Poster 36: Spatial RNA sequencing supports programmed death ligand 2 (PD-L2) as a potential immunotherapy target in high grade serous ovarian carcinoma (HGSOC)

Presenting Author: Elise Yates, MD – Houston Methodist Hospital (HMH) Department of Obstetrics and Gynecology

Topic: Translational Research

Objectives

Currently available PD-1 and PD-L1 immune checkpoint inhibitors have shown limited success in high grade serous ovarian carcinoma (HGSOC). There is increasing evidence for the significance of PD-L2, a PD-1 ligand expressed by antigen presenting cells (APC), and mainly by tumor associated M2 macrophages (CD163+), as a key player in the HGSOC immune microenvironment. Here we aimed to utilize the GeoMx Digital Spatial Profiling system to evaluate the presence of PD-L2 and APC in HGSOC across clinical stages and in normal adjacent tissue (NAT)

Methods

GeoMx spatial sequencing protocols were applied to HGSOC tumor microarrays (TMA) of different stages and whole transcriptome (RNA) was quantified using Illumina next generation sequencing (NGS). 47 cores were analyzed, including NAT (n=8), local (IA-IB, n=8), regional (IC-IIC, n=17), and distant HGSOC (IIIA+, n=14). The data was then imported into the GeoMx analysis pipeline and Q3 normalized data was used to perform immune deconvolution analysis. GeoMx SpatialDecon was used to estimate the abundance of immune cells using whole transcriptome analysis RNA expression as a proxy for cell type. Differences across stages were analyzed using a linear mixed model with BH multiple test correction and adjusted for region of interest (ROI) to account for inter-patient heterogeneity. A Kaplan-Meier plot of PD-L2 (PDCD1-LG2) progressionfree survival outcomes for all histologic subtypes of ovarian cancer (OvCa) was also created using kmplot.com.

Results

Expression of M2-macrophage marker CD163 and PD-L2 were significantly increased in the stroma among all stages of HGSOC compared to NAT (P=< 0.05) Generic macrophage marker CD68 was increased in local and distant stages. PD-1 (CD274) was not significantly different from NAT in the stroma among any stage of HGSOC. However, PD-1 expression was found to be increased in the NAT epithelium group when compared to local HGSOC epithelium. A Kaplan-Meier plot of PD-L2 expression in all subtypes and treatment pathways of OvCa demonstrated that high expressors have a much lower progression free survival versus low expressors (HR=1.41, p = 0.0022).

Conclusions

We found that macrophages are increasingly present in the HGSOC stroma across stages. Additionally, CD163 expression is increased versus NAT, demonstrating the importance of the M2-like macrophage phenotype in OvCa progression. Interestingly, we found that PD-L2, but not PD-L1 transcription, is significantly increased in the stroma of all stages of HGSOC versus NAT stroma. These findings were supported by a Kaplan-Meier plot of PD-L2 expression in all OvCa subtypes showing that the prognosis (progression-free survival) is poorer in high PD-L2 expressors. Therefore, PD-L2 serves as a potential immunotherapy marker for HGSOC. Future efforts should investigate the impact of PD-L2 expression on the success of anti-PD-1 therapies and the role of PD-L2 as a potential immunotherapy target.