a whole (tATM) as indicated by pan-cytokeratin expression, tumor nuclear compartment (nATM) as indicated by both DAPI and pan-cytokeratin positive, and cancer associated stromal (csATM) as indicated by vimentin-positive and pan-cytokeratin-negative. ATM expression levels within these compartments were correlated with clinical outcome.

Results: While tATM and nATM were significantly lower in tumors compared to normal breast epithelial tissues, csATM was significantly higher than the corresponding normal tissue compartment. In addition, the median expression level of both tATM and nATM were two to three-fold lower ($p < 0.001$) in HNBC than in HPBC. In both HNBC and HPBC cohorts, patients with low tATM, nATM and csATM tumors had significantly poorer survival outcomes than those with a high tATM, nATM and csATM, but this effect was more pronounced in HNBC. A multivariate analysis demonstrates these biomarkers predict survival independent of tumor size and lymph nodes status, only in HNBC cohort ($p < 0.001$).

Conclusions: Low ATM protein expression in both malignant tumor and stromal compartments likely contributes to the aggressive nature of breast cancer and is an independent prognostic factor associated with worse survival in HNBC patients.

No conflicts of interest

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Interleukin-8 in progression of ER-positive and ER-negative breast cancer patients

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Background: Interleukin-8 (IL-8) is a multifunctional cytokine, linked to cancer progression. Biological functions of IL-8 are associated with aggressive potential of estrogen receptor (ER)– breast cancer which is characterized with increased expression of IL-8. In contrast to that, less is known about the role and significance of IL-8 in ER+ breast cancer and about the influence of its expression on clinical course of disease in ER+ breast cancer patients.

Material and Methods: The study included 91 postmenopausal primary breast cancer patients (clinical stage I/II) with favorable clinicopathological parameters, mostly with negative lymph node status. These patients didn't receive any kind of adjuvant therapy according to valid protocol at that time. IL-8 levels were determined in primary tumor tissue homogenates by ELISA according to manufacturer's instructions (RayBio Human IL8 ELISA kit). The same primary tumor tissue homogenates were used for ER determination by classical biochemical method. ER levels $\geqslant 10$ fmol/mg were considered as positive.

Results: There was no statistically significant correlation ($p = 0.2$, Spearman rank order) between ER and IL-8 expression and no statistically significant difference between quantitative IL-8 levels in ER– and ER+ subgroups ($p = 0.06$ Mann Whitney test), although median values of IL-8 in ER-negative subgroup was 387 pg/mg versus 76 pg/mg in ER-positive subgroup. This implies that breast cancer patients with higher levels of IL-8 have lower level of ER. When patients were stratified according to their ER status and using the median IL-8 level for the whole group of breast cancer patients (88.82 pg/mg), it was obvious that IL-8 expression had no influence on survival of ER– breast cancer patients, but had significant influence on survival of ER+ breast cancer patients ($p = 0.04$, Log rank). Patients with ER+IL-8– phenotype had almost twice longer median relapse free survival time (150 months) than patients with ER+IL-8+ phenotype (85 months). Also, the number of relapses in ER+IL-8– subgroup was 18 versus 38 in ER+IL-8+ subgroup.

Conclusion: Our findings indicate the more complex association between ER and IL-8 in breast cancer and significant role of IL-8 in progression of ER+ tumors, in contrast to the previously accepted hypothesis that IL-8 expression is important feature which exclusively influence the course of disease in ER– breast cancer patients.

No conflicts of interest

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The effect of VEGF peptide vaccine on inhibition of metastasis in mice breast cancer model

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Introduction: Breast cancer is the most prevalent cancer in the women that cancer cells metastasis to different tissues and impair the function of them. Cancer cells for growth and development secret some metastatic factors that one of them is vascular endothelial growth factor (VEGF). Already peptide based vaccines are one of the immunotherapy approaches. In this study a peptide based vaccine designed and used for induction of immune system against of VEGF molecule.

Material and Methods: By bioinformatics, a part of the VEGF molecule was selected as a peptide based vaccine and conjugated with the KLH carrier. BALB/c mice divided into three groups that one group received peptide vaccine and then 4T1 breast cancer model was created, the second group were only 4T1 breast cancer model and third group were negative control. The mice serums were taken at intervals and the titration of serum IgG against VEGF was measured using ELISA. The weights of the mice in all of the groups as well as the primary tumor volume in vaccinated and tumoral group were measured respectively.

Results: The titer of anti-VEGF IgG had significantly increased in vaccinated group compared to tumoral group. Tumor growth in the vaccinated group compared to tumoral group showed the dramatic decrease. As well as the survival rate in the vaccinated group was more than the tumoral group and less than the negative control group.

Conclusion: The results of this research show that the designed peptide based vaccine to be able to inhibit the growth and spread of tumoral cells in murine model.

No conflicts of interest

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Immunohistochemical expression of carbonic anhydrase IX, cellular tumor antigen p53, and apoptosis regulator Bcl-2 in triple-negative breast cancer

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Background: Tumor hypoxia is an important indicator of malignant disease prognosis, associated with aggressive tumor growth, early metastasis and poor response to the treatment. In hypoxic conditions an increased expression of p53 can occur, along with induction of apoptosis via Bcl-2. Our previous study showed that the high expression of a hypoxic marker carbonic anhydrase IX (CA-IX) was a strong independent prognostic indicator for shorter overall (OS) and recurrence-free survival (RFS) in patients with invasive ductal breast carcinoma (Beketic-Oreskovic et al. Pathol Oncol Res, 2011; 17: 593–603).

Aim: The aim of this study was to examine the expression of a hypoxic marker carbonic anhydrase IX (CAIX), Bcl-2 as a marker of apoptosis, and tumor suppressor protein p53, as prognostic parameters in patients with triple negative breast cancer.

Material and Methods: Immunohistochemical expressions of CAIX, Bcl-2 and p53 were analyzed on paraffin-embedded tumor tissues from 64 female TNBC patients, and correlate with standard clinico-pathological parameters and patients' overall survival.

Results: Expression of Bcl-2 was in negative correlation with histological tumor grade ($p = 0.036$), while p53 expression was in positive correlation with both tumor grade and tumor size ($p = 0.033$ and $p = 0.010$, respectively). There was no significant correlation among the expressions of any examined markers. Patients with "high" tumor Bcl-2 expression (above cut-off values) have shorter OS (HR 4.31, 95% CI 0.50–37.44, $p = 0.020$), while there was no correlation between expression of CAIX, p53, KI-67 and patients' survival. In multivariate analysis Bcl-2 was shown to be an independent prognostic indicator for OS in TNBC patients (HR 10.45, 95% CI 2.23–49.02, $p = 0.003$).

Conclusions: High proportion of TNBC samples showed high expression of hypoxic marker CAIX, as well as tumor suppressor protein p53 and proliferation index KI-67. Increased expressions of CAIX, p53, and KI-67 were not connected with decreased patients' survival. However, patients with "high" tumor Bcl-2 expression have shorter overall survival, and expression of anti-apoptotic protein Bcl-2 was an independent prognostic indicator for decreased overall survival for patients with TNBC. With the "cut-off" values of Bcl-2 expression we were able to distinguish TNBC patients with better or worse prognosis.

No conflicts of interest