Code

Manifestation of MCMs expression in lung cancer TW cohort; Age, Stage, and mutation gene

1. Introduction

1.1 Background

Chen et al., 2020 suggests proteogenomic landscape of early stage lung adenocarcinoma in Taiwan cohort of mostly non-smokers, which reveals unique drivers and biomarkers. In this article, Minichromosome maintenance(MCM) proteins are mentioned for a moment that they are positively related to TP53 mutations. As engaged in cell cycle and DNA replication, they have shown to be dysregulated in lung cancer in many studies including Chen et al., 2020 and been considered as noticeable biomarker in NSCLC patients. (Huang et al., 2021) With TW cohort patients' profiles, especially in terms of Age, Stage, and mutation gene, I want to focus on the modalities of MCMs expression and confirm MCMs as potential biomarker pertaining to their role in tumorigenesis.

There are 9 genes in MCM family. MCM2-7 form a replicative helicase complex and serve as licensing factor for DNA replication to make sure that genomic DNA is replicated completely and accurately once during S phase in a single cell cycle. MCM8 and MCM9 form a dimer involved in homologous recombination repair. The ninth gene to encode an MCM domain is named as MCMDC2, but the function of the encoded protein is currently unknown. Excluding MCMDC2, they are separated in to two groups by function.

1.2 Aim

- Figure out the core function of MCM in lung cancer with comparisons by Age and Refined Stages
- Find out a significant mutation gene related to MCM and visualize MCM expression's distribution depending on the existence of the mutation

2. Utilizing Data Frames

2.1 Opening Data

Load packages needed for data manipulation and visualization.

Hide

```
library(dplyr)
library(tidyverse)
library(ggplot2)
library(ggridges)
library(RColorBrewer)
library(colorspace)
library(readxl)
library(cowplot)
```

Now open excel files. Below is the list of data I need.

- Patients' clinical profiles
- mRNA & Proteins expression results
- Proteomic subtypes analysis results

Hide

```
readxl::excel_sheets("~/Desktop/bsms222_116_choi/1-s2.0-S0092867420307431-mmc1.xlsx")
```

```
 [1] "description"                    "Table S1A_clinical_103patient"  "Table S1B_Cli
nicalStatisics"    "Table S1C_SNV"                  "Table S1D_transcriptome_log2TN"
 [6] "Table S1E_ProteomeLog2TN"       "Table S1F_PhosPepLog2TN"        "Table S1G_Pho
sSiteLog2TN"       "Table S1H_C>T"                  "Table S1I_C>A"
[11] "Table S1J_Nonsmoker TW TCGA"
```

Hide

```
# TS1A: Characteristics and clinical data of TW lung cancer patients
TS1A <- read_excel("~/Desktop/bsms222_116_choi/1-s2.0-S0092867420307431-mmc1.xlsx", s
heet = 2, na = "NA")

# TS1D: mRNA expression results
TS1D <- read_excel("~/Desktop/bsms222_116_choi/1-s2.0-S0092867420307431-mmc1.xlsx", s
heet = 5, na = "NA")

# TS1E: Proteomic expression results  normalized by column (patient) median
TS1E <- read_excel("~/Desktop/bsms222_116_choi/1-s2.0-S0092867420307431-mmc1.xlsx", s
heet = 6, na = "NA")
```

Hide

```
readxl::excel_sheets("~/Desktop/bsms222_116_choi/1-s2.0-S0092867420307431-mmc6.xlsx")
```

```
[1] "description"                   "S6A_annotated heatmap_subtype" "S6B_Fisher test
enrichments"   "S6C_IA cohort for validation"  "S6D_IB cohort for validation"
```

Hide

```
# TS6A: Heatmap of differentially regulated proteins in the three proteomic subtypes
TS6A <- read_excel("~/Desktop/bsms222_116_choi/1-s2.0-S0092867420307431-mmc6.xlsx", s
heet = 2, na = "NA")
```

2.2 Manipulating Data Frame

1) Transform TS1D & TS1E to tidy form

I want each observation in each patient is represented in one row. So I'll make a new column named "patient_id" then distribute expression results to columns "log2T/N_transcripts", "log2T/N_proteins" for TS1D and TS1E, respectively.

Hide

```
TS1D <- TS1D %>% gather("patient_id", "log2T/N_transcripts", 4:92)

TS1E <- TS1E %>% gather("patient_id", "log2T/N_proteins", 4:92)
```

2) Extract essential data from TS6A

TS6A seems quite complex as it contains both patients' proteomic subtypes profiles and log2 T/N ANOVA results.

Hide

```
# TS6A1: patients' proteomic subtypes profiles
# Take the data from row 1 to 79 and delete "Gene name", "ANOVA \r\np value", "ANOVA
 \r\nq-value", "k_means_4" which are all "NA".
# I want data in "id" column(row data) to be columns. So I use transpose(t) function
 and row_to_names function("janitor" package).

library(janitor)

TS6A1 <- TS6A[1:79,!(names(TS6A) %in% c("Gene name", "ANOVA \r\np value", "ANOVA \r\n
q-value", "k_means_4"))] %>%
        t() %>% as.data.frame() %>%
        row_to_names(row_number = 1)

# Then patient_id data become rownames. So I create patient_id column and reassign in
dex to rownames.
TS6A1$patient_id <- rownames(TS6A1)
rownames(TS6A1) <- 1:nrow(TS6A1)

# Lastly, to be in a tidy form, create columns for mutation genes and their counts.
# Make sure "mut_gene" column contains intact gene names in order to merge this data
 with others later on.
TS6A1 <- TS6A1 %>% select(patient_id, everything()) %>% gather("mut_gene", "count", 2
5:80)

TS6A1$mut_gene <- as.character(do.call(rbind.data.frame,
                                                    strsplit(TS6A1$mut_gene, '_'))[[1
]])
```

2.3 Merge Data Frame into a Single Comprehensive Data Frame

Now I have 4 data frames and I want to merge them into one, only containing data about MCM genes and proteins. First of all, let's look at MCM family genes(MCM2-9) in TS1E data.

