BoolNet Inference ovarian cancer cell A2780 (GSE52695)

RNA-seq analysis of Hypoxia and Normoxia cultured cancer cells (GSE52695)

Purpose: To study differential mRNA and precursor miRNA expression under hypoxia exposure in epithelial ovarian cancer cells. We treated ovarian cancer cell line A2780 under hypoxia condition at different time points. Normoxi acultured cells at corresponding time point were used as controls. Overall design A2780 cancer cells, cultured under hypoxia and normoxia. Time points tested: 6hr, 48hr, 6days.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52695

Rupaimoole R, Wu SY, Pradeep S, Ivan C et al. Hypoxia-mediated downregulation of miRNA biogenesis promotes tumour progression. Nat Commun 2014 Oct 29;5:5202. PMID: 25351346

```
packages_cran = c("igraph", "BoolNet", "BiocManager", "tidyverse", "fs", "effectsize")
# Install and load packages
package.check <- lapply(packages_cran, FUN = function(x) {
  if (!require(x, character.only = TRUE)) {
    install.packages(x, dependencies = TRUE)
    library(x, character.only = TRUE)
  }
})
packages_bioconductor = c("Biobase", "GEOquery", "vsn", "ArrayExpress", "illuminaHumanv3.db")
# Install and load packages
package.check <- lapply(packages_bioconductor, FUN = function(x) {
  if (!require(x, character.only = TRUE)) {
    BiocManager::install(x, dependencies = TRUE)
    library(x, character.only = TRUE)
  }
})
rm(package.check, packages_bioconductor, packages_cran)
```

Load the pre-processed data

```
load("../data/data.GSE52695.Rdata")
eset <- ExpressionSet(assayData = as.matrix(expr.GSE52695),
                      probeNames = row.names(expr.GSE52695))
expr.GSE52695 <- exprs(justvsn(eset))
```

Selecting the HIF Genes

```
# Selected genes from HIF Axis
hif.symbols <- c("TP53", "HIF1A", "EP300", "MDM2", "VHL")
```

```
# Select the probes and genes
expr.GSE52695.hif <- as.data.frame(expr.GSE52695) %>%
  rownames_to_column('symbol') %>%
  filter(symbol %in% hif.symbols) %>%
  arrange(symbol)
```

Example of Binarizing

```
cols <- (data.GSE52695$rep == 1)
ov_1 <-
expr.GSE52695.hif %>%
  dplyr::select(c("symbol", data.GSE52695$codes[cols])) %>% arrange(symbol) %>%
  arrange(symbol) %>%
  rename_at(vars(data.GSE52695$codes[cols]),
            ~paste0(substr(data.GSE52695$condition[cols],1,2),".",
                    data.GSE52695$time[cols],".",
                    substr(data.GSE52695$cell_line[cols],1,2)))
knitr::kable(ov_1)
```

symbol	no.6h.ov	no.2d.ov	no.6d.ov
EP300	2.472476	2.508330	2.928631
HIF1A	3.911729	3.921996	4.546781
MDM2	2.843657	2.988164	3.368365
TP53	2.397712	2.563628	3.616591
VHL	2.693130	2.480025	2.805941

```
binarizeTimeSeries(ov_1[,-1], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  add_column(symbol = ov_1$symbol, .before=0) %>%
  knitr::kable(.)
```

symbol	no.6h.ov	no.2d.ov	no.6d.ov
EP300	0	0	1
HIF1A	0	0	1
MDM2	0	0	1
TP53	0	0	1
VHL	1	0	1

```
binarizeTimeSeries(ov_1[,-1], method="kmeans")$binarizedMeasurements  %>%
  data.frame(.)  %>%
  aggregate(., list(symbol = ov_1$symbol), mean) %>%
  mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%
  rbind(., c("O2", 1,1,1)) %>%
  knitr::kable(.)
```

```
## Warning: `funs()` is deprecated as of dplyr 0.8.0.
```

```
## Please use a list of either functions or lambdas:
##
##   # Simple named list:
##   list(mean = mean, median = median)
##
##   # Auto named with `tibble::lst()`:
##   tibble::lst(mean, median)
##
##   # Using lambdas
##   list(~ mean(., trim = .2), ~ median(., na.rm = TRUE))
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_warnings()` to see where this warning was generated.
```

