Pseudo bulk chondrosarcoma analysis

Léa ROGUE

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This script analyze pseudo bulk scRNAseq the same manner the microarray part to see if it is coherent and to see the infiltrated tumors or not.

Load libraries

```
library(dplyr)
library(ComplexHeatmap)
library(colorRamp2)
```

Read data

```
df_tpm <- read.table("../../results/matrix_pseudo_bulk_tpm_normalized.tsv",
    sep = "\t", header = TRUE, check.names = F)

# Read CTA file
df_CTA <- read.table("../../data/CTA_list_clean.txt", header = FALSE)

# Read immune cells
df_immune_sign <- read.table("../../data/immune_cells_mhc_genes.tsv",
    header = FALSE, sep = "\t")

# Read list of CTA that impact survival probabilities
l_CTA_conv <- read.table("../../data/CTA_signif_coxph_conv_indiv.txt",
    sep = "\t", header = FALSE)$V1
```

Prepare data

```
df_tpm$CTA <- NA

# Rename col
colnames(df_CTA) <- c("SYMBOL")

# Df with CTA and whole genes by adding CTA in col CTA for
# CTA genes
df_tpm_CTA <- df_tpm %>%
    left_join(df_CTA, by = "SYMBOL") %>%
    mutate(CTA = ifelse(SYMBOL %in% df_CTA$SYMBOL, "CTA", NA))

# Reorganize columns
df_tpm_CTA <- df_tpm_CTA %>%
    select(SYMBOL, CTA, everything())

# Read immune cells genes
colnames(df_immune_sign) <- c("Signature", "Gene")
```

```r
# Merge df_CTA_whole and df_immune_sign to associate
# signatures
df_tpm_CTA_imm_cells <- merge(df_tpm_CTA, df_immune_sign, by.x = "SYMBOL",
    by.y = "Gene", all.x = TRUE)

# Reorganize the columns if needed
df_tpm_CTA_imm_cells <- df_tpm_CTA_imm_cells %>%
    select(SYMBOL, CTA, Signature, everything())

# Combine multiple signatures into one for each gene by
# concatenating with a comma
df_tpm_CTA_imm_cells_clean <- df_tpm_CTA_imm_cells %>%
    group_by(SYMBOL) %>%
    summarise(Signature = paste(unique(Signature), collapse = ", "),
        across(everything(), ~first(.)), .groups = "drop")

# Average the expression between same immune cells types
# Take rows with immune cells signature from normalized
# data
df_avg_immune_sign <- df_tpm_CTA_imm_cells %>%
    filter(Signature != "NA")

# Group by signature and calculate mean of expression
# values
df_avg_immune_sign_final <- df_avg_immune_sign %>%
    select(-c(SYMBOL, CTA)) %>%
    group_by(Signature) %>%
    summarise(across(where(is.numeric), \(x) mean(x,
        na.rm = TRUE)))
```

I. Relative immune cells expression

```r
# Create the heatmap
heatmap_data <- as.data.frame(df_avg_immune_sign_final)
rownames(heatmap_data) <- heatmap_data$Signature
heatmap_data <- heatmap_data[, -1]  # Remove the Signature column
heatmap_data <- log2(heatmap_data + 1)

# Calculate Z-scores
z_scores_row <- t(scale(t(heatmap_data)))

# Add columns
df_z_scores <- as.data.frame(z_scores_row)
colnames(df_z_scores) <- c("Low_L07", "Low_L28", "High_L31",
    "High_L44", "Ben_L49", "Med_L63", "Med_L80", "Low_L81", "Ded_L83")

colors <- colorRampPalette(c("blue", "white", "red"))(100)
Heatmap(as.matrix(df_z_scores), cluster_rows = TRUE, cluster_columns = TRUE,
    cluster_column_slices = TRUE, clustering_distance_columns = "euclidean",
    clustering_method_columns = "complete", show_column_dend = TRUE,
    col = colorRamp2(seq(-8, 8, length.out = 100), colors), border = NA,
```

```
    show_column_names = TRUE, column_names_gp = gpar(fontsize = 7),
    row_names_gp = gpar(fontsize = 7), heatmap_legend_param = list(title = "Expression Level"))
```