Hide

```
TS1E %>%
  filter(str_detect(Gene, "MCM[2-9]$")) %>%
  select(Gene, Protein) %>%
  unique()
```

Gene <chr>	Protein <chr>
MCM3	DNA replication licensing factor MCM3
MCM4	DNA replication licensing factor MCM4
MCM5	DNA replication licensing factor MCM5

Gene	Protein
<chr>	<chr>
MCM7	DNA replication licensing factor MCM7
MCM2	DNA replication licensing factor MCM2
MCM6	DNA replication licensing factor MCM6
MCM9	DNA helicase MCM9
MCM8	DNA helicase MCM8

8 rows

Then add a column “MCM_function” to TS1E by which genes are separated in to “DNA replication licensing factor” and “DNA recombination repair”.

Hide

```
TS1E_MCM <- TS1E %>%
  filter(str_detect(Gene, "MCM[2-9]$")) %>%
  mutate(MCM_function = case_when(
                        str_detect(Protein, "DNA replication licensing factor") %>% ~
"DNA replication licensing factor",
                        str_detect(Protein, "DNA helicase") %>% ~ "DNA recombination
 repair")) %>%
  select(-Accession)
```

Finally, create data frames only with MCM genes and essential columns.

Hide

```
MCM_genes <- TS1E_MCM %>% count(Gene) %>% .$Gene

TS1D_MCM <- TS1D %>%
  filter(gene %in% MCM_genes) %>%
  mutate(transcripts_median = Median) %>%
  select(patient_id, gene, `log2T/N_transcripts`, transcripts_median)

TS1DE_MCM <- merge(TS1D_MCM, TS1E_MCM,
      by.x = c("patient_id", "gene"),
      by.y = c("patient_id", "Gene"),
      all.x = T, all.y = T) %>%
  select(patient_id, gene, MCM_function, transcripts_median, `log2T/N_transcripts`, `
log2T/N_proteins`)

TS1_MCM <- merge(TS1A, TS1DE_MCM,
      by.x = "ID",
      by.y = "patient_id") %>%
  select(ID, gene, MCM_function, transcripts_median, `log2T/N_transcripts`, `log2T/N_
proteins`, Gender, Age, `Smoking Status`, Stage, EGFR_Status)

MCMFAM <- merge(TS6A1 %>% select(patient_id, `proteome_k3 (out of k4)`, mut_gene, cou
nt), TS1_MCM,
             by.x = c("patient_id"),
             by.y = c("ID"),
             all.x = T, all.y = T) %>%
      mutate(proteome_k3 = as.numeric(`proteome_k3 (out of k4)`)) %>%
      select(patient_id, gene, MCM_function, transcripts_median, `log2T/N_transcripts
`, `log2T/N_proteins`, mut_gene, count, everything(), -`proteome_k3 (out of k4)`)
```

3. Data Visualization

3.1 The core function of MCM in lung cancer with comparisons by Age and Refined Stages

By boxplots, the comparisons between groups with several categories are made. The data we need are 1) Age group and 2) refined tumor staging. Accord with refined class of stages in Chen et al., 2020, add “Age_group” & “refined_class” column to MCMFAM data frame.

[AGE GROUP]

- under 60: younger
- over or equal to 60: older

[REFINED STAGES]

- Stage IA: Overall stage IA with proteomic subtype 2 & 3
- Stage IA late-like: Overall stage IA with proteomic subtype 1
- Stage IB: Overall stage IB with proteomic subtype 2 & 3
- Stage IB late-like: Overall stage IB with proteomic subtype 1
- Late: Overall stage >= II

Hide

```
MCMFAM <- MCMFAM %>% mutate(Age_group = case_when(Age<60 ~ "younger", Age >= 60 ~ "older"),
                            refined_class = case_when(
                            Stage == "IA" & proteome_k3 %in% c(2, 3) ~ "IA",
                            Stage == "IB" & proteome_k3 %in% c(2, 3) ~ "IB",
                            Stage == "IA" & proteome_k3 == 1 ~ "IA_late-like",
                            Stage == "IB" & proteome_k3 == 1 ~ "IB_late-like",
                            Stage %in% c("IIA", "IIIA", "IIB", "IV", "IIIB") ~ "Late"
))
```

