The use of proteomics to analyse whole tumors and identify unique stroma cell targets for antibody-based therapeutics

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Introduction

Solid tumors comprises of two distinct compartments: cancer cells and the stroma that the cancer cells induce and are dispersed in. This stroma contains stromal (Fibroblasts, endothelial and pericyte cells) and infiltrating immune cells (Lymphocytes, macrophages, granulocytes and myeloid derived suppressor cells) (see Figure 1). Recent oncology research has implicated these stroma cells as promoters of tumor progression and there is thus a strong need to profile the cell membrane proteins present on these cells. We have conducted in-depth proteomic profiling of tumors from 14 different solid cancer indications[1] with varying degrees of stroma involvement to characterize their immune and stromal cell composition. Several novel stroma cell therapeutic targets were identified which if targeted by a therapeutic antibody have the potential to enhance the T cell immune response in tumors resistant to current immunotherapy.

Proteomics and Data Analysis

Clustering using a binary distance matrix and Ward scoring methods (see Figure 2) available in R (Version 3.2.0) were used to compare the proteomic expression[2] of a selection of fibroblast, T-cell and myeloid cell markers from isolated stroma and isolated immune cell samples including human dermal fibroblasts (HDF), Pan T-cells, CD4 T-cells and CD8 T-cells and myeloid derived cells (Pan Monocytes and CD14 Monocytes) against patient cancer samples. Analysis of the patient cancer samples discovered a number of novel target antigens expressed on immune cells, which exhibit high proteomic expression in cancer samples. In some samples with little T-cell infiltrate we find novel antigens expressed on myeloid derived cells.

Analysis of Target expression

OXBT189 expression on myeloid derived cells was validated by FACS analysis and IHC. Human peripheral blood mononuclear cells from normal donors were isolated by Ficoll separation from buffy coats. Anti- human CD11b, CD14 and CD33 APC conjugated antibodies were purchased from Becton Dickinson and utilized for FACS according to the manufacturer's directions. Anti-human OXBT189 antibody was purchased as a PE conjugate and used for FACS analysis according to the manufacturer's directions. IHC was performed on the Leica Bond Rx using anti-Human OXBT189 mAb at 1 ug/mL and 40 min heat-induced epitope retrieval in citrate buffer pH 6. Leica Bond Polymer Refine Detection kit was used.