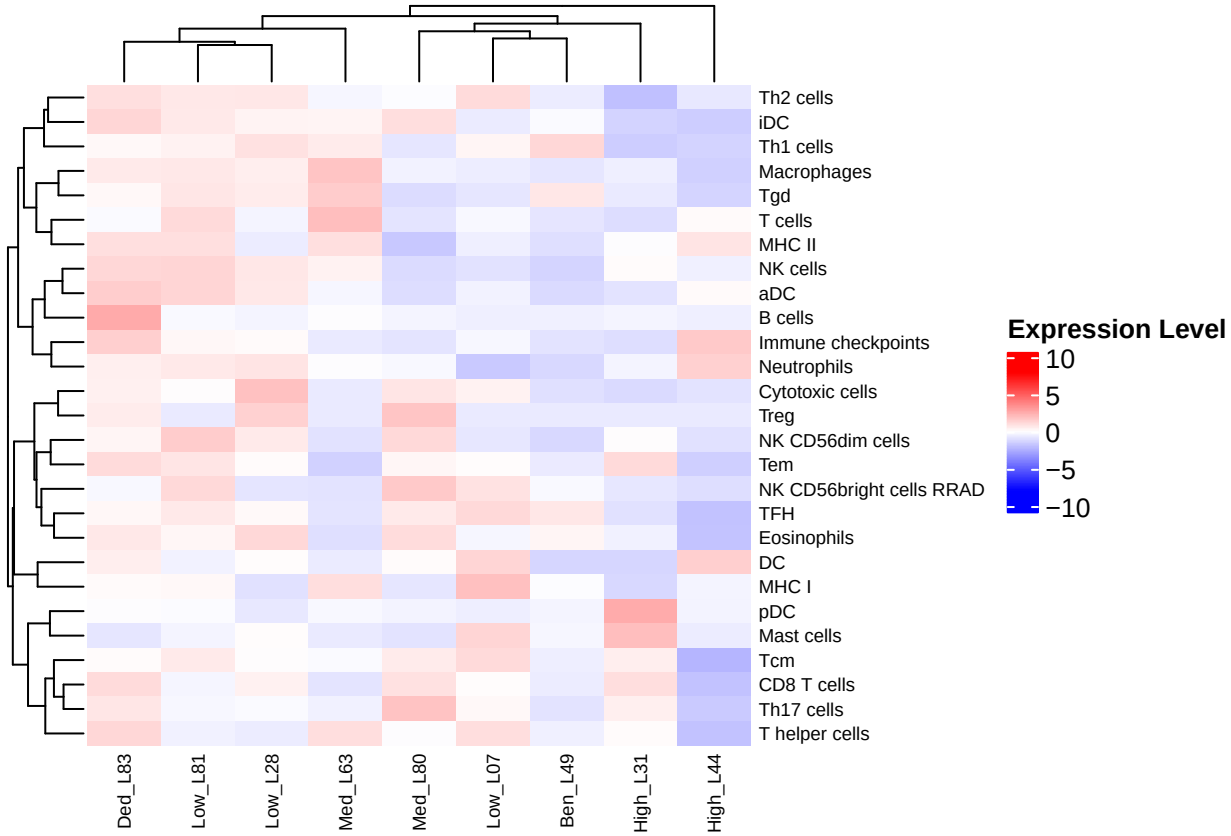


Figure 1: Relative immune cells expression heatmap

We see that the more infiltrated tumors are dedifferentiated and low tumors, contrary to the less infiltrated that are high grade.

