Assessment of Fas/FasL expression in the peripheral blood of patients with metastatic breast cancer: correlation with clinical outcome

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ABSTRACT

Background: The activation of Fas (CD95/APO-1) death receptor by Fas ligand (FasL/CD95L) can trigger a signal transduction pathway leading to apoptosis, which is a common pathway used by immune cells for tumor elimination. Cancer cells also exploit FasL expression to induce immune cell apoptosis, known as "Fas counterattack", and thus to increase their invasion and migration capacity. The role of Fas/FasL system in peripheral immune response needs further investigation.

Methods: PB was obtained from 98 patients with metastatic BC at baseline before first-line treatment. Fas/FasL expression was individually assessed on circulating tumor cells (CTCs) and peripheral blood mononuclear cells (PBMCs) by performing immunofluorescent stainings and using fluorescence microscopy.

Aim: To investigate the expression of Fas and FasL on CTCs and immune cells in the peripheral blood (PB) of BC patients.

Results: CTCs were detected in 26/98 (26.5%) patients. Fas+ CTCs and FasL+ CTCs were identified in 88.5% and 92.3% of CTC-positive patients, respectively, representing the 57.1% and 82.9% of total CTCs. Regarding co-expression at single cell level, Fas+/FasL+ CTCs were detected in 84.6% of patients, whereas Fas+/FasL- CTCs, Fas-/FasL+ CTCs and Fas-/FasL- CTCs were identified in 7.7%, 19.2% and 11.5% of patients, respectively. Regarding PBMCs, the Fas+/FasL+ phenotype was identified in 70.3%, of patients, whereas Fas-/FasL+ PBMCs and Fas-/FasL- PBMCs were detected in 24.2% and 5.5% of patients, respectively; interestingly the Fas+/FasL- expression pattern was not observed in any patient. A reduced progression-free survival (PFS) was demonstrated among CTC-positive as compared to CTC-negative patients (median PFS: 9.5 versus 13.4 months; p=0.004), and specifically those harboring Fas+/FasL+ CTCs (median PFS: 9.5 vs 13.4 months; p=0.009). Increased overall survival (OS) was demonstrated among patients harboring Fas+/FasL+ PBMCs, as compared to those with Fas-/FasL+ PBMCs and Fas-/FasL- PBMCs (median OS: 35.7 vs 25.9 vs 14.4 months; p=0.008).

Conclusion: The findings highlight the Fas/FasL signaling pathway as a putative mechanism of immune evasion and metastatic progression of BC. Their role as prognostic biomarkers in BC patients merits further investigation.

INTRODUCTION

Fas ligand (FasL/CD95L) triggers apoptotic cell death following ligation to its receptor, Fas (CD95), and this pathway is frequently used by immune cells to eliminate tumors (1). However cancer cells also exploit FasL expression to induce immune cell apoptosis, which is known as "Fas counterattack" (2). Fas/FasL expression has been demonstrated on breast tumors, providing prognostic information for BC patients (3). The role of Fas/FasL system in the peripheral immune response needs further investigation.

OBJECTIVES

We herein assessed the expression of Fas/FasL on circulating tumor cells (CTCs) and peripheral blood mononuclear cells (PBMCs) of metastatic BC patients, with the following aims:

- ***To investigate their distribution among the tumor and immune cell compartments in the peripheral blood of BC patients***
- ***To evaluate their prognostic role in BC***

METHODS AND MATERIALS

- **Peripheral blood (10ml) was obtained from 98 patients with metastatic BC at baseline before first-line treatment**
- **PBMCs were isolated and immunofluorescently stained for cytokeratins (CK; clones: AE1/AE3 & C11), Fas, FasL and dapi (1x10^6 PBMCs were analyzed per patient)**
- **Fas/FasL expression was individually assessed on single CTCs (CK+ cells) and PBMCs (CK-cells) using fluorescence microscopy**
- **The H1975 cell line served as positive control for Fas/FasL expression (Figure 1A); the detection of any expression was used to define positivity of CTCs and PBMCs for the respective markers**

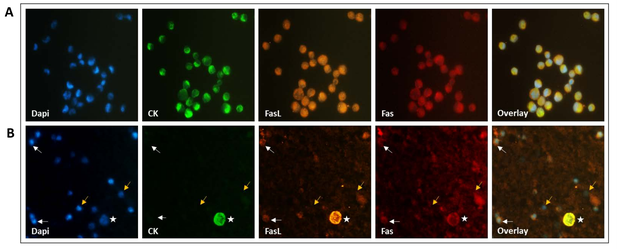

Figure 1: Representative images of Fas/FasL expression in H1975 control cell line (A) and in the peripheral blood of a metastatic BC patient (B). *A Fas+/FasL+ CTC (CK+ cell; indicated by star) among Fas+/FasL+ PBMCs (white arrows) and Fas-/FasL- PBMCs (yellow arrows), Ariol system (x400).*

