

Authors: Ricardo L. R. F. de La Roca[1], Francisco P. da Fonseca[2], Isabela W. Cunha[3], Stephania M. Bezerra[3]

Authors' Information:

[1] Oncology, Fundação Antonio Prudente - Hospital do Câncer A. C. Camargo, São Paulo - SP, Brazil.

[2] Division of Urology, Department of Pelvic Surgery.

[3] Department of Pathology, Post-graduation Course in Sciences.

Corresponding Author:
Ricardo L. R. Felts de la Roca. Al. Lorena, 131, cjs 85/87 São Paulo - SP. Brazil. E-mail: Ricardo@delarocaurologia.com.br
Francisco Paulo da Fonseca E-mail: fpf@uol.com.br
Isabela Werneck da Cunha E-mail: iwerneck@gmail.com
Stephania Martins Bezerra E-mail: stephaniab@gmail.com

Introduction: Positive surgical margins (PSMs) are common adverse factors to predict the outcome of a patient submitted to radical prostatectomy (PR). However, not all of these men will follow with biochemical (BCR) or clinical (CR) recurrence. Relationship between PSMs with these recurrent events has to be correlated with other clinicopathological findings in order to recognize more aggressive tumors in order to recommend complementary treatment to these selected patients. **Materials and Methods:** We retrospectively reviewed the outcome of 228 patients submitted to open retropubic RP between March 1991 and June 2008, where 161 had and 67 did not have PSMs. Minimum follow-up time was considered 2 years after surgery. BCR was considered when PSA ≥ 0.2 ng/ml. CR was determined when clinical evidence of tumor appeared. Chi-square test was used to correlate clinical and pathologic variables with PSMs. The estimated 5-year risk of BCR and CR in presence of PSMs was determined using the Kaplan-Meier method and compared to log-rank tests. **Results:** From the total of 228 patients, 161 (71%) had PSMs, while 67 (29%) had negative surgical margins (NSMs). Prostatic circumferential margin was the most common (43.4%) site. Univariate analysis showed statistically significant ($p < 0.001$) associations between the presence of PSMs and BCR, but not with CR ($p = 0.06$). Among 161 patients with PSMs, 61 (37.8%) presented BCR, while 100 (62.8%) did not. Predicting progression-free survival for 5 years, BCR was correlated with pathological stage; Gleason score; pre-treatment PSA; tumor volume in specimen; capsular and perineural invasion; presence and number of PSMs. RC correlated only with angiolymphatic invasion and Gleason score. Considering univariate analyses the clinicopathological factors predicting BCR for 5 years, results statistically significant links with prostate weight; pre-treatment PSA; Gleason score; pathological stage; tumor volume; PSMs; capsular and perineural invasion. Multivariate analysis, otherwise, evidence only Gleason score and percentage of tumor volume more than 20% of total specimen volume, as significant independent predictors of BCR. **Conclusion**: In univariate analyses, presence, number and localization of PSMs have consistent correlation with BCR following RP but on follow-up BCR occurred only in 38% of our patients with PSMs. In multivariate analyses, percentage tumor volume and Gleason score in the surgical specimen were the significant risk factors for BCR. Angiolymphatic invasion and Gleason score were significantly correlated with CR.

Keywords: biochemical recurrence, positive surgical margins, prostate cancer, radical prostatectomy, tumor volume percentage.

INTRODUCTION

The finding of positive surgical margins (PSMs) after radical prostatectomy (RP) implies that the cancer was not completely resected leading the surgeon to complementary treatment that can be: active surveillance, adjuvant radiotherapy or androgen-deprivation therapy. Many studies report that a PSM represents an independent predictor of biochemical recurrence (BCR) after RP. Otherwise, these studies have also shown that most men with PSMs do not develop BCR. In multivariate analysis, specimen Gleason score, pathological stage, percentage tumor volume in the surgical specimen and PSMs were all significant risk factors for BCR[1,2]. All of these factors have been previously associated with BCR. To verify the relationship between PSMs and BCR, we retrospectively studied the outcome of a group of patients submitted to RP, for clinically localized prostate adenocarcinoma (CaP), with and without PSMs, to observe if PSMs are predictive of BCR, correlated with other clinical and pathologic findings.

MATERIALS AND METHODS

Retrospectively we studied the outcome of 228 patients submitted to open retropubic RP, intended to cure a clinically localized CaP, by the staff of the Division of Urology, Pelvic Surgery Department, A.C. Camargo Cancer Center between March 1991 and June 2008, where 161 patients with PSMs were compared to a group of 67 patients without PSMs. We excluded from this study patients who received hormonal therapy or radiotherapy before the surgery, had involvement with seminal vesicles and/or had pelvic lymph nodes or stage T4 and incomplete or missing follow-up. It was considered that all patients had harboring organ-confined disease, along with the following: their plasmatic prostate-specific antigen (PSA), digital rectal examination, trans-rectal ultrasound, computerized tomography or nuclear magnetic resonance (high risk cases) results and anatomopathological study from the prostate biopsies. We recorded patients' ages and PSA levels prior to surgery, and also the following: pathological stage, weight, Gleason score, percentage of tumor volume, perineural and angiolymphatic invasion, capsular and extra-capsular involvement, the number and site (urethral, bladder neck, prostatic circumferential, skeletal muscle) of PSMs in the surgical specimen. Surgical margins were considered positive when tumor was seen on the inked surface of the surgical specimen.

The serum PSA levels after RP were measured every 4 months for 2 years and then every 6 months for 2 more years and annually thereafter. BCR was considered when the PSA reached level ≥ 0.2 ng/ml. CR was determined when clinical evidence of tumor was seen as a metastatic disease, or when PSA rose despite radiotherapy, hormone, or chemotherapy treatments. Minimum follow-up time was considered 2 years after surgery. Chi-square test was used to correlate clinical and pathologic variables with PSMs. The estimated 5-year risk of BCR and CR in presence of PSMs, was determined using the Kaplan-Meier method and compared to log-rank tests. All statistical tests were performed with $p < 0.05$ considered to indicate statistical significance with the aim of R free statistical software (www.r-project.org).

RESULTS

Follow-up of ≥ 5 years was available for 93 patients, and ≤ 5 years for 135 patients with 2 years being established as the minimum follow-up time after surgery (median follow-up 6.2 years). The mean age of patients was 64.5 years old, pre-treatment PSA plasmatic level ranging from 1.3 ng/ml to 78.8 ng/ml (median 8.47 ng/ml).

Prostate specimen was weighed ranging from 10 to 167 g (mean 44.77 g), and tumor volume was estimated in the specimen ranging from 0.5% to 100% of total prostate volume (mean 12.82%). Prostate capsule invasion (focal and extra capsular) was present in 123 cases and not in 105 cases, perineural invasion was present in 162 cases and not in 66 cases, angiolymphatic invasion was present in 18 cases and not in 210 cases.

Patients younger than 50 and older than 70 years old, showed higher incidence of PSMs. PSA pre-treatment ≥ 10 ng/ml, specimen Gleason score ≥ 7, pathological stage ≥ T2b, tumor volume ≥ 10% from the total volume of the specimen, capsular and perineural invasion when present, showed statistically significant associations with the occurrence of PSMs. Inversely, the weight of the prostate when ≤ than 60 g was more correlated with PSMs. From the total of 228 patients,161 (71%) had PSMs, while 67 (29%) had negative surgical margins (NSMs). From these with PSMs, 106 cases (46%) showed one margin, 44 (19%) two margins, and 11(5%) three margins. The prostatic circumferential site of margin was the most common site (43.4%) followed by the prostatic + urethral (apical) (14.9%), urethral (apical) (13.6%), and bladder neck (6.2%).

BCR occurred in 68 patients (30%), and not in 160 (70%), and clinical recurrence (CR) occurred in 10 (4%) and not in 218 (96%).

Univariate analysis showed statistically significant ($p < 0.001$) associations between the presence of PSMs and BCR, but not with CR ($p = 0.06$). BCR was seen in 68 patients, were those with NSMs corresponded to 7 (10.5%) cases, while PSMs were present in 61 (89.5%) cases.

Among 161 patients with PSMs, 61 (37.8%) presented BCR, while 100 (62.8%) did not. COX univariate analyses of several clinicopathological factors predicting progression-free survival BCR and CR at 5 years following RP correlated BCR progression-free survival with pathological stage, Gleason score, pre-treatment PSA, tumor volume in specimen, capsular and perineural invasion, presence of PSMs and number of PSMs. (Table 1).

Interestingly, the progression of free survival time for RC was correlated only with angiolymphatic invasion and Gleason score.

In univariate analyses, almost all predictors for BCR as prostate weight; pre-treatment PSA; Gleason score; pathological stage; tumor volume; PSMs; capsular involvement; and perineural invasion were statistically significant.

Multivariate analyses have correlated only Gleason score and tumor volume as statistically significant independent predictors of BCR. (Figure 1).

DISCUSSION

In contemporary series, PSMs are reported in 11-38% of patients undergoing RP. Multiples can be the prognostic factors pointed to the presence of PSMs ranging from clinicopathological factors to surgeons expertise and surgical techniques.

Our results correlate younger and elder age, pre-treatment PSA higher than 10 ng/ml, specimen Gleason score ≥ 7, smaller glands with weight ≤ 40 g, pathologic stage ≥ pT2b, percentage of tumor volume greater than 10% of surgical specimen, capsular and perineural invasion with the occurrence of PSMs in concordance with several authors[2] PSMs point a greater risk of biochemical progression but not all patients will suffer BCR. In our study, only 61 patients (38%) out of 161 patients with PSMs presented BCR, but similarly to literature, various PSMs sites, bladder neck and prostatic circumferential sites lead to worse outcomes, otherwise in our NSMs cases, BCR was only seen in 7 patients (10.5%).

It is extremely difficult to predict a PSM outcome, and as patients with PSMs are at greater risk of progression, the ability to stratify this risk needs to improve, along with other factors that may affect disease progression and survival Focal capsular or extensive extra-capsular involvement, were both correlated in our study with BCR. Other studies found that men with PSMs and no extra-capsular spread had a lower rate of recurrence than men with extra-capsular disease, but this was contradicted by the SEARCH database study group, who found that men with PSMs and no extra-capsular spread had a similar recurrence risk to those with extra-capsular disease regardless of margin status[3].

Table 1. Univariate and multivariate analyses of clinical and pathologic factors predictors of BCR at 5 years.

Characteristic		Univariate				Multivariate			
	n	RR	IC(95%)		Pr(> |z|)	RR	IC(95%)		Pr(> |z|)
Prostate weight	228	0.98	0.96	0.99	0.013	0.99	0.97	1.01	0.187
Patient age	228	0.99	0.96	1.03	0.737	0.99	0.95	1.02	0.489
Pre-operative PSA	228	1.039	1.021	1.056	0.0008	1.018	0.992	1.044	0.18
Gleason score					7.9E-05				0.005
≤ 6	124	1.00				1.00			
7	71	1.88	1.07	3.31	0.029	1.37	0.75	2.50	0.301
≥ 8	33	4.02	2.14	7.54	1.5E-05	3.13	1.57	6.25	0.001
Tumor Volume (%)	228	1.02	1.01	1.03	3.2E-05	1.02	1.00	1.03	0.022
Margins					0.003				0.653
negatives	67	1.00				1.00			
positives	161	3.51	1.51	8.13	0.003	1.47	0.27	7.96	0.653
Pathological stage					0.005				0.759
≤ *p* T2a	68	1.00				1.00			
≥ *p* T2b	160	3.09	1.41	6.77	0.005	0.78	0.16	3.81	0.759
Perineural invasion					0.009				0.533
no	67	1.00				1.00			
yes	151	3.24	1.47	7.12	0.003	1.62	0.67	3.90	0.284
extense	10	4.60	1.34	15.72	0.015	1.92	0.49	7.48	0.346
Capsular invasion					0.002				0.279
no	105	1.00				1.00			
yes focal	82	2.42	1.30	4.49	0.005	1.76	0.86	3.61	0.124
extracapsular	41	3.23	1.64	6.38	0.001	1.76	0.78	3.98	0.173
Angiolimphatic invasion					0.540				0.666
no	210	1.00				1.00			
yes	18	1.30	0.56	3.03	0.540	0.82	0.33	2.04	0.666

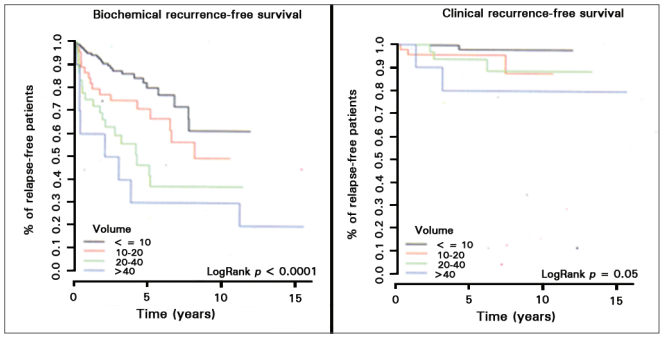

Figure 1. Probability of biochemical recurrence-free and clinical progression-free survival at 5-10 years according to percentage tumor volume in the specimen

Prostate weight, pre-treatment PSA, Gleason score, pathological stage, tumor volume, PSMs, capsular and perineural invasion in an univariate analysis were correlated with BCR in our results, in total concordance with the literature, but at multivariate analyses only Gleason score and tumor volume were statistically significant independent predictors of BCR.