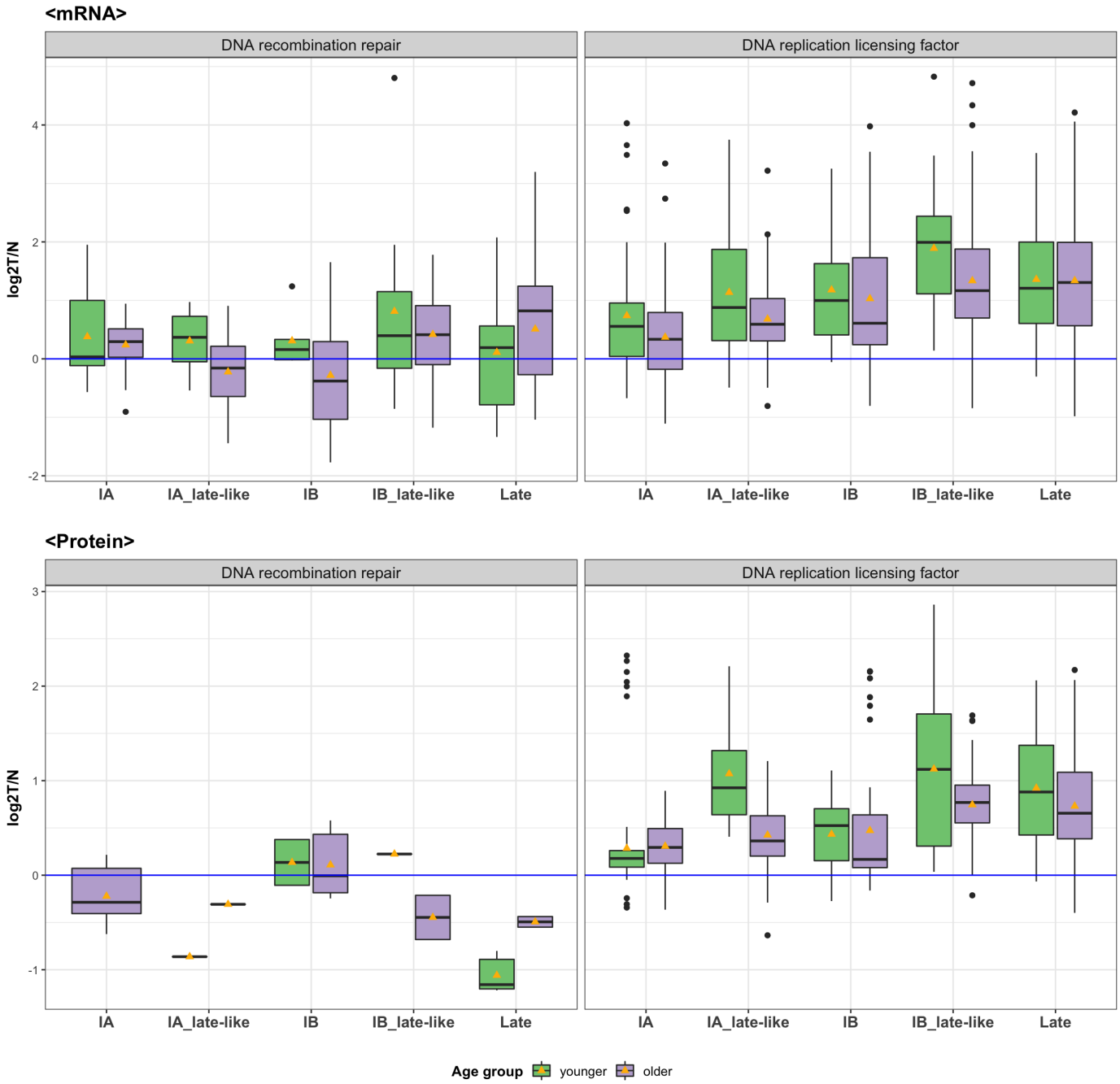
Draw plots of RNA & protein information with the separation by MCM_function.

Hide

```
# p1: log2T/N of mRNA transcripts compared by MCM function, Age, and Refined Stages
p1 <- MCMFAM %>%
  filter(!is.na(MCM_function) & !is.na(`log2T/N_transcripts`) & !is.na(refined_clas
s)) %>%
  select(-mut_gene) %>%
  unique() %>%
  ggplot(aes(refined_class, `log2T/N_transcripts`, fill = factor(Age_group, levels =
 c("younger", "older")))) +
  geom_boxplot() +
  stat_summary(fun = mean, geom = "point", size=2, shape = 17, color= "#FFB900",
                   position = position_dodge(width = .75)) +
  theme_bw() +
  theme(axis.text.x = element_text(size = 12, face = "bold"),
        axis.title.y = element_text(size = 12, face = "bold"),
        strip.text = element_text(size = 12),
        plot.title = element_text(size = 15, face = "bold")) +
  scale_fill_brewer(palette = "Accent") +
  geom_hline(yintercept = 0, col = "blue") +
  facet_grid(.~MCM_function) +
  xlab("") + ylab("log2T/N") + labs(title = "<mRNA>", fill = "Age group")


# p2: log2T/N of proteins compared by MCM function, Age, and Refined Stages
p2 <- MCMFAM %>%
  filter(!is.na(MCM_function) & !is.na(`log2T/N_proteins`) & !is.na(refined_class)) %
>%
  select(-mut_gene) %>%
  unique() %>%
  ggplot(aes(refined_class, `log2T/N_proteins`, fill = factor(Age_group, levels = c(
"younger", "older")))) +
  geom_boxplot() +
  stat_summary(fun = mean, geom = "point", size=2, shape = 17, color= "#FFB900",
                   position = position_dodge(width = .75)) +
  theme_bw() +
  theme(axis.text.x = element_text(size = 12, face = "bold"),
        axis.title.y = element_text(size = 12, face = "bold"),
        strip.text = element_text(size = 12),
        plot.title = element_text(size = 15, face = "bold"),
        legend.position = "bottom",
        legend.title = element_text(size=11, face = "bold"),
        legend.text = element_text(size=10)
        ) +
  scale_fill_brewer(palette = "Accent") +
  geom_hline(yintercept = 0, col = "blue") +
  facet_grid(.~MCM_function) +
  xlab("") + ylab("log2T/N") + labs(title = "<Protein>", fill = "Age group")


# Figure 1 as multi-panel plot
# Step1) p: combine p1 and p2
p <- plot_grid(p1+ theme(legend.position="none"),
          p2 + theme(legend.position="none"),
          ncol = 1)

# Step2) pl: combine p and legend
# The legend is shared by p1 and p2, which is why it was left out in p.
# Designate a legend particularly and add it below p.
```

```
legend <- get_legend(p2)

pl <- plot_grid(p, legend, ncol = 1, rel_heights = c(5, 0.15))

# Step3) Fig1: combine pl and title
title <- ggdraw() +
         draw_label( "Figure 1. The core function of MCM with comparison by Age and R
efined Stages",
                     fontface = 'bold',
                     size = 16,
                     x = 0.02, hjust = 0)

Fig1 <- plot_grid(title,pl,ncol = 1, rel_heights = c(.1, 3))
Fig1
```

