FUNCTIONAL CHARACTERIZATION OF GENES POTENTIALLY REGULATED BY ESTROGEN RECEPTOR AND/OR *Erbb2* ONCOGENE: IMPLICATIONS IN THE DIAGNOSIS, PROGNOSIS AND TREATMENT OF BREAST CANCER

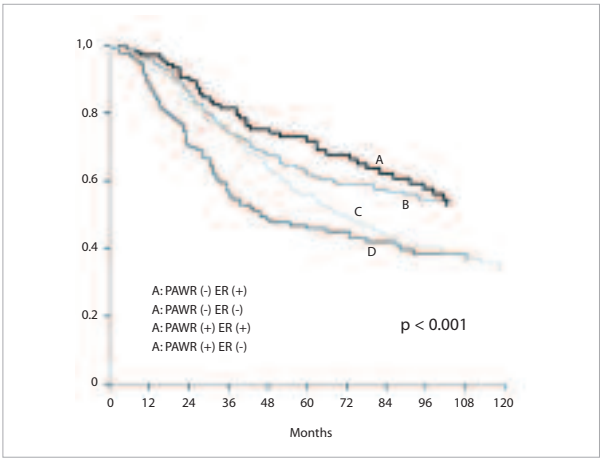
Maria Aparecida NAGAI

Medicine School / University of São Paulo (USP)

Breast cancer is the most commonly diagnosed neoplasm and the major source of morbidity and mortality among women worldwide. In Brazil, breast cancer is one of the most frequent malignancies in women and the leading cause of mortality. Data from the Ministry of Health estimates the occurrence of 49,400 new cases of breast cancer in 2008, representing an important health problem (INCA, Ministério da Saúde, 2008). Currently, the great deal of breast cancer research has been the identification of new diagnostic and prognostic factors for the disease and the understanding of critical signaling pathways involved in the carcinogenic process of the breast, which could provide the knowledge base for the identification of new therapeutic targets and new predictive markers of tumor sensitivity.

Using different techniques for gene expression analysis, such as DDRT-PCR, cDNA microarray, SAGE and Real Time PCR, in primary breast tumors regarding the presence or absence of ER and PR and two human mammary luminal cell lines expressing different levels of *Erbb2* before and after intensive exposure to docetaxel, we identified a large number of differentially expressed genes that could be considered as potential candidate markers for breast cancer. However, the functional role or clinical significance of these genes in breast cancer development and progression is still unknown.

The present study involves a group of qualified researchers with different expertise aiming to investigate the functional role and the prognostic value of the differentially expressed genes identified by our group, in breast cancer. To achieve these goals, six sub-projects will be performed. The first four are experimental studies involving the use of cell culture system in monolayers and in 3D, transient transfection with expression vectors, and suppression of gene expression by small interfering RNA (siRNA). In the fifth and sixth sub-projects, the Tissue Microarray technique (TMA) will be used to evaluate the clinical and prognostic significance of new candidate tumor markers in a large and well-characterized series of tumor samples and benign lesions of the breast. In addition, the expression of several panels of genes involved in different biological pathways will also be analyzed by IHC on TMAs.

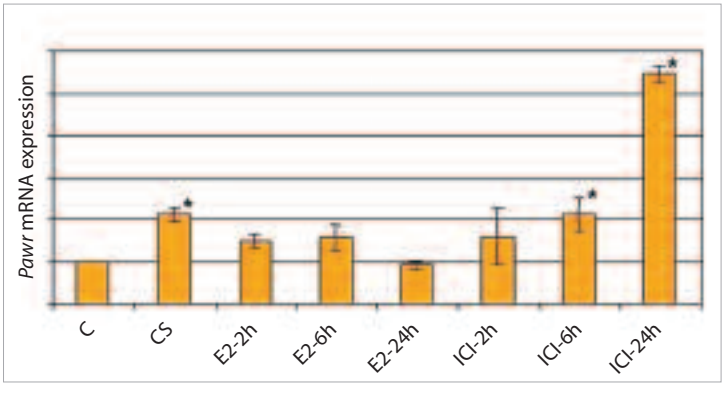


Kaplan-meier curves for long-term overall survival in breast cancer patients, stratified according to PAWR and ER protein expression. Tumors are classified in four categories according to the immunostaining status of PAWR and ER protein: PAWR-/ER-; PAWR-/ER+; PAWR+/ER-; PAWR+/ER+

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

To date we have preliminary results by real time RT-PCR confirming that several of the selected candidate genes are regulated by estrogen and/or growth factors, such as EGF (epidermal growth factor) in breast cancer cells, leading to the identification of a set of biomarkers candidates, whose expression in breast cancer cells, is selectively regulated by these cell signaling factors. We also showed that the transcripts of *Lrrc*49/*Thap*10 bidirectional gene pair are co-regulated by estrogen and that hypermethylation of the bidirectional promoter region simultaneously silences both genes in a subgroup of primary breast tumors. Moreover, among the genes evaluated in this study, the *Pawr* gene (*P*KC *a*poptosis *w*t1 *r*egulator gene; also named *Par*-4, *p*rostate *a*poptosis *r*esponse-4) that encodes a 38 KDa protein containing a death domain and a leucine zipper domain with pro-apoptotic activity, showed to be negatively modulated by estradiol and EGF in MCF-7 breast cancer cells. Our data using the pure antiestrogen ICI 182 780, also indicate that there might be a cross-talk between ER (estrogen receptor) and EGF pathways on the modulation of *Pawr*. In addition, immunohistochemical analysis of a wide panel of breast tumors was conducted to assess the prognostic value of the PAWR protein. By IHC on TMAs, fifty-two percent of the breast tumors showed positive PAWR protein expression. Significant associations were found between PAWR protein expression and advanced clinical stage, nuclear grade and the presence of estrogen receptor. Furthermore, PAWR protein expression was directly associated with shorter disease and overall survival of the patients. Although further studies are required to better characterize the biochemical and biological function of *Pawr*, our results suggest that down-regulation of *Pawr* expression might be involved in the regulation of cell proliferation and survival by estrogens and growth factors, and provide evidence that increased PAWR protein expression play role in breast cancer progression and could serve as useful prognostic marker of the disease outcome.

Effect of 17b-estradiol (E2) and fulvestrant (ICI 182 780) ont the Pawr *mRNA expression in MCF-7 breast cancer cells. *, p<0.05 treatment groups that showed higher* Pawr *mRNA expression than the control group*



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Maria Aparecida NAGAI

Faculdade de Medicina
Universidade de São Paulo (USP)
Avenida Dr. Arnaldo, 455 – Cerqueira César
01296903 – São Paulo, SP – Brasil

+55-11-30697292
nagai@usp.br