Gleason score reflects tumor aggressiveness, whereas cancer volume illustrates the extent of the lesion, so it can be hypothesized that high-grade cancer volume and percentage of high-grade cancer simultaneously reflect cancer invasion spread ability and their impact on outcome. Our results support other authors[4,5] conclusions as that high-grade cancer volume had the highest impact on recurrence-free survival in patients with surgically treated, pathologically organ-confined CaP or that prostate volume has prognostic value in pathologic T2 radical prostatectomy specimens thus pointing the importance of the percentage of tumor volume in the surgical specimen, rather than the presence of PSMs, as important predictor of BCR.

CONCLUSIONS

BCR occurred only in 38% of patients with PSMs. Globally RC was very rare, as it represented 4% of the total number of cases (PSMs and NSMs). In multivariate analyses, percentage tumor volume and Gleason

score in the surgical specimen were the independent significant prognostic risk factors for BCR, rather than the presence of PSMs, confirming the important value of these two pathological factors. Otherwise, angiolymphatic invasion and Gleason score were significantly correlated only with CR.

REFERENCES

1. Karakiewicz PI, Eastham JA, Graefen M et al.: Prognostic impact of positive surgical margins in surgically treated prostate cancer: multi-institutional assessment of 5831 patients. Urology 2005;66:1245-50
2. D'Amico AV, Whittington R, Malkowicz SB et al.: A multivariate analysis of clinical and pathological factors that predict for prostate specific antigen failure after radical prostatectomy for prostate cancer. J Urol.1995;154:131-8
3. Palisaar RJ, Graefen M, Karakiewicz PI, Hammerer PG, Huland E, Haese A, Fernandez S, Erbersdobler A, Henke RP, Huland H: Assessment of clinical and pathologic characteristics predisposing to disease recurrence following radical prostatectomy in men with pathologically organ-confined prostate cancer. Eur Urol. 2002Feb;41(2):155-61
4. Thompson IM 3rd, Salem S, Chang SS, Clark PE, Davis R, Herrell SD, Kordan Y, Baumgartner R, Phillips S, Smith JA Jr, Cookson MS, Barocas DA: Tumor volume as a predictor of adverse pathologic features and biochemical recurrence (BCR) in radical prostatectomy specimens: a tale of two methods. World J Urol. 2011Feb;29(1):15-20.
5. Cho IC, Kwon WA, Kim JE, Joung JY, Seo HK, Chung J, Park WS, Lee KH: Prostate volume has prognostic value only in pathologic T2 radical prostatectomy specimens. J Korean Med Sci. 2011Jun;26(6):807-13

#62
TITLE: PAR-4 AND BCL-2 EXPRESSION IN SALIVARY GLAND TUMORS

Authors: da Silva GM[1], Saavedra V[1], Lourenço SV[2], Ianez RF[1], Nagai MA[3], Kowalski LP[4], Soares FA[5], Cláudia Malheiros Coutinho-Camillo[1]

Authors' Information:

[1] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.

[3] Discipline of Oncology, Department of Radiology and Oncology, Medical School, University of São Paulo - SP, Brazil.

[4] Department of Otorhinolaryngology and Head and Neck Surgery, A.C. Camargo Cancer Center, São Paulo, Brazil.

[5] Department of Anatomic Pathology, A.C. Camargo Cancer Center, São Paulo and Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.

Corresponding Author:

da Silva GM. E-mail: gabi_madu@hotmail.com
Saavedra, V. E-mail: vick-saave@hotmail.com
Lourenço, SV. E-mail: silvialourenco@usp.br
Ianez, RF. E-mail: rere_fraga@hotmail.com
Nagai, MA. E-mail: nagai@usp.br
Kowalski, LP. E-mail: lp_kowalski@uol.com.br
Soares, FA. E-mail: soaresfernando@terra.com.br
Cláudia Malheiros Coutinho-Camillo. E-mail: claumcc@terra.com.br

Background: Pleomorphic adenoma is the most common benign tumor of major salivary glands. It shows a marked histological diversity with epithelial, myoepithelial, and mesenchymal components in a variety of patterns. Mucoepidermoid carcinoma is the most common malignant, locally invasive tumor of the salivary glands, especially of the parotid gland. Apoptosis is a genetically programmed form of cell death and aberrations of the apoptotic mechanisms that cause excessive or deficient programmed cell death have been linked to a wide array of pathologic conditions. **Objectives:** To characterize the expression of PAR-4 and Bcl-2 proteins and determine possible associations between the expression of these proteins and clinicopathologic features of salivary gland tumors. **Material and Methods:** Fifty cases of mucoepidermoid carcinoma and 50 cases of pleomorphic adenoma were analyzed by immunohistochemistry and the results were semi-quantitatively analyzed, considering the patterns and intensity of staining. **Results:** PAR-4 and Bcl-2 proteins were present in mucoepidermoid % of relapse-free patients and pleomorphic adenoma samples studied. Forty-eight out of 49 cases of pleomorphic adenoma (98.0%) showed positive nuclear and/or cytoplasmic staining of PAR-4 and 1 case (2.0%) showed negative staining. Nuclear positivity was observed in plasmocytoid cells and areas presenting squamous metaplasia also showed cytoplasmic staining. All 49 cases of mucoepidermoid carcinoma (100.0%) showed positive nuclear and/or cytoplasmic staining of PAR-4. Nuclear positivity was observed predominantly in intermediate cells and cytoplasmic/nuclear staining was observed in mucous and squamous cells. Thirty-three out of 50 cases of pleomorphic adenoma (66.0%) showed positive cytoplasmic staining of Bcl-2 and 17 cases (34.0%) showed negative staining. Positivity was observed predominantly near the tumor capsule and in plasmocytoid cells. Twenty out of 49 cases of mucoepidermoid carcinoma (40.8%) showed positive cytoplasmic staining of Bcl-2 and 29 cases (59.2%)

showed negative staining. Positivity was focally distributed. **Conclusions:** Our study provides evidence of apoptotic signaling: pleomorphic adenoma showed predominantly the expression of both Bcl-2 and PAR-4 proteins, which may act in the control of benign growth and in mucoepidermoid carcinomas proapoptotic mechanisms may be active; however, other proliferative mechanisms appear to be more important.

Funding Support: FFAPESP research grant 11/02051-6.

#64

TITLE: MMP16 POLYMORPHISM AS A POSSIBLE MARKER OF AGGRESSIVENESS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Authors: Frederico Omar Gleber-Netto[1], Dias THG[2,3], Nunes DN[3], Dias-Neto E[4]

Authors' Information:

[1] Laboratório de Genômica Médica, A.C. Camargo Cancer Center. São Paulo, Brazil.

[2] Laboratório de Neurociências (LIM-27), Instituto de Psiquiatria, Faculdade de Medicina da Universidade de São Paulo. São Paulo, Brazil.

[3] Head and Neck Genome Project (GENCAPO). Site: www.gencapo.famerp.br

Corresponding Author:

Frederico Omar Gleber-Netto E-mail: nettofog@hotmail.com
Dias THG. E-mail: thaishgd@gmail.com/etajara@hotmail.com
Nunes DN. E-mail: dianann@gmail.com
Dias-Neto E. E-mail: emmanuel@usp.br

Background: Nodal metastasis is the main prognostic factor for head and neck squamous cell carcinoma (HNSCC) patients. Nevertheless, the clinical diagnosis of this manifestation is limited using the current technology. Considering this, novel methods to diagnose and predict nodal metastasis (NM) are needed to improve patient management. **Objective:** We investigated possible associations of single nucleotide polymorphisms (SNP) in matrix metalloproteases (MMPs) genes and NM in HNSCC patients, aiming to determine new molecular markers of aggressiveness. **Study design:** Leukocyte DNA was extracted of HNSCC patients and SNPs more likely to have functional impact were evaluated using TaqMan assays in an ABI7500. A total of four genes/SNPs (MMP14/rs1042703; MMP16/rs2616490; MMP25/rs10431961; and TIMP-3/rs1065314) were investigated in 268 patients samples. SPSS 17.0 was used for statistical analysis. **Results:** The rs2616490 polymorphism in the MMP16 gene was significantly associated with nodal metastasis in oral cancer patients (p = 0.035) and with early development of nodal metastasis in laryngeal cancer patients (p = 0.038). **Conclusion:** The polymorphism in the MMP16 gene seems play an important role in HNSCC dissemination and might be a possible marker of nodal metastasis for oral cavity and larynx cancer patients.

Financial Support: FAPESP (2010/51168-0; 2011/13315-4)

#65

TITLE: EFFECT OF MELATONIN ON ANGIOGENESIS IN BREAST CANCER MICE MODEL

Authors: Jardim BV[1,2], Ferreira LC[1,2], Varma NR[3], Borin TF[1,4], Moschetta MG[1,4], Gelaleti GB[1,2], Leonel C[1,2], Maschio LB[1,2], Gonçalves NN[1,4], Lopes JR[1,2], Martins GR[1,4], Arbab AS[3], Zuccari DAPC[1,4]

Authors' Information:

[1] Laboratório de Investigação Molecular do Câncer, Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, SP, Brazil.

[2] Universidade Estadual Paulista (UNESP/IBILCE), São José do Rio Preto - SP, Brazil.

[3] Cellular and Molecular Imaging Laboratory, Department of Radiology, Henry Ford Hospital, Detroit, MI 48202, USA.

[4] Faculdade de Medicina de São José doRio Preto (FAMERP), São José do Rio Preto, SP, Brazil.

Corresponding Author:

Jardim, BV. E-mail: brunavj@hotmail.com
Ferreira, LC. E-mail: livinha_ferreira@hotmail.com
Varma, NR. E-mail: ravin@rad.hfh.edu
Borin, T.F. E-mail: thaiz80@yahoo.com.br
Moschetta, MG. E-mail: mary_na15@hotmail.com
Gelaleti, GB. E-mail: gabi_b_g@yahoo.com.br
Leonel, C. E-mail: camilaleonels_1@hotmail.com
Maschio, LB. E-mail: larissa_maschio@hotmail.com
Gonçalves, NN. E-mail: naiane_rp@yahoo.com.br
Lopes, JR. E-mail: jurlp@hotmail.com
Martins, GR. E-mail: grmartins@live.com
Arbab, AS. E-mail: saali@rad.hfh.edu
Zuccari, DAPC. E-mail: debora.zuccari@famerp.br

Background: Once a tumor lesion exceeds a few millimeters in diameter, hypoxia triggers a cascade of events to allow neovascularization and tumor progression (Weis; Cheresh, 2011). As neovascularization is essential for tumor growth and metastasis, controlling tumor-associated angiogenesis is a promising tactic in limiting cancer progression (ARBAB, 2012). Melatonin has been studied for their inhibitory properties on angiogenesis in the cancer (PARK et. al., 2010). **Objective:** In this study we evaluate the effects of melatonin treatment on tumor growth and angiogenesis in breast cancer mice model. **Methods:** MDA-MB-231 breast cancer cell line was cultured and cell viability was measured

by MTT assay after incubation with different concentrations of melatonin. We performed an *in vivo* study where cells were implanted in the mammary gland or flank of athymic nude mice. Mice were treated with 1mg of melatonin or vehicle daily, administered intraperitoneally 1 hour before room lighting was switched off. Tumors were measured weekly with a digital caliper and 20 proteins involved in angiogenesis were evaluated in mammary tumor tissues by Human Cytokine Antibody Array. **Results:** Melatonin *in vitro* treatment was able to significantly decrease cell viability. The breast cancer xenografts of nude mice treated with melatonin showed significantly smaller tumor after 21 days ($p < 0.05$). The mean tumor volume of control and treated animals were 282.00 ± 88.53 mm^3 and 144.90 ± 38.38 mm^3, respectively. The mean of tumor volume in control animals increased significantly from day 14 (118.90 ± 40.17 mm^3) to day 21 (282.00 ± 88.53 mm^3), which was not observed in the treated group ($p < 0.05$). Furthermore, there was tumor regression in an animal treated with melatonin (Day 7 = 27.38 mm^3; 8.79 mm^3 = Day 14, Day 21 = 4.8 mm^3), similar pattern was not seen in any of the control mouse. Semiquantitative densitometry analysis of membrane array indicated increased expression of epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 (IGF-I) in treated tumors compared to vehicle tumors ($p < 0.05$). **Conclusion:** Melatonin was able to reduce breast cancer cell viability *in vitro*. The treatment reduced tumor development in mice, showing to be effective against tumor growth. However, melatonin increased the expression of some growth factors, suggesting that melatonin´s anti-tumor action does not involve directly inhibition of these growth factors.

Financial Support: FAPESP

REFERENCES

1. Arbab AS. Activation of alternative pathways of angiogenesis and involvement of stem cells following anti-angiogenesis treatment in glioma. Histol Histopathol. 2012;27:5
2. Park SY et al. Melatonin suppresses tumor angiogenesis by inhibiting HIF-1α stabilization under hypoxia. J Pineal Res. 2010;48
3. Weis SM et al. Tumor angiogenesis: molecular pathways and therapeutic targets. Nat Med. 2011;17:11

#66

TITLE: GENOME-WIDE DNA METHYLATION PROFILING OF LEUKOCYTES REVEALS A DISTINCT PATTERN IN MELANOMA-PRONE PATIENTS HARBORING CDKN2A MUTATIONS

Authors: Araújo ESS[1], Vieira H[2], Brentani H[3], Achatz MIW[4], Moredo LF[5], Sá BCS5, Duprat JP[5], Carraro DM[1], Krepischi ACV[1]

Authors' Information:

[1] CIPE, A.C. Camargo Hospital, São Paulo, Brazil.
[2] Institute of Mathematics and Statistics, University of São Paulo, Brazil.
[3] Department and Institute of Psychiatry, Medical School, University of São Paulo, São Paulo, Brazil.
[4] Departament of Oncogenetics, A.C. Camargo Hospital, São Paulo, Brazil.
[5] Department of Skin Cancer, A.C. Camargo Hospital, São Paulo, Brazil.