Figure 1. The core function of MCM with comparison by Age and Refined Stages



[Additional Description]

- Horizontal blue lines are drawn on where y value is 0. As ‘log2T/N = 0’ signifies that the expression levels of tumor tissues and normal adjacent tissues are the same, upper part of the lines indicate

overexpression and under, low expression.
- Yellow triangle points are the means of each groups, which allow easier comparison between groups.

RESULTS

According to Figure 1, DNA licensing factor MCMs(MCM2-7) show substantial amount of expression, compared to DNA recombination repair MCMs(MCM8-9), in both mRNA transcripts and proteins. Focusing on DNA licensing factor data, MCMs' transcripts and proteins are generally expressed higher in younger age group than older group, and in IB late-like stage than other stages.

3.2 A significant mutation gene related to MCM and MCM expression's distribution depending on the existence of the mutation

One of important analysis from Figure 1 was that MCMs core function in lung cancer is DNA replication helicase and licensing factor and MCM2-7 are in charge. The next question dealt in Figure 2 is a related mutation gene and its correlation with MCM2-7. To find a related mutation, make a new data frame named "MCM_per_gene" which summarize averages of each MCMs expression quantities in case of genes' mutations defining proteomic subtypes in Table S6A.

Hide

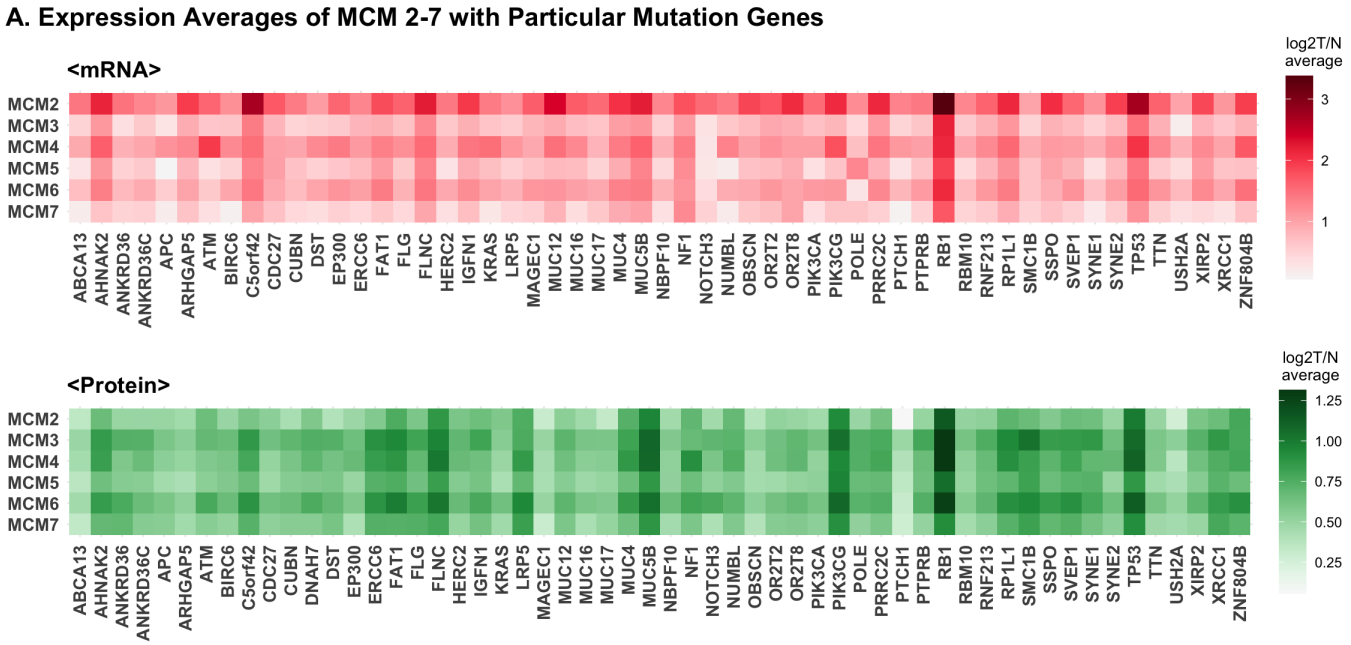
```
MCM_per_gene <- MCMFAM %>%
  filter(count > 0 & MCM_function == "DNA replication licensing factor") %>%
  select(gene, mut_gene, `log2T/N_transcripts`, `log2T/N_proteins`) %>%
  group_by(gene, mut_gene) %>%
  summarise(t_avg = mean(`log2T/N_transcripts`), p_avg = mean(`log2T/N_proteins`))
```

```
`summarise()` has grouped output by 'gene'. You can override using the `.groups` argu
ment.
```

To visualize expression level of MCM2-7 with mutation genes, heatmap is used and named as "Figure 2A".

