Gene Response Characterization of AKT inhibitors; Colorectal Cancer Cell Lines

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1 Motivation and Background

Cell lines are widely used as *in vitro* systems to characterize the genetic response of chemical compounds. They are a crucial step for therapeutic proposals. However, contradictory results are getting from different cell lines whose try to study the same biological system.

The report by [5] shows the results from two experiments (GSE18232, GSE18005) that could be used to determine the gene response to AKT inhibitors in *in vitro systems*.

To assess the effect of AKT inhibitors, 5 chemical compounds which act as AKT ihibitors (group 1) were tested versus 4 chemical compounds which are not characterized as direct AKT1 inhibitors (group 2) over three different cell lines (HCT116, HT29 and SW480). One independent model was performed by each gene (N=11,853).

In this example, we try to evaluate the AKT1 gene expression response after applying two groups of different compounds.

2 Data Modelling

The complete model from the data can be written as follows;
$X_{gijk} = \mu_g + A_{gi} + B_{gj(i)} + C_{gk} + AC_{gik} + e_{gijk}$

1. X_{gijk} represents the kth measurement of the gth gene expression of jth chemical compound of the ith group evaluated in the $k-th$ cell line.
2. μ_g the mean of expression of the gth gene
3. A_{gi} the effect of the ith group
4. $B_{gj(i)}$ the effect of the jth compound within the ith group
5. C_{gk} the effect of the kth cell-line
6. AC_{gki} the effect of the kth cell-line
7. e_{gijk} the measurement error term.

It is assumed that A_{gi} is a fixed effect whileas $B_{gj(i)}$ and e_{gijk}´s are normal distributed with 0 expected value and σ_B, σ_e respectively, and that these two sequences of random variables are independent of each other. The data were evaluated by analysis of variance (ANOVA) based on this nested model.

3 Usage

3.1 The data

The processed data is supplied in the VARCOMPCI package from GSE18232 and GSE18005 experiments. The data can be easy loaded as follows;

```
> library(varcompci)
> data(dataAKT_I)
> data(deadAKT_I)
> dim(dataAKT_I)

[1] 11853    29

> head(dataAKT_I[,c(1:3)])

                X          EBI_ID HCT116_Ly294001.log2Ratio
1 ENSG00000168209 ENSG00000168209                    -2.864
2 ENSG00000101255 ENSG00000101255                    -2.820
3 ENSG00000153234 ENSG00000153234                     2.744
4 ENSG00000069482 ENSG00000069482                    -2.622
5 ENSG00000146278 ENSG00000146278                     2.456
6 ENSG00000100867 ENSG00000100867                     2.166

> dim(deadAKT_I)

[1] 27  5

> head(deadAKT_I)

  X                        NAME CL Q G
1 1   HCT116_Ly294001.log2Ratio  1 1 1
2 2        HCT116_SH5.log2Ratio  1 2 1
3 3        HCT116_SH6.log2Ratio  1 3 1
4 4 HCT116_Wortmannin.log2Ratio  1 4 1
5 5     HT29_Ly294001.log2Ratio  2 1 1
6 6          HT29_SH5.log2Ratio  2 2 1

>
```

3.2 The effects over AKT1 gene

The first point is to compare the classical R outputs with VARCOMPCI package.