symbol	no.6h.ov	no.2d.ov	no.6d.ov
EP300	0	0	1
HIF1A	0	0	1
MDM2	0	0	1
TP53	0	0	1
VHL	1	0	1
O2	1	1	1

```
# Function to binarize according an consensus mean of probes, add the O2 state and rename columns
binNetHy <- function(b){

  cols <- data.GSE52695$codes %in% names(b)

    binarizeTimeSeries(b[,-1], method="kmeans")$binarizedMeasurements  %>%
    as.data.frame(.)  %>%
    aggregate(., list(symbol = b$symbol), mean) %>%  # mean of binarized probes
    mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%  # consensus with a bies to 1 (>= 0.5)
    rbind(., c("O2",0,0,0)) %>%
      rename_at(vars(data.GSE52695$codes[cols] ),
              ~paste0(substr(data.GSE52695$condition[cols],1,2),".",
                      data.GSE52695$time[cols],".",
                      substr(data.GSE52695$cell_line[cols],1,2), ".",
                      data.GSE52695$rep[cols])) %>%
    column_to_rownames("symbol")
}

binNetNo <- function(b){
    cols <- data.GSE52695$codes %in% names(b)

    binarizeTimeSeries(b[,-1], method="kmeans")$binarizedMeasurements  %>%
    as.data.frame(.)  %>%
    aggregate(., list(symbol = b$symbol), mean) %>%  # mean of binarized probes
    mutate_at(vars(-symbol), funs(ifelse(. >= 0.5, 1, 0))) %>%  # consensus with a bies to 1 (>= 0.5)
    rbind(., c("O2",1,1,1)) %>%
      rename_at(vars(data.GSE52695$codes[cols] ),
              ~paste0(substr(data.GSE52695$condition[cols],1,2),".",
                      data.GSE52695$time[cols],".",
                      substr(data.GSE52695$cell_line[cols],1,2), ".",
                      data.GSE52695$rep[cols])) %>%
```

```
    column_to_rownames("symbol")
}

ov.1 <-
expr.GSE52695.hif %>%
  dplyr::select(c("symbol", data.GSE52695$codes[data.GSE52695$rep == 1]))  %>%
  binNetNo(.)

ov.2 <-
expr.GSE52695.hif %>%
  dplyr::select(c("symbol", data.GSE52695$codes[data.GSE52695$rep == 2]))  %>%
  binNetNo(.)

ov.3 <-
expr.GSE52695.hif %>%
  dplyr::select(c("symbol", data.GSE52695$codes[data.GSE52695$rep == 3]))  %>%
  binNetHy(.)

ov.4 <-
expr.GSE52695.hif %>%
  dplyr::select(c("symbol", data.GSE52695$codes[data.GSE52695$rep == 4]))  %>%
  binNetHy(.)
```