Hide

```
# p3: MCM2-7 mRNAs log2T/N averages of patients with specific mutation genes
p3 <- MCM_per_gene %>%
  filter(!is.na(t_avg)) %>%
  ggplot(aes(mut_gene, reorder(gene, desc(gene)), fill = t_avg)) +
  geom_tile() +
  scale_fill_continuous_sequential("Reds 3") +
  theme_minimal() +
  theme(axis.text.x = element_text(size = 11, face = "bold",angle = 90, hjust = 1, vj
ust = 0.5),
        axis.text.y = element_text(size = 11, face = "bold"),
        plot.title = element_text(size = 14, face = "bold"),
        legend.title = element_text(size = 10, hjust = 0.5),
        legend.key.height = unit(9, "mm")) +
  coord_fixed(ratio = 1) +
  xlab("") + ylab("") + labs(title = "<mRNA>", fill = "log2T/N\naverage")

# p4: MCM2-7 Proteins log2T/N averages of patients with specific mutation genes
p4 <- MCM_per_gene %>%
  filter(!is.na(p_avg)) %>%
  ggplot(aes(mut_gene, reorder(gene, desc(gene)), fill = p_avg)) +
  geom_tile() +
  scale_fill_continuous_sequential("Greens 3") +
  theme_minimal() +
  theme(axis.text.x = element_text(size = 11, face = "bold",angle = 90, hjust = 1, vj
ust = 0.5),
        axis.text.y = element_text(size = 11, face = "bold"),
        plot.title = element_text(size = 14, face = "bold"),
        legend.title = element_text(size = 10, hjust = 0.5),
        legend.key.height = unit(9, "mm")) +
  coord_fixed(ratio = 1) +
  xlab("") + ylab("") + labs(title = "<Protein>", fill = "log2T/N\naverage")

# Define the title of Figure 2A and combine it with p3&p4.
title <- ggdraw() +
         draw_label( "A. Expression Averages of MCM 2-7 with Particular Mutation Gene
s",
                     fontface = 'bold',
                     size = 16,
                     x = 0.02, hjust = 0)

Fig2A <- plot_grid(title, p3, p4, ncol = 1, rel_heights = c(.2, 1, 1))
Fig2A
```



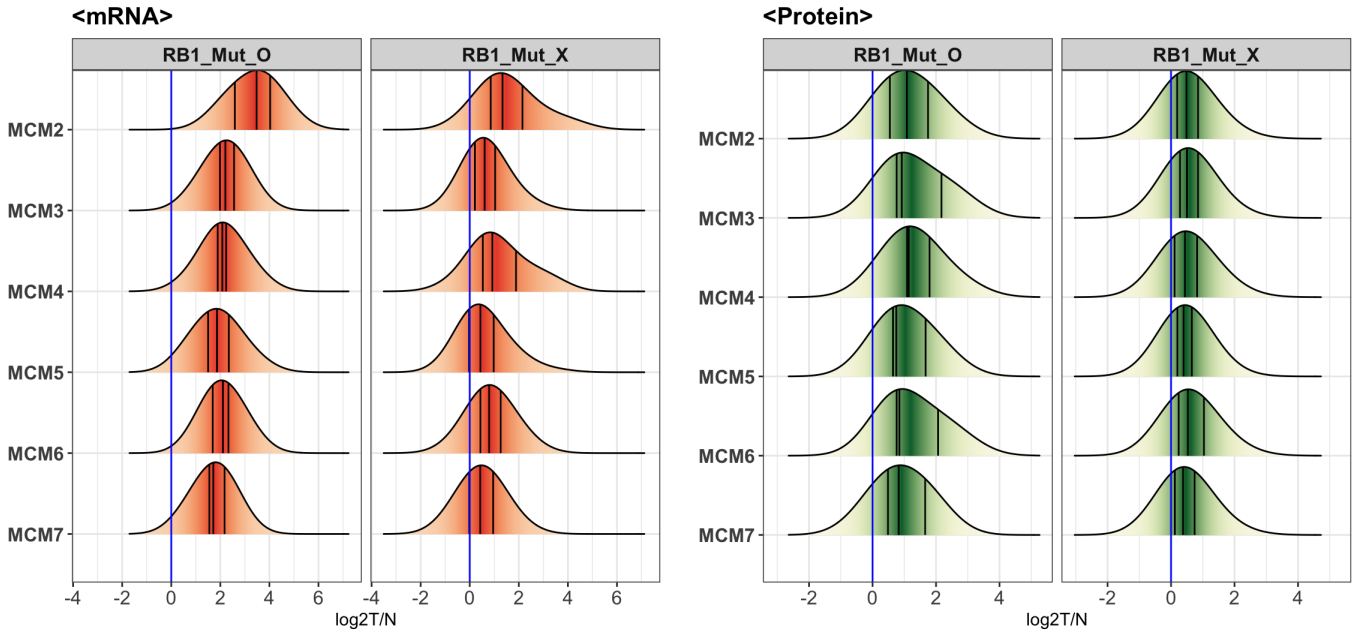
RESULTS

The figure implies that patients with mutation genes like RB1 and TP53 averagely show high level of MCMs expression of both mRNAs and proteins. However, it is already stated in Chen et al., 2020 that TP53 mutation and MCM 2-7 are positively related. The new finding is $RB1$ even with higher MCM 2-7 expression averages than TP53. So now I want to see MCM2-7 expression's distribution depending on the existence of RB1 mutation. With density ridges, visualize distribution of MCM2-7 expression respectively comparing the status of RB1 mutation.

