Data-driven identification of transcription factor networks over time in indicating a shifted cellular homeostasis

STA130 - Final Project

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Introduction

Goals And Motivation

- Decrease or reduce the growth and expansion of malignant cells
- Controlling the transcription factor network using the data set given [1]
- Identify movement from deleterious to healthy phenotype overtime

Research Questions & Categories of Analysis

1) Can we predict cellular phenotype outcomes 'Y' values from transcription factors (TF)? $\rightarrow$ Linear Regression
2) At time 't' in experimental condition, what TF are most predictive of cellular values/states? $\rightarrow$ Classification Trees
3) Do protein levels in experimental condition 'X' change over time 't'? $\rightarrow$ Two Sample Hypothesis Testing

Data Set

- Overall 22 Levels of Transcription Factors (AP-1)
- 4 Phenotype Indicators (MiTFg, Sox10, NGFR, AXL)
- Others: Time point, Drugs type, Dose id, Dosage, Repetition

Table 1: Data Wrangling

AXL	Sox10	Timepoint	Drugs	dose_id	Doses	Rep
3.536432	3.686878	0.5	0	1	0	1
3.732794	3.668114	0.5	0	1	0	1
3.609001	3.781692	0.5	0	1	0	1
3.223876	3.700308	0.5	0	1	0	1
3.600571	3.755307	0.5	0	1	0	1

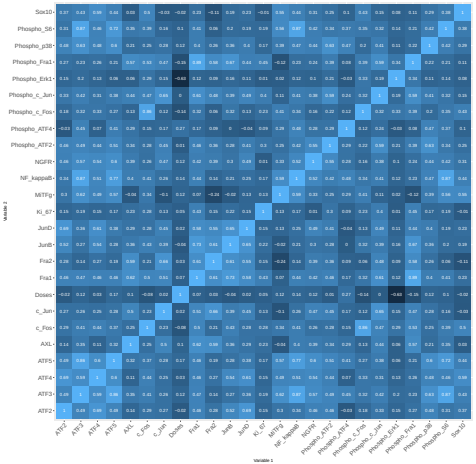
- Total Observations: 540792 (exclude NA's)
- Time point (exclude any characters)
- Doses [exclude any unit ('uM' and '+' sign)]
- Change the name of drugs to 0 and 1. (0 for 'Vem' and 1 for 'Vem + Tram')

Initial Data Analysis

- Empirically determining HIGH/LOW levels of the specific proteins that determine the phenotype of a melanoma cell:

Cellular Phenotype	MiTFg	NGFR	SOX10	AXL
Undifferentiated	LOW	LOW	LOW	HIGH
Neural crest-like	LOW	HIGH	HIGH	HIGH
Transitory	HIGH	HIGH	HIGH	LOW
Melanocytic	HIGH	LOW	LOW	LOW

- Correlation Matrix (Drug 'Vem' at Timepoint 0.5 h)



Linear Regression

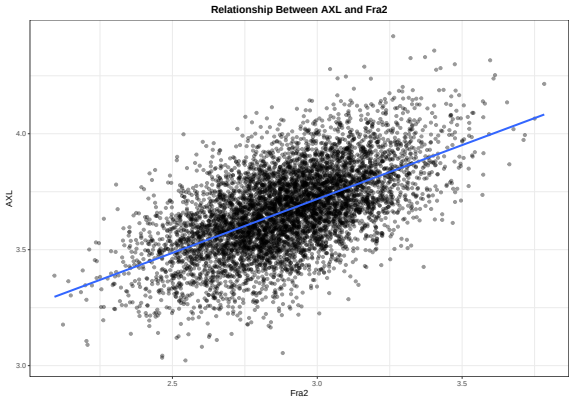
Research Question:

Can we predict cellular phenotype outcomes 'Y' values from transcription factors (TF)?

Methods:

1. Subset the data frame to include time point 0.5 hours and Drug Vem
2. Categorize the phenotypic outcomes using empirically determined HIGH/LOW distinctions of the 4 specific proteins
3. Extract the transcription factor(s) that are the mostly correlated with each of the 4 specific proteins (using the correlation matrix)
4. Use the linear regression method to identify the most significant transcription factor
5. Plot the transcription factor against the protein, observing the effects on phenotypic outcome
6. Train-test to assess accuracy of model

Identifying Predictors (Linear Regressions)

