COMPREHENSIVE EVALUATION OF PD-L1 EXPRESSION IN CIRCULATING TUMOR CELLS (CTCs) AND PLASMA-DERIVED EXOSOMES IN TRIPLE NEGATIVE BREAST CANCER PATIENTS

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ΕΛΛΗΝΙΚΟ ΣΥΝΕΔΡΙΟ ΚΛΙΝΙΚΗΣ ΟΓΚΟΛΟΓΙΑΣ 31ο ΕΣΚΟ

ABSTRACT

Introduction: Triple-negative (TN) is the most aggressive subtype of breast cancer (BC) accounting for approximately 15% of all BC cases. Available target therapies are limited and often insufficient for these patients. Liquid biopsy is a non-invasive method for identifying cancer biomarkers and potential therapeutic targets in body fluids, examining different components such as circulating tumor cells (CTCs), nucleic acids, and extracellular vesicles/exosomes.

Aim: Our study aimed to evaluate the presence of programmed cell death ligand 1(PD-L1) in CTCs and plasma-derived exosomes, from TNBC patients, and address their clinical relevance.

Methods: Our research enrolled 35 TNBC patients and 12 healthy donors. CTCs were isolated by ficoll density gradient centrifugation, followed by triple immunofluorescence experiments and VyCAP platform analysis. Additionally, exosomes were isolated from the plasma of the same cohort of patients and characterized by transmission electron microscopy and Western blot analysis.

Results: CTCs were detected in 20% of cases. Notably, PD-L1 was identified in 6 out of 7 CK-positive patients (85.7%), while PD-L1 negative CTCs were detected only in 1 case (14.3%). The $CK^{+}PD\text{-}L1^{+}CD45^{-}$ phenotype was associated with shorter progression-free survival (PFS) (log-rank p=0.006, HR=7.8). On the other hand, PD-L1 exosomal expression in patients was lower compared to healthy donors. However, in 22.9% of patients, PD-L1 exosomal expression exceeded the median observed in healthy donors and was associated with significantly poorer PFS (log-rank p=0.046, HR=6.4). Finally, the combination of ($CK^{+}PD\text{-}L1^{+}CD45^{-}$) phenotype in CTCs and PD-L1 exosomal overexpression showcased a statistically significant worse PFS (log-rank p=0.018, HR=6.4).

Conclusions: Overexpression of PD-L1 in either CTCs or exosomes is associated with worse clinical outcome. Furthermore, the combined evaluation of exosomes and CTCs provides an interesting approach to classify patients at higher risk of relapse. Furthermore, PD-L1 overexpression could potentially serve as a therapeutic target for these patients.

INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed cancer among females, with more than 2 million new cases annually. Triple Negative BC (TNBC) is the most aggressive BC subtype, with current treatment options being limited and frequently inadequate[1]. Liquid Biopsy (LB) enables non-invasive identification of new cancer biomarkers and provides innovative tools for efficient disease and therapy monitoring. CTCs hold an established clinical value in LB for many different cancer types, including TNBC, while exosomes have gained increasing attention for their promising potential in cancer research and clinical applications.

Immune checkpoint molecules, such as Programmed Death Ligand 1 (PD-L1), regulate immune responses by preventing autoimmunity. Some cancer cells exploit this mechanism by overexpressing PD-L1, allowing them to evade immune recognition and elimination[2].

Previous studies from our group have highlighted the correlation between PD-L1 expression in CTCs and clinical outcomes in TNBC, lung, and prostate cancer patients[2,3]. Regarding exosomes many questions remain to be answered considering the role of PD-L1 overexpression with current studies yielding limited and contradictory findings[4].

This study aimed to evaluate the expression of PD-L1 in CTCs and plasma-derived exosomes from TNBC patients, while also investigating its clinical relevance and association with disease progression and patient outcome.