Hide

```
# p5: mRNA expression distribution of MCM 2-7 depending on RB1 mutation
p5 <- MCMFAM %>% filter(!is.na(`log2T/N_transcripts`) & mut_gene == "RB1" & MCM_funct
ion == "DNA replication licensing factor") %>%
           mutate(RB1_mut = case_when(count == 0 ~ "RB1_Mut_X", count >0 ~ "RB1_Mut_
O")) %>%
           ggplot(aes(`log2T/N_transcripts`, reorder(gene, desc(gene)), fill=0.5-abs(
0.5-stat(ecdf)))) +
           theme_bw() +
           theme(strip.text = element_text(size = 12, face = "bold"),
                 plot.title = element_text(size = 15, face = "bold"),
                 axis.text.x = element_text(size = 12),
                 axis.text.y = element_text(size = 12, face = "bold")) +
           scale_color_continuous_sequential("Peach") +
           scale_fill_continuous_sequential("Peach") +
           stat_density_ridges(geom = "density_ridges_gradient",
                               bandwidth = 0.8, scale = 0.9,
                               calc_ecdf=TRUE, show.legend = F, quantile_lines = T) +
           geom_vline(xintercept = 0, col = "blue") +
           facet_wrap(.~RB1_mut)+
           xlab("log2T/N") + ylab("") + labs(title = "<mRNA>", size = 5)

# p6: Protein expression distribution of MCM 2-7 depending on RB1 mutation
p6 <- MCMFAM %>% filter(!is.na(`log2T/N_proteins`) & mut_gene == "RB1" & MCM_function
== "DNA replication licensing factor") %>%
           mutate(RB1_mut = case_when(count == 0 ~ "RB1_Mut_X", count >0 ~ "RB1_Mut_
O")) %>%
           ggplot(aes(`log2T/N_proteins`, reorder(gene, desc(gene)), fill=0.5-abs(0.5
-stat(ecdf)))) +
           theme_bw() +
           theme(strip.text = element_text(size = 12, face = "bold"),
                 plot.title = element_text(size = 15, face = "bold"),
                 axis.text.x = element_text(size = 12),
                 axis.text.y = element_text(size = 12, face = "bold")) +
           scale_color_continuous_sequential("Green-Yellow") +
           scale_fill_continuous_sequential("Green-Yellow") +
           stat_density_ridges(geom = "density_ridges_gradient",
                               bandwidth = 0.8, scale = 0.9,
                               calc_ecdf=TRUE, show.legend = F, quantile_lines = T) +
           geom_vline(xintercept = 0, col = "blue") +
           facet_wrap(.~RB1_mut)+
           xlab("log2T/N") + ylab("") + labs(title = "<Protein>", size = 5)

# p_RB1: combine p5 & p6
p_RB1 <- plot_grid(p5, p6, ncol = 2)

# Fig2B: combine p_RB1 & title
title <- ggdraw() +
         draw_label( "B. Expression Distribution of MCM 2-7 depending on RB1 Mutatio
n",
                     fontface = 'bold',
                     size = 16,
                     x = 0.02, hjust = 0)

Fig2B <- plot_grid(title, NULL, p_RB1, ncol = 1, rel_heights = c(.3, .1, 5))
Fig2B
```

B. Expression Distribution of MCM 2-7 depending on RB1 Mutation



[Additional Description]

- Vertical blue lines are drawn on where x value is 0. As 'log2T/N = 0' signifies that the expression levels of tumor tissues and normal adjacent tissues are the same, right part of the lines indicate overexpression and left, low expression.
- Color gradient implicates density; the darker the higher density.
- 3 black lines of each density plots state quantile(25%, 50%, 75%).

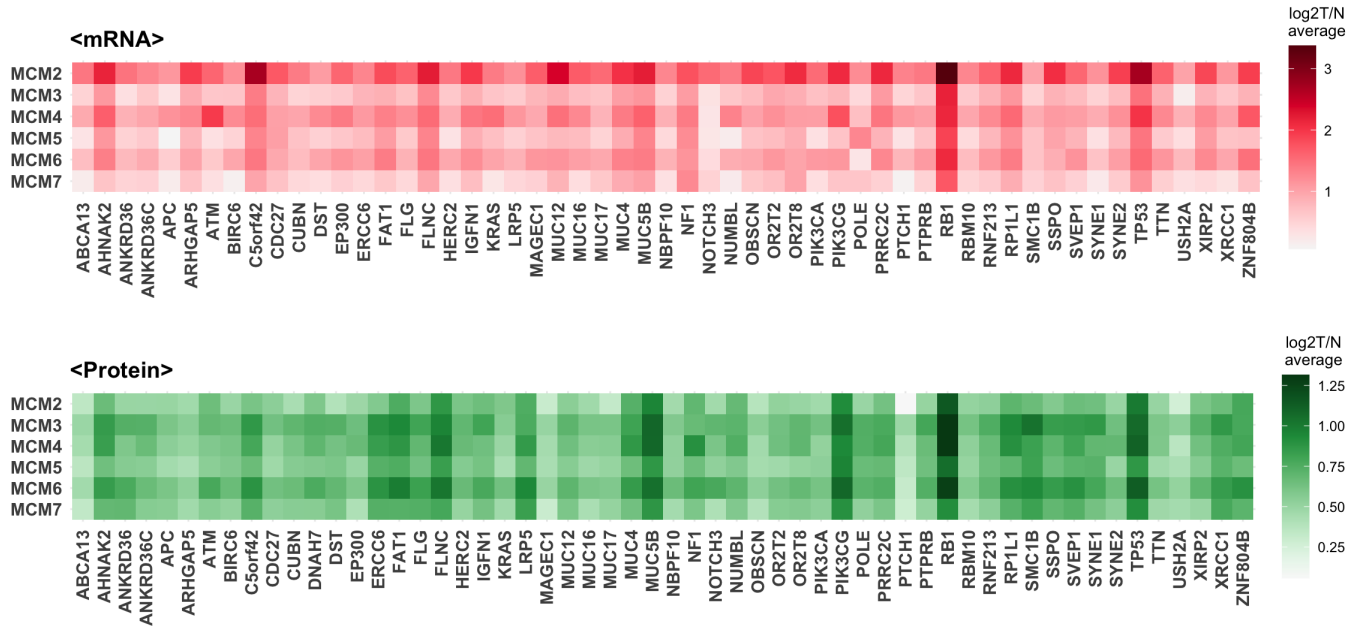
Combine Fig2A and Fig2B to Fig2.

