SeekGene | Decoding Unknown of Life with Novel Single-cell Technology

Scan Me

The application of scFAST-seq in tumour research

The Combination of Single-cell Mutation and Expression Analysis Uncovers the MYSTERIES of Tumour

BACKGROUND

Projects like TCGA and ICGC had depicted mutation patterns in various cancer tissues, but the tumour microenvironment remains unclear. Although the human tumour atlas network (HTAN), which utilizes 3' single-cell transcriptome-seq technology, has provided valuable information on the cellular composition, it cannot detect mutations, leaving many questions and assumptions in tumour research, for example:

1. Do driver mutations really occur in just tumour cells?
2. If some mutations make cancer cells sensitive to certain treatments, why do some patients still have no response to those treatments?
3. Tumours are the result of accumulated gene mutations. How many mutations do normal cells need to accumulate to become tumour cells, and what functional impact do these mutations have on tumour cells?
4. When multiple mutations or co-mutations occur, are they in the same group of cancer cells or different ones, and how can we distinguish when considering therapeutic methods for patients?
5. What is the underlying mechanism of tumour metastasis, which tumour clone contributes to the metastasis, what are their characteristics, and how can we control it?
6. Is acquired resistance caused by new mutations or transcriptional reprogramming during therapy?
7. In the era of precision medicine, how can we effectively select appropriate drug targets and tailor effective treatments based on the specific characteristics of individual patients?

Tumours exhibit dual heterogeneity in mutation and expression, where **mutations** are the "**cause**" and **phenotype** is the "**effect**". Detecting both mutations and expression within single cells is essential for a comprehensive understanding of challenging questions. The innovative **scFAST-seq** technology captures **full-length RNAs** with **random primers** to detect **both mutation and expression**, providing **novel** insights for scientific research on **tumour** development and metastasis.

1. Mutation-induced functional changes and indications for combination therapy

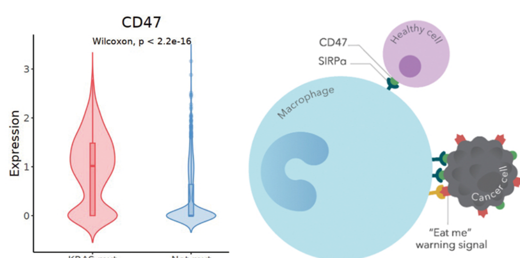

Figure 1. Expression of CD47 in cells with KRAS^{mut} mutants and KRAS^{WT}. Compared to KRAS^{WT}, CD47 is significantly upregulated in KRAS^{mut}, which releases "Don't eat me" signals to macrophages and achieves immune escape. This implies combined CD47-targeted drugs may lead to better therapeutic effects.

Application:
Mutation-induced changes in cell expression and function, screening drug target databases, exploring combination therapy targets, and achieving personalized precision treatment.

2. Locating co-mutations at the single-cell level

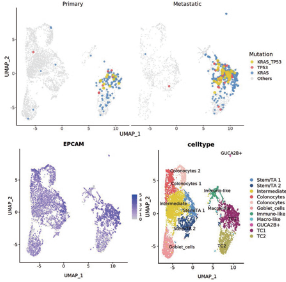

Figure 2. Differences in mutations between primary and metastatic tumours. Metastatic tumour shows a higher number of tumour cells with KRAS and TP53 co-mutations compared to primary tumour, suggesting that cells with KRAS and TP53 co-mutations may have a high metastatic potential (yellow dots represent a cell with both KRAS and TP53 mutations).

Application:
Investigate the impact of co-mutation on cell function and provide new perspectives for cancer therapies.

3.Tracing mutated cell subpopulations

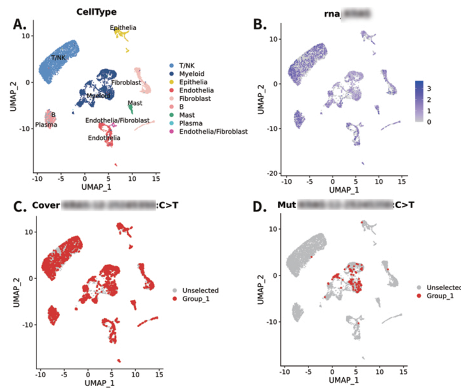

Figure 3. A drug-related mutation at the Y position of gene X is specifically present in "non-tumour cells".
A. Cell clusters and annotation;
B. Expression of gene X in different cell groups;
C. Coverage of the Y position of gene X in single cells: each dot represents a cell, with red dots indicating detection of the Y position sequence of gene X in the cell, and gray dots indicating no coverage of the Y position of gene X in the cell.
D. The Y position mutation of gene X is specifically present in non-epithelial cells (the gene and site information is not shown because the data has not been published).

Application: This gives us a hint that precision medicine guidance should not solely rely on the detection of mutations, but also on the identification of the cells carrying these mutations.

4. Research on the process of tumour occurrence and development

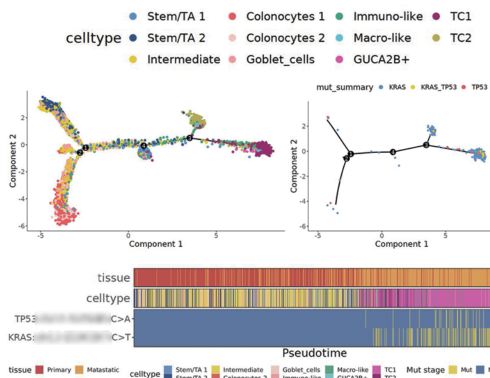

Figure 4. Mutation accumulation along the trajectory from normal epithelial cells to tumour cells. Meanwhile, the trend of changes in gene expression along the trajectory can be analyzed.

Applications

-Describe the dynamic evolution process of mutation accumulation; search for biomarkers related to early screening, diagnosis, and prognosis of tumours.
-Use PDX/organoid models to perform scFAST-seq detection before and after drug treatment; explore drug sensitivity-related biomarkers; study drug-resistant mutations or transcriptional reprogramming that occur during the drug resistance progress.

Comparison

	3' scRNA-seq	scFAST-seq
Cell partitioning method	Digital Droplet / Microwell	Digital Droplet
Core technology	**OligodT**s of Barcoded Beads capture RNA molecules with polyA tails (mainly mRNAs)	**Random primers** capture RNA molecules (± polyA tailed RNA)
Sequencing platform	Illumina / MGI, PE150	Illumina / MGI, PE150
Sequencing fragments	3' end of mRNA (<1/10 of full-length)	Full-length RNA sequences
RNA detected	Mainly mRNAs and a small amount of lncRNAs with polyA tails	mRNAs, **lncRNA**s, **pathogen** RNAs, etc.
Analysis dimension	Gene expression	Gene expression; SNP/InDel **mutation**, Gene **fusion**; **Noncoding** RNA regulation; **Pathogen** transcripts like viral RNA
Cost-performance	Medium: Basic expression information	High: Comprehensive Information with Low Cost

scFAST-seq, self-developed by Beijing SeekGene BioSciences Co., Ltd