



Metastatic Carcinoma Cells Floating in Pleural Effusion Devour PD-1+ Lymphocytes

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Abstract

We studied the interaction of PD-1⁺ lymphocytes with Ovary Carcinoma Cancer Cells (OCC) in several patients with pleural metastasis using immunocytochemical staining of pleural liquid. In addition to the intact separate T-cells, we discovered ubiquitous PD-1⁺-OCC aggregates in which the lymphocytes showed significant signs of cell defects and degradation in the environment of the otherwise intact OCC. Formation of such complexes and subsequent destruction of lymphocytes may be a pathway to provide the nutritional supply and support further growth of cancer cells in plank tonic state, in absence of vascularization and angiogenesis, relevant to both progression of lung metastases and some primary tumors such as small cell lung carcinoma.

Keywords

Ovarian Carcinoma; T-Lymphocytes; Pleural Effusion

Introduction

High aggression of the metastatic tumors, particularly ubiquitous in the lungs, remains one of the major problems in managing cancer progression and treatment. For example, the pleural metastatic expression of Ki-67 is high whereas p53 is lower than in primary tumors [1]. However, an establishment and growth of early metastatic formations are still poorly understood and not really amenable to prevention. For the most part the metastatic tumor proliferation is considered in context of interaction with the related stromal cells, vascularization and angiogenesis.

Tumor Infiltrating Lymphocytes (TIL) are promising agents in cancer therapy as indicated by ongoing clinical trials involving autologous isolates causing tumor lysis [2]. Development of the PD-1⁺ sub-population of activated lymphocytes has been linked to cancer progression and various drug strategies emerged related to the so-called immune checkpoint inhibitors (e.g., Keytruda) blocking this receptor. In this report, driven by the interest in metastatic tumors and the role of T-cells, we looked into interactions of plank tonic cancer cells with circulating lymphocytes in pleural isolates of several patients with metastatic ovary carcinoma and discovered widespread PD-1⁺-OCC aggregates that are terminally destructive for lymphocytes but not damaging to OCC.

Methods

A fine needle aspiration biopsy was taken from three ovary carcinoma cancer patients with pleural and ascitic metastases. The pleural effusions were incubated for one hour and then sludge moved on smears. Cytological smears were fixed with a mixture of alcohol-acetone in a 1:1 ratio for 10 min and air-dried and endogenous peroxidase was inactivated with 1% sodium azide (Merck) for 15 min. Then smears were washed twice by water and left for five minutes in Tris-NaCl buffer (pH 7.6). After that, the field for immunohistochemical analysis was surrounded with a hydrophobic pen (DakoCytomation) before the application of pig serum (Novocastra). The anti-PD-1 monoclonal antibodies (NCL-HPp, Novocastra), directed against PD-1 antigens, were applied after incubation with serum (30 min at room temperature), and the preparation was incubated for an hour at 37°C. At the end of labeling with the first antibodies, the preparations were washed twice with the buffer for five minutes and pigs biotinylated antibodies (DakoCytomation) directed against anti-PD-1 antibodies were applied. The second antibody preparations (cover slip impression) were incubated for 15 min at room temperature. The next step of the immunohistochemical procedure was preceded by washing preparations in two shifts buffer; coating was for 10 min at room temperature in an imaging system that consisted of a soluble complex—avidin and biotinylated horseradish peroxidase (DakoCytomation). The 3,3'-Diaminobenzidine

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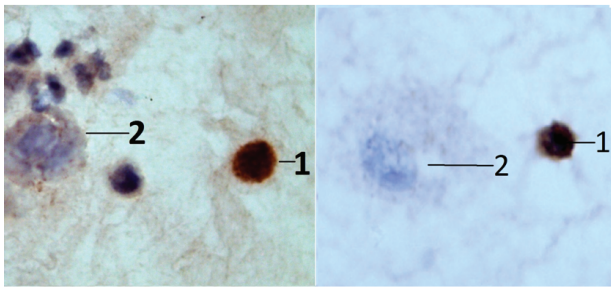


Figure 1: Intact PD-1⁺ T-cells (1) and OCC (2)