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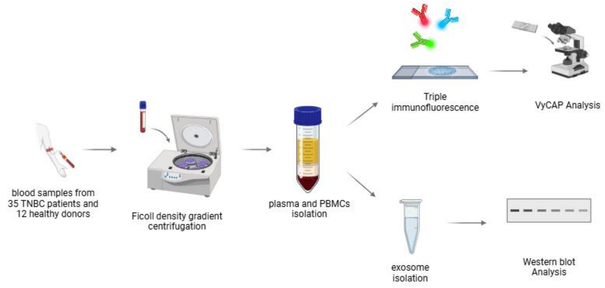
MATERIALS & METHODS



RESULTS

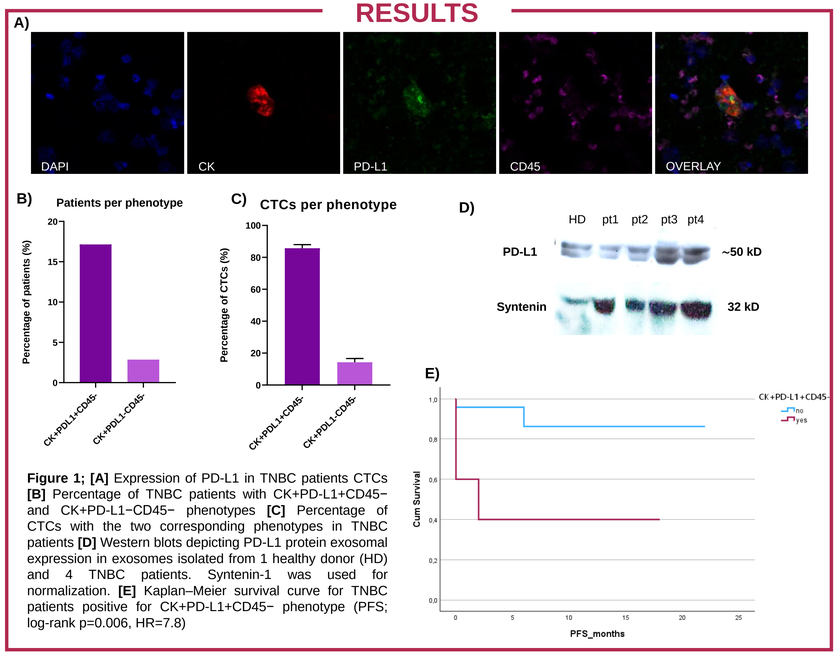
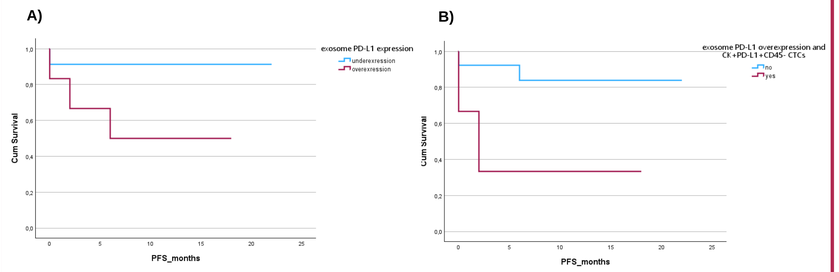


Figure 1; [A] Expression of PD-L1 in TNBC patients CTCs **[B]** Percentage of TNBC patients with CK+PD-L1+CD45− and CK+PD-L1−CD45− phenotypes **[C]** Percentage of CTCs with the two corresponding phenotypes in TNBC patients **[D]** Western blots depicting PD-L1 protein exosomal expression in exosomes isolated from 1 healthy donor (HD) and 4 TNBC patients. Syntenin-1 was used for normalization. **[E]** Kaplan–Meier survival curve for TNBC patients positive for CK+PD-L1+CD45− phenotype (PFS; log-rank p=0.006, HR=7.8)

RESULTS



Figures 2; [A] Kaplan–Meier survival curve for TNBC patients with PD-L1 protein exosomal overexpression (PFS; log-rank p=0.046, HR=6.4) **[B]** Kaplan–Meier survival curve for TNBC patients PD-L1 protein exosomal overexpression and CK+PD-L1+CD45− CTC phenotype (PFS; log-rank p=0.018, HR=6.4).

CONCLUSIONS

- **PD-L1 was overexpressed in the majority of the isolated CTCs from TNBC patients**
- **The expression of PD-L1 in CTCs was related to poorer PFS (log-rank p=0,006)**
- **Exosomal overexpression of PD-L1 in TNBC patients was also a biomarker for poorer PFS (log-rank p=0.046)**
- **Combined analysis of CTCs and Exosomes regarding PD-L1 overexpression was associated with poorer PFS (log-rank p=0.018).**
- **Expression of PD-L1 in CTCs and exosomes is an interesting biomarker in TNBC patients and a potential therapeutic target.**

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