3.2.1 A classical ANOVA model with R

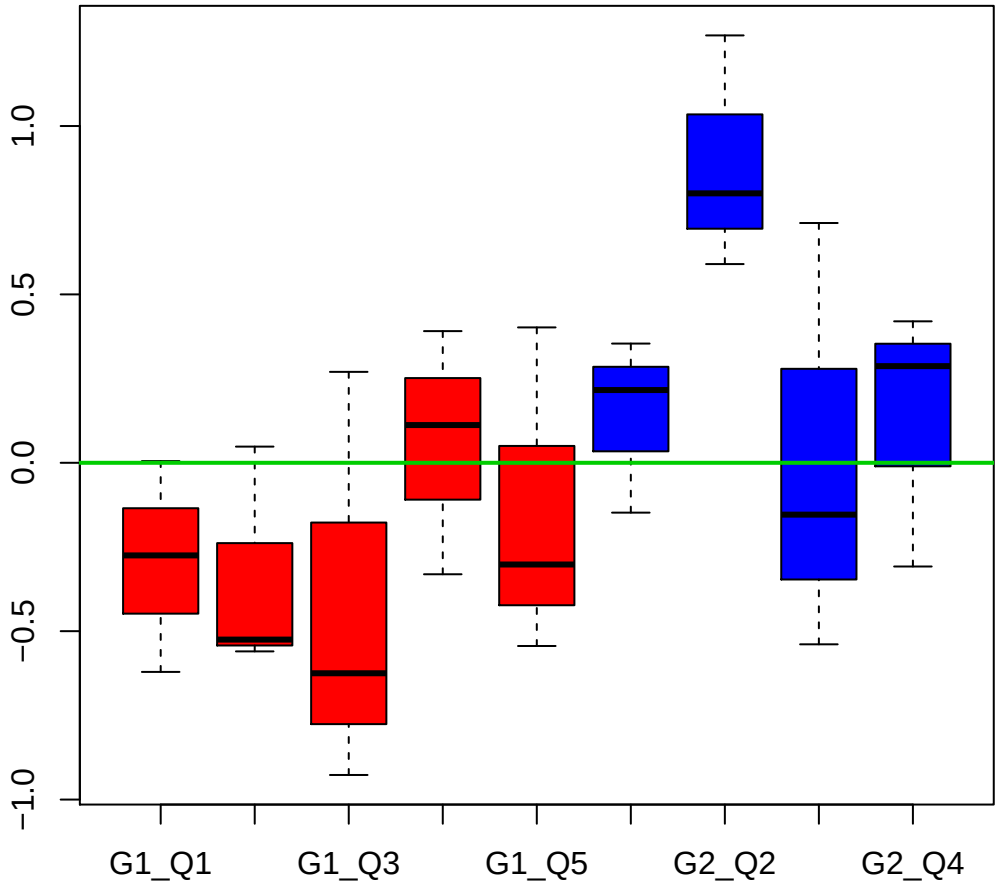
The chemical compounds from group 1 are known inhibitors of AKT1 gene. In this section we attempted to evaluate the compound effects on AKT1 over the three cell lines under study.

```
> row.names(dataAKT_I)<-as.character(dataAKT_I[,1])
> akt1<-dataAKT_I["ENSG00000142208",]
> #akt1
> a_akt1<-aov(t(akt1[1,as.character(deadAKT_I$NAME)])~ as.factor(deadAKT_I$CL)*as.factor(deadAKT_I$G)
> summary(a_akt1)
```

```
                                                Df Sum Sq Mean Sq F value
as.factor(deadAKT_I$CL)                          2 0.2314  0.1157   0.615
as.factor(deadAKT_I$G)                           1 1.8285  1.8285   9.711
as.factor(deadAKT_I$Q)                           4 0.8130  0.2033   1.080
as.factor(deadAKT_I$CL):as.factor(deadAKT_I$G)   2 0.5833  0.2916   1.549
as.factor(deadAKT_I$G):as.factor(deadAKT_I$Q)    3 1.0742  0.3581   1.902
Residuals                                       14 2.6360  0.1883
                                                 Pr(>F)
as.factor(deadAKT_I$CL)                         0.55483
as.factor(deadAKT_I$G)                          0.00758 **
as.factor(deadAKT_I$Q)                          0.40365
as.factor(deadAKT_I$CL):as.factor(deadAKT_I$G) 0.24677
as.factor(deadAKT_I$G):as.factor(deadAKT_I$Q)  0.17573
Residuals
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

A group effect is detected and plotted as follow;

