MAGE-A3 & PD-L1 Expression in Lung Cancer Tumor Microenvironment

MAGE-A3 and PD-L1 protein expression in lung cancer a look at the immune cells' in tumor microenvironment

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Abstract

This lab's previous studies highlight the co-expression of PD-L1 (CD274) and MAGE-A3 in non-small cell lung cancer (NSCLC) and squamous cell lung cancer, with around 50% of the screened tumors exhibiting this co-expression. Additionally, this study also identified MAGE-A3 as a secreted protein using CytoSections. A sandwich ELISA was developed for MAGE-A3 and demonstrated that serum levels of MAGE-A3 protein were significantly elevated in lung cancer patients compared to matched controls. This statistically significant difference suggests that MAGE-A3 could serve as a valuable biomarker for lung cancer diagnosis and prognosis. Such findings may also provide insights into the tumor microenvironment and the immune evasion mechanisms facilitated by PD-L1 and MAGE-A3 co-expression, potentially guiding the development of targeted therapies or combination strategies in the treatment of lung cancer. In this study, the lung cancers were screened with CD1C, CD163, CD20, CD31, CD3E, CD4, CD68, CD8A, FOXP3 immune markers previously evaluated with PD-L1 to characterize the tumor micro-environment where PDL1 and MAGE-A3 protein are co-expressed versus when only MAGE-A3 is expressed in lung cancer. MAGE-A3 was detected on positive CD3e and suggesting a role in regulating immune response. This study offers insights into the tumor microenvironment, a new view of immune escape in lung cancer, and the potential of MAGE-A3 as a diagnostic/prognostic biomarker and therapeutic target, particularly in combination with immune checkpoint inhibitors.

Introduction

In a previous study, the protein expression patterns of PD-L1 (CD274) and the cancer-testis antigen MAGE-A3 were examined in non-small cell lung cancer (NSCLC) and squamous cell lung cancer using sequential tissue sections. Both proteins were found to be present in a substantial number of cases. Notably, high levels of PD-L1 and MAGE-A3 expression were observed in immune cells located adjacent to the tumor or within regions densely populated with immune cells. Further investigations demonstrated that MAGE-A3 is a secreted protein, initially identified using CytoSections controls. This finding was subsequently confirmed with a sandwich ELISA assay developed in our lab, which specifically detects MAGE-A3 without cross-reactivity to the other 11 members of the MAGE-A family. Figure 2 shows that serum levels of MAGE-A3 protein were significantly elevated in lung cancer patients compared to matched controls. This statistically significant difference suggests that MAGE-A3 could serve as a valuable biomarker for lung cancer diagnosis and prognosis. This study investigates MAGE-A3–positive tumors to better understand the immune evasion mechanisms potentially facilitated by the presence of MAGE-A3 in immune cells. We screened MAGE-A3–positive lung cancer samples using a panel of immune cell markers—including CD1c, CD163, CD20, CD31, CD3E, CD4, CD68, CD8A, FOXP3, and PD-L1—using a double-stain immunohistochemistry (IHC) assay. Our goal was to characterize the immune cells within the tumor microenvironment that express high levels of MAGE-A3. The results showed that some immune cells expressing CD3E, CD4, CD8A, and CD20 were positive for MAGE-A3. Notably, CD31 was the most strongly associated marker, with significant co-expression observed in MAGE-A3–positive immune cells. These findings offer new insights into the tumor microenvironment and suggest that MAGE-A3 may play a role in promoting immune evasion in lung cancer by modulating immune cell behavior.

Design & Methods

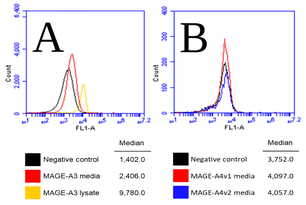
Immunocytochemistry

Tissues were sourced from OriGene's bank, collected from major U.S. medical centers under IRB and HIPAA-compliant protocols. Manual IHC staining was performed on paraffin-embedded CytoSections and FFPE tissues using anti-MAGE-A3 (OTIG9) and anti-PD-L1 (UMAB228) antibodies. Heat-induced epitope retrieval (HIER) with OriGene-Citrate pH6.0 or TEE buffer was applied for CD1C, CD163, CD20, CD31, CD3E, CD4, CD68, CD8A, and FOXP3. Detection used OriGene's Polink-DS-MM-Hu A Kit DS203-18 per manufacturer instructions.

Flow Cytometry

HEK293T cells in 10-cm dishes were transiently transfected with PEI and MAGE-A3 or MAGE-A4 TrueORF cDNA. Post-transfection media was collected, concentrated, and applied to untransfected cells to deliver secreted MAGE-A3/A4 proteins. After 48 hours, cells were fixed in 10% NBF for 2 hours and blocked with FACS buffer (1% BSA, 2% FBS, 0.002% NaN_3, PBS). Flow cytometry was performed using FITC-conjugated anti-DDK antibody (1:1000) in dilution buffer (0.1% NaN_3, 3% BSA, PBS). Gating used negative controls, and fluorescence was detected in channel 1.