Hide

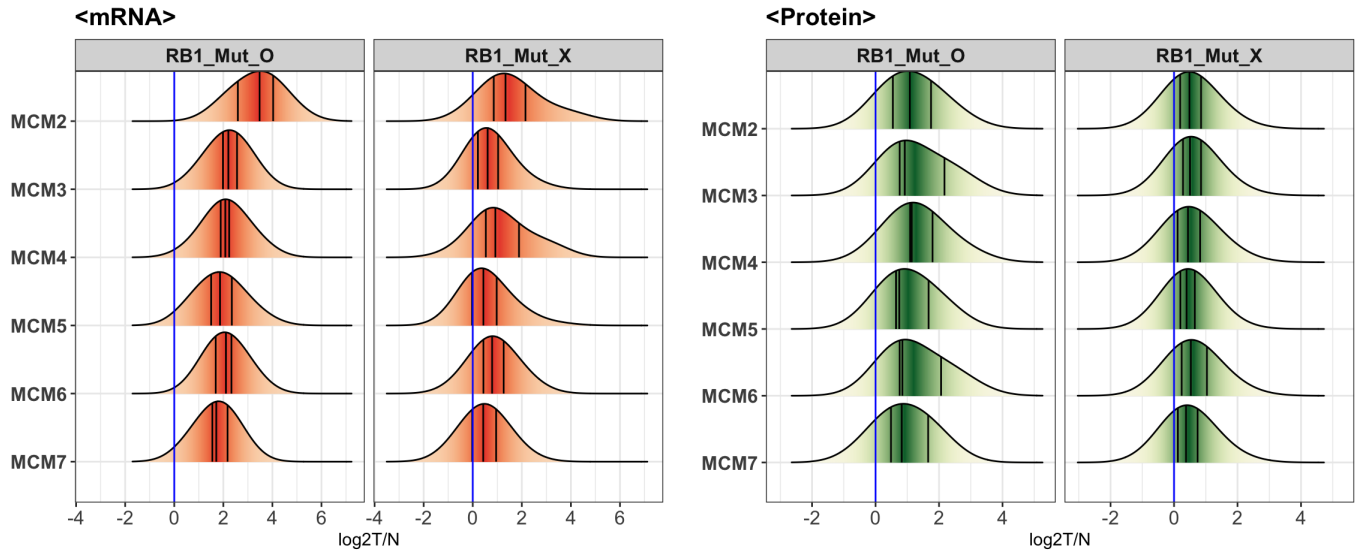
```
title <- ggdraw() +
         draw_label( "Figure 2. A Significant Mutation Gene Related to MCM",
                     fontface = 'bold',
                     size = 17,
                     x = 0.02, hjust = 0)
p_mut <- plot_grid(Fig2A, Fig2B, ncol = 1, rel_heights = c(6,5))
Fig2 <- plot_grid(title, p_mut, ncol = 1, rel_heights = c(.2, 5))
Fig2
```

Figure 2. A Significant Mutation Gene Related to MCM

A. Expression Averages of MCM 2-7 with Particular Mutation Genes



B. Expression Distribution of MCM 2-7 depending on RB1 Mutation



RESULTS

Through Figure 2A, RB1 turns out that it most significantly related to MCM2-7. To observe more specific distribution depending on the RB1 mutation status, density ridges were plotted as Figure 2B. MCM 2-7 seem to be more highly expressed with RB1 mutation, as comparing the quantile lines distances from blue lines. Specifically, MCM 2 and MCM 3 are markedly expressed than other MCMs in transcripts and proteins respectively.

