

# Project 4

Project 4 focuses on understanding the mechanisms of immune remodeling in cervical cancer (CC) and identifying potential therapeutic targets; [paper1](#), [paper2](#). Cervical cancer progression involves complex interactions between immune cells and tumor cells, leading to an immunosuppressive microenvironment. To investigate these interactions, this study utilizes scRNA-seq data from normal cervix, high-grade squamous intraepithelial lesions (HSIL), and cervical cancer tissues to analyze transcriptional responses in immune cells.

Unsupervised clustering of transcriptional profiles revealed distinct immune cell populations and their interactions with tumor cells. Differences in immune cell states were linked to the progression of cervical cancer and the establishment of an immunosuppressive microenvironment. Specifically, the study identified unique HPV-related epithelial clusters and critical node genes that regulate disease progression. The transition from normal cervix to HSIL and cervical cancer was marked by changes in immune cell populations, including T cells, dendritic cells, and macrophages.

Further analysis identified key immune cell subsets and their roles in shaping the tumor microenvironment. Network analyses demonstrated that immune cell interactions influence functional T cell programs and systemic immune coordination. This study provides insights into how immune remodeling impacts cervical cancer progression and highlights transcriptional markers that could be used to predict patient outcomes and guide therapeutic strategies.

## Available data

These samples represent different stages and conditions of cervical tissues, providing a comprehensive dataset for analyzing the progression from normal cervix to cervical cancer.

1. Normal Cervix without HPV (NO\_HPV):
  - N\_HPV\_NEG\_1
  - N\_HPV\_NEG\_2
2. Normal Cervix with HPV (N\_HPV):
  - N\_1
  - N\_2
3. High-Grade Squamous Intraepithelial Lesions with HPV (HSIL\_HPV):
  - HSIL\_1
  - HSIL\_2
4. Cervical Cancer with HPV (CA\_HPV):
  - SCC\_4
  - SCC\_5

- ADC\_6

Data has been downloaded and prepared for you from [GEO GSE208653](#).

In order to download the data, run:

Code starts here:

```
wget https://single-cell-transcriptomics.s3.eu-central-1.amazonaws.com/projects/data/project4.tar.gz  
tar -xzvf project4.tar.gz
```

Code ends here

After extracting, a directory project4 appears with the following content:

```
.  
|   └── data  
|       ├── ADC_6  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── HSIL_1  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── HSIL_2  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── N_1  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── N_2  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── N HPV NEG_1  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── N HPV NEG_2  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       ├── SCC_4  
|       |   ├── barcodes.tsv.gz  
|       |   ├── features.tsv.gz  
|       |   └── matrix.mtx.gz  
|       └── SCC_5
```

```
└── barcodes.tsv.gz  
└── features.tsv.gz  
└── matrix.mtx.gz  
└── paper1.pdf  
└── paper2.pdf
```

10 directories, 29 files

Now create a new project in the project1 directory (Project (None) > New Project ...), and create Seurat object from the count matrices:

Code starts here:

```
library(Seurat)  
# vector of paths to all sample directories  
datadirs <- list.files(path = "data", full.names = TRUE)  
  
# get the sample names  
# replace underscores with hyphen to correctly extract sample names later on  
names(datadirs) <- basename(datadirs) |> gsub("_", "-", x = _)  
  
# for now, we only take the HPV negative and cervical cancer samples  
datadirs <- datadirs[c("N-HPV-NEG-1", "N-HPV-NEG-2", "SCC-4", "SCC-5")]  
  
# create a large sparse matrix from all count data  
sparse_matrix <- Seurat::Read10X(data.dir = datadirs)  
  
# create a seurat object from sparse matrix  
seu <- Seurat::CreateSeuratObject(counts = sparse_matrix,  
                                    project = "CervicalCancerStudy")
```

Code ends here

### Project exercise

with this dataset, go through the steps we have performed during the course, and try to reproduce the results provided in the paper. Pay specific attention to quality control, clustering and annotation.

### Tips

- For mitochondrial genes, ribosomal genes and hemoglobin genes you can use the following patterns: "<sup>A</sup>MT-", "<sup>A</sup>RP[SL]" and "<sup>A</sup>HB[^P]".
- Work iterative; meaning that based on results of an analysis, adjust the previous analysis. For example, if clustering is not according to cell types, try to adjust the number of components or the resolution.
- Please read the methods section of the paper.

- If the code for data analysis is available, try to adapt it (for specific parameters).
- Check the supplementary figures.
- Try to understand if they used some other tools for the data analysis.