Corresponding Author:

Érica Sara Souza de Araújo. E-mail: erica.sara.araujo@gmail.com
Henrique Vieira. E-mail: enrikevieira@gmail.com
Helena Brentani. E-mail: helena.brentani@gmail.com
Maria I. W. Achatz. E-mail: misabel.achatz@uol.com.br
Luciana F. Moredo. E-mail: lufacure@gmail.com
Bianca C. S. Sá. E-mail: bianca.sa@terra.com.br
João P. Duprat. E-mail: joaopduprat@gmail.com
Dirce M. Carraro. E-mail: dirce.carraro@accamargo.org.br
Ana C.V. Krepischi. E-mail: ana.krepischi@gmail.com

Introduction: Melanoma is a highly aggressive tumor accounting for 75% of skin cancer deaths. Up to 40% of the hereditary melanoma cases are due to germline mutations in the CDKN2A gene. In melanoma development, melanocytes transformation is mediated by genetic and epigenetic mutations; currently, no aberrant DNA methylation was associated with melanoma predisposition. **Objective:** Investigating if the presence of a germline CDKN2A mutation could induce genome-wide DNA methylation changes in melanoma patients harboring these mutations. **Material and Methods:** We analyzed the leukocyte methylomes of 9 melanoma-prone patients carrying CDKN2A mutations and compared with data from 9 controls paired by sex and age. Genome-wide DNA methylation profiling was obtained using the Infinium HumanMethylation450 BeadChip platform (Illumina), which interrogates methylation levels of ~450,000 CpG dinucleotides distributed across the genome. Differential methylation analysis was performed using the GenomeStudio software. **Results and Discussion:** In the CDKN2A-mutated group, 412 differentially methylated CpGs were found, 256 of them affecting 225 RefSeq genes. Notably, the VTRNA2-1 gene, a non-coding RNA, exhibited a pattern of hypomethylation in 10 CpGs mapped at the promoter region. Recently, VTRNA2-1 was characterized as a tumor suppressor gene whose DNA methylation pattern is related to outcome in acute myeloid leukemia patients (Treppendahl, et al., 2012). **Conclusion:** The group of melanoma-prone patients carrying CDKN2A mutations

showed a distinctive epigenomic profile compared to controls. These epimutations may be due to the presence of a constitutive CDKN2A mutation, and could possibly impact the disease. Further studies are necessary to elucidate the role of epimutations in melanoma patients, notably the hypomethylation pattern of VTRNA2-1.

Financial Support: CAPES and FAPESP

REFERENCE

1. Treppendahl MB et al. Allelic methylation levels of the noncoding VTRNA2-1 located on chromosome 5q31.1 predict outcome in AML. Blood 2012; 119:1.

#67

TITLE: DIFFERENTIAL EXPRESSION PROFILE OF PAPILLARY THYROID CARCINOMA HARBORING *BRAFV600E* MUTATION

Authors: MC Barros Filho[1], FA Marchi[2], CA Pinto[1], SR Rogatto[1,3], LP Kowalski[1]

Authors' Information:

[1] International Research Center/A.C. Camargo Hospital, São Paulo, Brazil.

[2] São Paulo University, São Paulo, Brazil

[3] Faculty of Medicine/UNESP, Botucatu - SP. Brazil.

Corresponding Author:

Mateus de Camargo Barros Filho. E-mail: mateuscbarros@gmail.com

International Research Center/A.C. Camargo Hospital, Taguá Street 440, São Paulo. Brazil.

MC Barros Filho. E-mail: mateuscbarros@gmail.com

FA Marchi. E-mail: fmarchibr@hotmail.com

CA Pinto. E-mail: calopes@accamargo.org.br

SR Rogatto. E-mail: rogatto@fmb.unesp.br

LP Kowalski. E-mail: lp_kowalski@uol.com.br

Background: The most prevalent genetic alterations in papillary thyroid carcinoma (PTC) leads to MAPK signaling pathway activation, including point mutations in BRAF and RAS and RET rearrangements (RET/PTC). BRAF mutation (BRAFV600E) is found in 35-70% of PTC and is frequently associated with tumor aggressiveness. **Objectives:** Identify transcripts and molecular pathways according to BRAF mutation status and compare the findings with clinical data. **Methods:** Mutation status of BRAFV600E was evaluated in 231 PTCs by pyrosequencing. All cases were also tested for RET/PTC inversion by RT-qPCR. Sixty-one tumors were evaluated by oligoarray expression using Sure Print G3 8 x 60K slides (Agilent Technologies). Multi-group Significance Analysis of Microarray (SAM) was applied according to BRAFV600E allele frequency. The results were compared with GEO Database (two PTC studies with BRAF mutation status were available). Networks and functional analysis were generated through IPA software (Ingenuity® Systems). Ten transcripts were further assessed by RT-qPCR in 50 samples previously analyzed by microarray and in 25 independent samples. **Results:** More than 15% of BRAF mutated alleles were detected in 129 out of 231 cases (55.8%). The alteration was associated with classic variant of PTC. Large-scale expression analysis using unsupervised hierarchical clustering revealed a distinct expression profile of PTC presenting high frequency of BRAFV600E alleles (> 30%) or RET/PTC inversion. The supervised analysis (SAM multi-class) revealed 400 transcripts (FDR 0%). Fifty-eight transcripts were also identified using public microarray data. Cell-to-cell signaling and interaction was the most important molecular network, and neuregulin signaling was the major canonical pathway. Down-regulation of ZMAT, HGD and ELMO1 and up-regulation of GGCT, PROS1, PDLIM4, ERBB3 and RIMS2 in BRAFV600E tumors were confirmed by RT-qPCR in both dependent and independent validations sets. **Conclusion:** A distinct expression profile was observed according to the percentage of BRAF mutated alleles or RET/PTC inversion. Cases BRAF mutated presented a signature of genes associated with neuregulin signaling pathway. In overall, these findings could be useful to better stratify these patients and provide additional support for identification of therapeutic targets that can be relevant in clinical practice.

#68

TITLE: A CUSTOMIZED APPROACH FOR SCREENING GERMLINE MUTATIONS IN *BRCA1* GENE IN BREAST CANCER PATIENTS BY NEXT GENERATION SEQUENCES IN 454-GS JUNIOR PLATFORM

Authors: Rocha CAP[1], Ferreira EN[1], Figueiredo MCP[1], and Carraro DM[1,2]

Authors' Information:

[1] Laboratory of Genomics and Molecular Biology. International Center for Research, A.C. Camargo Cancer Center, SP, Brazil.

[2] National Institute of Science and Technology in Oncogenomics (INCITO), São Paulo, SP, Brazil.

Corresponding Author:

Rocha CAP. E-mail: camila.aviler.rocha@usp.br

Introduction: Germline mutations in the tumor suppressor genes BRCA1 and BRCA2 is associated with a high risk of breast cancer onset. The identification of individuals

carrying mutations in these genes is extremely important for the clinical management of the patient and the family relatives. However, due to the high cost of complete sequencing of these genes, the molecular screening is limited to a small number of patients. In this sense, the next generation sequencers represent a valuable approach, allowing the analysis of large genomic regions of different samples in parallel. In this study we propose to establish a customized approach for complete sequencing the BRCA1 gene by parallel sequencing in GS Junior® platform (454-Roche). **Methods:** Eleven breast cancer triple negative patients, previously screened for BRCA1 mutations by capillary sequencing (Carraro et al., 2013), were selected for this study. All exons of BRCA1 gene were amplified by PCR. The amplicons of each patient were pooled, coupled to specific adapters containing 6 nucleotide barcodes and sequenced in GS Junior platform. **Results:** A total of 118,918 sequences from 6 samples were generated. After removal of low quality and polyclonal sequences, 47,830 reads remained. Next, the sequences of each patient were identified by the barcodes and then aligned to the genomic sequence of BRCA1 gene. We observed a heterogeneous coverage of exons, where some exons, such as 8, 9, 11, 12, 19 and 20 were deeply coverage (more than 50 reads) and others (exons 13, 16, 17 and 24) were covered by only few reads. This fact could be a consequence of using a wide range of amplicons size (200pb to 600pb) in the same sequencing run. Finally, we compared the sequences generated from GS Junior and capillary sequencing. From the 5 patients previously reported to contain a genomic alteration in BRCA1 gene, 2 were confirmed: a single nucleotide change leading to a missense alteration (1186 A > G in sample 2003) and one insertion leading to in-frame mutation (5385 insC in sample 2017). The other 3 patients did not presented coverage in the altered region. No alteration was observed in the wild-type patient. **Conclusion:** The customized approach for sequencing BRCA1 gene in GS Junior platform was validated and both single nucleotide alterations and indels we confirmed. We are improving the approach by sequencing PCR fragments of similar size (from 200bp to 350pb) and expect to obtain homogeneous coverage for all exons.

Financial Support: CNPq

REFERENCES

1. Carraro DM, Ferreira EN, de Campos Molina G, Puga RD, Abrantes EF, Trapé AP, Ekhardt BL, Nunes DN, Brentani MM, Arap W, Pasqualini R, Brentani H, Dias-Neto E, Brentani RR. Poly (A)+ transcriptome assessment of ERBB2-induced alterations in breast cell lines. PLoS One 2011, 6: e21022.

#69

TITLE: GERMLINE MUTATIONS IN THE PREDISPOSITION TO CUTANEOUS MELANOMA: INVESTIGATING *CDKN2A* MICRODELETIONS AND SCREENING OF THE *MITF* VARIANT E318K

Authors: Talita Aguiar[1], Érica S. S. Araújo[1], Maria I. W. Achatz[2], Luciana F. Moredo[3], Bianca C. S. Sá[3], João P. Duprat[3], Dirce M. Carraro[1], Ana C.V. Krepischi[1]

Authors' Information:
1 CIPE, A.C. Camargo Hospital, São Paulo, Brazil
2 Departament of Oncogenetics, A.C. Camargo Hospital, São Paulo, Brazil
3 Departament of Oncogenetics, A.C. Camargo Hospital, São Paulo, Brazil

Corresponding Author:
tata_fmaguiar@hotmail.com

Melanoma is an aggressive tumor that accounts for 75% of the deaths by skin cancer, and the majority of the cases are sporadic; however, some individuals in the population have a high predisposition to develop melanoma due to the presence of a constitutive mutation in their genomes. There are two major genes exhibiting high penetrant mutations in cutaneous melanoma susceptibility: CDKN2A (encoding p16 and p14 proteins), and CDK4. p16 mutations are far more common, accounting for approximately 40% of familial cases, and also by multiple melanomas, whereas CDK4 and p14 mutations were detected in a few families. Therefore, many cases of high predisposition to melanoma, as the negative point mutations in these two major genes, have no clear etiology of the disease. Other types of germline genomic alterations, as CDKN2A deletions, may predispose to melanoma, and are not always detected by conventional sequencing. A recent study detected a 4q13 submicroscopic duplication segregating with the disease in a family; this duplication contains a group of genes previously linked to melanoma, including CXCL1 and IL8. Additionally, independent studies identified a germline mutation in the gene MITF predisposing to familial melanoma, as well as the sporadic melanoma and renal cell carcinoma. MITF is a known oncogene specific to melanomas, and the variant identified would be a predisposing allele conferring intermediate risk. We propose to investigate these rare factors of genetic predisposition to melanoma (deletions and duplications of CDKN2A, and the MITF variant E318K) in a cohort of Brazilian patients from families with a diagnosis of hereditary melanoma, negative for mutations in key genes such as CDKN2A and CDK4. Twenty-five controls were screening of the MITF variant E318K, and one heterozygous carrier was detected.

#70

TITLE: GENE EXPRESSION PROFILES ASSOCIATED TO ORAL SQUAMOUS CELL CARCINOMA DEVELOPMENT

Authors: Oliveira-Costa JP[1], Carvalho AF[2], Buim MEC[3], Kowalski LP[4], Carraro DM[2], Soares FA[5], Ribeiro-Silva A[1].

Authors' Information:

[1] Department of Pathology and Forensic Medicine, Ribeirao Preto School of Medicine, University of São Paulo, Brazil and Department of Anatomic Pathology, A.C. Camargo Cancer Center, Brazil.

[2] Laboratory of Genetics and Molecular Biology, A.C. Camargo Cancer Center, Brazil.

[3] University Nove de Julho, Brazil.

[4] Department of Head and Neck Surgery and Otorhinolaryngology, A.C. Camargo Cancer Center.

[5] Department of Anatomic Pathology, A.C. Camargo Cancer Center, Brazil.