```
> aux<-paste(deadAKT_I$G, deadAKT_I$Q,sep="")
> lab<-c("G1_Q1","G1_Q2", "G1_Q3", "G1_Q4", "G1_Q5","G2_Q1", "G2_Q2", "G2_Q3", "G2_Q4")
> col_lab<-c(rep(2,5),rep(4,4))
> boxplot(t(akt1[1,as.character(deadAKT_I$NAME)])~as.factor(aux), names=lab, col=col_lab)
> abline(h=0, col=3, lwd=2)
```



A clear repressive effect, as expected, on AKT1 gene is oberved for group 1 except for Q4 compound, whileas an effect around 0 or over where found in group 2. Note that some of the compounds in group 2 can act, indirectly, as activators of AKT1.

Taking account that Krech T et al 2010 [5] cited that the unexpected non-inhibitor effect observed in compound Q4 (Wolframin) must be interpreted carefully because of not working in the optimal conditions for this compound, we could eliminate Q4 to get a balanced ANOVA in further studies.

Then, a classical balanced ANOVA could be calculated as follows;

```
> deadAKT_I$eff<-as.vector(aux)
> deadAKT_I2<-subset(deadAKT_I, eff!="14")
> #deadAKT_I2<-subset(deadAKT_I, eff!="23")
> #deadAKT_I2<-subset(deadAKT_I2, eff!="25")
> deadAKT_I2$Q[deadAKT_I2$eff=="15"]<-4
> #deadAKT_I2$Q[deadAKT_I2$Q==5]<-2
```

```
> a_akt1_2<-aov(t(akt1[1,as.character(deadAKT_I2$NAME)])~ as.factor(deadAKT_I2$CL)*as.factor(deadAKT_
> summary(a_akt1_2)

                                                Df Sum Sq Mean Sq F value
as.factor(deadAKT_I2$CL)                         2 0.1473  0.0737   0.412
as.factor(deadAKT_I2$G)                          1 2.1319  2.1319  11.931
as.factor(deadAKT_I2$Q)                          3 0.7403  0.2468   1.381
as.factor(deadAKT_I2$CL):as.factor(deadAKT_I2$G)  2 0.8941  0.4471   2.502
as.factor(deadAKT_I2$G):as.factor(deadAKT_I2$Q)   3 0.8327  0.2776   1.553
Residuals                                       12 2.1441  0.1787
                                                 Pr(>F)
as.factor(deadAKT_I2$CL)                        0.67114
as.factor(deadAKT_I2$G)                         0.00477 **
as.factor(deadAKT_I2$Q)                         0.29586
as.factor(deadAKT_I2$CL):as.factor(deadAKT_I2$G) 0.12352
as.factor(deadAKT_I2$G):as.factor(deadAKT_I2$Q)  0.25173
Residuals
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> #coef(a_akt1_2)
```

3.2.2 A VARCOMPCI overview

We can use VARCOMPCI to test the compound effect over AKT1 gene. The compound effect could be considered as a random factor in the model. To reach that, it is necessary to create an unique dataset for AKT1;

```
> varcomp_akt<-data.frame(t(akt1[1,as.character(deadAKT_I$NAME)]))
> varcomp_akt$G<-as.vector(deadAKT_I$G)
> varcomp_akt$Q<-as.vector(deadAKT_I$Q)
> #varcomp_akt$Q<-as.vector(deadAKT_I$eff)
> varcomp_akt$CL<-as.vector(deadAKT_I$CL)
> varcomp_akt

                               ENSG00000142208 G Q CL
HCT116_Ly294001.log2Ratio               -0.275 1 1  1
HCT116_SH5.log2Ratio                    -0.560 1 2  1
HCT116_SH6.log2Ratio                    -0.927 1 3  1
HCT116_Wortmannin.log2Ratio              0.391 1 4  1
HT29_Ly294001.log2Ratio                  0.005 1 1  2
HT29_SH5.log2Ratio                       0.048 1 2  2
HT29_SH6.log2Ratio                       0.270 1 3  2
HT29_Wortmannin.log2Ratio               -0.331 1 4  2
SW480_Ly294001.log2Ratio                -0.621 1 1  3
SW480_SH5.log2Ratio                     -0.525 1 2  3
SW480_SH6.log2Ratio                     -0.625 1 3  3
SW480_Wortmannin.log2Ratio               0.112 1 4  3
HCT116_AG1478.log2Ratio                  0.354 2 1  1
HCT116_PD98059.log2Ratio                 0.800 2 2  1
HCT116_SulindacSulfide.log2Ratio         0.402 1 5  1
HCT116_SulindacSulfone.log2Ratio         0.287 2 4  1
HCT116_U0126.log2Ratio                   0.712 2 3  1
```

