MAIN PUBLICATIONS

Achatz MI, Olivier M, Calvez FL, Martel-Planche G, Lopes A, Rossi BM, Ashton-Prolla P, Vargas FR, Casali da Rocha JC, Vettore AL, Hainaut P. 2007. Response to "Germline TP53 R337H mutation is not sufficient to establish Li-Fraumeni or Li-Fraumeni-like syndrome", by Ribeiro et al. *Cancer Lett*. **247(2)**:356-8.

Stolf BS, Santos MM, Simao DF, Diaz JP, Cristo EB, Hirata R Jr, Curado MP, Neves EJ, Kowalski LP, Carvalho AF. 2006. Class distinction between follicular adenomasand follicular carcinomas of the thyroid gland on the basis of their signatureexpression. *Cancer.* **106(9)**:1891-900.

Folgueira MA, Carraro DM, Brentani H, Patrao DF, Barbosa EM, Netto MM, Caldeira JR, Katayama ML, Soares FA, Oliveira CT, Reis LF, Kaiano JH, Camargo LP, Vencio RZ, Snitcovsky IM, Makdissi FB, e Silva PJ, Goes JC, Brentani MM. 2005. Gene expression profile associated with response to doxorubicin-based therapy in breast cancer. *Clin Cancer Res*. **11**:7434-43.

Gomes LI, Esteves GH, Carvalho AF, Cristo EB, Hirata R Jr, Martins WK, Marques SM, Camargo LP, Brentani H, Pelosof A, Zitron C, Sallum RA, Montagnini A, Soares FA, Neves EJ, Reis LF. 2005. Expression profile of malignant and nonmalignant lesions of esophagus and stomach: differential activity of functional modules related to inflammation and lipid metabolism. *Cancer Res.* **65**:7127-36.

Reis EM, Ojopi EP, Alberto FL, Rahal P, Tsukumo F, Mancini UM, Guimaraes GS,Thompson GM, Camacho C, Miracca E, Carvalho AL, Machado AA, Paquola AC, Cerutti JM, da Silva AM, Pereira GG, Valentini SR, Nagai MA, Kowalski LP, Verjovski-Almeida S, Tajara EH, Dias-Neto E, Bengtson MH, Canevari RA, Carazzolle MF, Colin C, Costa FF, Costa MC, Estecio MR, Esteves LI, Federico MH, Guimaraes PE, Hackel C, Kimura ET, Leoni SG, Maciel RM, Maistro S, Mangone FR, Massirer KB, Matsuo SE, Nobrega FG, Nobrega MP, Nunes DN, Nunes F, Pandolfi JR, Pardini MI, Pasini FS, Peres T, Rainho CA, dos Reis PP, Rodrigus-Lisoni FC, Rogatto SR, dos Santos A, dos Santos PC, Sogayar MC, Zanelli CF. 2005. Head and Neck Annotation Consortium. Large-scale transcriptome analyses reveal new genetic marker candidates of head, neck, and thyroid cancer. *Cancer Res*. **65**:1693-9.

Stolf BS, Abreu CM, Mahler-Araujo MB, Dellamano M, Martins WK, de Carvalho MB, Curado MP, Diaz JP, Fabri A, Brentani H, Carvalho AF, Soares FA, Kowalski LP, Hirata Jr. R, Reis LF. 2005. Expression profile of malignant and nonmalignant diseases of the thyroid gland reveals altered expression of a common set of genes in goiter and papillary carcinomas. *Cancer Lett*. **227**:59-73.

Meireles SI, Cristo EB, Carvalho AF, Hirata Jr. R, Pelosof A, Gomes LI, Martins WK,Begnami MD, Zitron C, Montagnini AL, Soares FA, Neves EJ, Reis LF. 2004. Molecular classifiers for gastric cancer and nonmalignant diseases of the gastric mucosa. *Cancer Res*. **64**:1255-65.

