APPLICATION NOTE

Automated approach to quantify single molecule in complex tissues

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Summary

Accurate RNA quantification at the single-cell level is critical for understanding the dynamics of gene expression and regulation across space and time. Single molecule in situ hybridization (ISH) such as RNAscope® provides spatial and quantitative measurements of individual transcripts; therefore, it can be used to explore differential gene expression among a heterogenous cell population if combined with cell identity information. Such analysis is not straightforward, and in this perspective we developed an efficient analysis method to enable the quantification of transcripts in a cell specific manner.

Approach

Here we present the automatic mRNA transcription quantification platform to allow automatic detection of gene transcript signals, immunofluorescence signals and precise segmentation of single cells. This approach can accurately and efficiently quantify cell-specific single-molecule RNA though integration with deep-learning methodology.

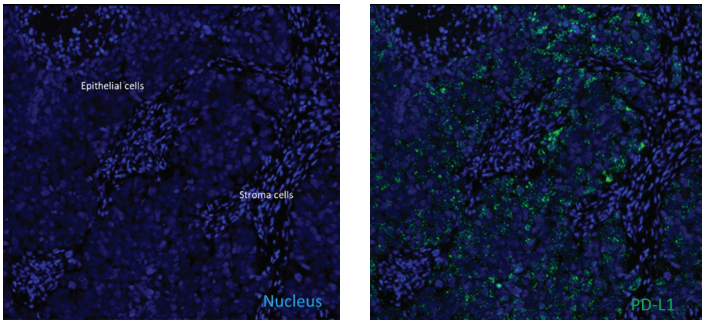

FIGURE 1. Differential distribution of PD-L1 RNAs in NSCLC tissue section

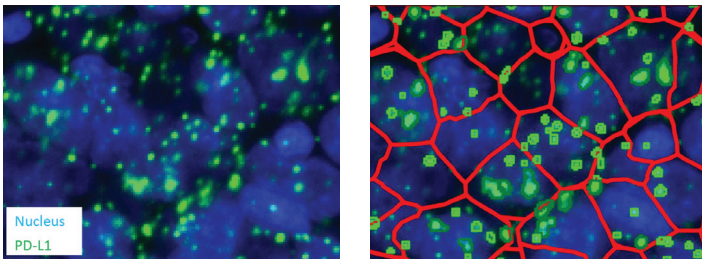

FIGURE 2. Single-cell detection of PD-L1 transcript

Case Illustration Study

In the present study, we automatically quantified PD-L1 drug target transcript in FFPE non-small cell lung cancer (NSCLC) tissue further treated with pembrolizumab that progressed on therapy.

Cell segmentation and single-cell molecule detection

Individual PD-L1 RNA molecules were imaged in fixed cells by hybridization with oligonucleotide probes followed by computational identification of the fluorescent signals **(Figure 1)**. DNA staining was also performed. Differences in expression levels were seen across the tumor with PD-L1 staining mostly located in the tumoral area of the NSCLC tissue consistent with the expected expression of PD-L1 in tumor epithelial cells.

Single-cell detection of the PD-L1 transcripts was then automatically performed on QuPath. First a deep learning algorithm was applied to segment the cell based on DNA staining, followed by a subcellular segmentation to detect single molecule based on the size of the fluorescent dots **(Figure 2)**.

Quantification

The analysis of RNAscope results involves quantification of the number of fluorescent dots in each cell within the tissue. Each dot represents one RNA molecule, and thus the number of dots is indicative of the number of RNA molecules present. It is noteworthy that sometimes the dots can be found in clusters, which makes them difficult to distinguish separately. To overcome this challenge and estimate the correct number of dots in a cluster, the area of the latter is divided by the size of the dot. With the molecular weight of the transcripts of interest, one can obtain the mass of the molecules in each cell. Concentration can then easily be calculated based on the volume of the cell correlated to the area of the cell and the thickness of the tissue section. In one example the concentration of the transcripts was 5 pg/ml in a positive cell.

Application

In situ analysis of RNA in single cells at single-molecule sensitivity in clinical specimens is a valuable tool in the era of personalized medicine and offers a realistic alternative for measuring drug target expression. Here we present the use of RNAscope for the quantification of RNA targets in the tumor microenvironment (TME) that are involved in tumor immunology and immunotherapy. Detecting RNA biomarker expression at the single-cell level while preserving spatial information is critical to understanding cellular organization and cell-to-cell interactions in the cancer-immunity cycle.

Specifically, this detection aids in:

- Localization of specific immune cell types (i.e., cytotoxic lymphocytes and regulatory T cells) in the TME
- Determining spatial relationships between different cell types in the TME
- Characterization of secreted proteins (i.e., cytokines and chemokines)
- Evaluation of immune function in TME beyond enumeration of tumor infiltrating lymphocytes.

Contact us for more information.

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