The survival of patients with only PR receptors or no receptors (ER–PR–EGFR–) within breast cancer tissue do not differ significantly from the parameters found in the reference variable ER+ PR+EGFR–, RR for DFS and OS are, respectively, less than 1 (0.63 and 0.26) or only slightly greater than 1 (1.07 and 1.16).

**174** POSTER

**Significance of MAGE-A gene expression in primary breast cancer**

Y. Lee[1], H. Park[1], J. Jung[1], G. Hwang[1], N. Lee[2], B. Kim[2], W. Hae[2], J. Suh[2]. [1]*Kyungpook national university hospital, Surgery, Daegu, Korea;* [2]*Kyungpook national university hospital, Clinical pathology, Daegu, Korea*

**Background:** The melanoma antigen (MAGE)-A genes are expressed in various malignant tissues including breast cancer, but not in normal tissues other than the placenta and testis. MAGE-A family consists of several subtypes from MAGE-1 to MAGE-12. We intended to investigate the significance and the correlation with clinicopathological factors of MAGE-A gene expression in primary breast cancer.

**Methods:** We collected 23 breast cancer tissues and 12 breast benign lesion tissues and kept at −70° until total RNA isolation. MAGE gene expression was assessed by nested reverse transcription-polymerase chain reaction assay using Cancer Hunter Core kit (iC&G Co., Daegu, Korea) which contained multiple MAGEs recognizing primers that can bind to the sequences of cDNA of MAGE-1, -2, -3, -4, -5 and -6 together. We determined estrogen receptor (ER), progesterone receptor (PR), P53 and c-erb B2 status by immunohistochemistry.

**Results:** The MAGE gene expression was positive in 15 (65.2%) of 23 cancer tissues, which correlated with the tumor size (P=0.0086) and inversely correlated with the ER status (P=0.0007). No association was observed for MAGE-A gene expression and lymph node metastasis, grade, TNM stage, PR, P53 and c-erb B2 status. The expression of MAGE genes is not recognized in benign tissues.

**Conclusions:** Our data suggest that the MAGE-A gene expression in primary breast cancer relates to potential involvement in tumor progression and may be associated with unfavorable prognosis. The detection of multiple MAGE gene expression together seems to be more useful for the diagnosis of MAGE-expressing cancers.

**175** POSTER

**Transforming growth factor-beta1 and steroid receptor status in breast cancer**

N. Todorovic-Rakovic[1], V. Ivanovic[2], M. Demajo[3], Z. Neskovic-Konstantinovic[4], D. Nikolic-Vukosavljevic[1]. [1]*Institute for Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade, Serbia;* [2]*Institute for Nuclear Science "Vinca", Laboratory for Radiobiology and Molecular Genetics, Belgrade, Serbia;* [3]*Institute for Nuclear Science "Vinca", Laboratory for Molecular Biology and Endocrinology, Belgrade, Serbia;* [4]*Institute for Oncology and Radiology of Serbia, Department of Clinical Oncology, Belgrade, Serbia*

**Background:** In despite of controversial results of transforming growth factor-beta1 (TGF-beta1) role and importance in breast cancer, it is generally accepted that TGF-beta1 is molecular biomarker of breast cancer progression. It is suggested that estrogen receptor (ER) and progesterone receptor (PR) phenotypes as markers of breast cancer estrogen dependence, are inherently different in relation to biological and clinical features. The aim of this study was to examine the relationship between the expression of TGF-beta1 and ER/PR status.

**Materials and methods:** This study included 52 breast cancer patients (I, II, III and IV stage) and 36 healthy women donors, as controls. Informed consent was obtained from each woman, according to the National Health Regulations. Determination of TGF-beta1 levels in platelet-poor plasma samples, was performed with commercial Quantikine ELISA kit (R&D,USA). Steroid receptor content was determined by classical biochemical ligand binding assay.

**Results:** Statistically significant higher expression of TGF-beta1 was found in breast cancer patients in relation to healthy donors. In that analyzed breast cancer group of patients, expression of TGF-beta1, there was a statistically significant difference between PR-positive and PR-negative subgroups, as well as between ERPR-negative and ERPR-positive subgroups. Higher levels of TGF-beta1 were found within unfavorable steroid receptor phenotypes. It is important to point out that in separated group of patients with metastasis (stage IV) statistically significant higher levels of TGF-beta1 were found even in the ER-negative subgroup as well, in comparison with ER-positive subgroup.

**Conclusion:** These findings indicate that TGF-beta1 could be considered as a marker of progression of disease and possible marker of more aggressive hormonally independent breast cancer (ER–, PR–, ER–PR–).