Brentani H, Caballero OL, Camargo AA, Da Silva AM, Da Silva WA Jr, Neto ED, Grivet M, Gruber A, Guimaraes PE, Hide W, Iseli C, Jongeneel CV, Kelso J, Nagai MA, Ojopi EP, Osorio EC, Reis EM, Riggins GJ, Simpson AJ, De Souza S, Stevenson BJ, Strausberg RL, Tajara EH, Verjovski-Almeida S, Acencio ML, Bengtson MH, Bettoni F, Bodmer WF, Briones MR, Camargo LP, Cavenee W, Cerutti JM, Andrade LE, Dos Santos PC, Costa MC, Da Silva IT, Estecio MR, Sa Ferreira K, Furnari FB, Faria M Jr, Galante PA, Guimaraes GS, Holanda AJ, Kimura ET, Leerkes MR, Lu X, Maciel RM, Martins EA, Massirer KB, Melo AS, Mestriner CA, Miracca EC, Miranda LL, Nobrega FG, Oliveira PS, Paquola AC, Pandolfi JR, De Moura Campos Pardini MI, Passetti F, Quackenbush J, Schnabel B, Sogayar MC, Souza JE, Valentini SR, Zaiats AC, Amaral EJ, Arnaldi LA, De Araujo AG, De Bessa SA, Bicknell DC, De Camaro ME, Carraro DM, Carrer H, Carvalho AF, Colin C, Costa F, Curcio C, Da Silva ID, Da Silva NP, Dellamano M, El-Dorry H, Espreafico EM, Ferreira AJ, Ferreira CA, Fortes MA, Gama AH, Giannella-Neto D, Giannella ML, Giorgi RR, Goldman GH, Goldman MH, Hackel C, Ho PL, Kimura EM, Kowalski LP, Krieger JE, Leite LC, Lopes A, Luna AM, Mackay A, Mari SK, Marques AA, Martins WK, Montagnini A, Neto MM, Nascimento AL, Neville AM, Nóbrega MP, O'Hare MJ, Otsuka AY, De Melo AI, Paco-Larson ML, Pereira GG, Da Silva NP, Pesquero JB, Pessoa JG, Rahal P, Rainho CA, Rodrigues V, Rogatto SR, Romano CM, Romeiro JG, Rossi BM, Rusticci M, De Sa RG, Sant' Anna SC, Sarmazo ML, E Lima De Silva TC, Soares FA, De Fatima Sonati M, De Freitas Sousa J, Queiroz D, Valente V, Vettore AL, Villanova FE, Zago MA, Zalcberg H. 2003. The generation and utilization of a cancer-oriented representation of the human transcriptome by using expressed sequence tags. *Proc Natl Acad Sci USA*. **100(23)**:13418-23.

Camargo AA, Samaia HP, Dias-Neto E, Simao DF, Migotto IA, Briones MR, Costa FF, Nagai MA, Verjovski-Almeida S, Zago MA, Andrade LE, Carrer H, El-Dorry HF, Espreafico EM, Habr-Gama A, Giannella-Neto D, Goldman GH, Gruber A, Hackel C, Kimura ET, Maciel RM, Marie SK, Martins EA, Nobrega MP, Paco-Larson ML, Pardini MI, Pereira GG, Pesquero JB, Rodrigues V, Rogatto SR, da Silva ID, Sogayar MC, Sonati MF, Tajara EH, Valentini SR, Alberto FL, Amaral ME, Aneas I, Arnaldi LA, de Assis AM, Bengtson MH, Bergamo NA, Bombonato V, de Camargo ME, Canevari RA, Carraro DM, Cerutti JM, Correa ML, Correa RF, Costa MC, Curcio C, Hokama PO, Ferreira AJ, Furuzawa GK, Gushiken T, Ho PL, Kimura E, Krieger JE, Leite LC, Majumder P, Marins M, Marques ER, Melo AS, Melo M, Mestriner CA, Miracca EC, Miranda DC, Nascimento AL, Nobrega FG, Ojopi EP, Pandolfi JR, Pessoa LG, Prevedel AC, Rahal P, Rainho CA, Reis EM, Ribeiro ML, da Ros N, de Sa RG, Sales MM, Sant'anna SC, dos Santos ML, da Silva AM, da Silva NP, Silva WA Jr, da Silveira RA, Sousa JF, Stecconi D, Tsukumo F, Valente V, Soares F, Moreira ES, Nunes DN, Correa RG, Zalcberg H, Carvalho AF, Reis LF, Brentani RR, Simpson AJ, de Souza SJ. 2001. The contribution of 700,000 ORF sequence tags to the definition of the human transcriptome. *Proc Natl Acad Sci USA*. **98**:12103-8.

Pignon JP, Bourhis J, Domenge C, Designé L. Contribution of Kowalski LP. et al. 2000. Chemotherapy added to locoregional treatment for head and neck squamouscell carcinoma: three meta-analyses of updated individual data. *Lancet.* **355**:949-53.