Corresponding Author:

João Paulo Oliveira-Costa (Presenting author). E-mail: oliveiracostajp@me.com

Alex Fiorini de Carvalho. E-mail: alexficar@gmail.com

Marcilei Eliza Chavichiolli Buim. E-mail: mebuim@hotmail.com

Luiz Paulo Kowalski. E-mail: lp_kowalski@uol.com.br

Dirce Maria Carraro. E-mail: dirce.carraro@cipe.accamargo.org.br

Fernando Augusto Soares. E-mail: soaresfernando@terra.com.br

Alfredo Ribeiro-Silva. E-mail: arsilva@fmrp.usp.br

Introduction: Oral squamous cell carcinoma (OSCC) development is still a poorly understood phenomenon, in terms of gene expression profiles which might be involved in alterations leading to high invasiveness and aggressive behavior. Also, in terms of tumor size, it is still unclear how gene expression changes during OSCC development. **Methods:** Using 36 samples of OSCC cases, we performed cDNA and lincRNA microarrays, comprising whole human genome and more than 7000 lincRNAs, in order to identify gene profiles with similar behavior throughout T1 to T4 tumor staging. Also, 10 differentially expressed genes were tested individually using qRT-PCR. **Results:** We found 7 different gene expression profiles, either with a rise in expression levels (as seen in Figure 1), or decrease in expression levels, between T1, T2, T3 and T4 TNM staging. The seven different gene expression profiles showed 58 differentially expressed genes, including an increased expression of immune response-related family genes, and decreased expression of zinc finger proteins. In RT-PCR experiments, we found significantly correlations between CD274 (PD-L1) and tumor size (p = 0.043), lymphatic invasion (p = 0.047) and metastatic lymph node (p = 0.001); BLNK and perineural invasion (p = 0.021); ABL2 and tumor size (p = 0.039); HOXB9 and tumor size (p = 0.026); and between ZNF813 and tumor size (p = 0.039). **Conclusion:** Our study shows the existence of several genes with similar expression patterns during OSCC development, and also points to novel genes, which has not yet been implicated in oral carcinogenesis, providing new potential targets in oral cancer research.

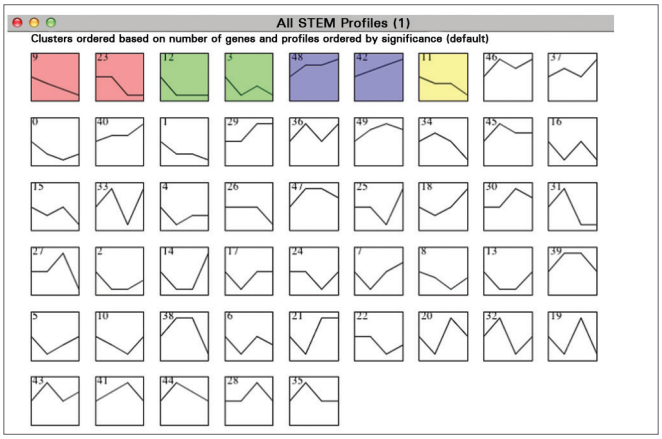

Figure 1. Gene expression profiles with similar behavior through T1 to T4 stages.

Funding Support: FAPESP (2010/08400-0 and 2010/08637-0).

#71

TITLE: EVALUATION OF THE RELATIONSHIP BETWEEN E-CADHERIN EXPRESSION AND PROLIFERATIVE ACTIVITY AT THE INVASIVE FRONT OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Authors: Juliano Jampietro[1], Yukie Sato-Kuwabara[1], José Humberto H.T.G. Fregnani[2], Felipe Coimbra[1], Fernando Augusto Soares[1]

Authors' Information:

[1] A.C. Camargo Câncer Center, São Paulo, Brazil.

[2] Hospital do Câncer Barretos, Barretos, Brazil.

Introduction: Esophageal squamous cell carcinoma (ESCC) has a poor prognosis mainly because it is usually in an advanced stage at the time of diagnosis. The epithelium-mesenchyme transition (EMT), when epithelial cells loss their original characteristics and acquire mesenchymal phenotype, is indicated first by the loss of E-cadherin expression. The EMT is considered the main event in tumor progression and metastasis. *In vitro* studies have demonstrated that E-cadherin downregulation can lead to cell proliferation. This finding has not been evaluated in human tumors. This study aimed to elucidate whether tumor cells that have undergone EMT at the invasive front of the ESCC are in proliferative state. **Material and Methods:** Samples of 58 ESCC cases

were double stained by immunohistochemistry (IHQ) for E-cadherin (BD Bioscience) and Ki67 (ROCHE) antibodies using the Automated System Ventana BenchMark XT (ROCHE). IHC analysis was performed using Aperio ScanScope XT (APERIO) with Color Deconvolution algorithm. In each case, E-cadherin and Ki67 expression were evaluated in the tumor core and at the invasive front separately. Positivity was divided into strong, moderate and weak positive according to intensity staining. For each case a score was given based on the formula with the percentages of each positivity group [Score = 1x (% Weak) + 2x (% Moderate) + 3x (% Strong)]. **Results and Discussion:** Analysis of the IHQ double-stain for E-cadherin showed that 51 (87.9%) cases had a high expression at the tumor core when compared with invasive front and in 7 (12.1%) cases were found high expression at the invasion front ($p < 0.001$). The median values for E-cadherin double-stain scores were 184.2 and 163.0 at invasive front and tumor core, respectively ($p < 0.001$). Analyses of the Ki67 double-stain showed that 19 (32.7%) cases showed a high expression at tumor core and 39 (67.3%) cases showed a high expression at the invasive front ($p < 0.001$). Proliferation rates, represented by Ki67 expression, showed median values of 8.1 and 6.8 at the invasive front and tumor core, respectively ($p = 0.052$). Spearman correlation between the IHQ double and single-stain was moderately positive ($p < 0.001$). **Conclusion:** Tumor cells at the invasive front showed an increased proliferation rate as they lose the E-cadherin expression and at the tumor core showed a higher expression of E-cadherin and a lower expression of Ki67. However, some cases showed a different behavior with a higher expression of E-cadherin at the invasive front and an elevated rate proliferation at tumor core. A more complex mechanism in the dynamics of E-cadherin and Ki67 expressions could be acting during EMT. Further studies are necessary to the better understanding of the complexity of this mechanism.

#72
TITLE: QUANTITATIVE ANALYSIS OF THE EXPRESSION OF 165 AND 165_{xxx}B ISOFORMS OF VEGF IN BREAST CANCER BY QPCR

Authors: Castro R[1], Biselli-Chicote PM[1], Castro TB[1], Regiani VR[1], Neto DS[2], Francisco JLE[3], Pavarino EC[1], Zuccari DAPC[4], Goloni-Bertollo EM[1]

Authors' Information:

[1] Research Unit in Genetics and Molecular Biology, Faculty of Medicine of São José do Rio Preto, São Paulo, Brazil.

[2] Pathology Service, Hospital de Base, São Jose do Rio Preto, São Paulo, Brazil.

[3] Department of Gynecology and Obstetrics, Hospital de Base, São José do Rio Preto, São Paulo, Brazil.

[4] Research Laboratory in Molecular Cancer, Faculty of Medicine of São José do Rio Preto, São Paulo, Brazil.

Corresponding Author:

Castro, TB. E-mail: E-mail: tialfi_@hotmail.com

Biselli-Chicote, PM. E-mail: patriciabiselli@famerp.br

Regiani, VR. E-mail: vregiani@yahoo.com.br

Neto, DS. E-mail: dalisius@bol.com.br

Francisco, JLE. E-mail: joseluiz.fancisco@hospitaldebase.com.br

Pavarino, EC. E-mail: erika@fmaerp.br

Zuccari, DAPC. E-mail: E-mail: debora.zuccari@famerp.br

Goloni-Bertollo, EM. E-mail: eny.goloni@famerp.br

Background: Cancer is the second leading cause of death in the world after cardiovascular diseases and breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide, accounting for 23% of the total new cancer cases and 14% of the total cancer deaths in 2008. About half breast cancer cases and 60% of the deaths are estimated to occur in economically developing countries. Angiogenesis plays a critical role in local growth of solid tumors and subsequently in the process of distant spread. Numerous studies have demonstrated the importance of angiogenesis in cancer. Nevertheless, 3' alternative splice site selection in exon 8 of VEGF gene results in a sister family of isoforms, $VEGF_{xxx}b$, which are anti-angiogenic and downregulated in tumor tissues. **Objectives:** To evaluate the differences in the expression of isoforms 165 and 165b of the VEGF gene in breast tumor tissue compared to normal breast tissue. **Methodology:** We quantitatively analyzed the expression of pro-angiogenic and anti-angiogenic VEGF isoforms in breast carcinoma and adjacent normal tissue samples. For that purpose, total RNA from 40 tumor samples and their respective margins were obtained and synthesized cDNA from. We designed and synthesized primers and specific probes for each isoform, which were used for the analyses of expression by real time PCR. **Results:** Positive correlation was observed between the expression values of VEGF165 and $VEGF165_{xxx}b$ in breast tumors ($p < 0.001$), but the gene expression $VEGF165_{xxx}b$, it was observed that a relative increase over normal samples showed no statistical significance of diverging several authors in the literature. A bigger sample size might help in more advanced studies and collaborate to better development of researches on tumor angiogenesis involving VEGF gene. Studies approaching control of VEGF splicing in order to promote the selection of the distal splicing site (anti-angiogenic $165_{xxx}b$) instead of proximal site (pro-angiogenic 165) might promote an efficient therapy for breast cancer.

Financial Support: Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP

REFERENCES

1. Hilmi C, Guyot M, Pagès G. VEGF spliced variants: possible role of anti-angiogenesis therapy. J Nucleic Acids. 2012;
2. Catena et al.: VEGF121b and VEGF165b are weakly angiogenic isoforms of VEGF-A. Molecular Cancer. 2010 9:320.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011 Mar-Apr; 61(2):69-90.

#73

TITLE: BIOBANKING PRACTICE: LOW CONCENTRATION OF STORED RNA AFFECTS INTEGRITY

Authors: Olivieri EHR[1], Franco LA[2], Pereira RG[2], Mota LDC[1], Campos AHJFM[3], Carraro DM[1,2,4]

Authors' Information:

[1] DNA & RNA Bank, A.C. Camargo Biobank (ACCB), A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] Laboratory of Genomics and Molecular Biology, A.C. Camargo Cancer Center, São Paulo, Brazil.

[3] Tumor Bank, A.C. Camargo Biobank (ACCB), A.C. Camargo Cancer Center, São Paulo, Brazil.

[4] National Institute of Science and Technology in Oncogenomics (INCITO), São Paulo, SP, Brazil.

Corresponding Author:

Eloisa Helena Ribeiro Olivieri. E-mail: eloisa.ribeiro@accamargo.org.br

Luana de Andrade Franco. E-mail: luana.biology@gmail.com

Rafael Gomes Pereira. E-mail: rafarmabio@hotmail.com

Louise Danielle de Carvalho Mota. E-mail: louise.mota@accamargo.org.br

Antônio Hugo J. F. M. Campos. E-mail: ahcampos@accamargo.org.br

Dirce Maria Carraro. E-mail: dirce.carraro@cipe.accamargo.org.br

Background: Biobanking plays an important role in translational cancer research[1-3]. The impact of tissue and RNA storage is not well documented. The storage temperature and period of time the tissues and purified RNA aliquots are frozen can directly impact RNA preservation. **Methods:** Here, we assessed the RNA integrity of frozen tissues that were stored at -140°C for distinct time intervals of up to seven years and the preservation of RNA stored at -80°C when diluted at either 250 ng/µl or 25 ng/µl for 4 years with repeated freezing and thawing. Additionally, we also generated a profile of the total RNA collection of the A.C. Camargo Cancer Center Biobank. The RIN (RNA integrity number) was used to evaluate RNA quality. **Results:** The integrity of the RNA aliquots stored at a dilution of 250 ng/µl and at -80°C was preserved throughout the different time intervals. However, statistically significant differences in degradation were observed in RNA stored at a dilution of 25 ng/µl after only eight months of storage at -80°C. We also observed that ovary and stomach samples had the greatest RNA degradation compared with the total RNA integrity of tissues of distinct topographies. **Conclusions:** Our results showed that both the temperature of preservation and the concentration of the RNA aliquot should be strictly controlled by the biobank staff involved in macromolecule purification. Moreover, we showed that in general, the A.C. Camargo Cancer Center Biobank maintains a high quality RNA collection (average RNA integrity number (RIN) algorithm above 7 for most topographies). This result demonstrates that these samples will be useful for gene expression analysis by virtue of adherence to optimal standard operating procedures for both tissue and macromolecule laboratories.

REFERENCES

1. Riegman PH et al. Biobanking for better healthcare. Mol Oncol 2008;2:213-222.
2. Barnes RO et al. Influence of evolution in tumor biobanking on the interpretation of translational research. Cancer Epidemiol Biomarkers Prev 2008;17:3344-3350.
3. Campos AHJFM et al. The Value of a Tumor Bank in the Development of Cancer Research in Brazil: 13 Years of Experience at the A.C. Camargo Hospital. Biopreservation and Biobanking 2012;168-173.

#74

TITLE: POLO-LIKE KINASE 1 AS NEW THERAPEUTIC TARGET FOR MEDULLOBLASTOMA

Authors: Pezuk JA[1], Manzini PMR[1], Oliveira JC[1], Morales AG[1], Queiroz RP[2], Suazo VK[2], Scrideli CA[2], Valera ET[2], Brassesco MS[2], Tone, LG[2]

Authors' Information:

[1] Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil.

[2] Department of Pediatrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil.

Corresponding Author:

Julia Alejandra Pezuk. E-mail: juliapezuk@usp.br

Avenida Bandeirantes, nº 3900, Monte Alegre. Ribeirão Preto São Paulo - SP. Brazil.