```
HT29_AG1478.log2Ratio                      -0.148 2 1  2
HT29_PD98059.log2Ratio                      0.590 2 2  2
HT29_SulindacSulfide.log2Ratio             -0.544 1 5  2
HT29_SulindacSulfone.log2Ratio             -0.308 2 4  2
HT29_U0126.log2Ratio                       -0.154 2 3  2
SW480_AG1478.log2Ratio                      0.216 2 1  3
SW480_PD98059.log2Ratio                     1.269 2 2  3
SW480_SulindacSulfide.log2Ratio            -0.302 1 5  3
SW480_SulindacSulfone.log2Ratio             0.420 2 4  3
SW480_U01262.log2Ratio                     -0.539 2 3  3
```

The design matrix can be defined as follow,

```
> Matrix=cbind(c(1,0),c(0,1))
> Matrix

     [,1] [,2]
[1,]    1    0
[2,]    0    1
```

Applying the varcompci function to get an ANOVA for crossing effects;

```
> totvar=c("Q","CL")
> response="ENSG00000142208"
> dsn="varcomp_akt"
> x<-varcompci(dsn="varcomp_akt",response=response, totvar=totvar, Matrix=Matrix)
> x["ANOVA"]

      df      SS      MS       F    Pval
Q      4 0.86104 0.21526 1.28179 0.40785
CL     2 0.23144 0.11572 0.68907 0.59204
Q:CL   8 1.34350 0.16794 0.42601 0.87559
resid 12 4.73056 0.39421      NA      NA
```

The random effect estimates are obtained from;

```
> x["EMS"]

      EMS
Q     "var(Resid) + 1.8var(Q:CL) + 5.4var(Q)"
CL    "var(Resid) + 1.8var(Q:CL) + 9var(CL)"
Q:CL  "var(Resid) + 1.8var(Q:CL)"
resid "var(Resid)"

> x["CI"]

      Method       LB Estimate      UB
Q      TBGJL -0.07641  0.00876 0.29548
CL     TBGJL -0.05609  -0.0058 0.48641
Q:CL   TBGJL -0.50448 -0.12571 0.13554
resid  Exact  0.20271  0.39421  1.0742
```

A nested model with VARCOMPCI;

```
> totvar=c("G","Q")
> Matrix=cbind(c(0,0),c(1,1))
> x<-varcompci(dsn="varcomp_akt",response=response, totvar=totvar, Matrix=Matrix)
> x["ANOVA"]

      df      SS      MS       F    Pval
G      1 1.82853 1.82853 6.78213 0.23340
G:Q    7 1.88727 0.26961 1.40635 0.33203
resid 18 3.45075 0.19171      NA      NA

> x["EMS"]

      EMS
G     "var(Resid) + 2.7var(G:Q) + Q(G)"
G:Q   "var(Resid) + 2.7var(G:Q)"
resid "var(Resid)"

> x["CI"]

      Method       LB Estimate      UB
G:Q    TBGJL -0.07195  0.02885 0.34072
resid  Exact  0.10946  0.19171 0.41925
```

Similar results were obtained when we used balanced ANOVA data.

```
> varcomp_akt<-data.frame(t(akt1[1,as.character(deadAKT_I2$NAME)]))
> varcomp_akt$G<-as.vector(deadAKT_I2$G)
> varcomp_akt$Q<-as.vector(deadAKT_I2$Q)
> #varcomp_akt$Q<-as.vector(deadAKT_I2$eff)
>
> varcomp_akt$CL<-as.vector(deadAKT_I2$CL)
>

> Matrix=cbind(c(1,0),c(0,1))
> totvar=c("Q","CL")
> response="ENSG00000142208"
> dsn="varcomp_akt"
> x<-varcompci(dsn="varcomp_akt",response=response, totvar=totvar, Matrix=Matrix)
> x["ANOVA"]

      df      SS      MS       F    Pval
Q      3 0.74034 0.24678 1.43710 0.38644
CL     2 0.14735 0.07367 0.42903 0.69978
Q:CL   6 1.03032 0.17172 0.41440 0.84607
resid 12 4.97256 0.41438      NA      NA