II. Relative expression of CTAs

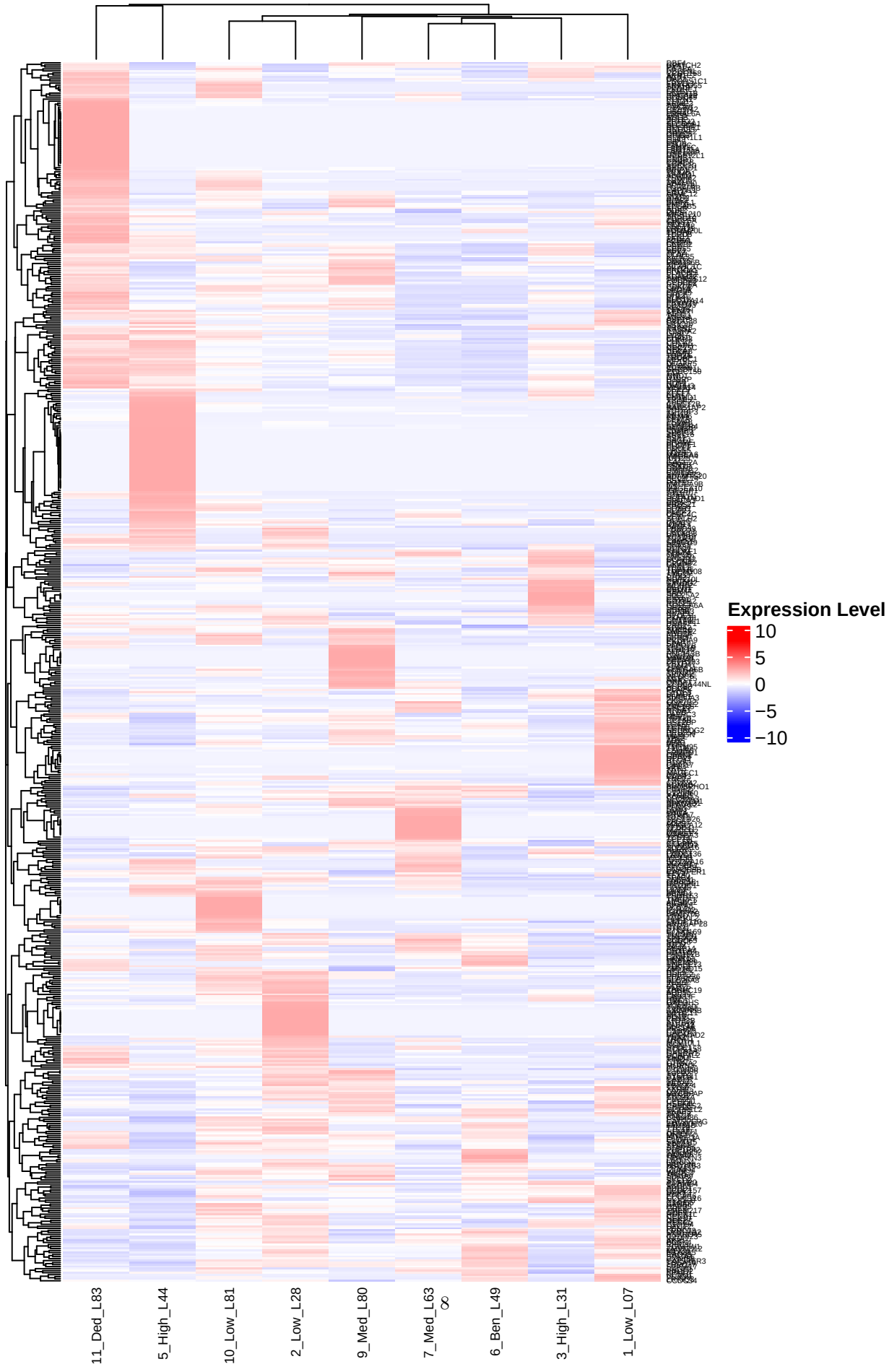
```
# Create the heatmap
cta_data <- subset(df_tpm_CTA, df_tpm_CTA$CTA == "CTA")
rownames(cta_data) <- cta_data$SYMBOL
cta_data <- cta_data[, -c(1, 2)]
cta_data <- log2(cta_data + 1)

# Calculate Z-scores
z_scores_row <- t(scale(t(cta_data)))

# Add columns
df_z_scores <- as.data.frame(z_scores_row)

Heatmap(as.matrix(df_z_scores), cluster_rows = TRUE, cluster_columns = TRUE,
```

```
    cluster_column_slices = TRUE, clustering_distance_columns = "euclidean",
    clustering_method_columns = "complete", show_column_dend = TRUE,
    col = colorRamp2(seq(-8, 8, length.out = 100), colors), border = NA,
    show_column_names = TRUE, column_names_gp = gpar(fontsize = 7),
    row_names_gp = gpar(fontsize = 4), heatmap_legend_param = list(title = "Expression Level"))
```


Expression Level
10
5
0
−5
−10
11_Ded_L83
5_High_L44
10_Low_L81
2_Low_L28
9_Med_L80
7_Med_L63
6_Ben_L49
3_High_L31
1_Low_L07