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Laser Capture Microdissection System Veritas™

The Antonio Prudente Cancer Care Center (APCCC), based at Cancer Hospital, in São Paulo, was approved by FAPESP in 2000. Its primary goal is to contribute to the advancement of cancer prevention, diagnosis and treatment. Following the best standards recognized worldwide, we aim to develop new tools to improve patient care. In 2000, research projects were primarily focused on gene discovery, mutation detection, differential expression between normal and tumor tissues, and epidemiology of HPV.

During its first five years, the APCCC got involved in a major sequencing effort, financed by FAPESP and the Ludwig Institute for Cancer Research (LICR), the Human Cancer Genome Project (HCGP). This project ended in 2001, with a significant contribution to the human transcriptome, producing an excess of 1 million ESTs generated from normal and tumor tissues. The APCCC contributed with more than 99% of tissue-derived RNA. Because of that, APCCC developed procedures and protocols for the creation of a tumor bank that allows the extraction of high quality DNA/RNA/protein.

At the same time, efforts were also dedicated to the establishment of cDNA microarray and tissue microarray platforms. Finally, we also invested heavily on bioinformatics in order to have these platforms integrated with our tumor bank and samples linked to clinical data, and acquired the expertise to analyze both platforms. This integrated effort for quantitative analysis of transcripts was the major achievement during that period and enabled us to identify new molecular markers for diagnosis and prognosis.

For the 2005-2008 period, we focused our research project on a small number of tumors (Head and neck, Sarcomas, Wilms' Tumor, and Breast). Thus, taking advantage of the previously built platforms, we were in a position to address clinically relevant questions: Can we improve diagnosis? Can we evaluate prognosis? Can we predict response to therapy? For the second period of RIDC (2005-2008) we succeeded in the identification of new diagnostic and prognostic markers for the proposed tumors.

MAIN RESEARCH TOPICS

During its existence, the APCCC has been characterized by the effort to bring together basic researchers and medical staff in order to produce new advances in tumor diagnosis and etiology, prognostic markers, and tools to predict response to therapy. It is a definition of translational research with the aim to benefit our patients.

APCCC has been working with gene expression transcriptomic studies. We participated in HCGP working with ORESTES and contributed with more than one million sequences of different tumors and their normal counterparts (Camargo AA et al. 2001. *Proc Natl Acad Sci USA*. **98(21)**:12103-8; Brentani H et al. 2003. *Proc Natl Acad Sci USA*. **100(23)**:13418-23). We worked with SAGE on establishing different mathematical models of analysis (Vêncio RZ et al. 2007. *BMC Bioinformatics*. **8**:246; Barrera J et al. 2007. *BMC Bioinformatics*. **8**:169), and have also been working with microarrays contributing with important classifiers that may help in clinical practice.

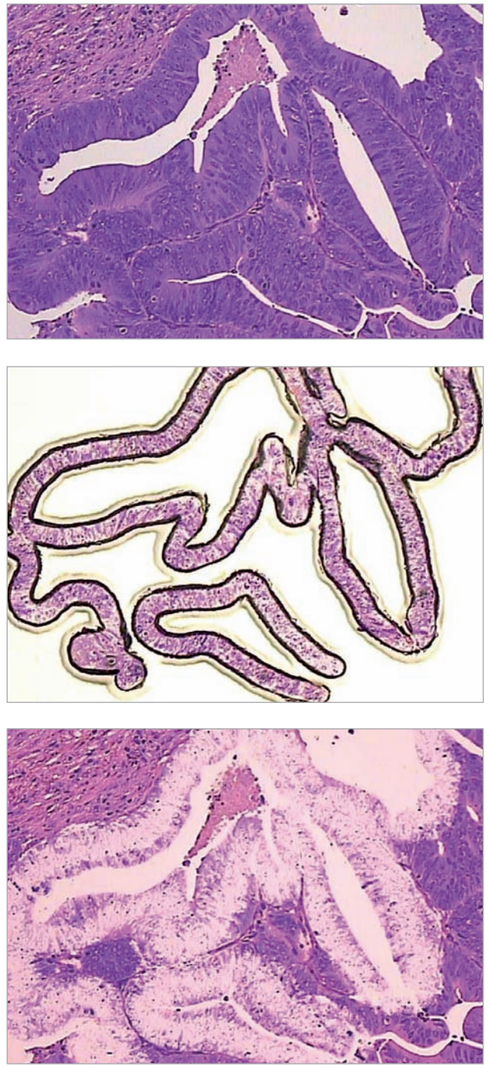
Several facilities have been organized as follows: tumor and DNA/RNA bank, DNA/RNA extraction facility, TMA facility, and gene expression facility. We them focused our efforts on four types of tumor: head and neck carcinomas, sarcomas, Wilms' tumor, and breast carcinomas.