> x["EMS"]

      EMS
Q     "var(Resid) + 2var(Q:CL) + 6var(Q)"
CL    "var(Resid) + 2var(Q:CL) + 8var(CL)"
Q:CL  "var(Resid) + 2var(Q:CL)"
resid "var(Resid)"
```

```
> totvar=c("G","Q")
> Matrix=cbind(c(0,0),c(1,1))
> x<-varcompci(dsn="varcomp_akt",response=response, totvar=totvar, Matrix=Matrix)
> x["ANOVA"]

      df      SS      MS       F    Pval
G      1 2.13189 2.13189 8.13155 0.21472
G:Q    6 1.57305 0.26218 1.31679 0.37340
resid 16 3.18562 0.19910      NA      NA

> x["EMS"]

      EMS
G     "var(Resid) + 3var(G:Q) + Q(G)"
G:Q   "var(Resid) + 3var(G:Q)"
resid "var(Resid)"

>
```

Finally, we can conclude that exists a clear group effect. Although, no effects to compounds or cell lines were found, we must be careful with the conclusions due to small sample size and the fact that no replicates were provided. Anyway, varcompci provides a framework to easily test nested and crossed anova models with random effects.

3.3 Testing other genes effects with VARCOMPCI

The Krech et T al 2010 [5] study provides information for other gene effects. Here we used VARCOMPCI to test them. Firstly we must find the ensembl identifier for each one. This can be provided using biomart from R;

```
> hugos<-c("NUSAP1", "GAPDH","ASPM","PRC1", "CENPF")
> inf<- c("ENSG00000137804", "ENSG00000111640", "ENSG00000066279", "ENSG00000198901","ENSG0000011772
> inf<-cbind(inf, hugos)
> inf

     inf               hugos
[1,] "ENSG00000137804" "NUSAP1"
[2,] "ENSG00000111640" "GAPDH"
[3,] "ENSG00000066279" "ASPM"
[4,] "ENSG00000198901" "PRC1"
[5,] "ENSG00000117724" "CENPF"
```

3.3.1 Testing Cell Line and Group effect

In the following code, we only show the ANOVA tables since the Cell Line effects were not found to be statistically significant and it is possible to get negative values for the variance parameter estimates.

```
> for(k in 1:dim(inf)[1]){
+ varcomp_k<-data.frame(t(dataAKT_I[as.character(inf[k,1]),as.character(deadAKT_I2$NAME)]))
+ varcomp_k$G<-as.vector(deadAKT_I2$G)
+ varcomp_k$Q<-as.vector(deadAKT_I2$Q)
+ #varcomp_k$Q<-as.vector(deadAKT_I2$eff)
+ varcomp_k$CL<-as.vector(deadAKT_I2$CL)
+ totvar=c("G","CL")
```

```
+ Matrix=cbind(c(1,0),c(0,1))
+ response<-names(varcomp_k)[1]
+ x<-varcompci(dsn="varcomp_k",response=response, totvar=totvar, Matrix=Matrix)
+ print(inf[k,2])
+ print(x["ANOVA"])
+ #print(varcompci(dsn="varcomp_k",response=response, totvar=totvar, Matrix=Matrix,vecprint=c("EMS","
+ }