**176** POSTER

**Loss of fhit expression is associated with higher malignant phenotypes in breast cancer**

V. Sanna[1], P. Cossu Rocca[2], A. Spanu[3], M. Sarobba[1], G. Sanna[1], A. Mura[2], G. Mura[2], A. Farris[1]. [1]*University, Medical Oncology, Sassari, Italy;* [2]*University, Department of Pathology, Sassari, Italy;* [3]*University, Nuclear Medicine, Sassari, Italy*

**Background:** Breast cancer originates trough a series of genetic events, with a multistep pattern which includes alterations of tumor suppressor gene. Fhit gene has been identificated as a gene with a role of suppression in human cells.

**Method:** 225 breast tumor have been analyzed about the expression of fhit protein with an himmunohistochemical assay.

**Results:** In the present study was noted a negative-weak expression of fhit protein in 46% of patients, according to the criteria of fhit immunohistochemical assay score, and a moderate–strong reaction in 54%. As regards fhit vs grading we had 9 (39%) cases with negative–weak reaction and 14 (61%) with a moderate or strong reaction among G1; instead among G2 fhit positive staining moderate or strong was detected in 42 cases (45%) and reduced (negative–weak) in 21 (72%). Among G3 fhit expression was moderate–strong in 8 (28%) cases and weak negative in 21 cases (72%). Fhit protein is widely reduced in poor differentiated carcinomas. As regards fhit protein expression and nodal status we we detected a weak negative expression in 34 cases (63%) and a moderate strong expression in 20 cases (37%). On the contrary a weak negative reaction was detected in 48 cases (44%) and a moderate strong in 62 (56%) among N–. Therefore if nodal status is positive fhit protein is more often alterated. Finally we had examined fhit vs hormonal receptors. 73 cases (42%) of ER-positive had a negative–weak reaction and 101 (58%) a moderate–strong reaction. Instead 21 cases (73%) of Er-negative was negative–weak and 11 (27%) was moderate–strong. In a similar way among patients with a pg-positive status fhit expression was weak–negative in 64 cases (43%) and moderate–strong in 85% (57%). Among patients with pg-negative status a strong moderate reaction was detected in 27 cases (40%) and weak–negative in 40 (60%) cases. That is fhit protein is more expressed in patients with a positive status of hormone receptors.

**Conclusions:** In our study Fhit expression was inversely correlated in a statistically significant manner with histological grade (c2=6.2 for 2 grad of liberty, $p<0.05$) negative nodal status (c2=4.6; $p<0.05$) negative ER receptors (c2=12.3; $p<0.01$) negative PG receptors (c2=4.5; $p<0.05$). Loss of fhit expression could be associated with higher malignant phenotypes and appear to be a prognostic factor in breast cancer.

**177** POSTER

**Mammaglobin A, a novel marker of minimal residual disease in early stages breast cancer**

F. Janku[1], Z. Kleibl[2], J. Novotny[1], P. Tesarova[1], L. Petruzelka[1], M. Safanda[1], P. Mares[1], M. Zemanova[1], B. Matous[1]. [1]*Charles University, 1st Faculty of Medicine, Department of Oncology, Praha, Czech Republic;* [2]*Charles University, 1st Faculty of Medicine, Institute of Biochemistry and Experimental Oncology, Praha, Czech Republic*

**Background:** Mammaglobin A, in contrast to other factors, is a breast specific member of uteroglobin gene family. Expression is restricted to normal and neoplastic breast epithelium. We examined Mammaglobin A expression using RT-PCR in the bone marrow of patients with breast cancer.

**Methods:** There were examined bone marrow aspirates of 37 patients (median age 51) with stage I (40%), II (58%) and III (2%) breast cancer who underwent either immediate complete curative surgery or neoadjuvant therapy with subsequent radical procedure. mRNA was isolated using QIAamp RNA blood mini kit (Qiagen®). Subsequently two-step nested RT-PCR for the expression of Mammaglobin A was performed.

**Results:** Mammaglobin A was detected in samples from 5 (14%) out of 37 patients. With a median follow-up of 24 month (range: 4–32) we noted only 2 recurrences. However, one was observed in the patient with positive bone marrow. These data are obviously too immature to observe statistical differences.

**Conclusion:** We have shown that RT-PCR assay for Mammaglobin A may be used for detection of occult breast cancer cells in the bone marrow. Clinical and prognostic value of this method should be further investigated in the prospective fashion. At the meeting we will demonstrate updated results of 65 patients.

Acknowledgement: Supported by a grant IGA MZ CR NC/6672-3.