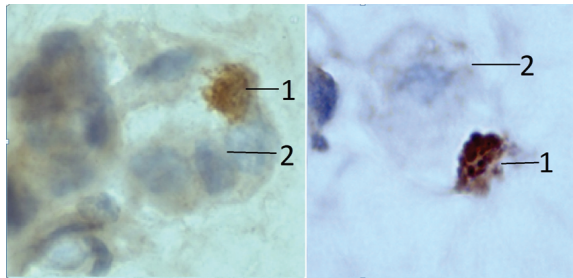


Figure 2: Damaged PD-1⁺ T-cells (1) phagocytized by OCC (2)

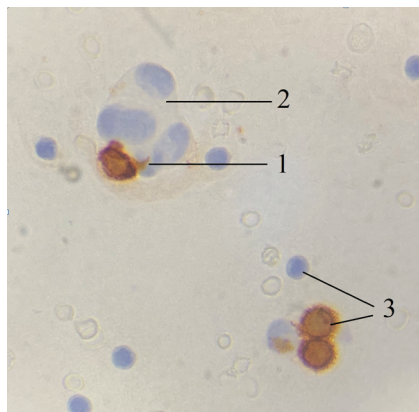


Figure 3: Damaged PD-1⁺ T-cells (1) phagocytized by OCC (2) and intact T-cells (3)

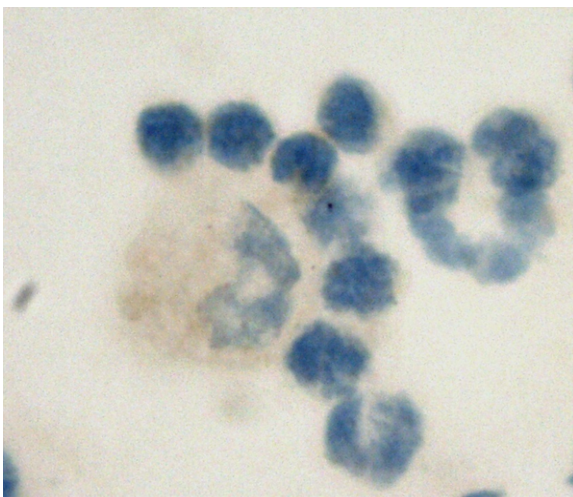


Figure 4: Damaged OCC cell

(DAB) in the format of Novocastra was used as a substrate for the manifestation of immunohistochemical reaction. Cover slip impression was additionally stained with haematoxylin. Analysis of the preparations (cover slip impression) was carried out using Leica DM 4000 B optical microscope with the immersion objective ($\times 100$). We visualized a total of 793 unbound pleural PD-1⁺ T-cells and PD-1⁺ OCC aggregates.

Results and Discussion

In the analyzed cytological mounts, PD-1⁺ lymphocytes were seen as both separate cells and in association with OCC. The total cell counts are presented in the Table 1. The separate PD-1⁺ lymphocytes looked normal and had a distinct spherical structure, granularity, and correct regular shape with defined contours, without any signs of cytoplasmic membrane destruction (Figure 1,3). In comparison, PD-1⁺ lymphocytes attached to the edges of OCC appeared damaged. They displayed major defects in the cell's shape that were associated with uneven PD staining and abnormality in chromatin integrity (Figure 2). We also observed what appeared to be remnants of the T-cells in cytoplasmic compartments of otherwise intact OCC, visible as more faint grainy formations (Figure 2). This led us to propose that PD-1⁺ lymphocytes get destructed and digested by intercellular interaction with OCC keeping cancer cells normal. In contrast, the interactions of PD-1⁺ lymphocytes with OCC (Table 2) overwhelmingly led to oncolytic alterations in OCC, possibly apoptotic, visualized as changes in the chromatin structure manifested in occurrence of the compacted fragments at the edge of cytoplasmic compartment (Figure 4).

Conclusions

Based on the immunocytochemical observations in a small group of metastatic ovary carcinoma patients, PD-1⁺ cells form complexes with plank tonic cancer cells in lungs and undergo phagocytosis while leaving OCC intact. Such interactions may be responsible for supporting proliferation and nutritional supply of the cancer cells and possibly relevant to both metastatic lung formations and Small Cell Lung Cancer (SCLC) that is typically presented by the floating clusters of the cancer cells as a major attribute [3].

Conflicts of Interest

The authors declare that there is no conflict of interest.

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