4. Discussion

Hide

```
plot_grid(Fig1, Fig2)
```

Figure 1. The core function of MCM with comparison by Age and Refined Stages

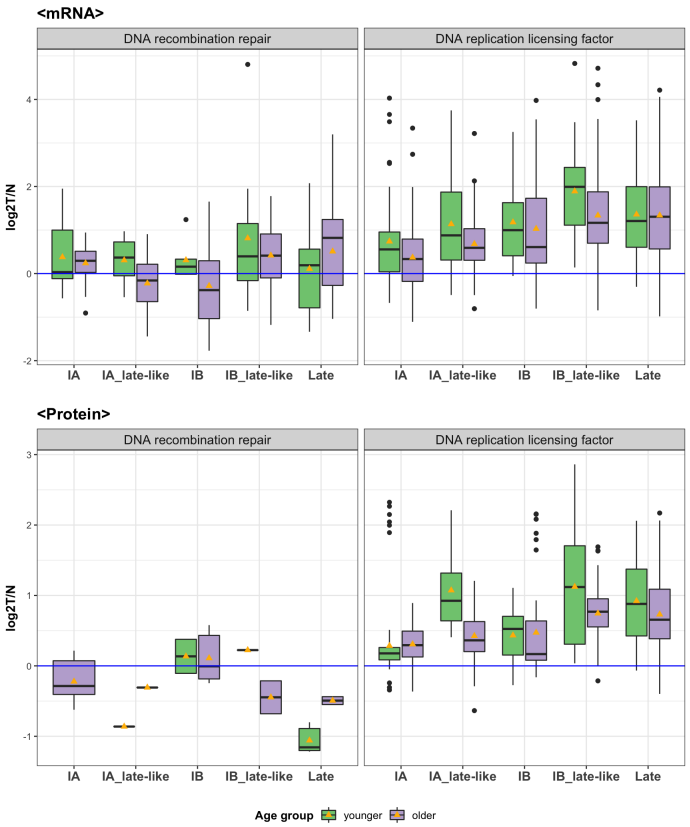
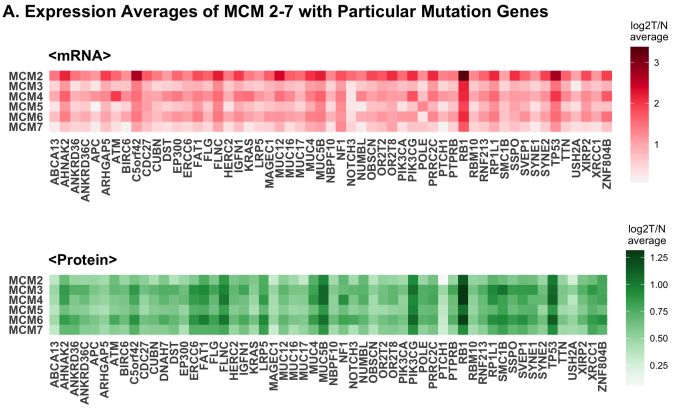
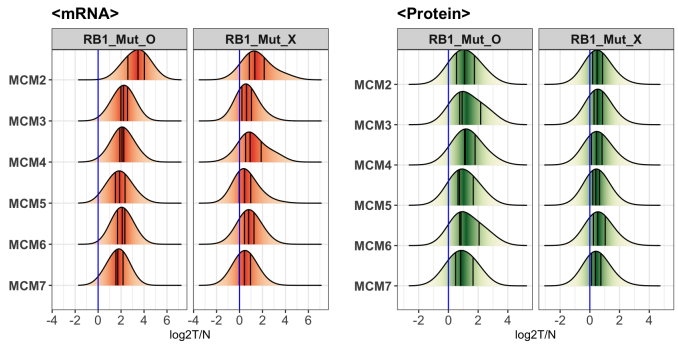


Figure 2. A Significant Mutation Gene Related to MCM

A. Expression Averages of MCM 2-7 with Particular Mutation Genes



B. Expression Distribution of MCM 2-7 depending on RB1 Mutation



The figures display overall manifestation of MCMs expression in TW cohort in terms of Age, Stage and mutation gene. To summarize the results, major MCM's role is a replicative helicase as well as a licensing factor for DNA replication. They are highly expressed in younger patients and patients with IB late-like lung cancer stage. Also, RB1 mutation has close relationship with MCM over-expression in lung cancer, especially with high MCM2 transcripts and MCM3 proteins. Correspondingly, MCM2-7 and RB1 are known to play crucial role in cell-cycle G1 phase balancing genome integrity. Formation of pre-replicative complex(pre-RC) is the first step in preparation for DNA replicatioin and MCM2-7 complex is a part of pre-RC. Then RB1 acts as tumor suppressor that it controls progression from G1 to S phase. (Haland et al., 2015) G1 phase and its check point have significance in tumorigenesis, its related factors are considered as potential biomarkers or cancer therapy targets. So it is expected that RB1 mutation cause uncontrolled, continuous cell cycle and provokes pre-RC including MCM expressed in high level. However, the plots are not enough to show complex interaction and phosphorylation associated with other factors engaged in G1 phase, such as CDCs and CDKs. Also they do not include other characteristics of patients, including smoking status, Sex, and many other mutated genes. Nevertheless the figures above represent much to confirm MCMs are deserve consideration in lung cancer with relation to RB1, a very well-known tumor suppressor, and suggest features of patient series with chances to show effectiveness when targeting MCMs for cancer therapy.

Reference

1. Chen et al.(2020), Proteogenomics of Non-smoking Lung Cancer in East Asia Delineates Molecular Signatures of Pathogenesis and Progression, Cell, Volume 182(1), Pages 226-244

2. Huang et al.(2021), Potential Prospective Biomarkers for Non-small Cell Lung Cancer: Mini-Chromosome Maintenance Proteins, Frontier in Genetics, https://doi.org/10.3389/fgene.2021.587017 (https://doi.org/10.3389/fgene.2021.587017)

3. MCM family(HGCN): https://www.genenames.org/data/genegroup/#!/group/1085 (https://www.genenames.org/data/genegroup/#!/group/1085)

4. Haland et al.(2015), Simultaneous measurement of passage through the restriction point and MCM loading in single cells, Nucleic Acids Research, Volume 43(22), https://doi.org/10.1093/nar/gkv744 (https://doi.org/10.1093/nar/gkv744)