Efficient single-cell sequencing for full-length transcripts to decipher subclonal structure of melanoma

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INTRODUCTION

• Intratumoral heterogeneity is driven by expansion of mutant subclones, each with a distinct metastatic potential and ability to evade therapy
• Subclonal evolution has a profound impact on therapeutic outcomes
• Subclones can be characterized and traced by phylogenetic analysis of mutations in single cells
• Unfortunately, single-cell full-length transcript sequencing (scRNAseq) datasets that allow for mutation calling are sparse

OBJECTIVE & METHODS

Objective: Identify mutations that drive subclonal evolution and therapeutic outcomes

M4 Mouse Melanoma Cell Line

Subcutaneous Implantation

Immune Checkpoint Blockade

Tumor regression

Relapsed

• Subcutaneous implantation of the genetically heterogeneous and phenotypically diverse M4 mouse melanoma (Perez-Guijarro et al. Nature Med 2020) into syngeneic mice, followed by immune checkpoint blockade treatment (anti-CTLA-4)
• Isolation of single cells from M4 melanoma tumors, followed by preparation of full-length transcript cDNA libraries using plexWell Rapid Single Cell and Smart-seq2 protocols
• Use Triscell (Rashidi Mehrabadi et al. bioRxiv 2021) to build phylogeny trees on the single-cell mutation data

RESULTS

1 Anti-CTLA-4 caused significant growth delay of M4 melanoma

The experimental setting is depicted in the objective and methods. Three tumors from the isotype control group (blue) and four tumors from anti-CTLA-4 treatment group (red) were harvested, and subjected to single-cell sequencing. m108 and half of the m117 cells were prepared with the plexWell Rapid Single Cell protocol while the other tumors (and half of m117 cells) were prepared for sequencing with the Smart-seq2 library preparation protocol.

2 Comparable data quality from plexWell and Smart-seq2 methods

The distribution of mitochondrial genes and unique counts of reads on genes are similar between single-cell data generated by the plexWell Rapid Single Cell and Smart-seq2 library preparation methods.

3 Trees built on scRNAseq mutation data of the control and anti-CTLA-4 treated M4 melanoma from Smart-seq2 library preparation

Each leaf represents a group of cells with similar mutational profiles. The number of somatic de novo mutations is shown for each node. Subclones corresponding to the counterpart in the trees generated from Smart-seq2 data were identified. Importantly, the driver mutations and seeding mutations of each subclone were identical to those in the trees generated from Smart-seq2 data. The inverse correlation between Axl and Mitf levels is also observed here.

4 Tree built on scRNAseq mutation data of the control group M4 melanoma from plexWell library preparation

5 Summary of mutations driving subclonal development

The driver mutations were those recurrently detected from all of the trees. The subclones were denoted at the end of each branch (color-coded font).

6 Model of subclonal development in M4 melanoma

At early stages of carcinogenesis, KRAS activation induces senescence. Later mutations activate either YAP/TAED or mTOR pathways to allowing cells to escape from senescence, transforming the cells. Mutations at different genes in the same pathways may happen in individual cells, resulting in individual subclones.

CONCLUSIONS

• We demonstrated that Triscell accurately generates phylogenies based on scRNAseq mutations, allowing the tracing of subclonal evolution in tumors, thus providing a tool that can be leveraged in the clinic to uncover mechanisms of tumor cell evolution
• Trees generated from the smart-Seq2 and plexWell rapid datasets have corresponding subclonal structures. Interestingly, each subclone has distinct developmental state and sensitivity to immunotherapy
• Our results suggest that mutations of different genes may be related downstream signaling, resulting in the development of subclones
• This study highlights the importance of using scRNAseq to investigate tumor cell evolution and the benefits of using efficient library preparation method to generate high-quality datasets

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