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

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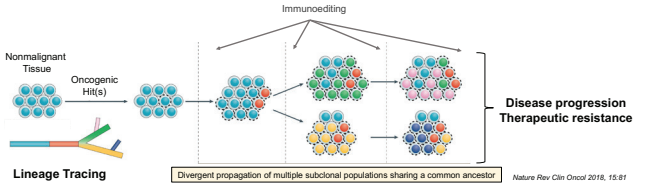
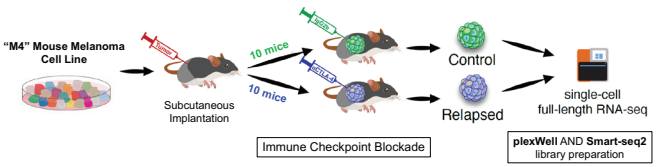
INTRODUCTION

- 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 sparce

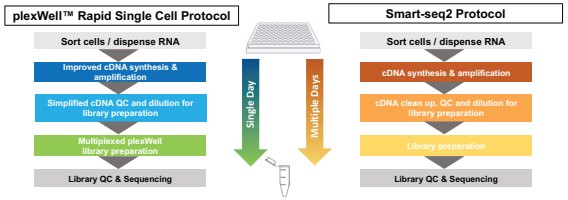


OBJECTIVE & METHODS

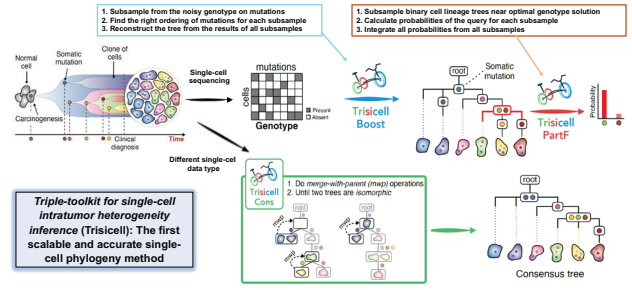
Objective: Identify mutations that drive subclonal evolution and therapeutic outcomes



- Subcutaneous implantation of the genetically heterogeneous and phenotypically diverse M4 mouse melanoma (*Pérez-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

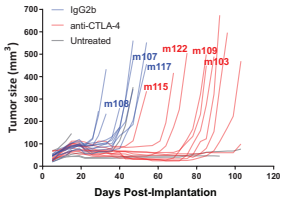


- Use Trisicell (*Rashidi Mehrabadi et al.* bioRxiv 2021) to build phylogeny trees on the single-cell mutation data



RESULTS

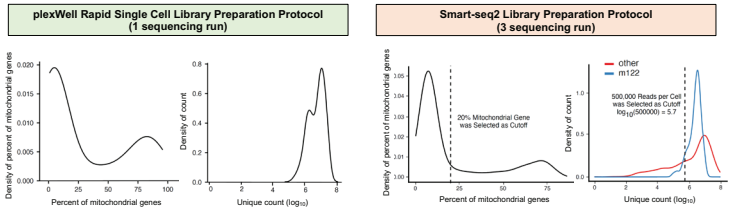
1 Anti-CTLA-4 caused significant growth delay of M4 melanoma



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.

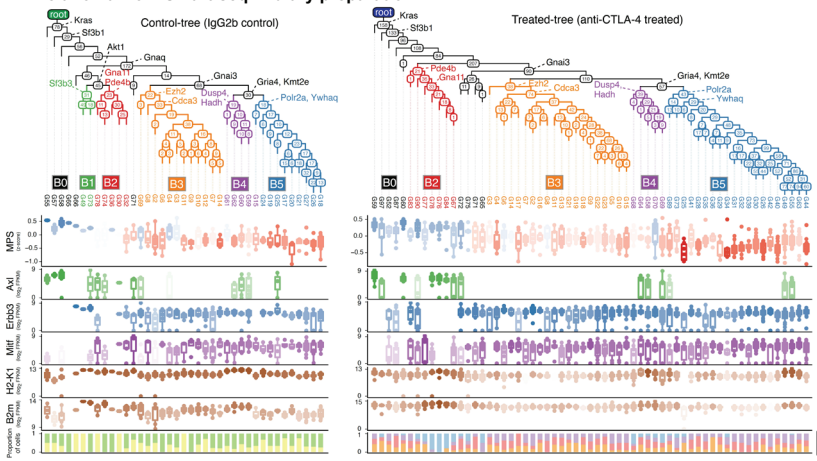
Tumor	m107	m108	m117	m115	m122	m103	m109
# of cells	192	96	192	192	96	192	192

2 Comparable data quality from plexWell and Smart-seq2 methods

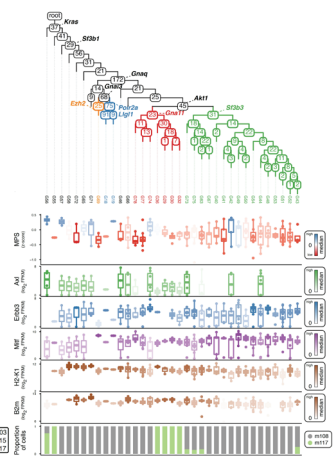


The distribution of mitochondria genes and unique counts of reads on genes are similar between single-cell data generated by the plexWell Rapid Single Cell and Smart-seq2 library preparation methods.

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



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

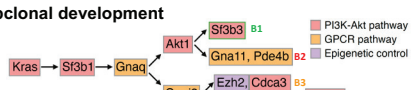


Each leaf represents a group of cells with similar mutational profiles. The number of somatic de novo mutations is shown for each node. Subclones corresponding to the counterpart in the trees generated from Smart-seq2 data were identified. Importantly, the driver mutations and seeding mutations of each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

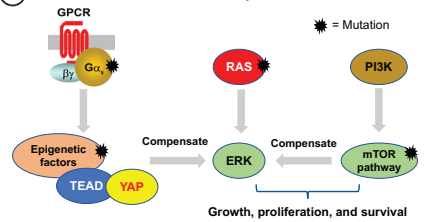
Each leaf represents a group of cells with similar mutational profiles. The number of somatic de novo mutations is shown for each node. The seeding mutations in the trunk and subtrees were identified, and the corresponding subclones in the control and anti-CTLA-4 treated tumors were subtrees with the same color code. Each subclones exhibited a distinct melanocytic plasticity signature (MPS) score, expression of neural crest development markers (Axl, ErbB3, and Mitf), and antigen presentation genes (H2-K1 and B2m), as shown under the trees. Notice that subclones B1 and left subtree of B2 were eliminated after anti-CTLA-4 treatment.

5 Summary of mutations driving subclonal development

The driver mutations were 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/TEAD 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 Trisicell accurately generates phylogenies based on scRNA 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 activate 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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