Determining the contributions of tumour-related genes to the Undifferentiated Pleomorphic Sarcoma anti-tumour microenvironment

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Abstract

Sarcoma survival rates have remained relatively stagnant in comparison to other cancers in recent decades. There is vast heterogeneity across and within sarcoma subtypes which convolutes classification and treatment. Accordingly, it is critical to integrate recent medical advances, such as immunotherapy, into the standard of care for sarcoma. Our group discovered that some sarcomas, including some Undifferentiated Pleomorphic Sarcomas (UPS) cases, contain an immune infiltrate and express variable levels of the immunosuppressive ligand Programmed Cell Death Ligand 1 (PD-L1). A survival advantage was found in only the UPS cases that expressed high PD-L1 levels. The factors underlying this improved clinical outcome are likely related to the tumour immune microenvironment (TME) however the tumour-related mechanisms that influence immune infiltration in UPS are unclear.

A list of genes that were differentially expressed and unique to PD-L1 high UPS cases was determined. Among these, STAT1 was of particular interest. I hypothesize that tumour-related genes associated with PD-L1 in UPS support an anti-tumour TME and ultimately, aid in tumour suppression. STAT1 is co-expressed with PD-L1 and may be related to tumour infiltration by immune cells. This study will create and characterize primary sarcoma cell lines to investigate the potential tumor-related roles of PD-L1 and STAT1 in the UPS TME.

Introduction

- PD-L1 expression in solid tumours and immune infiltration was discovered through immunohistochemistry staining in multiple sarcoma subtypes.
- By multivariate analysis, a better clinical outcome was associated with UPS cases that express higher levels of PD-L1.
- RNA-seq determined genes that were differentially expressed between PD-L1^{high} and low cases in UPS which may be linked to clinical outcome.
- An Ingenuity Pathway Analysis (IPA) indicated that genes within the TH1 pathway, including STAT1, were significantly activate within the genes uniquely expressed in UPS PD-L1-high cases.

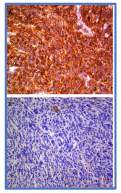


Figure 1. Immunohistochemistry stains of 2 cases; (A) a UPS with high and (B) low PD-L1 expression.

Overall Survival Analysis by Cox Proportional Hazards Model for Patients with UPS (n=29)

Prognostic Factor	Multivariate RR	95% CI	P-value#
PD-L1 Low vs High	6.27	1.05-79.1	0.035
Size >9cm vs <=9cm	1.45	0.3-8.82	
Gender Male vs Female	0.63	0.06-5.86	
Age at Diagnosis	1.03	0.96-1.14	

Inference by Firth-type bias correction

Table 1. In multivariate analysis of 29 non-metastatic UPS patients that included established prognostic factors, OS tended to be better in the group with high PD-L1 expression compared to those with low PD-L1 expression[1].

Hypothesis and Aims

I hypothesize that tumour-related PD-L1 and co-expressed STAT1 support an anti-tumour microenvironment through cytokine signaling that is unique to UPS

1. Create and characterize primary cell lines at the gene and cytokine level.
2. Identify how cell-line secreted cytokines impact immune migration and;
3. Determine if PD-L1 and STAT1 over or underexpression impacts global gene expression and alters the functional consequences of cytokine output

Methods

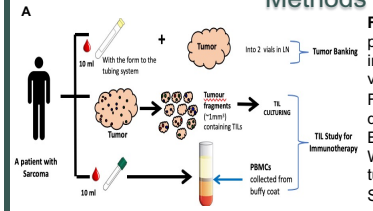


Figure 2. The methods of sample processing and cell line validation used in Aim 1. A) Untreated tumours are viably preserved with matched PBMCs. Fragments are further physically dissociated and expanded in vitro. B) Bulk tumour and paired blood were Whole Exome Sequenced to identify tumour-specific variations. PCR and Sanger sequencing determine if that mutation is retained in the cell line.

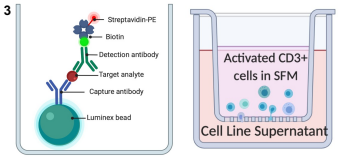


Figure 3. The characterizing methods used in Aim 1. qPCR assayed the gene expression of PD-L1 and related genes. A 14-plex Luminex assay quantified the cytokines in cell line supernatant. In Aim 2, a migration assay using CD3+ cells will determine the trafficking potential of tumour-secreted cytokines.

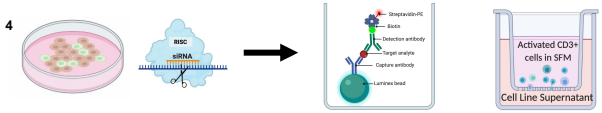


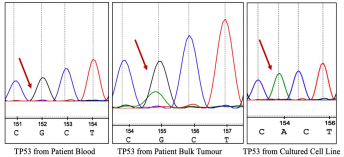
Figure 4. In Aim 3, cell line gene expression of PD-L1 and STAT1 will be modified and RNAseq, cytokine blots and supernatant-based functional assays will determine how each gene influences global gene expression, cytokine secretion and immune cell trafficking.

Results

Initiating primary sarcoma cell lines required optimization. The used methods included the use of viably-preserved samples that were physically dissociated and plated. 3 cell lines were selected for future aims.

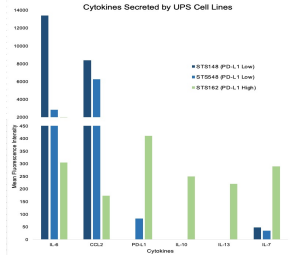
Case Name	Diagnosis	PD-L1 Status	PD-L1 qPCR Expression
STS 148	UPS	Low	0.3
STS 548	UPS	Low	0.16
STS 162	UPS	High	1.04

Case	Subtype & PD-L1 Status	gene symbol	Variation	VAF	Tumour AD (Ref/Alt)	Effect
STS162	UPS PD-L1 High	TP53	G524A	0.19	44/10	nonsynonymous SNV



Tumour specific variations (TSV) for each case were identified from WES data. Using PCR of the gene in question and sanger sequencing, multiple TSVs have been retained in 3 cell lines.

Figure 5. (left) Case STS162 retained a TSV after 15 passages as identified by PCR and Sanger Sequencing.



Cell line supernatant was collected after 48 hours from wells with 5x10^5 cells/ml. A 14-plex Luminex assay suggests IL-6 and CCL2 were more highly secreted by the UPS PD-L1 low cases, while IL-10, IL-7 and IL-13 were more available in the UPS PD-L1 high supernatant.

Figure 6. (left) 3 cell line supernatant samples were analyzed for 14 different cytokines, determined by mean fluorescence intensity.

Conclusions and Future Plans

The heterogeneity of sarcomas has compromised *in vitro* studies in comparison to other malignancies, which is why characterizing primary cell lines was essential. Here, we show that tumour cultures were successfully grown from viably-preserved patient samples where physical dissociation is the preferred method. A validation pipeline demonstrated that cell lines can retain tumour specific mutations over time. A Luminex assay suggests that UPS cell line have distinct secretory profiles. Future experiments include identifying the immune trafficking potential of the cytokine profiles associated with UPS PD-L1 high and low cell lines. These experiments aim to shed light on the differences in the UPS PD-L1 high and low microenvironments.

Acknowledgements and References

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