Immune checkpoints and epithelial to mesenchymal transition related molecules in triple negative breast cancer patients' circulating tumor cells and their clinical impact

Vasileios Vardas[1]
Anastasios Tolios[1], Athina Christopoulou[2], Vassilis Georgoulias[3], Anastasia Xagara[4], Filippos Koinis[4], Athanasios Kotsakis[4] and Galatea Kallergi[1]
[1] Laboratory of Biochemistry/Metastatic Signaling, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, GR-26504 Patras, Greece
[2] Oncology Unit, ST Andrews General Hospital of Patras, GR-26332 Patras
[3] Hellenic Oncology Research Group (HORG), GR-11526 Athens
[4] Laboratory of Oncology, Faculty of Medicine, School of Health Sciences, University of Thessaly, GR 41110, Larissa, Greece

Background & objectives

Triple negative (TN) represents 15% of all breast cancers and it is the most aggressive breast cancer (BC) subtype. There is a limitation in targeted therapies for these patients, leading to an unmet need for new biomarkers. A better understanding of the molecular mechanisms that govern metastasis and the identification of early therapeutic approaches to prevent the dissemination of tumor cells in TN breast cancer (TNBC) patients is highly important. The present study aimed to investigate the expression of immune checkpoint molecules (PD-L1, CTLA-4) and Epithelial to Mesenchymal transition (EMT) related proteins (GLU and VIM) in TNBC patients' CTCs and assess their relationship to disease severity and clinical outcome.

Methods

Eighty-three patients were enrolled in this study: 52 TNBC and 31 luminal. Of these patients, 53 were in early stage, while 30 had metastatic disease. PBMC's cytospins were prepared from these TNBC patients, using Ficoll-Hypaque density gradient centrifugation. Protein expression was identified by immunofluorescence staining experiments and VyCap analysis.

Results

All the examined proteins were upregulated in TNBC patients. The expression of $GLU^+VIM^+CK^+$ phenotype was higher (47%) in metastatic TNBC compared to early TNBC patients (20%) (p = 0.043). Among all the BC patients, a significant correlation was found between $PD\text{-}L1^+CD45^-CK^+$ and $CTLA\text{-}4^+CD45^-CK^+$ phenotypes (Spearman test, p = 0.029) implying an important role of dual inhibition in BC. Finally, the phenotypes $GLU^+VIM^+CK^+$ and $PD\text{-}L1^+CD45^-CK^+$ were associated with shorter Overall Survival (OS) in TNBC patients (OS: log rank p = 0.048, HR = 2.9, OS: log rank $p < 0.001$, HR = 8.48, respectively).

Conclusion

Thus, PD-L1, CTLA-4, GLU, and VIM constitute significant biomarkers in TNBC associated with patients' outcome, providing new therapeutic targets for this difficult breast cancer subtype.