TTG-RCode

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R code used to generate figures and tables in the manuscript: Topological Tumor Graphs: a graph-based spatial model to infer stromal recruitment for immunosuppression in melanoma histology

Load data

```r
load("TTG_data.Rdata")
```

Tables

Table 1

```r
# Association of stromal features with AJCC stage (stage_var)
attach(measDataCompSurv)
kruskal.test(strm_percent ~ stage_var, data= measDataCompSurv)
kruskal.test(strml_clustering ~stage_var, data= measDataCompSurv)
kruskal.test(strml_barrier ~stage_var, data= measDataCompSurv)


# Association of stromal features with Breslow thickness (breslow_depth_value)
hist(breslow_depth_value)
cor.test (breslow_depth_value, strm_percent, method="pearson",use = "complete.obs")
cor.test (breslow_depth_value, strml_clustering, method="pearson",use = "complete.obs")
cor.test (breslow_depth_value, strml_barrier, method="pearson",use = "complete.obs")


# Association of stromal features with  ulceration (ulcer_status)
t.test(strm_percent ~ ulcer_status)
t.test(strml_clustering ~ ulcer_status)
t.test(strml_barrier ~ ulcer_status)


# Association of stromal features with  NRAS/BRAF mutation status (mutn_categ)
kruskal.test(strm_percent~mutn_categ, data=measDataCompSurv)
kruskal.test(strml_clustering~mutn_categ, data=measDataCompSurv)
kruskal.test(strml_barrier~mutn_categ, data=measDataCompSurv)


# Association of stromal features withtumour type (tumour_type)
```

```
wilcox.test(strml_clustering ~ tumour_type, data= measDataCompSurv)
wilcox.test(strml_barrier ~ tumour_type, data= measDataCompSurv)
```

Table 2

```
### Stromal Barrier
# Create quartiles of stromal barrier
TTG_data$strmbar_quartile <- with(TTG_data, cut(strml_barrier,
                                                                breaks=quantile(strml_barrier, probs=se
                                                                include.lowest=TRUE,
                                                                labels = 1:4)
)


TTG_data$strmbar_quartile2only [strmbar_quartile == "1"] <- "Low barrier"
TTG_data$strmbar_quartile2only [strmbar_quartile== "4"] <- "High barrier"

# univariate Cox-Proportional Hazards test for 1st and 4th quartiles of stromal barrier
univarcox_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile, data=TTG_data)
summary(univarcox_barrierquartile)

#  multivariate
# adjust for lymphocyte%
adj_lympercent_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile + lym_perc
summary(adj_lympercent_barrierquartile)

# adjust for stromal%
adj_strmpercent_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile + strm_pe
summary(adj_strmpercent_barrierquartile)

# adjust for breslow and ulceration
adj_breslowulcer_barrierquartile <- coxph(Surv(times, patient.vital_status) ~ strmbar_quartile + breslow
summary(adj_breslowulcer_barrierquartile)



### Stromal Clustering
# defining low and gigh stromal clustering using pre-defined threshold of  0.590253
TTG_data$strml_clustering_hcateg [TTG_data$strml_clustering > 0.590253] <- "2"
TTG_data$strml_clustering_hcateg [TTG_data$strml_clustering <= 0.590253] <- "1"


# univariate Cox-Proportional Hazards test for categorical stromal clustering groups
univarcox_clustering_hcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg, data=
summary(univarcox_clustering_hcateg)

#  multivariate
# adjust for lymphocyte%
adj_lympercent_clusterhcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg + lym_
summary(adj_lympercent_clusterhcateg)

# adjust for stromal%
```

```r
adj_strmpercent_clusterhcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg + st
summary(adj_strmpercent_clusterhcateg)

# adjust for breslow and ulceration
adj_breslowulcer_clusterhcateg <- coxph(Surv(times, patient.vital_status) ~ strml_clustering_hcateg + b
summary(adj_breslowulcer_clusterhcateg)


### Mixed model: stromalbarrier + stromalclustering
time <- TTG_data$times
status <- TTG_data$patient.vital_status
sfit <- survfit(Surv(time, status)~ clustbar_combo, data=TTG_data)
### cox proprtional hazards test
combo_cox <- coxph(Surv(times, patient.vital_status) ~ clustbar_combo, data=TTG_data)
summary(combo_cox)
```

Figures

Figure 3a

```r
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install("RTCGA.clinical")
library(RTCGA.clinical)
install.packages("tidyverse")
library(tidyverse)
install.packages("survival")
library(survival)
install.packages("survminer")
library(survminer)
install.packages("survMisc")
library(survMisc)
install.packages("ggplot2")
library(ggplot2)
install.packages("ggpubr")
library(ggpubr)
install.packages("magrittr")
library(magrittr)
```

```r
### survival plot for stromal barrier
sfit2 <- survfit(Surv(times, patient.vital_status)~ strmbar_quartile2only, data=TTG_data)
colorpal3 = c("firebrick1", "royalblue3")

ggsurv2 <- ggsurvplot(sfit2, risk.table = TRUE, fontsize = 5, xlim = c(0, 3650),break.time.by=1000, lege
                      palette = colorpal3,
                      ylab= "Overall Survival",
                      title = "Stromal Barrier",
                      font.title = c(20, "plain"),
                      legend.labs = c ("high barrier", "low barrier"),
                      font.x = c(20, "plain"),
                      font.y = c(20, "plain"),
                      font.tickslab = c(16, "plain"),
                      tables.col = "strata",
                      tables.y.text = FALSE,
                      legend.title = "",
)

ggsurv2$plot <- ggsurv2$plot +
  theme(legend.text = element_text(size = 14, color = "black")) +
  theme(plot.title = element_text(hjust = 0.5, face = "bold"))
```