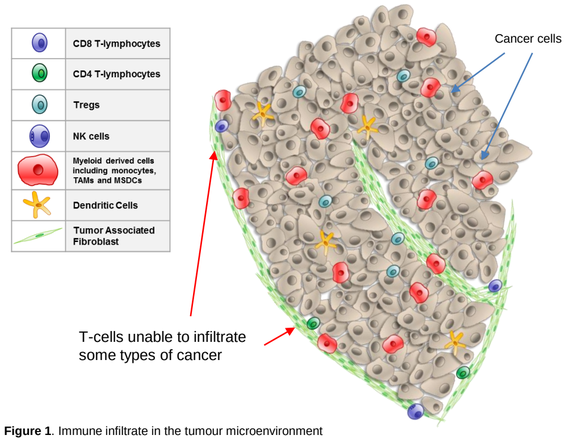

Figure 1. Immune infiltrate in the tumour microenvironment

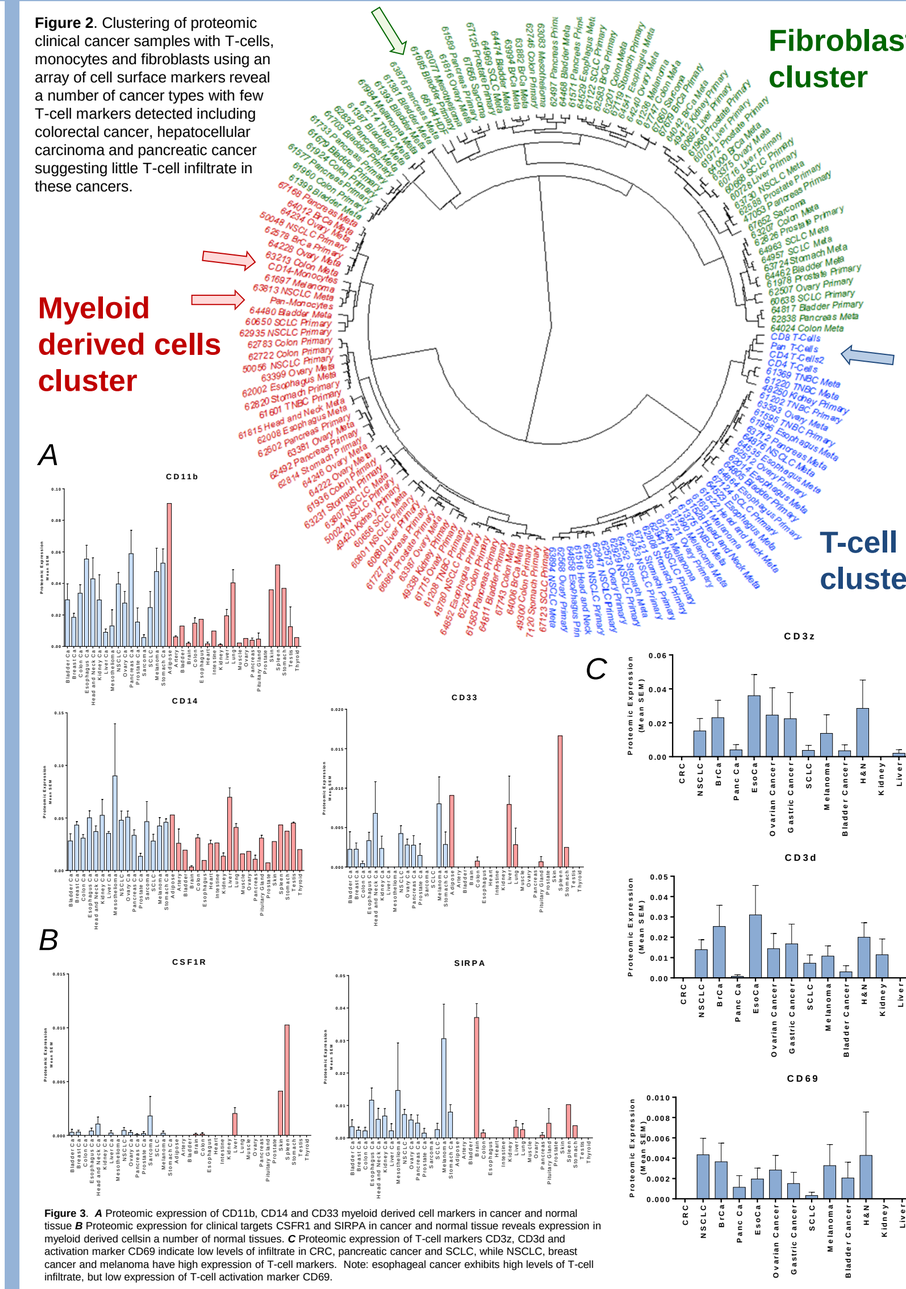

Figure 2. Clustering of proteomic clinical cancer samples with T-cells, monocytes and fibroblasts using an array of cell surface markers reveal a number of cancer types with few T-cell markers detected including colorectal cancer, hepatocellular carcinoma and pancreatic cancer suggesting little T-cell infiltrate in these cancers.

Figure 3. ***A*** Proteomic expression of CD11b, CD14 and CD33 myeloid derived cell markers in cancer and normal tissue ***B*** Proteomic expression for clinical targets CSFR1 and SIRPA in cancer and normal tissue reveals expression in myeloid derived cellsin a number of normal tissues. ***C*** Proteomic expression of T-cell markers CD3z, CD3d and activation marker CD69 indicate low levels of infiltrate in CRC, pancreatic cancer and SCLC, while NSCLC, breast cancer and melanoma have high expression of T-cell markers. Note: esophageal cancer exhibits high levels of T-cell infiltrate, but low expression of T-cell activation marker CD69.