Manzini PMR. E-mail: primanzini@yahoo.com.br

Oliveira JC. E-mail: jaqbiomed@yahoo.com.br

Morales AG. E-mail: dremorales@yahoo.com.br

Queiroz RP. E-mail: rosane@fmrp.usp.br

Suazo VK. E-mail: veridiana@fmrp.usp.br

Scrideli CA. E-mail: cascrideli @hcrp.fmrp.usp.br

Valera ET. E-mail: elvisvalera@hotmail.com

Brassesco MS. E-mail: solbrassesco@usp.br

Tone LG. E-mail: lgtone@fmrp.usp.br

Purpose: Medulloblastoma (MB) is the most common embryonic tumor of the central nervous system in childhood. Even though survival rates attain over 50%, treatment causes functional and cognitive sequels on patients; thus, alternative treatments are still needed. Polo-like kinase 1 (PLK1) is a serine-threonine kinase involved in cell cycle progression that has already been associated with cell proliferation and tumor prognosis. The aim of this study was to evaluate the effects PLK1 inhibition by several specific inhibitors on medulloblastoma cell lines. **Methods:** We tested and compared the *in vitro* antitumor activities of four different Polo-like kinase 1 inhibitors (PLK1) (BI 2536, BI 6727, GW843682X and GSK461364), against UW402, UW473 and ONS-76 MB cell lines. Cells were treated with different concentrations for 24, 48 and 72 hours. Proliferation, colony formation capacity, apoptosis and cell cycle dynamics assays were performed and results analyzed by one-way ANOVA. **Results:** The inhibition of PLK1 with BI 2536, BI 6727, GSK461364 and GW843682X showed an efficient decrease ($p < 0.05$) in cell proliferation and cell renewal. Moreover, cell cycle analysis demonstrated G2/M arrest, along with increased number of cells in mitosis ($p < 0.05$) for all drugs with a corresponding increase in apoptosis rates ($p < 0.05$). **Conclusion:** Even though the four drugs tested showed anticancer activity, GSK461364 was more efficient. These results emphasize the potential of PLK1 inhibition improve medulloblastoma outcome and point to GSK461364 as a strong candidate for future therapeutic intervention.

Financial Support: FAPESP (2011/01026-8 & 2011/11287-3).

#75
TITLE: NUCLEAR UNPHOSPHORYLATED STAT3 IS OVEREXPRESSED IN GLIOBLASTOMA MULTIFORME AND CORRELATES WITH POOR PROGNOSIS

Authors: Rodrigues BR[1], Queiroz NGT[1], Roffé M[1], Lopes MH[2], Cunha IW[1], Martins VM[1]

Authors' Information:

[1] A.C. Camargo Cancer Center, Centro Internacional de Pesquisa, Instituto Nacional de Oncogenômica and Instituto Nacional de Neurociência Translacional, São Paulo.

[2] Departamento de Biologia Celular e do Desenvolvimento, Instituto de Ciências Biomédicas, Universidade de São Paulo.

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor. Patients diagnosed with GBM have a poor prognosis and therapeutic approaches are very limited in these tumors. Aberrant phosphorylation of signal transducer and activator of transcription 3 (STAT3) on a single tyrosine (705) promotes oncogenic transformation in a variety of tissues. Recently, roles for unphosphorylated STAT3 have been described in many tumors, but its role in GBM is still limited. The aim of this study was to evaluate, the expression and cellular distribution of STAT3 and its phosphorylated forms pSTAT3 (Tyr705) and pSTAT3 (Ser727) in GBMs compared to gliomas of grade II and III and non-neoplastic tissue. Immunohistochemistry experiments were performed for the total and phosphorylated forms of STAT3 in tissue microarrays (TMA) containing 85 GBM, 35 gliomas grade II, 12 gliomas grade III and 14 non-neoplastic brain tissue. The expression of total STAT3 was higher in GBM than any other tumor or non-tumor samples ($p < 0.0001$). In addition, nuclear STAT3 is also higher in GBM ($p < 0.0001$). STAT3 phosphorylation is associated with the protein translocation to the nucleus; however, any alteration in the phosphorylated forms of STAT3 was observed when GBM samples were compared to other gliomas or non-neoplastic tissues. Thus, indicating that higher levels of nuclear STAT3 are not related with its phosphorylation. Remarkably, a direct correlation was found between GBM samples positive for nuclear STAT3 and tumor recurrence ($p = 0.0004$, Pearson Chi Square) and also with a shorter overall survival in these patients ($p = 0.0195$). Therefore, the nuclear translocation of unphosphorylated STAT3 deserves further studies since it may represent relevant therapeutic targets for GBM.

Financial Suport: Supported by FAPESP and National Institute of Translational Oncogenomics and National Institute of Translational Neurosciences.

REFERENCES

1. Yang J et al. Novel Roles of Unphosphorylated STAT3 in Oncogenesis and Transcriptional Regulation. Cancer Res 2005;65:939-947
2. De la Iglesia, N et al. STAT3 regulation of glioblastoma pathogenesis. Curr Mol Med. 2009;5:580-90

#76
TITLE: HOMOLOGOUS RECOMBINATION MARKERS IN MCF-7 CELLS BMI-1 POSITIVE AND SILENCED

Authors: Giórgia Gobbi da Silveira[1], Celes MRN[2], Camillo CMC[3], Soares FA[3], Rossi MA[1], Ribeiro-Silva A[1]

Authors' Information:

[1] University of São Paulo, Ribeirão Preto, Brazil.

[2] Federal University of Goias, Goiania, Brazil.

[3] A.C. Camargo Cancer Hospital, São Paulo, Brazil.

Corresponding Author:
Giórgia Gobbi da Silveira (presenting author). E-mail: giorgia.silveira@gmail.com
Celes MRN. E-mail: rubia.celes@gmail.com
Camillo CMC. E-mail: claumcc@terra.com.br
Soares FA. E-mail: soaresfernando@terra.com.br
Rossi MA (in memorian)
Ribeiro-Silva A. E-mail: arsilva@fmrp.usp.br

Introduction: Homologous recombination (HR) is one of double-strand break (DSB) repair pathways. During HR, a sequence lost in a double-stranded DNA is repaired by a physical exchange by the same sequence of a second copy of the DNA. Errors during homologous recombination are among the causes of breast cancer in patients with mutations in the genes BRCA1 and BRCA2, also associated to failures in coordinating the response to DNA damage, possibly influenced by the loss of p53 function. BRCA1 and BRCA2, associated with the RAD51 protein, accumulates in DNA damage foci after signaling H2AX, a DNA damage marker that accumulates in lesion foci, associated to ATM/ATR pathway, leading to DNA repair. Topoisomerase IIIβ (TopIIIβ) removes HR intermediates before the segregation of chromosomes, preventing damage to the structure of the cellular DNA. BMI -1 is a Polycomb group protein which is able to induce telomerase activity, enabling the immortalization of epithelial cells. Immortalized cells have shown to be more susceptible to double-strand breaks. The role of proteins involved in HR, in breast carcinomas positive for BMI-1, remains to be investigated. Thus, our objective was to evaluate the relationship between BMI-1 and regulatory proteins of homologous recombination. **Methods:** We cultivated MCF-7 cells, which constitutively express the BMI-1. From this culture, two groups was assembled, the BMI-1 positive and silenced BMI-1, both in triplicate. For the analysis of gene expression was used qRT-PCR assays, in order to analyze the expression of genes associated with HR (BRCA1 and 2, ATM, ATR, p53, TopIIIβ, RAD51 and H2AX) in the presence and absence of BMI-1. **Results:** Real-Time PCR assay showed that BMI positive cells have high expression of BRCA1 and 2, ATM, ATR, p53, TopIIIβ, RAD51 and H2AX compared to silenced cell. **Conclusion:** Our results suggest higher damage and higher presence of HR proteins in BMI-1 positive cells, thus indicating that HR might be an important pathway in BMI-1 positive breast carcinomas.

Financial Support: FAPESP (2011/23042-5), FAPESP (2011/22849-2)

#77

TITLE: RISK FACTORS FOR TOLERANCE IN BIOCHEMOTHERAPY AND INTERLEKIN-2 IN HIGH DOSE TREATMENT OF METASTATIC MELANOMA IN ICU

Authors: Emele VCR[1], Medeiros P[1], Caruso P[1]

Authors' Information:
[1] Hospital A. C. Camargo. São Paulo - SP. Brazil.

Corresponding Author:
Valéria Cristina Rodrigues Emele. E-mail: valeria_emele@yahoo.com.br
Pedro Medeiros Jr. E-mail: pedromedjr@yahoo.com
Pedro Caruso. E-mail: pedcaruso@gmail.com

Background: The incidence of melanoma continues to rise gradually and remains a malignancy with high mortality, disproportionately affecting young adults. Since the start of the usage of Dacarbazine, many therapeutic options have been studied as other treatment alternatives in an attempt to achieve better results, including better disease-free survival and better survival rates. The treatment with biochemotherapy and interleukin-2 in high doses (HD IL-2) are currently used and have been widely studied and modified to improve the results and also to manage and control the toxicity, side effects and complications resulting from these treatments. We have noted that these effects are severe and lead some patients to not tolerate the treatment proposed, which may impact the results. The objectives of this study are to investigate the risk factors for tolerance to a cycle of HD IL-2 and biochemotherapy and to describe the toxicity, side effects, complications and survival to treatment with HD IL-2 and biochemotherapy. This retrospective study included 31 patients on 111 treatment cycles with the gathering of demographic and clinical data. **Results:** The treatment with biochemotherapy is better tolerated compared to the treatment with HD IL-2. The toxicity associated with treatment with HD IL-2 comprises high elevation of C-reactive Protein, mild renal toxicity, grade-3 hepatic toxicity and hematologic toxicity with grade-3 leucopenia and thrombocytopenia. The toxicity associated with treatment with biochemotherapy includes grade-3 liver toxicity, grade 3-4 hematologic toxicity, in which leucopenia was prevalent. Fever was the most prevalent and severe side effect in the treatment with biochemotherapy, with nausea in grade 1-2, vomiting, loss of appetite, itching and diarrhea reaching grades 3 to 4 for both treatments. Complications such as vasoactive drugs, need for oxygen and weight gain were

prevalent in the treatment with interleukin-2. The median survival was 28.9 ± 3.7 for HD IL-2, 8.4 ± 1.5 for biochemotherapy and 10.6 ± 2.8 for patients who underwent both treatments. **Conclusions:** Out of all the factors associated with tolerance to the treatment, the only significant was the treatment with HD IL-2. Hematologic toxicities, hepatic and gastroenterology were prevalent in the two types of treatments, as well as fever and itch. There was predominance of complications in treatment with HD IL-2. The median survival was higher in the treatment with HD IL-2

#79

TITLE: LYMPHANGIOGENESIS IN ORAL SQUAMOUS CELL CARCINOMA AND ASSOCIATION WITH CLINICOPATHOLOGICAL CHARACTERISTICS

Authors: Elen Alves de Sousa[1], Lourenço SV[2], Kowalski LP[3], Soares FA[4], Coutinho-Camillo CM[1]

Authors' Information:

[1] Department of Anatomic Pathology, Hospital A.C Camargo, São Paulo - SP. Brazil.

[2] Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.

[3] Department of Otorhinolaryngology and Head and Neck Surgery, Hospital A.C. Camargo, São Paulo, Brazil.

[4] Department of Anatomic Pathology, Hospital A.C. Camargo, São Paulo and Department of General Pathology, Dental School, University of São Paulo, São Paulo, Brazil.

Corresponding Author:

Elen Alves de Sousa E-mail: elenpkn@yahoo.com.br
Lourenço, SV. E-mail: silvialourenco.70@terra.com.br
Kowalski, LP. E-mail: lp_kowalski@uol.com.br
Soares, FA. E-mail: soaresfernando@terra.com.br
Coutinho-Camillo CM. E-mail: claumcc@terra.com.br

Background: Head and neck carcinoma is the fifth most prevalent cancer worldwide and squamous cell carcinoma (SCC) constitutes at least 90% of all oral malignancies. Many clinical and pathological factors have been associated with local recurrence of oral carcinoma, such as disease stage, lymph node metastasis and perineural invasion. Lymph node involvement is the strongest prognostic factor for survival of patients with carcinoma of the head and neck. Lymphangiogenesis is the growth of new lymphatic vessels. However, the initial mechanism of lymphatic spread is not yet established. **Objectives:** To investigate the process of lymphangiogenesis in oral squamous cell carcinoma samples and associate the expression of VEGF-C and VEGFR3 proteins with clinicopathological characteristics of the tumors. **Material and Methods:** Fifty-three tumors and 26 positive lymph nodes (from 22 out of the 53 cases) were analyzed by immunohistochemistry using VEGF-C and VEGFR3. The results were analyzed semi-quantitatively using conventional optical microscopy, considering the pattern and intensity of staining. **Results:** VEGF-C was expressed in a well-established cytoplasmic pattern in the central part of the tumor. There is loss of protein expression in areas of invasion front. Positive VEGF-C expression was observed in 21 out of 53 cases (39.6%) and in 6 out of 26 lymph nodes (23.0%) and no expression was observed in 32 out of 53 cases (60.4%) and 20 out of the 26 lymph nodes (77.0%). VEGFR3 protein was predominantly expressed in keratinocytes in central islands of tumor, with positive microvessels in areas of inflammatory infiltrate in lymph node parenchyma. Positive expression of VEGFR3 was observed in 9 out of 48 cases (18.7%) and eight out of 26 lymph nodes (30.8%) and loss of expression was observed in 39 out of 48 cases (81.3%) and 18 out of the 26 lymph nodes (69.2%). VEGFR3 positivity in peritumoral microvessels was observed in 67 samples (42 cases and 25 lymph nodes), predominantly in samples that present negative expression of this protein. **Conclusions:** Our study provides evidence of mechanisms of lymphangiogenesis signaling in oral squamous cell carcinoma.

