

Week 3 Report

Chen, Pin-Jui

Sep 26, 2025

Index

- 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.

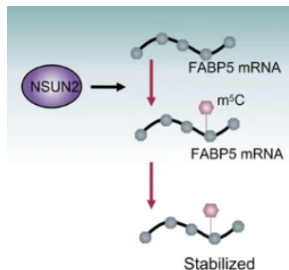


Figure 1: Illustration of NSUN2 mechanism[1]

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(增生), metastasis(轉移), and chemoresistance(化療抗藥性)

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

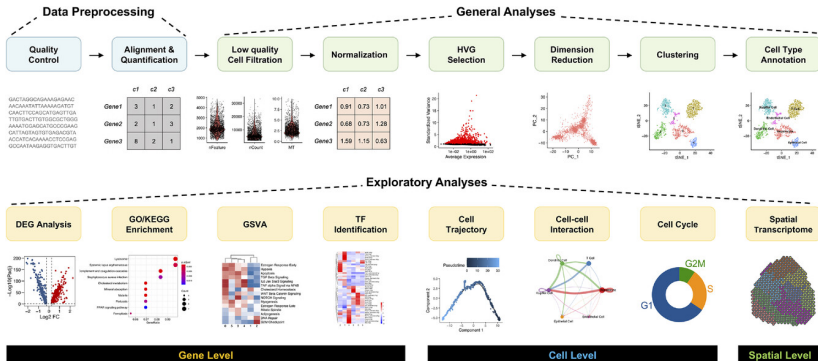
- Determine if SNPs are related to cancer stage
 - T-test (for continuous covariates)
 - Chi-squared test (for categorical variables)
- Assess the independent effect of SNPs
 - Multiple unconditional logistic regression model
 - Adjusted Odds Ratio (AOR) with 95% confidence interval

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..

參考文獻 I

- [1] Min Yang et al. “NSUN2 promotes osteosarcoma progression by enhancing the stability of FABP5 mRNA via m5C methylation”. In: *Cell Death & Disease* 14.2 (2023), p. 125.
- [2] Mayumi Okamoto et al. “Frequent increased gene copy number and high protein expression of tRNA (cytosine-5-)-methyltransferase (NSUN2) in human cancers”. In: *DNA Cell Biol* 31.5 (2012), pp. 660–671.
- [3] M. Li, N. Du, and P. Tang. “The oncogenic role of NSUN2 in lung adenocarcinoma by stabilizing CCT5 mRNA via a YBX1-dependent m5C modification”. In: *Mol Cell Biochem* (2025). Epub ahead of print.

參考文獻 II

- [4] G. Zhang et al. “NSUN2 stimulates tumor progression via enhancing TIAM2 mRNA stability in pancreatic cancer”. In: *Cell Death Discov* 9.1 (2023), p. 219.
- [5] S. Yang et al. “RNA 5-Methylcytosine regulators are associated with cell adhesion and predict prognosis of endometrial cancer”. In: *Translational Cancer Research* 12.10 (Oct. 2023), pp. 2556–2571.
- [6] Li-Chuan Hung et al. “NSUN2 rs13181449 variant decreases the risk of oral cancer development”. In: *International Journal of Medical Sciences* 22.12 (2025), pp. 3022–3031.

參考文獻 III

- [7] Lei Lin et al. "NSUN2 gene rs13181449 C>T polymorphism reduces neuroblastoma risk". In: *Gene* 854 (2023), p. 147120. ISSN: 0378-1119. DOI: <https://doi.org/10.1016/j.gene.2022.147120>. URL: <https://www.sciencedirect.com/science/article/pii/S0378111922009404>.
- [8] Dragomirka Jovic et al. "Single-cell RNA sequencing technologies and applications: A brief overview". In: *Clinical and translational medicine* 12.3 (2022), e694.