

# Week 3 Report

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- ① 探討 RNA 甲基轉移酶 NSUN2 基金多型性與子宮內膜癌其臨床特徵之影響
- ② Single-cell RNA sequencing technologies
  - Single-cell analysis
  - Applications of single-cell RNA sequencing

# What is NSUN2

- **NSUN2** is a gene.
- The gene is transcribed into mRNA, which is then translated into **NSUN2 protein**.
- The **NSUN2 protein** belongs to the RNA methyltransferase family, which adds  $m^5C$  (5-甲基胞嘧啶) modifications to tRNA and mRNA, helping RNA stability and regulating gene expression.

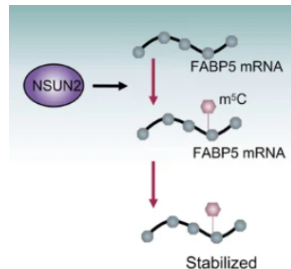


Figure 1: Illustration of NSUN2 mechanism[1]

# NSUN2 and Cancer

- Cancer cells:
  - NSUN2 overexpressed [2].
  - Stabilizes oncogenic mRNAs like
    - Lung Adenocarcinoma [3]
    - Pancreatic Cancer [4]
    - Uterine Corpus Endometrial Carcinoma [5]
  - Promotes proliferation, metastasis, and chemoresistance

# NSUN2 Gene Polymorphisms

- SNP, Single Nucleotide Polymorphism (單一核苷酸多型性)
  - A single-base change in DNA sequence (e.g., C/T, A/G).
  - May influence gene expression or protein function.
- In this plan, we will examine four key NSUN2 SNPs:
  - rs4702373 (C/T)
  - rs166049 (T/G)
  - rs13181449 (C/T)
  - rs8192120 (C/A)

Some studies have shown that NSUN2 rs13181449 C>T is associated with a reduced risk of cancer, including oral cavity squamous cell carcinoma [6] and neuroblastoma [7].

# Statistical Methods

## Relationship between cancer stage and NSUN2 SNPs

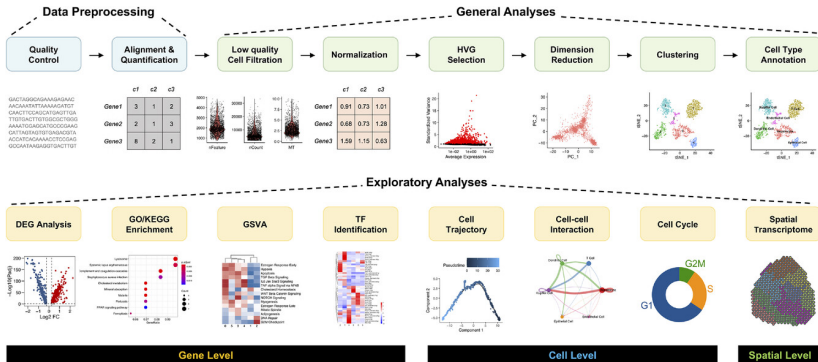
- Determine if SNPs are related to cancer stage
  - T-test (for continuous covariates)
  - Chi-squared test (for categorical variables)
- Assess the independent effect of SNPs
  - Multiple unconditional logistic regression model
  - Adjusted Odds Ratio (AOR) with 95% confidence interval

# scRNA-seq

The procedures of scRNA-seq mainly include [8]

- ① Single-cell isolation and capture
- ② Cell lysis
- ③ Reverse transcription (conversion of their RNA into cDNA)
- ④ cDNA amplification
- ⑤ Library preparation

# Single cell RNA Analysis Method





# Single cell RNA Analysis

- The single-cell RNA sequencing data set is high-dimensional.
- Most genes in each cell belong to housekeeping ones, as they are characterized by **no significant changes** in the expression level between cells, and their presence tends to obscure the real biological signals.
- The subsets of features that exhibit high cell-to-cell variation in the data set are also called **highly variable genes (HVGs)**.
- A high-quality HVGs should include genes that can distinguish different cell types, and the quality of HVGs has a significant effect on the precision of clustering.

# Single cell RNA Analysis

- **Batch-effect correction** : **deepMNN** achieves higher accuracy than commonly used methods, particularly for large-scale datasets.
- **Dimensionality reduction** : **PCA, t-SNE and UMAP**. UMAP in high-dimensional cytology and single-cell RNA sequencing is better than t-SNE.
- **Clustering** : **SC3** and **Seurat** performed better and **Seurat** being several orders of magnitude faster.

# Exploratory Analysis

To robustly reveal functional bias and biological significance of specific cell populations, it is necessary to perform **functional enrichment analyses** on a targeted differentially expressed gene set.

- Functional enrichment analysis
- Transcription factor inference
- Pseudo-time analysis
- Cell-cell communication
- Cell cycle analysis

# scRNA-seq analysis on tumours

A scRNA-seq analysis can distinguish functionally healthy cells from cancer cells at various developmental stages of tumours.

## Challenge :

- Their microenvironment contains variety of tumour and non-tumour cells in different states and stages.
- The same section of a tumour might be very different if the biopsy was taken under different times and conditions.
- Single-cell gene expression data often contain a lot of noises, which leads to batch effect.

Tumour microenvironments are infiltrated with the **immune cell types**. So far, there are more advanced approaches reported on alterations of immune cells in tumours.

# scRNA-seq analysis on tumours

In tumours, different cell types (including tumour cells) **communicate actively** through signaling pathways.

scRNA-seq can also study how tumours evolve. This evolution affects tumour growth and the development of traits like drug resistance.

By revealing small groups of treatment-resistant cells, scRNA-seq helps guide therapy choices and enables more precise, personalized treatment..