Financial Support: CAPES

#80

TITLE: CHROMOSOMAL IMBALANCES DETECTED BY ARRAY CGH REVEALED A NEW POTENTIAL CANDIDATE REGION ON 7P22.3 FOR HEREDITARY COLORECTAL CARCINOMA

Authors: Villacis RAR[1], Santos MMM[2], Rossi BM[3], Carraro DM[1], Kowalski LP[4], Rogatto SR[1,5]

Authors' Information:

[1] CIPE - Research Center, A.C. Camargo Hospital, Rua Taguá 440, São Paulo, SP, Brazil.

[2] Department of Pelvic Surgery, A.C. Camargo Hospital, São Paulo - SP, Brazil.

[3] Department of Oncogenetic, Barretos Cancer Hospital, Barretos, SP, Brazil.

[4] Department of Head and Neck Surgery and Otorhinolaryngology, A.C. Camargo Hospital, São Paulo, SP, Brazil.

[5] Department of Urology, Faculty of Medicine, UNESP, Botucatu, SP, Brazil.

Corresponding Author:

Villacis RAR. E-mail: andrevillacis@yahoo.com.br
Santos MMM. E-mail: erikammsantos@gmail.com
Rossi BM. E-mail: rossibm@gmail.com
Carraro DM. E-mail: dirce.carraro@accamargo.org.br
Kowalski LP. E-mail: lp_kowalski@uol.com.br
Rogatto SR. E-mail: rogatto@fmb.unesp.br

Colorectal cancer (CRC) is one of the most common tumors around the world and includes about 30% of cases with a hereditary component. The main hereditary disease of CRC is Lynch Syndrome (LS), which is caused by germline mutations in mismatch repair (MMR) genes. Approximately 50% of patients that meet the Amsterdam Criteria did not show mutations in MMR genes, suggesting the association of alterations in other genes to predisposition to CRC. We used the array-based CGH 4 x 180K platform (Agilent Technologies) to evaluate germline copy number variations (CNVs) in 58 patients with LS without pathogenic mutations in MMR genes. The Feature Extraction and the Genomic Workbench software (statistical algorithm ADM-2 and sensitivity threshold of 6.0) were used to extract the genomic data and perform the analyses, respectively. It was found 381 CNVs (6.6 ± 4.8 CNVs/individual), 170 genomic gains and 211 losses. The data were compared with three databases of genomic variants (DGV, DGVa and dbVar) and a reference Brazilian dataset of 100 healthy individuals. We identified 88 rare CNVs (detected in < 5% of reference cases or < 20 cases in other databases) in 45 cases and 20 new rare (not previously described) CNVs in 17 cases. Four rare CNVs mapped at 6p11.2, 7p22.3, 11q13.2 and 14q23.1 were found in at least four cases. In particular, chromosome 7 encompassed 19% (17/88) of all rare CNVs detected. Eight cases presented 11 CNVs mapped on 7p22.3 comprising two genes. In addition, we also evaluated 10 single nucleotide polymorphisms (SNPs) classified as high CRC risk in 50 out of 58 cases using the TaqMan SNPs genotyping assay (rs961253, rs3802842, rs4444235, rs4779584, rs4939827, rs6983267, rs9929218, rs10411210, rs10795668 and rs16892766). Eight risk alleles were found in more than 50% of the cases. Each patient presented at least four alleles of CRC risk. The results suggested that CNVs and SNPs could be involved in hereditary predisposition to CRC.

Financial Support: FAPESP, CNPq, CHIBCHA Project.

#81

TITLE: EXPRESSION OF GROWTH AND CELL PROLIFERATION FACTORS IN GASTRIC CARCINOMAS: THE ASSOCIATION WITH POOR OVERALL SURVIVAL ON PATIENTS WITH INTESTINAL TYPE

Authors: Michelle M. Barcelos Baldoni[1], Milton José de Barros e Silva[1], Wilson Luiz Costa Junior[1], Felipe José Fernandez Coimbra[1], Alessandro Landskron Diniz[1], Heber Salvador de Castro Ribeiro[1], Clovis Antonio Lopes Pinto[1], Maria D. Begnami[1]

Authors' Information:

[1] Instituição: A.C. Camargo Cancer Center.

Corresponding Author:

Michelle Maccarini Barcelos Baldoni. E-mail: michellebarcelos@hotmail.com

Coimbra, FJF. E-mail: coimbra.felipe@uol.com.br

Pinto, CAL. E-mail: coipinto@uol.com.br

Begnami, MD. E-mail: mariadirlei@gmail.com

Background: Gastric carcinomas (GC) express a variety of growth factors and cell proliferation acting in the mechanisms of tumor invasion and spread. The expression of angiogenic factors such as vascular endothelial growth factor (VEGF) has been demonstrated in diffuse type GC, whereas expression of growth factors is associated with intestinal-type carcinomas. **Objective:** To study the immunohistochemical expression of proteins associated with growth factors and cell proliferation in GC. **Methods:** We studied 400 GC, arranged in duplicates in 2 blocks of tissue microarray (TMA). Immunohistochemistry was performed using antibodies: c-MET (Novocastra), TGFbetaI (Santa Cruz), TGFbetaII (Santa Cruz), c-erbB-2 (Dako) and VEGF (Santa Cruz). Cases were considered positive for TGFbetaI, TGFbetaII and VEGF when cytoplasmic staining was observed in more than 10% of tumor cells. Positive cases for c-met and c-erbB-2 were detected when strong staining on the membrane cells was observed in more than 10% of neoplastic cells. **Results:** Expression of TGFbetaI, TGFbetaII and VEGF were detected in 314/385 (81%), 370/382 (96%) and 333/366 (90%) of GC. 333/376 (88%) cases were positive for c-met and 54/385 (14%) for c-erbB-2. Intestinal type carcinomas were more often positive for TGFbetaII ($p = 0.01$), VEGF ($p = 0.001$), c-met ($p = 0.01$), and c-erbB-2 ($p = 0.001$). According to univariate statistical analyzes expressions of TGFbeta II, c-met and c-erbB-2 were independent factors associated with overall survival of patients with GC. **Conclusions:** The proteins associated with growth factors and cell proliferations are differentially expressed in intestinal type of GC. Our findings pointed them out as biological factors associated with worse prognosis and important therapeutic targets.

#82

TITLE: EPIDERMOID CARCINOMA ARISING FROM SYRINGOMATOUS ADENOMA OF THE NIPPLE: CASE REPORT AND REVIEW OF THE LITERATURE

Authors: Floriano Rodrigues Riva Neto[1], Cavalcante JM[1], Lima LGCA[1], Andrade VP[1], Nascimento AG[1], Pinto CAL[1], Osorio CABT[1]

Authors' Information:

[1] A C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:

Cyntia Aparecido Bueno e Toledo. E-mail: cabtoledo@accamargo.org.br

Introduction: Syringomatous adenoma of the nipple (SAN) is a rare breast lesion, mimicking histological characters of skin syringoma. Primary breast squamous cell carcinoma is also a rare lesion, especially in the nipple. **Case Report:** A previously health 64-year-old woman was admitted with scaling, hardening, redness and retraction of the right nipple for the last 4 months, without discharge. Mammography displayed a nodular lesion measuring 11 x 10 x 9 mm. Percutaneous biopsy showed an infiltrative sclerosing lesion, composed of small tear-shaped, two-layered typical cuboidal cell ducts, closely related to areas that displayed extensive squamous cell differentiation with minimal atypia evolving to areas of clear well-differentiated invasive squamous cell carcinoma (sCC), indicating that sCC arose from the syringomatous adenoma of the nipple. **Discussion:** SAN is a rare breast lesion that typically presents as a nipple or subareolar mass. Average age of presentation is 40 years, admitting a broad variation. It is an infiltrative lesion composed of bland epithelial cells arranged in small angulated glandular comma-shapped structures, disposed in a solid or chordonal fashion. Squamous cell nests and cysts within a fibrous stroma are also seen. Despite its infiltrative nature, this is a benign lesion, albeit recurrent. Malignant transformation of the squamous cell component has been reported as a rare event. **Conclusion:** Despite its rarity, SAN can be associated with malignant neoplasms, as SCC. This should be a differential diagnosis of nipple lesions.

#83

TITLE: EPIDERMOID CARCINOMA ARISING FROM SYRINGOMATOUS ADENOMA OF THE NIPPLE: CASE REPORT AND REVIEW OF THE LITERATURE

Authors: Floriano Rodrigues Riva Neto[1], Cavalcante JM[2], Lima LGCA[3], Andrade VP[1], Nascimento AG[1], Pinto CAL[1], Osório CABT[1]

Authors' Information:

[1] Hospital A.C. Camargo Cancer Center - Departamento de Anatomia Patológica São Paulo - SP. Brazil.

[2] ICC Ceará. Brazil

[3] A.C. Camargo CC São Paulo - SP. Brazil.

Corresponding Author:

Floriano Rodrigues Riva Neto. E-mail: florianoriva@gmail.com
Cavalcante, JM. E-mail: jujumagalhaescavalcante@yahoo.com.br
Lima, LGCA. E-mail: luiz_calima@yahoo.com.br
Andrade, VP. E-mail: victor.andrade@accamargo.org.br
Pinto, CAL. E-mail: calopes@accamargo.org.br
Osório, CABT. E-mail: cynthiaosorio@gmail.com

Goal of the project: The early diagnosis in cancer, mostly that kind of cancer that has a way of prevention and a predictable carcinogenesis. **Historic background:** The A.C. Camargo Cancer Center Prevention Program began, in fact, as a legacy of the first notable creator of the voluntary service Carmen Prudente. Start around 2007, with employers and people from the voluntary service inviting friends and relatives to visit the hospital, watch a lecture about the most important topics about cancer prevention and after all, do some tests, a kind of annual checkup. The project was very unpretentious, but became strong with the fast growth of the institution. **The current scenario:** Around 25 thousand people are being covered every year. The lecture about prevention is given in nonprofit organizations and underserved communities. People are invited to do metabolic and cancer screening. Each oncology department has a personal attendance guideline to guide the prevention program and uniform the medical appointment. The tests in the metabolic screening are: hemogram, lipidogram, thyroid function, kidney function, hepatic function and hepatitis serology test. The cancer screening for women includes the pap, the mammography and the fecal occult blood, for men: PSA and fecal occult blood test. All the patients receive their results in a medical appointment, and this step is very important because many others complimentary tests can be indicated for each patients according their complains. In the sequence, if everything is normal, the patients receive a discharge. If they only have metabolic or cardiovascular alterations, they are forwarded to a primary and public health system. Finally if they need something more to investigate, the prevention program is able to do: prostatic, cervix and breast biopsy, colonoscopy, colposcopy, upper aero digestive endoscopy, and all kind of imaging tests. **Results:** Last year, the project covered 25 thousand people, 40% are men and 60% are women. More than 50% are over 40 years old. The cancer rate was 0.6%. A total of 150 cases (44 cases of urologic cancer, 38 cases of breast cancer, 16 cases of head and neck cancer, 16 cases of colorectal cancer, 15 cases of skin cancer, 10 cases of gyn cancer, 9 digestive cancer, 1 lung and 1 sarcoma).

The next challenges: Organize a real and effective database, reducing bias of selection and cover more people.

#84

TITLE: THE A.C. CAMARGO CANCER CENTER PREVENTION PROGRAM

Authors: Patricia M. Peresi[1], Thiago Celestino Chulam[1], Paulo Sergio Aleixo[1], Fernanda Sulian[1], Lizania Tesser Albardeiro[1]

Authors' Information:

[1] A.C. Camargo Cancer Center - São Paulo - Brazil. Departamento de Medicina Comunitaria e prevenção de cancer.

Corresponding Author:

Patricia M. Peresi. E-mail: peresi@yahoo.com.br
Thiago Celestino Chulam. E-mail: thiagochulam@yahoo.com.br
Paulo Sergio Aleixo. E-mail: paulo.aleixo@accamargo.org.br
Fernanda Sulian. E-mail: fernanda.sulian@hcancer.org.br
Lizania Tesser Albardeiro. E-mail: lizania.albardeiro@hcancer.org.br

Goal of the Project: The early diagnosis in cancer, mostly the types of cancer that yield to prevention and a predictable carcinogenesis. **Historic Background:** The A.C. Camargo Cancer Center Prevention Program began, in fact, as a legacy of the first notable creator of the voluntary service: Carmen Prudente. The program started in 2007, with employers and people from the voluntary service inviting friends and relatives to visit the hospital, watch a lecture about the most important topics about cancer prevention, and afterwards participate in a type of annual checkup. The project initially was very modest, but quickly grew together with the institution. **The Current Scenario:** Around 25 thousand people participate every year. The lecture about prevention is given in nonprofit organizations and underserved communities. People are invited to do metabolic and cancer screenings. Each oncology department has a personal attendance guideline to guide the prevention program and standardize the medical appointment. The tests in the metabolic screening are: hemogram, lipidogram, thyroid function, kidney function, hepatic function and hepatitis serology test. The cancer screening for women includes a pap test, mammogram and a fecal occult blood test. The screening for men includes both a PSA and fecal occult blood test. All the patients receive their results in a medical appointment, a very important step because many other complimentary tests can be indicated for each patient according to the results. In this step, if everything is normal the patient receives a discharge. If they only have metabolic or cardiovascular alterations, they are forwarded to a primary and public health system. Finally if further investigation is needed, the prevention program is able to do: prostatic, cervix and breast biopsy, colonoscopy, colposcopy, upper aero digestive endoscopy, and all types of imaging tests. **Results:** Last year, the project included 25 thousand people, 40% men and 60% women. More than 50% were over 40 years old. The cancer rate was 0.6%. A total of 150 cases (44 cases of urologic cancer, 38 cases of breast cancer, 16 cases of head and neck cancer, 16 cases of colorectal cancer, 15 cases of skin cancer, 10 cases of gynecological cancer, 9 digestive cancer, 1 lung and 1 sarcoma). **The Next Challenges:** Organize real and effective databases, reducing bias of selection and the inclusion of more people.

