INTRODUCING MOLECULAR PIXELATION: UNRaveling the changes in spatial distribution of immune cell surface proteins upon treatment with RITUXIMAB

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Background

Understanding of up and down regulation of genes, post-transcriptional changes as well as variations in protein translation are insufficient to fully comprehend onset of disease and disease progression as well as response to treatment.

The cell surface proteome is spatially dynamic and changes with the state of the cell, which in turn determines its activity in health and disease. Protein spatial architecture enables cell-cell communication, mobility, structure, and immunological activities. Molecular Pixelation enables the study of these fundamental aspects of immune cell biology at an unprecedented scale enabling data driven research into immunology, drug development and future diagnostics.

Methods

Pixelgen Technologies has developed the Molecular Pixelation workflow1 for single cell analysis of immune cells which generates location data on spatial cell surface proteins. Delivering 76 immune-cell-specific proteins with spatial resolution in a multiplex assay panel.

The Molecular Pixelation protocol (figure 1) is initiated with antibody-oligo conjugates binding to proteins on the surface of PFA-fixed cells. This is followed by two rounds of pixelation. Pixelation A involves the addition of DNA-pixels A, where each A pixel binds to many antibody-oligo conjugates in proximity, generating small connected protein neighbourhoods followed by a round of Pixelation B, connecting the protein neighbourhoods into a protein map. This protein map is amplified and converted into a Illumina compatible next-generation sequencing (NGS) library.

The NGS FASTQ files are imported to Pixelgen’s software Pixelator, undergoing several steps of QC and analysis. The read sequences are built to produce a graph, ultimately generating a network of protein connections. Each graph is a reconstruction of the surface of a cell.

Results

One of the major mechanisms of action for rituximab is antibody-dependent cellular cytotoxicity (ADCC), which is mediated by natural killer (NK) cells e.g. Rituximab clusters CD20 on B-cell cancers, recognized by NK-cells activating ADCC. This is clearly shown by the Molecular Pixelation Polarity scores which were significantly elevated in Rituximab stimulated samples compared to controls. Orthogonal comparison with fluorescent microscopy for Rituximab was performed on fixed cells to validate the spatial distribution of the target proteins upon stimulation (figure 2).

Conclusions

This work presents the applicability of Molecular Pixelation for single-cell analyses as a multiplexed, 3D spatial proteomics method, without any dedicated instrumentation. As illustrated, high protein multiplexing with spatial dimensions can provide insights into essential processes in immune therapies of cancer.

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Abstract 174 Figure 1

Abstract 174 Figure 2

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