epithelial ovarian cancer cell line A2780

```
# epithelial ovarian cancer cell line A2780 - 3 time-points, 4 replicates
ov.1.net <- reconstructNetwork(ov.1, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
ov.2.net <- reconstructNetwork(ov.2, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
ov.3.net <- reconstructNetwork(ov.3, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)
ov.4.net <- reconstructNetwork(ov.4, method="bestfit",returnPBN=TRUE,readableFunctions=TRUE)

ov.1.p <- plotNetworkWiring(ov.1.net, plotIt=F)
ov.2.p <- plotNetworkWiring(ov.2.net, plotIt=F)
ov.3.p <- plotNetworkWiring(ov.3.net, plotIt=F)
ov.4.p <- plotNetworkWiring(ov.4.net, plotIt=F)

# ov.all <- reconstructNetwork(list(ov.1.net,ov.2.net,ov.3.net,ov.4.net),method="bestfit",returnPBN=TRU
# ov.all.p <- plotNetworkWiring(ov.all, plotIt=F)
```

```
# A2780 ovarian - 3 steps, replicate 1
print(ov.1.net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
```

```
## EP300 = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!VHL) ( probability: 1, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 1 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## O2 = 1
```

```
# A2780 ovarian - 3 steps, replicate 2
print(ov.2.net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = (!VHL) ( probability: 0.25, error: 0)
## HIF1A = (!MDM2) ( probability: 0.25, error: 0)
## HIF1A = (!HIF1A) ( probability: 0.25, error: 0)
## HIF1A = (!EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = (!VHL) ( probability: 0.25, error: 0)
## TP53 = (!MDM2) ( probability: 0.25, error: 0)
## TP53 = (!HIF1A) ( probability: 0.25, error: 0)
## TP53 = (!EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 1 ( probability: 1, error: 0)
##
```

```
## Knocked-out and over-expressed genes:
## EP300 = 0
## MDM2 = 0
## VHL = 0
## O2 = 1
```

```
# A2780 ovarian - 3 steps, replicate 3
print(ov.3.net)
```

```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
##
## Alternative transition functions for gene EP300:
## EP300 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53) ( probability: 0.25, error: 0)
## VHL = (MDM2) ( probability: 0.25, error: 0)
## VHL = (HIF1A) ( probability: 0.25, error: 0)
## VHL = (EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## EP300 = 0
## HIF1A = 0
## MDM2 = 0
## TP53 = 1
## O2 = 0
```

```
# A2780 ovarian - 3 steps, replicate 4
print(ov.3.net)
```

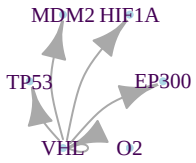
```
## Probabilistic Boolean network with 6 genes
##
## Involved genes:
## EP300 HIF1A MDM2 TP53 VHL O2
##
## Transition functions:
```

```
##
## Alternative transition functions for gene EP300:
## EP300 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene HIF1A:
## HIF1A = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene MDM2:
## MDM2 = 0 ( probability: 1, error: 0)
##
## Alternative transition functions for gene TP53:
## TP53 = 1 ( probability: 1, error: 0)
##
## Alternative transition functions for gene VHL:
## VHL = (!TP53) ( probability: 0.25, error: 0)
## VHL = (MDM2) ( probability: 0.25, error: 0)
## VHL = (HIF1A) ( probability: 0.25, error: 0)
## VHL = (EP300) ( probability: 0.25, error: 0)
##
## Alternative transition functions for gene O2:
## O2 = 0 ( probability: 1, error: 0)
##
## Knocked-out and over-expressed genes:
## EP300 = 0
## HIF1A = 0
## MDM2 = 0
## TP53 = 1
## O2 = 0
```

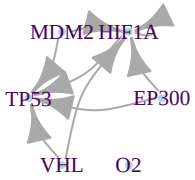
```
# # A2780 ovarian - 3 steps, all replicates
# print(ov.all.p)
```

```
# A2780 ovarian cancer - 4 time-points
par(mfrow = c(1,4))
plot(ov.1.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", layou
     main="A2780 ovarian\n 3 steps, replicate 1")
plot(ov.2.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", layou
     main="A2780 ovarian\n 3 steps, replicate 2")
plot(ov.3.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", layou
     main="A2780 ovarian\n 3 steps, replicate 3")
plot(ov.4.p, vertex.label.color="#440154ff", vertex.color="lightblue", vertex.frame.color="white", layou
     main="A2780 ovarian\n 3 steps, replicate 4")
```

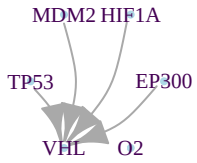
A2780 ovarian
3 steps, replicate 1



A2780 ovarian
3 steps, replicate 2



A2780 ovarian
3 steps, replicate 3



A2780 ovarian
3 steps, replicate 4