Figure 1 MAGE-A3, and MAGE-A4 flow cytometry results using FITC and median fluorescence channel 1 absorbance (FL1-A).



A. Comparison of FL1-A of cells in media containing secreted MAGE-A3 from transfection to negative control and MAGE-A3 over-expression protein lysate.
B. Comparison of FL1-A of cells in media containing secreted MAGE-A4 variant 1 and MAGE-A4 variant 2 to negative control

Figure 2 Serum MAGE-A3 concentration in control and lung cancer patients
* p<0.05 compared to control group

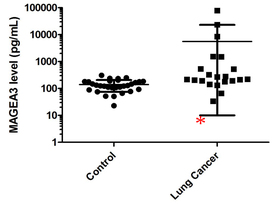
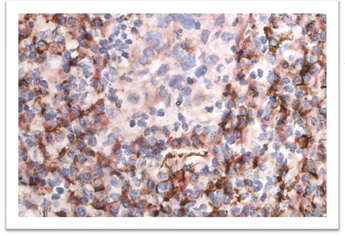


Figure 3 MAGE-A3 & CD31 Enlarged



Results

Figure 4 Double stain of Lung Cancer with MAGE-A3 (red) & PD-L1 (brown). Tumor immune cells show rare overlap with both proteins except in the tumor associated macrophages shown with red arrow.

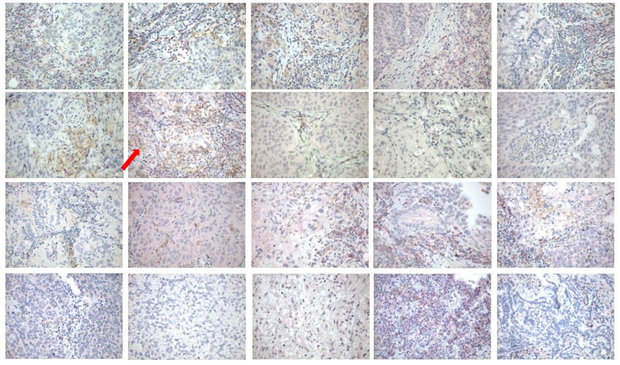


Figure 5 Double stain of lung cancer with MAGE-A3 (red) & CD31 (brown). Tumor immune cells show significant overlap with both proteins while the tumor cells remain positive for just MAGE-A3

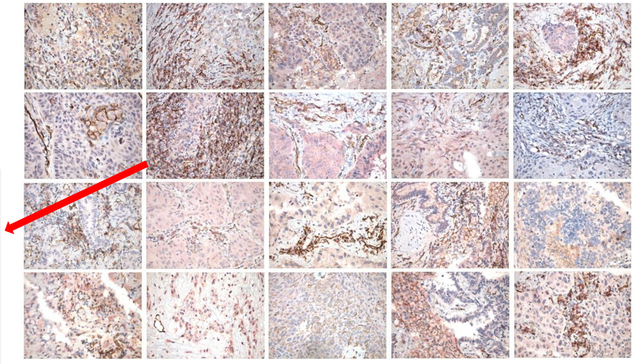
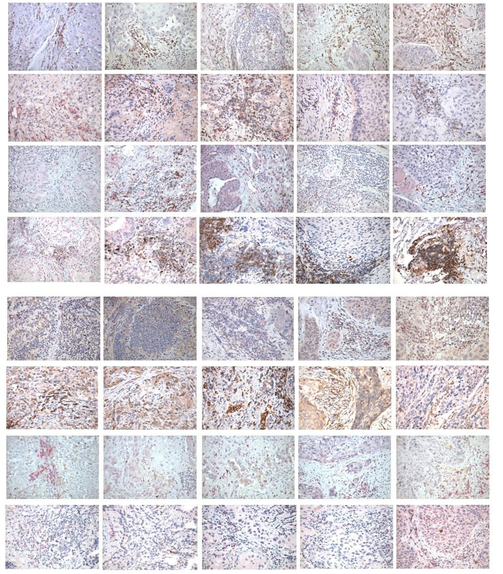


Figure 6 Double stain of Lung Cancer with MAGE-A3 (red) & immune cell marker in brown OnlyCD3e, CD4, CD8a, & CD20 <5% Overlap with MAGE-A3, the other immune cell markers show rare or no overlap



Conclusion

Our previous studies have shown that MAGE-A3 is secreted and detectable in immune cells when tumors are MAGE-A3 positive. This study demonstrates that PD-L1 and MAGE-A3 when co-expressed in lung cancer, there was notable overlap observed in alveolar macrophages expressing both markers. CD31, also known as PECAM-1, is a protein typically expressed by endothelial cells, as well as various immune cells including T cells, B cells, dendritic cells, and natural killer cells. Interestingly, in this study we found MAGE-A3 is often present with CD31 is expressed in tumor-associated immune cells. However, we did not see the level of overlap in the other immune cell markers evaluated. While CD31 is traditionally regarded as an endothelial marker, it also plays key roles in immune cell trafficking, activation, and intercellular interactions. New question what is MAGE-A3 role in these activities.