   hugos
"NUSAP1"
      df       SS      MS       F    Pval
G      1  0.61568 0.61568 1.75876 0.41131
CL     2  1.41396 0.70698 2.01956 0.33117
G:CL   2  0.70013 0.35007 0.27973 0.78142
resid 18 22.52617 1.25145      NA      NA
  hugos
"GAPDH"
      df      SS      MS       F    Pval
G      1 0.04010 0.04010 0.44607 0.62513
CL     2 0.78971 0.39486 4.39250 0.18544
G:CL   2 0.17979 0.08989 1.92699 0.34165
resid 18 0.83969 0.04665      NA      NA
 hugos
"ASPM"
      df       SS      MS       F    Pval
G      1  3.73434 3.73434 1.02007 0.49684
CL     2 11.99294 5.99647 1.63799 0.37908
G:CL   2  7.32173 3.66086 1.42578 0.41224
resid 18 46.21733 2.56763      NA      NA
 hugos
"PRC1"
      df       SS      MS       F    Pval
G      1  1.14581 1.14581 0.51306 0.60430
CL     2  0.38889 0.19444 0.08707 0.91991
G:CL   2  4.46657 2.23328 0.88627 0.53015
resid 18 45.35782 2.51988      NA      NA
  hugos
"CENPF"
      df      SS      MS       F    Pval
G      1 1.65900 1.65900 2.64238 0.35110
CL     2 0.19333 0.09666 0.15396 0.86658
G:CL   2 1.25569 0.62785 1.53486 0.39450
resid 18 7.36304 0.40906      NA      NA

>
```

3.3.2 Testing Compound and Group effect

In the following code, we showed ANOVA tables and estimates because of the significance of some Compound Effects.

```
> for(k in 1:dim(inf)[1]){
+ varcomp_k<-data.frame(t(dataAKT_I[as.character(inf[k,1]),as.character(deadAKT_I2$NAME)]))
```

```
+ varcomp_k$G<-as.vector(deadAKT_I2$G)
+ varcomp_k$Q<-as.vector(deadAKT_I2$Q)
+ #varcomp_k$Q<-as.vector(deadAKT_I2$eff)
+ varcomp_k$CL<-as.vector(deadAKT_I2$CL)
+ totvar=c("G","Q")
+ Matrix=cbind(c(0,0),c(1,1))
+ response<-names(varcomp_k)[1]
+ x<-varcompci(dsn="varcomp_k",response=response, totvar=totvar, Matrix=Matrix)
+ print(inf[k,2])
+ print(x["ANOVA"])
+ #print(x["EMS"])
+ print(x["CI"])
+ }

   hugos
"NUSAP1"
      df       SS      MS       F    Pval
G      1  0.61568 0.61568 0.21565 0.72323
G:Q    6 17.13003 2.85501 6.08238 0.02252
resid 16  7.51023 0.46939      NA      NA
      Method      LB Estimate      UB
G:Q    TBGJL 0.20446  0.79521 4.45017
resid  Exact 0.26036  0.46939 1.08723
  hugos
"GAPDH"
      df      SS      MS       F    Pval
G      1 0.04010 0.04010 0.41948 0.63411
G:Q    6 0.57354 0.09559 1.23776 0.40114
resid 16 1.23565 0.07723      NA      NA
      Method       LB Estimate      UB
G:Q    TBGJL -0.03233  0.00612 0.12786
resid  Exact  0.04284  0.07723 0.17888
 hugos
"ASPM"
      df       SS      MS       F    Pval
G      1  3.73434 3.73434 0.81731 0.53205
G:Q    6 27.41440 4.56907 1.91788 0.22397
resid 16 38.11758 2.38235      NA      NA
      Method       LB Estimate      UB
G:Q    TBGJL -0.63506  0.72891 6.55731
resid  Exact  1.32145  2.38235 5.51816
 hugos
"PRC1"
      df       SS      MS        F    Pval
G      1  1.14581 1.14581  0.16906 0.75166
G:Q    6 40.66651 6.77775 11.35924 0.00467
resid 16  9.54676 0.59667       NA      NA
      Method      LB Estimate       UB
G:Q    TBGJL 0.71706  2.06036 10.74575
resid  Exact 0.33096  0.59667  1.38205
  hugos
"CENPF"
```

```
      df      SS     MS       F    Pval
G      1 1.65900 1.6590 2.37408 0.36649
G:Q    6 4.19279 0.6988 2.42047 0.15312
resid 16 4.61926 0.2887      NA      NA
      Method       LB Estimate      UB
G:Q    TBGJL -0.04804   0.1367 1.02891
resid  Exact  0.16014   0.2887 0.66872