RESULTS

1. Frequency of Fas/FasL expression on CTCs

- **CTCs were detected in 26.5% of patients (total CTC count: n=70).**
- **Fas+ CTCs and FasL+ CTCs were identified in 88.5% and 92.3% of CTC-positive patients, respectively, and represented the 57.1% and 82.9% of total CTCs (Figure 2).**

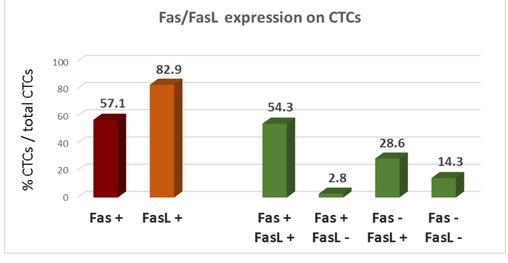

Figure 2. Distribution of Fas/FasL expression on CTCs of patients with metastatic BC (n=98).

- **Regarding co-expression at single cell level, Fas+/FasL+ CTCs were detected in 84.6% of patients, and represented the 54.3% of total CTCs (Figure 1B, Figure 2).**

2. Frequency of Fas/FasL expression on PBMCs

- **Fas+ PBMCs and FasL+ PBMCs were detected in 70.3% and 94.5% of patients, respectively, with a mean percentage per patient: 23.8% and 30.9%, respectively (Figure 1B, Figure 3A).**

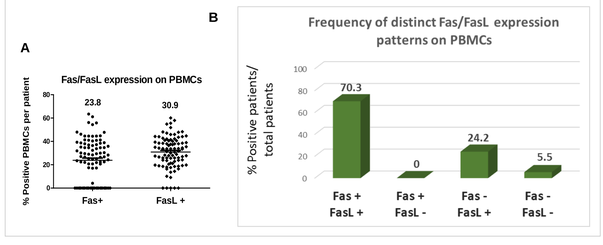

Figure 3. Distribution of Fas/FasL expression on PBMCs of patients with metastatic BC (n=96). A) *Percentage of positivity per patient; lines correspond to mean values,* ***B)*** *Frequency of distinct phenotypes among patients.*

- **The Fas+/FasL+ phenotype was identified on PBMCs in 70.3% of patients (Figure 3B); no Fas+/FasL- PBMCs were observed in any patient.**

3. Clinical relevance of Fas/FasL expression on CTCs and PBMCs

- **A reduced progression-free survival (PFS) was demonstrated among patients with Fas+/FasL+ CTCs, as compared to those with no detectable Fas+/FasL+ CTCs (median PFS: 9.5 vs 13.4 months; p=0.009) (Figure 4A).**
- **Increased overall survival (OS) was observed among patients harboring the Fas+/FasL+ phenotype on PBMCs, as compared to patients with Fas-/FasL+ PBMCs and Fas-/FasL- PBMCs (median OS: 35.7 vs 25.9 vs 14.4 months; p=0.008) (Figure 4B).**

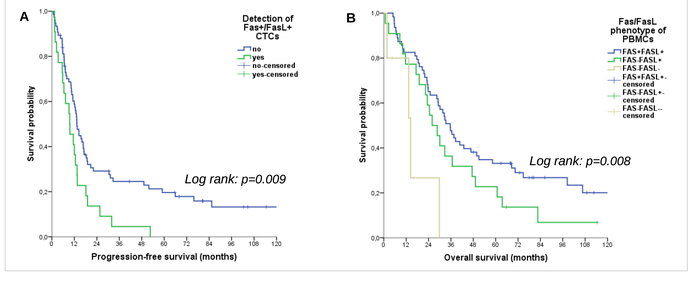

Figure 4. Kaplan-Meier curves for PFS (A) and OS (B) of metastatic BC patients (n=96), based on the Fas/FasL expression status of CTCs and PBMCs

CONCLUSIONS

- **Fas/FasL are frequently expressed on CTCs and PBMCs of metastatic BC patients, and provide significant prognostic information.**
- **The findings highlight the Fas/FasL signaling pathway as a putative mechanism of immune evasion and metastatic progression of BC.**
- **The role of Fas/FasL as prognostic biomarkers in BC patients merits further investigation.**

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