#85
TITLE: EVALUATION OF ANGIOGENIC FACTORS AS PROGNOSTIC MARKERS IN BREAST CANCER

Authors: Maschio LB[1,3], Jardim BV[1,3], Moschetta MG[1,4], Ferreira LC[1,3], Gonçalves NN[1,4], Gelaleti GB[1,3], Leonel C[1,3], Lopes JR[1,3], Borin TF[1,4], Martins GR[1,4], Jampietro J[2,5], Soares FA[2,5], mZuccari DAPC[1,4]

Authors' Information:
[1] Laboratório de Investigação Molecular no Câncer, Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, SP, Brazil.
[2] A.C. Camargo Cancer Center, Centro Internacional de Pesquisa e Ensino (CIPE), São Paulo - SP. Brazil.
[3] Universidade Estadual Paulista (UNESP/IBILCE), São José do Rio Preto - SP. Brazil.
[4] Faculdade de Medicina de São José doRio Preto (FAMERP), São José do Rio Preto, SP, Brazil.
[5] A.C. Camargo Cancer Center, São Paulo - SP, Brazil.

Corresponding Author:
Maschio, LB. E-mail: larissa_maschio@hotmail.com
Jardim, BV. E-mail: brunavj@hotmail.com
Moschetta, MG. E-mail: mary_na15@hotmail.com
Ferreira, LC. E-mail: livinha_ferreira@hotmail.com
Gonçalves, NN. E-mail: naiane_rp@yahoo.com.br
Gelaleti, GB. E-mail: gabi_b_g@yahoo.com.br
Leonel, C. E-mail: camilaleonels_1@hotmail.com
Lopes, JR. E-mail: jurlp@hotmail.com
Borin, TF. E-mail: thaiz80@yahoo.com.br
Martins, GR. E-mail: grmartins@live.com
Jampietro, J. E-mail: julianojampietro@gmail.com
Soares, FA. E-mail: fasoares@me.br
Zuccari, DAPC. E-mail: debora.zuccari@famerp.br

Background: Tumor angiogenesis is quantified by measuring vascular density through endothelial cell markers and growth factors. The vascular endothelial growth factor (VEGF) acts in the regulation of vascular permeability, stimulates growth, migration and invasion of endothelial cells, while VEGFR-2, is used to mediate their effects. CD34 is expressed on capillary endothelial cells, staining more strongly neoplastic endothelium, thus assists in the identification of breast tumors with a more aggressive phenotype. In addition, the Factor-VIII appears in angiogenesis by intratumoral vascularization. **Objectives:** To evaluate the individual expression of VEGF, VEGFR-2 markers, and then jointly with the double staining of CD34 and Factor VIII in breast cancer. **Methodology:** We selected tumor fragments of 60 women with invasive ductal carcinoma, attended to at the Clinic of Obstetrics and Gynecology, Hospital de Base, at the Medical School of São José do Rio Preto in the years 2000-2006. The expression of proteins were detected by immunohistochemistry with the primary antibodies anti-VEGF, anti-VEGFR2 (Santa Cruz Biotecnology) dilutions 1:300 and 1:50, respectively, and quantified by optical densitometry.

Further, the results were statistically compared with the clinicopathological parameters, treatment and prognosis of patients. In a sample group of 23 patients, we performed double staining of anti-fator VIII and anti-CD34 (Dako antibodies), diluted 1:200 and 1:500, respectively, and then were qualitatively evaluated with VEGF and FLK-1. **Results:** The evaluation of VEGF and VEGFR-2 showed significance between high expression of VEGF with smaller tumor size, low proliferative rate (Ki-67), absence of lymph node involvement, positivity for the p53 tumor suppressor gene and progesterone receptor (PR) ($p < 0.05$). Overexpression of the VEGFR-2 was detected in women with high cellular proliferation (Ki-67) and smaller tumor ($p < 0.05$). The expression of these proteins with double staining showed that 26% of patients had poor prognosis and of these, 66% had high expression of at least one of the proteins evaluated by marking CD34-and Factor VIII. **Conclusion:** The results reflect the complexity of the role of these proteins in the angiogenic process when evaluated separately, however when assessed together confirms the formation of new blood vessels in the tumor region, thereby supporting their use as prognostic markers in breast cancer.

Financial Support: *Fundação de Amparo a Pesquisa do Estado de São Paulo* - FAPESP.

REFERENCES

1. Carpini, JD et al. Vascular endothelial growth factor and its relationship to the prognosis and treatment of breast, ovari an, and cervical cancer. Angiogenesis. 2010;13:1.
2. PARK, SY et al. Melatonin suppresses tumor angiogenesis by inhibiting HIF-1α stabilization under hypoxia. J. Pineal Res. 2010;48:2.
3. GUO, S et al. Vascular endothelial growth factor receptor-2 in breast cancer. Biochim Biophys Acta. 2010;1860:1.

#87

TITLE: DIFFERENTIAL METHYLATION PROFILE IS RELATED WITH POOR PROGNOSIS AND HPV INFECTION IN PENILE CARCINOMAS PATIENTS

Authors: Hellen Kuasne[1,2], Ilce MS Cólus[3], Hector Hernandez-Vargas[4], Mateus C Barros-Filho[1], Fábio A Marchi[1], Gustavo C Guimarães[1], Cristovam Scapulatempo Neto[5], Fernando A Soares[1], Zdenco Herceg[4], Silvia R Rogatto[1,6]

Authors' Information:

[1] A.C. Camargo Cancer Center, São Paulo, SP, Brazil.
[2] Institute of Biosciences, Department of Genetic, UNESP, Botucatu - SP. Brazil.
[3] Department of General Biology, Londrina State University, Londrina - PR. Brazil.
[4] Epigenetics Group, International Agency for Research on Cancer (IARC); Lyon, France.
[5] Barretos Cancer Hospital, Barretos, SP, Brazil.
[6] Department of Urology, Faculty of Medicine, UNESP, Botucatu - SP. Brazil.

Corresponding Author:
Hellen Kuasne. E-mail: hellenkuasne@hotmail.com

Background: Penile carcinoma (PeCa) is an important public health problem in poor and developing countries. Despite the unpredictable behavior and aggressive treatment, there are few data on genetic and epigenetic alterations reported in PeCa. The aim of this study was to identify epigenetic alterations and its association with prognosis in PeCa patients. **Patients and Methods:** PeCa samples were collected from 36 patients. Two independent methods were used to evaluate the methylation profile: enrichment of methylated region (MCip) (MethylMiner - Invitrogen) and enrichment of unmethylated region (digested with restriction enzyme McrBC). Samples were hybridized in a 244K Human DNA Methylation Microarray platform (Agilent Technologies). Genomic Workbench Standard (v 5.0.14) and BRB array tools software were used to analyze the data. Only probes were considered with a p value < 0.001 and FDR < 0.05. **Results:** HPV positivity was detected in 43% of cases (Linear Array HPV Test Genotyping - Roche), mainly for 16 subtype. Involvement of lymph nodes, advanced clinical stage (III and IV), tumors with histological grades 2 and 3 and absence of HPV infection were associated with shorter overall survival and disease free survival. The comparison among methylation profile and histological tumor grade revealed significant differences in grade 1 (well differentiated), grade 2 (intermediate) and grade 3 (poorly differentiated). The heat map graphic analysis revealed that tumors grade 2 and 3 were similar and epigenetically different from grade 1 tumors. One hundred twenty-two genes hypermethylated and 110 hypomethylated, respectively, were found between tumor grades 1 and 3. A differential methylation profile was also found in the HPV positive versus HPV negative cases. The main networks associated with HPV positive cases were immunological response and Rb pathway. HPV negative samples presented an association with genes involved in regulation of stem cells, development and differentiation. **Conclusions:** Our results showed that tumors present a differential methylation signature related to prognosis. A differential methylation profile was found according to HPV status (positive or negative), indicating at least two disrupted pathways, one related to viral infection and other associated with transcriptional regulation of stem cells.

Keywords: methylation profile, penile carcinoma, poor prognosis, pyrosequencing.

Financial Support: FAPESP, CNPq and CAPES.

#88

TITLE: ASSESSMENT OF RENAL FUNCTION IN CANCER OUTPATIENTS WHO HAD BEEN TREATED WITH ANTICANCER DRUGS BEFORE AND AFTER ADMINISTRATION OF NONIONIC LOW-OSMOLAR IODINATED CONTRAST MEDIA

Authors: Melo JIF[1], Chojniak R[1], Bitencourt AGV[1]

Authors' Information:
[1] A.C. Camargo Cancer Center.

Corresponding Author:
Rubens Chojniak. E-mail: chojniak@uol.com.br
Street Professor. Antônio Prudente, 211 - São Paulo, SP - Zip Code: 01509-010.
João Italo Fortalesa de Melo. E-mail: italo_me@outlook.com
Almir Galvão Vieira Bitencourt. E-mail: almirgvb@yahoo.com.br

Context: Computed tomography (CT) is often used in the evaluation of cancer patients. The intravascular administration of iodinated contrast media (ICM) is required and may result in adverse reactions, including contrast-induced nephropathy (CIN). Treatment with anticancer drugs (AD) is one of the longest and harmful therapies in medicine with nephrotoxic potential, which generates a concern, especially related to administration of ICM in such patients. **Objective:** Identify the incidence rates of CIN in cancer outpatients who underwent CT with use of nonionic low osmolar IMC, who had undergone treatment with AD, through the measurement of serum markers of renal injury. **Methodology:** Prospective study of 239 outpatients that were subjected to CT using nonionic low osmolar ICM. Serum creatinine, C-reactive protein (CRP), cystatin C and microalbuminuria were analyzed before and after contrast administration. The glomerular filtration rate (GFR) was estimated by the MRDR and Cockroft-Gault formulas, which use serum creatine, and the Larsson formula, which uses serum cystatin C. **Results:** Of the 239 included patients, 168 were treated with AD and 67 were not. The age of patients ranged from 14 to 82 years (mean = 53.4 +/-14.8 years), and 57.7% were female. None of the patients presented clinically significant CIN. There was no statistically significant difference in the results of patients who had already been treated with AD and those who were not subjected to this treatment, as shown in Table 1. **Conclusions:** There was no significant kidney damage related to the use nonionic low osmolar ICM in cancer outpatients, regardless of the history of previous treatment with AD.

Financial Support: FAPESP

#89

TITLE: PREVALENCE OF GERMLINE MUTATION P.R337H IN THE TP53 GENE IN FAMILIES WITH MULTIPLE CASES OF CANCER

Authors: Andrade KC[1,2], Santiago KM[1], Nobrega A[1], Fortes FP[1], Achatz, MI[1]

Authors' Information:
[1] Fundação Antônio Prudente - A.C. Camargo Cancer Center - São Paulo - SP. Brazil.
[2] UNESP - Universidade Estadual Paulista "Júlio de Mesquita Filho" - Campus Botucatu - SP. Brazil.

Corresponding Author:
Andrade, KC. E-mail: kelvincesar.an@gmail.com
Achatz, MI. E-mail: miachatz@gmail.com
Santiago, KM. E-mail: karinamirsant@gmail.com
Nobrega, A. E-mail: amandapfranca@gmail.com
Fortes, FP. E-mail: ferpfortes@yahoo.com.br

Background: Germline mutations in the TP53 tumor suppressor gene are associated to the Li-Fraumeni Syndrome (LFS) and its variants (LFL). They predispose individuals for early onset tumors, including soft tissue sarcoma, pre-menopausal breast cancer, central nervous system tumors and adrenocortical carcinoma. In Brazil, there is a high frequency of a germline TP53 mutation (p.R337H, CGC > CAC; exon 10) in South and Southeastern population due to a founder effect. It is estimated to be present in 0.3% of the local population, but only a few families have been diagnosed with such alteration. Therefore, the development of suitable criteria to select probable carriers will enable not only the identification of at-risk families, but also provide suitable screening and early detection. **Objective:** To determine the efficacy of wide criteria for detection of p.R337H carriers in Brazil. **Methods:** In this study, 31 patients from the Oncogenetics Department, A.C. Camargo Cancer Center, São Paulo, Brazil, were selected and tested for p.R337H mutation. Criteria for inclusion were: (1) more than three family members with cancer AND (2) at least one of them under age 50 AND (3) two of them being first or second degree relatives. Germline DNA was extracted from peripheral blood and analyzed by Restriction Fragment Length Polymorphism (RFLP) using the Hhal restriction enzyme. The confirmation of positive finding was done through direct sequencing of exon 10. **Results:** One out of 31 patients (3.22%) was found to carry the p.R337H mutation. The patient developed ductal invasive breast cancer at age 47 and invasive adenocarcinoma of the lung at age 48. In addition, an extensive cancer family history was referred (Figure 1). The proband will receive genetic counseling and will be included in follow-up protocol for TP53 germline mutation carriers. **Discussion:** One family who initially did not fulfill LFS/LFS criteria was found to be carrier of the

Table 1. Descriptive statistics of the difference between post and pre-contrast and result (*p*-value) of the comparison between groups.