>
```

3.4 VARCOMPCI Conclusions for AKT Inhibitors study

The group of AKT1 inhibitors decressed the expression of AKT1 gene without large Cell Lines (CL) effect neither Compound (Q) effect. Then AKT1 expression can be modelling as follows;
$X_{gijk} = \mu_g + e_{gijk}$

1. X_{gijk} represents the kth measurement of the gth gene expression of jth chemical compound of the ith group evaluated in the $k-th$ cell line.
2. μ_g the mean of expression of the gth gene
3. e_{gijk} the measurement error term.

It is assumed that A_{gi} is a fixed effect whileas e_{gijk} is normal distributed with 0 expected value and standard deviation σ_e.

To estimate the effects we could use a standard balanced ANOVA computed in R;

```
> a_akt1_2<-aov(t(akt1[1,as.character(deadAKT_I2$NAME)])~  as.factor(deadAKT_I2$G))
> summary(a_akt1_2)

                        Df Sum Sq Mean Sq F value  Pr(>F)
as.factor(deadAKT_I2$G)  1  2.132  2.1319   9.856 0.00476 **
Residuals               22  4.759  0.2163
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> coef(a_akt1_2)

             (Intercept) as.factor(deadAKT_I2$G)2
              -0.3045000                0.5960833
```

This means that an ihnibition effect in AKT1 gene is around -0.3 whileas the gene expression under the second group of Compounds is increasing around 0.3. The estimation of σ_e is given by the ANOVA table (Mean squares) and is;

```
> sqrt(0.2163)

[1] 0.4650806
```

For the other genes under study, we did not detect a Cell Line effect; however it is not possible to discard a Compound effect over the gene expression. For this reason the best model to represent the data, must be written as follows;
$X_{gijk} = \mu_g + B_{gj(i)} + e_{gijk}$

1. X_{gijk} represents the kth measurement of the gth gene expression of jth chemical compound of the ith group evaluated in the $k-th$ cell line.

2. μ_g the mean of expression of the gth gene

3. $B_{gj(i)}$ the effect of the jth compound within the ith group

4. e_{gijk} the measurement error term.

It is assumed that A_{gi} is a fixed effect whileas $B_{gj(i)}$ and e_{gijk}´s are normal distributed with 0 expected value and σ_B, σ_e respectively, and that these two sequences of random variables are independent of each other.

The global gene effect, μ_g, could be estimated applying a mean function;

```
> akt_2<-dataAKT_I[as.character(inf[,1]),as.character(deadAKT_I2$NAME)]
> apply(akt_2,1, mean)

ENSG00000137804 ENSG00000111640 ENSG00000066279 ENSG00000198901 ENSG00000117724
    -0.62433333     -0.05304167     -1.14087500     -1.07025000     -0.51500000
```

A global down regulation is found for these genes as comented in Krech et al study [5].

$B_{gj(i)}$ and e_{gijk} estimates are provided by VARCOMPCI. Estimation of $(e_{gijk})^2$ is provided by VARCOMPCI ANOVA table (Mean Squares) whileas estimation for $B_{gj(i)}$ is provided by VARCOMPCI components of variance. For example, for PRC1 gene, a global effect μ_g is estimated by using the mean with a value of -1.07 with total variance of $B_{gj(i)} + e_{gijk} = 2.06 + 0.597$.

4 Note

We prepared the files and classified the compounds as described in Krech T el al. 2010 [5]. In addition other supporting information about the role of the compounds were extract from the literature. We found evidence for AKT1 direct inhibition through PIK3/AKT pathway (group 1) for: LY294001 (Q1, [1]); SH-5 (Q2,[6]), SH6 (Q3, [8]), Wortmannin (Q4,[1]) and SulindacSulfide (Q5, [7]).

Additional information provided evidences for non-direct inhibition of AKT1 for the following compounds (group 2); AG1478 (inhibitor of EGF receptor tyrosine kinase, Q1, [3]), PD98059 (inhibitors of the MAPK3/MAPK1 signaling pathways, Q2, [2]), SulindacSulfone (Increased psca promoter activity, Q3, [9]), U0126 (MEK-inhibitor, Q4, [4]).

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