The head and neck tumors front has been approached in order to recognize predictors of response for chemo/radiotherapy in larynx squamous cell carcinoma. By using biopsies taken before treatment, the gene expression profile of a group of 21 responders was compared with a second group of 14 non-responders. After mathematical analysis, four trios of genes were identified that could predict responsiveness to treatment.

We have also analyzed molecular signatures in sarcomas. Mesenchymal tumors are unusual, but they have significant morbidity and mortality. Our main effort in the last three years was to identify classifiers able to separate locally aggressive tumors but without ability to develop metastasis from potential metastatic sarcomas. We have used fibromatosis as a tumor model with high local aggressiveness and fibrosarcomas as a model of metastatic sarcomas.

The third branch of the Center is related to molecular markers as predictors of adverse outcome in Wilms' tumors. For this, we have tested blastemal predominant Wilms' tumors sensible and resistant to chemotherapy. By using SAGE, we have selected 14 differentially expressed genes.

Finally, the breast carcinoma front was approached by two different projects. One of them studied the validation of *Adam*23 hypermethylation (HyMe) as an independent prognostic factor, and the second aims to explore the transcriptional variability caused by alternative splicing to identify breast carcinoma-associated splicing variants.



Laser Capture Microdissection Procedure

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

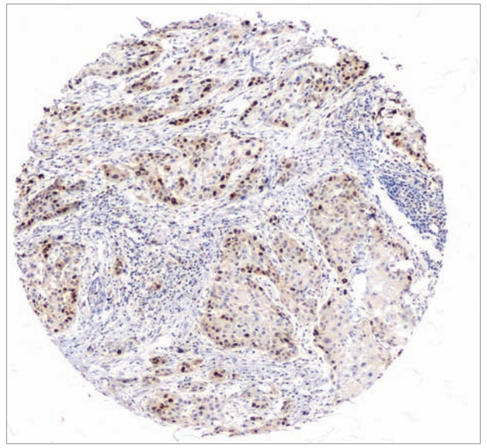
Our main research focus is on translation research in order to improve diagnosis and prognosis of the tumors, and identify predictors of treatment response. Nowadays our aims are concentrated on tumors of head and neck, soft tissues, breast and Wilms' tumor.

The breast carcinoma front was approached by two different projects. One of them studied the validation of *Adam*23 hypermethylation (HyMe) as an independent prognostic factor, and comprised three segments: *Adam*23 regulation of the activation of avb3 integrin; *Adam*23 HyMe in plasma samples from breast cancer patients; and *Adam*23 HyMe and detection of micrometastasis in sentinel lymph nodes. The second project in the breast carcinoma section aims to explore the transcriptional variability caused by alternative splicing to identify breast carcinoma-associated splicing variants.

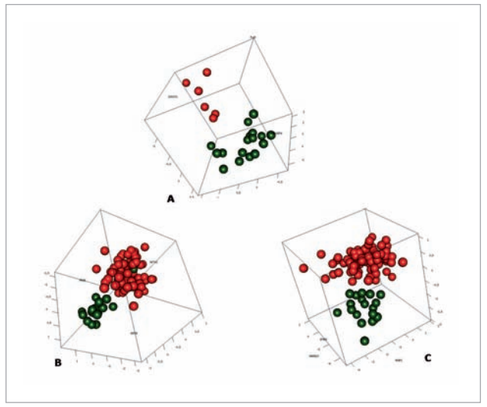
Also, we have analyzed molecular signatures in sarcomas. The main effort is to identify classifiers that are able to separate locally aggressive tumors, but without ability to develop metastasis from potential metastatic sarcomas.

A third segment of APCCC's proposal is related to predictors of response for chemo/radiotherapy in larynx squamous cell carcinoma. After mathematical analysis, four trios of genes were identified that could predict responsiveness to the treatment.

The last project is related to molecular markers as predictors of adverse outcome in Wilms' tumors. For this, we have tested blastemal predominant Wilms' tumors sensible and resistant to chemotherapy. Five genes showed a differential expression between relapsed and non-relapsed WT samples with statistical significance ($p<0.05$). All of them were over-expressed in nonrelapsed WT samples. Trios of classifiers were exhaustively searched among the 5 genes using the qRT-PCR data, and 2 trios were promising predictors of adverse outcome in WT, correctly separating 95% of the samples.



Topoisomerase positivity in squamous cell carcinoma



Scatter plot showing differences in gene expression between mesenchymal tumors

More than 10,000 samples in the Tissue Microarrays (TMA) of different organs and different tumors