Variable	Anticancer drugs = No	Anticancer drugs = Yes					*p*-value						
	n[(1)]	Mean	SD	Mín	Avg	Max	n[(1)]	Mean	SD	Mín	Avg	Max	
Creatinine mg/dL	67	0.032	0.142	-0.280	0.000	0.440	156	0.024	0.140	-0.810	0.025	0.510	0.0819
Microalbuminuria	65	-2.8	160.0	-968.0	-1.0	790.1	155	-9.8	60.2	-606.1	0.0	149.3	0.598
Cystatin C	45	-0.004	0.137	-0.450	0.010	0.260	88	-0.002	0.158	-1.190	0.000	0.210	0.975
CRP	65	-0.037	0.264	-1.380	0.000	0.580	156	0.019	1.251	-6.780	-0.010	7.910	0.579
Creatinine urinary	65	0.504	0.692	-1.070	0.430	2.550	155	0.439	0.776	-3.550	0.420	4.250	0.569
Estimated clearance: MDRD	67	-3.3	14.4	-57.3	0.0	31.2	156	-3.7	17.6	-51.4	-3.9	60.2	0.684
Estimated clearance: Cockroft-Gault	67	-2.8	12.8	-34.0	0.0	25.0	156	-3.4	16.0	-58.1	-3.5	54.6	0.635
Cystatin C (Larsson)	45	1.9	19.2	-33.7	-3.3	50.9	88	0.2	18.3	-31.4	0.0	88.6	0.898

[(1)] We considered only patients who had test results both pre and post-contrast; Mean; SD = Standard deviation; Min = Minimum; Median = Avg, Max = Maximum.

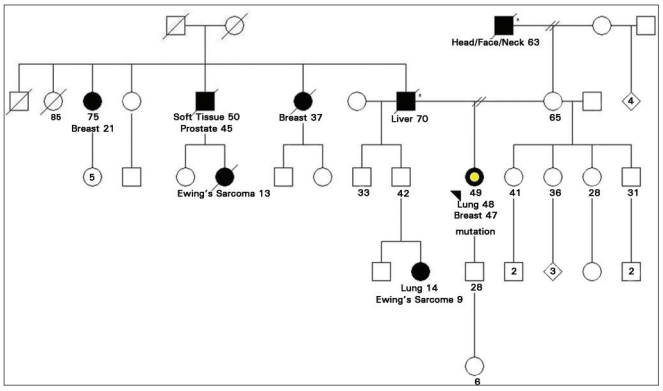

Figure 1. Pedigree of the family affected by the germline mutation p.R337H.

p.R337H germline TP53 mutation. This result shows that the proposed criteria may detect families with this mutation, but further studies including a larger group of families will be useful to define its effectiveness. Also, in order to improve sensibility, the inclusion of more stringent criteria will be needed.

Keywords: Li-Fraumeni syndrome, p.R337H, TP53.

Financial Support: PIBIC, CNPq - National Council for Scientific and Technological Development.

REFERENCES

1. Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med. 1969 Oct; 71(4):747-52.
2. Achatz MI, Olivier M, Le Calvez F et al. The TP53 mutation, R337H, is associated with Li-Fraumeni and Li-Fraumeni-like syndromes in Brazilian families. Cancer Lett. 2007 Jan 8;245(1-2):96-102.
3. Garritano S, Gemignani F, Palmero EI, et al. Detailed haplotype analysis at the TP53 locus in p.R337H mutation carriers in the population of Southern Brazil: evidence for a founder effect. Hum Mutat 2010 Feb; 31(2):143-50.

#90

TITLE: POLYMORPHISMS IN GENES OF FOLATE METABOLISM PATHWAY ASSOCIATED TO THE DEVELOPMENT OF HEAD AND NECK CANCER: A CASE REPORT

Authors: Castro TB[1], Galbiatti ALS[1], Castro R[1], Fernandes GMM[1], Raposo LS[2], Maniglia JV[2], Pavarino EC[1], Goloni-Bertollo EM[1]

Authors' Information:

[1] Research Unit in Genetics and Molecular Biology; Faculty of Medicine of São José do Rio Preto, São Paulo, Brazil.

[2] Faculty of Medicine of São José do Rio Preto, São Paulo, Brazil; Department of Otorhinolaringology and Head and Neck Surgery, Base Hospital, São José do Rio Preto, São Paulo, Brazil.

Background: Head and neck cancer is considered the fifth most common type in the world and is associated with a high mortality rate when diagnosed in advanced stages. The most common histological type (90% of cases) is squamous cell carcinoma. The main risk factors include the consumption of tobacco and alcohol, viral infections, especially with the Epstein-Barr virus and human papillomavirus and deficiencies of vitamins and micronutrients such as folate. The most affected group is male with advanced age (average 60 years). The excessive consumption of alcohol can affect nutrient absorption by the intestine, causing major nutritional deficiencies and modifying metabolic pathways, such as via folate, that is responsible for insertion of methyl groups in DNA methylation and is important for synthesis of purine and pyrimidines. The xenobiotic pathway is another which may play an important role in carcinogensis. The machinery of xenobiotic metabolism comprises two types of enzymes: cytochrome P450 (CYPs) enzymes that mediate Phase I or oxidative metabolism and microsomal epoxide hydrolases (EPHX1), which convert many compounds to highly reactive metabolites in carcinogenic substances. Polymorphisms in genes that encode enzymes involved in xenobiotic metabolism could modify the function of these enzymes, resulting in the improper activation or deto-

xification of smoke and alcohol metabolites. In this case report we evaluated 11 polymorphisms in 8 genes of two pathways of metabolism, folate and xenobiotics in a patient diagnosed with tumor Head and Neck Squamous Cell Carcinoma at 20 years of age and died during treatment. The tumor (T4N0M0) by pathologic examination of biopsy was positive for HPV infection. The patient reported not consuming alcoholic drink and tobacco. In folate pathway were evaluated 5 genes and 7 SNPs (DHFR -19pb, SHMT1 C1420T, DNMT3B -149C > T, DNMT3B -283T > C, DNMT3B -579G > T, MTHFR C677T and MTR A2756G) in xenobiotic metabolism pathway were evaluated 6 SNPs in 3 genes (CYP1A1 T3801C *2A, CYP1A1 *2C A2455G, CYP2E1 *5B C1019T, CYP2E1 *6 A7766T, EPHX1 Tyr113His and EPHX1 His139Arg). **Methods:** Real-Time PCR and Conventional PCR techniques were used for genotyping. **Results:** Found in the polymorphic allele in 6 of 7 SNPs in folate pathway and in all SNPs analyzed in xenobiotics pathway were homozygous for wild type. **Discussion:** Alterations in genes of folate pathway may play an important role in carcinogenesis of head and neck.

REFERENCES

1. Jemal A et al. Global cancer statistics. CA Cancer J Clin. 2011 Mar-Apr; 61(2):69-90. Epub 2011 Feb 4. Erratum in: CA Cancer J Clin. 2011 Mar-Apr; 61(2):134.
2. Azad AK et al. Validation of genetic sequence variants as prognostic factors in early-stage head and neck squamous cell cancer survival. Clin Cancer Res. 2012 Jan 1;18(1):196-206.
3. PYTYNIA KB et al. Matched-pair analysis of survival of never smokers and ever smokers with squamous cell carcinoma of the head and neck. J Clin Oncol, 22,3981-88,2004.

#91
TITLE: EVALUATION OF THE RELATIONSHIP BETWEEN BACTERIAL INFECTION, SPECIFIC CANCER SURVIVAL AND CLINICAL COURSE IN PATIENTS WITH COLORECTAL CANCER AND BREAST CANCER

Authors: Attiê R[1], Chinen LTD[2], De Lima VCC[2]

Authors' Information:

[1] Department of Pharmacy - A.C. Camargo Cancer Center, São Paulo, Brazil.

[2] Department of Medical Oncology - A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:

Attiê R. E-mail: regina.attie@hcancer.org.br
Chinen LTD. E-mail: ltdchinen@gmail.com
de Lima VCC. E-mail: vladcclima@gmail.com

Background: Inflammation has been linked to cancer since the 19th century and has been studied for a better understanding of prevention and treatment. Some infectious agents are already recognized as major components of chronic infections that are associated with cancer. Although there are several studies about the relationship between inflammation and cancer, other studies have suggested that infection may have a protective effect for cancer, some of these being used in therapy, such as BCG (*Bacillus Calmette-Guérin*) in bladder cancer. **Objective:** As many controversies are found in the literature, this study had as objective to verify the role of bacterial infections in the clinical evolution of patients undergoing treatment or treated with colorectal cancer and breast cancer. **Methods:** A retrospective cohort study analyzed data from January 2006 to April 2010 to better understand the clinical evolution of patients who were hospitalized and reported bacterial infections with etiologic agents isolated in colorectal cancer and breast cancer. In addition to records of the SAME, the data of the Hospital Infection Control Service (SCIH) were also used. **Results:** From a total of 2,595 SCIH records, 214 patients were analyzed and divided into two groups: infected and control (non-infected). For patients with colorectal cancer, the higher the number of infections, the worse survival was. Neutrophil count was associated with worse cancer-specific survival. Infection remained as an independent predictor of worse specific cancer survival, along with clinical staging (p = 0.01). Regarding breast cancer, an independent predictor was found in patients who underwent surgical-only treatment, who had worse cancer-specific survival compared to patients who have other associated treatments. **Conclusions:** Through the analysis of data, we concluded that the presence of infection decreases cancer-specific survival.

REFERENCES

1. Arnold IC, Hitzler I, Müller A. The immunomodulatory properties of helicobacter pylori confer protection against allergic and chronic inflammatory disorders. Front Cell Infect Microbiol 2012;2:10.
2. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539-45.
3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436-44.

#92
TITLE: CYTOLOGY IN AUTOPSY AS AN ADJUVANT METHOD FOR ACCURATE ON SITE DIAGNOSIS

Authors: Dias MVS[1], Silva BC[1], Batista JRB[1], Martins VR[1]

Authors' Information:
[1] International Research Center, A.C. Camargo Cancer Center, São Paulo, Brazil.

Corresponding Author:
Marcelo Luiz Balancin. E-mail: mlbalancin@me.com

Background: Autopsy is crucial for public health databases. Despite all modern techniques, about 30% of autopsies diagnose an unsuspected disease, including cancer, which could have changed clinical decisions. Cytology is a simple, fast and cheap method, uncommonly used in autopsies. **Objective:** We will discuss the role of cytology as a fast and cheap method to diagnose unsuspected cancer in autopsies performed at ISCMSP. **Cases:** (1) A 60-year-old woman died after 5-days in the ICU due to bilateral pneumonia and respiratory failure. During the autopsy, a 15.0 cm necrotic mass covering the psoas muscle extending to the acetabulum was seen. Exfoliative cytology was performed and showed a poorly differentiated metastatic squamous cell carcinoma. Immunohistochemistry staining revealed CK7 and p16 positivity, consistent with endocervical origin. Hysterectomy had been performed without a cancer diagnosis. (2) A 70-year-old man with respiratory failure died after massive pneumonia. A 5.0 cm pediculated and mucinous lesion was found in the urinary bladder fundus. Exfoliative cytology revealed monomorphic atypical glandular cells arranged in branched papillary structures consistent with bladder adenocarcinoma. **Discussion:** Cytology dramatically changed the cause of death on death certificates on these two cases. To date, 8 studies have been published on English-language literature discussing the role of cytodiagnosis on autopsy practice and fewer than 2% of necropsies centers do it routinely. Cytology can potentially improve immediate clinico-pathological correlation in autopsies as well as contribute to more accurate death certificates.

#93

TITLE: SEARCH FOR POTENTIAL TUMOR BIOMARKERS FOR PENILE SQUAMOUS CELL CARCINOMA BY 2D DIFFERENCE GEL ELECTROPHORESIS TECHNIQUE

Authors: Adriana Bulgarelli[1], Elisângela de Jesus Silva[1], Nilson Antonio Assunção[2], Fernando Augusto Soares[1], José Vassallo[1]

Authors' Information:
[1] A. C. Camargo Cancer Center, São Paulo, Brazil.
[2] Universidade Federal de São Paulo - UNIFESP, São Paulo, Brazil.

Corresponding Author:
Adriana Bulgarelli. E-mail: adriana.bulgarelli@gmail.com

Penile squamous cell carcinoma (SCC) corresponds to 2.1% of malignant tumors in Brazilian men. Five-year survival rate drops dramatically in patients with lymph node metastasis. Therefore, the search for more robust prognostic markers and those predictive of response to therapy are warranted. Proteomics represent a powerful tool for this purpose. In the present study, it was our objective to determine the differential protein pattern of penile SCC. This approach may reveal potential tumor markers. Six samples, three of penile SCC and three of normal penile epithelium (NPE) were compared, using two-dimensional gel electrophoresis. All gel images were analyzed using the ImageMaster 2D Platinum 7.0 software. A total of 23 proteins were found differentially expressed in the six samples. Compared with NPE, 15 were overexpressed and 8 underexpressed in penile SCC ($p < 0.05$). Additionally, 264 spots were identified only in penile SCC and 150 were identified only in normal tissue ($p < 0.05$). Our data provide a novel proteomic approach to investigate the protein profile of penile SCC and might represent the basis for further studies on the characterization of these proteins, some of which may be of potential clinical value.