Copy-number profiling of single, digitally-sorted PD-L1 positive cells in formalin-fixed, paraffin-embedded NSCLC tissues

Giulio Bassi1, Chiara Bolognesi1, Chiara Mangano1, Claudio Forcato1, Alberto Ferrarini1, Valentina Del Monaco1, Paola Tononi1, Genny Busoni1, Gianni Medoro1, Nicolò Manaresi1, Francesca Fontana1

1 Menarini Silicon Biosystems SpA, Bologna, Italy

Abstract # 5607
Booth # 2615

Introduction

The use of immune checkpoint blockade targeting the programmed death ligand-1 (PD-L1) has shown promising early clinical activity in NSCLC tumor types. The effectiveness of these therapies depends on different aspects of tumor biology such as the expression of immune checkpoint proteins and the presence of infiltrating active immune cells. Here we present a method for the digital isolation of single PD-L1+ cells from FFPE tissues, along with their genetic characterization for the identification of novel biomarkers.

Methods

FFPE tissue sections from NSCLC patients, tested for PD-L1 expression using IHC VENTANA PD-L1 (SP142) Assay (Fig.1), were dissociated down to cell suspension, and immunofluorescently labelled using Abs to Keratin, Vimentin and PD-L1-SP263. Four cell populations (Ker+/PD-L1+, Ker+/PD-L1-, Vim+/PD-L1+ and Vim+/PD-L1+) were identified at the DEPArray™ platform and pure single cells along with pools of precise number (range 43-121) of cells were isolated (Fig.2). Recovered cells were whole genome amplified (AmpliTV™ WGA kit), and genome-wide copy-number aberrations (CNA) profiles were obtained by AmpliTV™ LowPass kit compatible with Ion Torrent™ platforms.

Results

Qualitative observations of the simultaneous staining of PD-L1 antigen and tumor and stromal-associated antigens at single cell level provided a starting point to define potential differences associated to different cell-types by means of single-cell LowPass (LP) whole genome sequencing (WGS). For both patients, in the tumor populations numerous gains and losses were clearly identified along the genome; Keratin+/PD-L1+ and Keratin+/PD-L1- pooled cells displayed comparable profiles. Notably, in both patients copy gains were identified on chromosome 9 with a variable range of alleles (3 to 6) on the same PD-L1 locus All the stromal (Vimentin+PD-L1+/-) cells showed flat profiles (Fig.3a). A hierarchical unsupervised clustering clearly separates normal stromal cell, described by a flat profile, from the group of keratin positive single-cells PD-L1+ / PD-L1- characterized by an overabundance of gains and losses, confirming the aberrant nature of tumor cells (Fig.3b,c).

Conclusion

This study demonstrates an innovative approach for isolation and genetic profiling of single pure PD-L1+ cells from dissociated FFPE tissue. Data show that there isn’t a direct correlation between copy-number of PD-L1 gene and its expression, suggesting other mechanisms of regulation (i.e. epigenetic, transcriptional, post-transcriptional). DEPArray™ sorting combined with low-pass whole genome sequencing enables high quality genome-wide profiling of pure single cells and pools isolated from phenotypically distinct populations in FFPE tumor tissue.