Figure 3b

```
###  survival plot for stromal clustering
sfit1 <- survfit(Surv(time, status)~ TTG_data$strml_clustering_hcateg, data=TTG_data)
colorpal3 = c("firebrick1", "royalblue3")

ggsurv1 <- ggsurvplot(sfit1, risk.table = TRUE, fontsize = 5, xlim = c(0, 3650),break.time.by=1000, lege
                      palette = colorpal3,
                      title = "Stromal Clustering",
                      font.title = c(20, "plain"),
                      ylab= "Overall Survival",
                      legend.labs = c ("high clustering", "low clustering"),
                      font.x = c(20, "plain"),
                      font.y = c(20, "plain"),
                      font.tickslab = c(16, "plain"),
                      tables.col = "strata",
                      tables.y.text = FALSE,
                      legend.title = "",
)

ggsurv1$plot <- ggsurv1$plot +
  theme(legend.text = element_text(size = 14, color = "black")) +
  theme(plot.title = element_text(hjust = 0.5, face = "bold"))
```

Figure 3c

```
### scatter plot of stromal barrier versis clustering
### create groups so that the 'missing' values (2nd and 3rd quartiles) are in grey
TTG_data$clustbar_combo [strml_clustering_hcateg== "1" & strmbar_quartile=="1"] <- "Low cluster/ Low ba
TTG_data$clustbar_combo [strml_clustering_hcateg== "1" & strmbar_quartile=="4"] <- "Low cluster/ high ba
TTG_data$clustbar_combo [strml_clustering_hcateg== "2" & strmbar_quartile=="1"] <- "high cluster/ low ba
TTG_data$clustbar_combo [strml_clustering_hcateg== "2" & strmbar_quartile=="4"] <- "high cluster/ high 
TTG_data$clustbar_combo [strml_clustering_hcateg== "1" & strmbar_quartile=="2"] <- "none"
TTG_data$clustbar_combo [strml_clustering_hcateg== "1" & strmbar_quartile=="3"] <- "none"
TTG_data$clustbar_combo [strml_clustering_hcateg== "2" & strmbar_quartile=="2"] <- "none"
TTG_data$clustbar_combo [strml_clustering_hcateg== "2" & strmbar_quartile=="3"] <- "none"
TTG_data$clustbar_combo <- as.factor(TTG_data$clustbar_combo)
table (TTG_data$clustbar_combo)
```

```
color_easy <- c("firebrick1", "pink", "steelblue1", "royalblue3", "ivory3")[TTG_data$clustbar_combo]
plot(TTG_data$strml_barrier, TTG_data$strml_clustering, col=color_easy, pch=16,
     panel.first = grid(col = "ivory4"),
     xlab="Stromal Barrier",
     ylab = "Stromal Clustering",
     cex.lab=1.3,cex.axis=1.2)
```

Figure 3d

```
###  KM plot of the 4 groups defined in Fig 3c
time <- TTG_data$times
status <- TTG_data$patient.vital_status

sfit <- survfit(Surv(time, status)~ clustbar_combo, data=TTG_data)
colorpal = brewer.pal(n=4, name="BrBG")
colorpal = brewer.pal(n=4, name="RdYlBu")
colorpal2 = c("firebrick1", "pink", "steelblue1", "royalblue3")


ggsurv <- ggsurvplot(sfit, risk.table = TRUE, fontsize = 5, xlim = c(0, 3650),break.time.by=1000, legend
                     palette = colorpal2,
                     ylab= "Overall Survival",
                     font.title = c(20, "plain"),
                     legend.labs = c ("high clustering, high barrier", "high clustering, low barrier",
                     font.x = c(20, "plain"),
                     font.y = c(20, "plain"),
                     font.tickslab = c(16, "plain"),
                     tables.col = "strata",
                     tables.y.text = FALSE,
                     legend.title = "",
)

ggsurv$plot <- ggsurv$plot +
  theme(legend.text = element_text(size = 14, color = "black"))
ggsurv
```

Figure 3e

```
###  lymphocyte% across these 4 groups defined in Fig 3c
boxplot (TTG_data$lym_percent ~ TTG_data$clustbar_combo,
         col= "white",
         border = colorpal2,
         outline=FALSE,
         ylab= "Lymphocyte%", cex.lab=1.5,
         cex.axis = 1.5,
         frame=F)

stripchart(TTG_data$lym_percent ~ TTG_data$clustbar_combo, data = TTG_data,
           vertical = TRUE, method = "jitter",
            pch = 16, col = c("firebrick1", "pink", "steelblue1", "royalblue3") , bg = "black",
            add = TRUE)

###  Tukey test to do pairwise comparisons
tt <- TukeyHSD(clustbarcombo_lympho_aov)
tt$`TTG_data$clustbar_combo` [,"p adj"] #this gives full pvalues
```

