

## MAIN PUBLICATIONS

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## Antonio Prudente Cancer Care Center (APCCC)

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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.

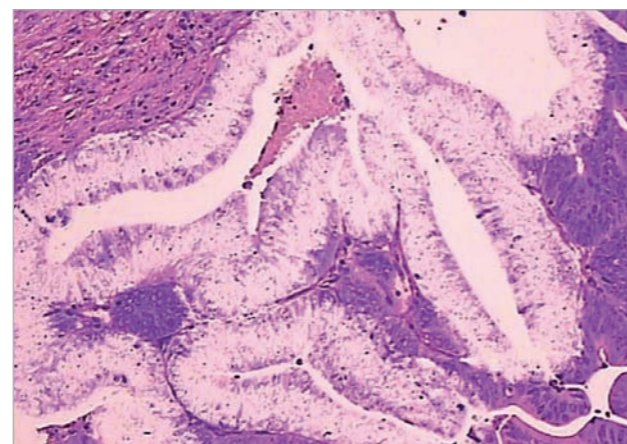
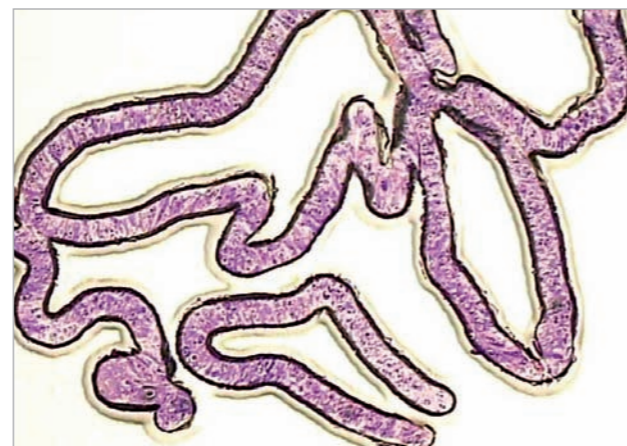
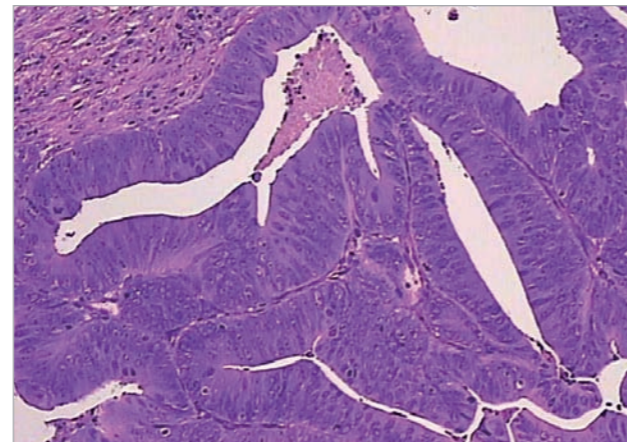
Several facilities have been organized as follows: tumor and DNA/RNA bank, DNA/RNA extraction facility, TMA facility, and gene expression facility. We then focused our efforts on four types of tumor: head and neck carcinomas, sarcomas, Wilms' tumor, and breast carcinomas.

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 *Adam23* 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

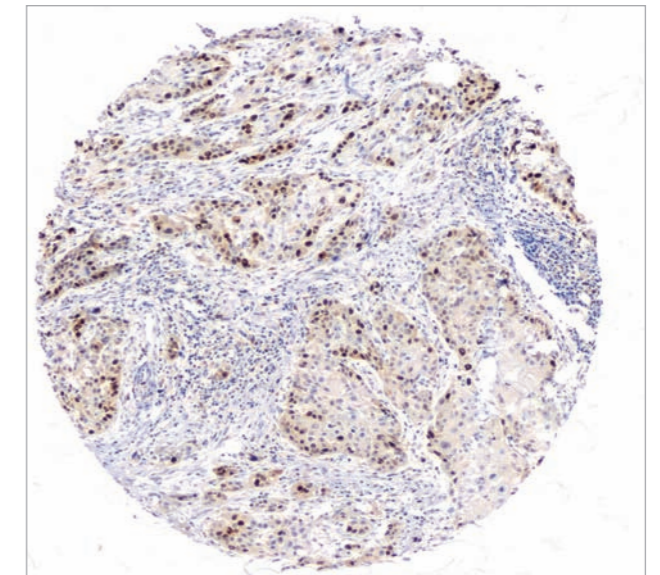
Our main research focus is on translation research in order to improve diagnosis and prognosis of the tumors, and identify predictors of treatment response. Nowadays our aims are concentrated on tumors of head and neck, soft tissues, breast and Wilms' tumor.

The breast carcinoma front was approached by two different projects. One of them studied the validation of *Adam23* hypermethylation (HyMe) as an independent prognostic factor, and comprised three segments: *Adam23* regulation of the activation of *avb3* integrin; *Adam23* HyMe in plasma samples from breast cancer patients; and *Adam23* 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.

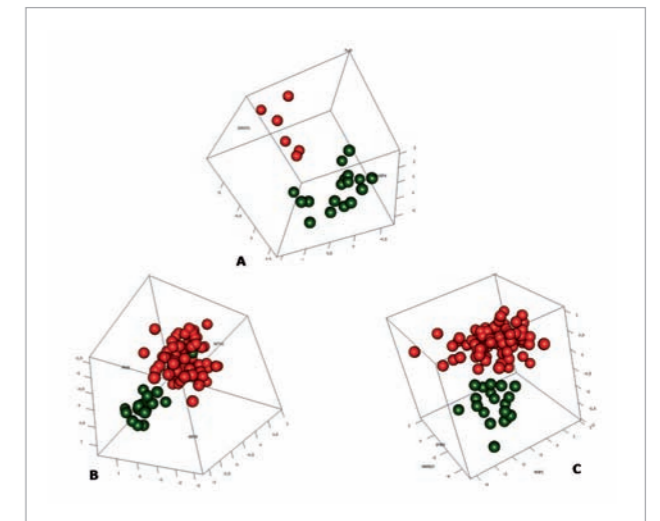
Also, we have analyzed molecular signatures in sarcomas. The main effort is to identify classifiers that are able to separate locally aggressive tumors, but without ability to develop metastasis from potential metastatic sarcomas.

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