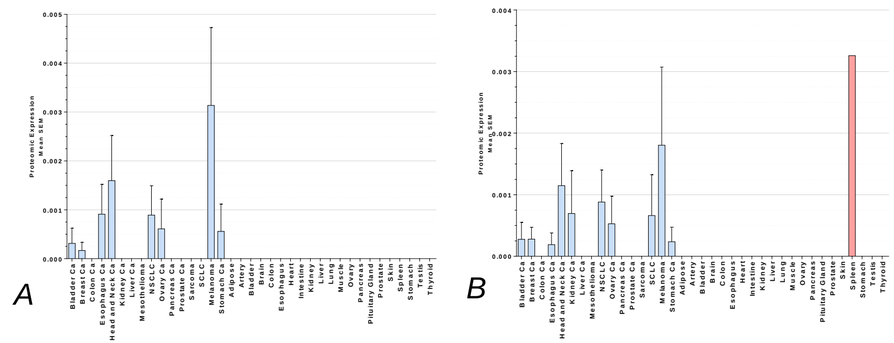

Figure 4. Proteomic expression in cancer samples for IO targets. ***A*** OXBT186 is expressed on T-cells and also shows expression in cancers with high T-cell infiltrate ***B.*** OXBT189 is expressed on myeloid derived cells, in addition to T-cells. Proteomic analysis reveals high expression in a broad range of cancer samples including those with and without high T-cell infiltrate.

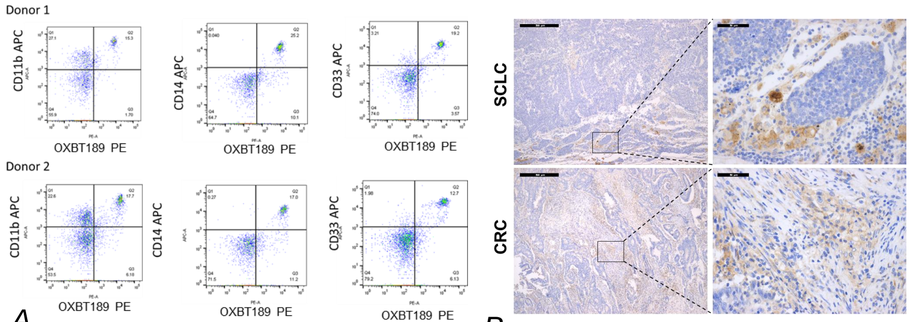

Figure 5 ***A*. OXBT189 is expressed on myeloid derived cells from human peripheral blood.** Human peripheral blood mononuclear cells from normal donors were isolated and used for FACS analysis. Double-positive CD11b, CD14 and CD33 vs. OXBT189 populations are present for both donors. ***B.*** IHC analysis reveals OXBT189 is expressed on myeloid derived cells in the stroma of small cell lung cancer and colorectal cancer samples. Scale bars, left = 500 µm; right = 50 µm.

Conclusions

Proteomics provides a unique profile of the tumour microenvironment (TME). Using proteomic profiling, we have been able to analyse the expression of known and unknown cell surface immune cell markers in the TME. This provides a novel approach for the discovery of potential I-O targets and holds the possibility that it may provide a means to predict the effectiveness of current or future I-O therapies. Using this approach, we have found novel I-O targets including, but not limited to, the examples seen in Figure 4 and Figure 5. These targets are expressed on a range of immune cells, including T-cells and myeloid derived cells, in the stroma of the tumour microenvironment, with expression confirmed by IHC and/or FACS.

The proteomic data has shown that some cancers appear to lack the T-cell infiltrate needed for current I-O therapies to elicit a clinical response. For those cancers where there is little or no detectable T-cell infiltration, targets on other immune cells which may block T cell migration/activation are available for novel I-O directed therapies.

1. OGAP®
2. Kast *et al.* Improved proteomic quantitation for the validation of therapeutic targets (2013) ASMS 61st meeting Poster session MP25: 497