Figure 4a

```
install.packages("readxl")
library("readxl")

boxplot(ratioLymArea ~ strmbar_quartile2only, data = TTG_data)
boxplot(ratioLymArea ~ strml_clustering_hcateg, data = TTG_data) #1= low clustering, 2= high clustering
```

Figure 4b

```
###  compare stromal clustering and barrier across 3 immunephenotypeS described by Tamborero et al
# Immune phenotypeS 1 (‘low cytotoxicity’): Cluster 1
# Immune phenotypeS 2 (‘intermediate cytotoxicity’): Cluster 2
# Immune phenotypeS 3 (‘high cytotoxicity’): Cluster 3

TTG_data$Immune.phenotypeS <- as.factor(TTG_data$Immune.phenotypeS)
kruskal.test(strml_barrier ~ Immune.phenotypeS, data = TTG_data)
kruskal.test(strml_clustering ~ Immune.phenotypeS, data = TTG_data)

###  dunntest for groupwise comparison of stromal barrier across 3 immune phenotypes
dunn.test::dunn.test(strml_barrier, Immune.phenotypeS, method ="Hochberg")

###  plot strmbar across 3 immune phenotypes
colorpal = c ("firebrick4", "firebrick3", "indianred1")
clus <- c("Cluster 1 \n n=216","Cluster 2 \n n=86","Cluster 3 \n n=72")
###  altering border width to accomodate ticks
par(mar = c(5,5,5,5),
    mgp = c(4, 2.5, 0),
    mai = c(2.0, 1.5, 0.8, 0.4))


boxplot(strml_barrier ~ Immune.phenotypeS, data = TTG_data,
        main="",
        xlab="Immune phenotypes",
        ylab="Stromal barrier",
        outcol= "white",
        border= colorpal,
        cex.lab=1.5,
        cex.axis = 1.5,
        names=c("Cluster 1 \n n=216","Cluster 2 \n n=86","Cluster 3 \n n=72"),
        frame= F)
```

```r
stripchart(strml_barrier ~ Immune.phenotypeS,
           vertical = TRUE,
           data = TTG_data,
           method = "jitter",
           add = TRUE, pch = 16,
           col = colorpal)
```

Figure 4c

```r
###  multiple wilcoxon (mann whitney) tests for all immune scores: low vs high stromal barrier
wilcoxtests_tombimm <- lapply(
  TTG_data[, 70:85],
  function(x) {wilcox.test(x ~ strmbar_quartile2only, data = TTG_data) })
wilcoxtests_tombimm


###  multiple wilcoxon (man whitney) tests for all immune scores: low vs high stromal clustering
wilcoxtests_tambimm_clust <- lapply(
  TTG_data[, 70:85],
  function(x) {wilcox.test(x ~ strml_clustering_hcateg, data = TTG_data) })
wilcoxtests_tambimm_clust


###  Boxplots
###  Treg
boxplot(Treg ~ strmbar_quartile2only, data = TTG_data,
        main="",
        ylab="T-reg score",
        ylim = c(-0.7, 0.8),
        xlab = "",
        outcol= "white",
        border= "cyan3",
        cex.lab=1.5,
        cex.axis = 1.5,
        frame=F)

text(x= 1.5, y= 0.5, labels= "p=0.04", cex = 1.5)

stripchart(Treg ~ strmbar_quartile2only,
           vertical = TRUE,
           data = TTG_data,
           method = "jitter",
           add = TRUE, pch = 16,
           col = "cyan2")

###  Temm
boxplot(Tem ~ strmbar_quartile2only, data = TTG_data,
        main="",
        ylab="T-effector memory score",
        ylim = c(-0.7, 0.8),
        xlab = "",
        outcol= "white",
        border=  "darkturquoise",
        cex.lab=1.5,
        cex.axis = 1.5,
        frame=F)
text(x= 1.5, y= 0.5, labels= "p=0.003", cex = 1.5)

stripchart(Tem ~ strmbar_quartile2only,
           vertical = TRUE,
           data = TTG_data,
           method = "jitter",
           add = TRUE, pch = 16,
           col =  "darkturquoise")



###  CD8 t cells
boxplot(`CD8+ T` ~ strml_clustering_hcateg, data = TTG_data,
        main="",
        ylab="CD8 T-cell score",
        xlab = "",
        outcol= "white",
        border= "aquamarine2",
        cex.lab=1.5,
        cex.axis = 1.5,
        frame = F)
text(x= 1.5, y= 0.5, labels= "p=0.03", cex = 1.5)

stripchart(`CD8+ T` ~ strml_clustering_hcateg,
           vertical = TRUE,
           data = TTG_data,
           method = "jitter",
           add = TRUE, pch = 16,
           col = "aquamarine2")
```