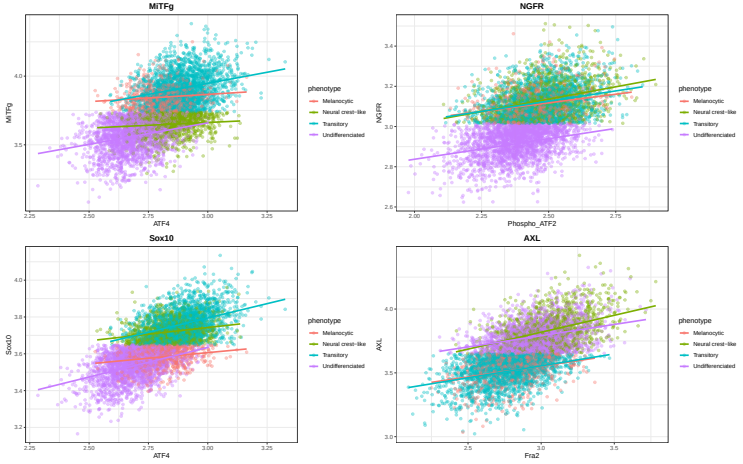


Equation:

- Initial Condition for each Drug

Term	Estimate	p-value	Correlation
(Intercept)	0.66037444	2.657616e-104	1
Fra1	0.54898424	2.247034e-269	0.62
Fra2	0.27987459	0.000000e+00	0.49
Phospho_Fra1	0.02822788	4.107424e-03	0.57

Results and Prediction (Linear Regressions)



What the results tell us:

- MiTFg:
- NGFR
- Sox10
- AXL

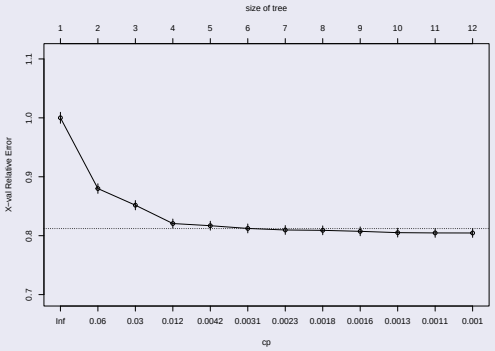
Classification Trees

Research Question:

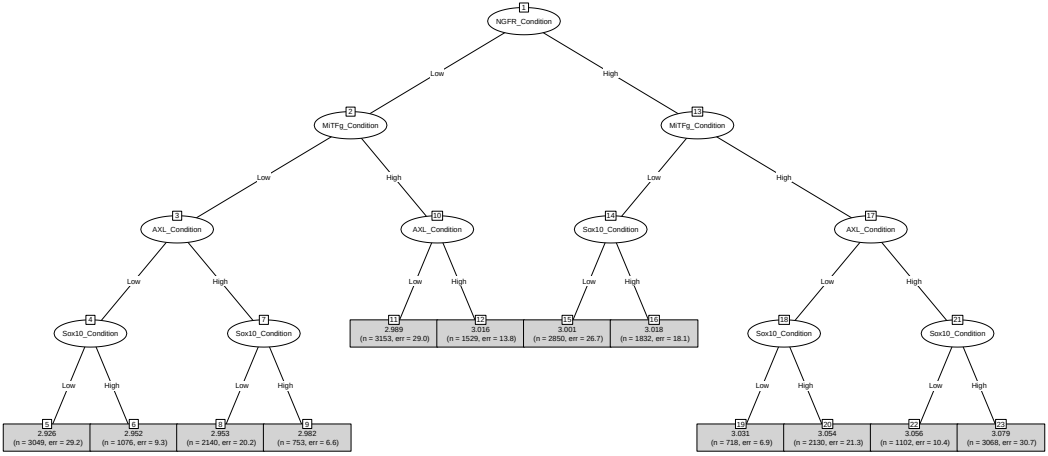
- At time 't' in experimental condition, what TF are most predictive of cellular values/states?

Methods:

1. Separate the data frame for time point 0.5 and 15 hours
2. Separate the time point into drugs 0 and 1
3. Each drug 0 and 1 creates the new columns for 4 phenotype indicators conditions (High/Low)
4. Check all phenotypes of 0.5h drugs 0 and 1 to identify any condition matches



Classification Trees example : Phospho_p38



Results (Classification Trees)

- Time point = 0.5h Drug = 0

AP-1	Error	Condition	#Observations
Phospho_ATF2	26.3	Undifferentiated	2140
Phospho_p38	6.9	Melanocytic	718
NF_kappaB	5.7	Melanocytic	718

- Time point = 0.5h Drug = 1

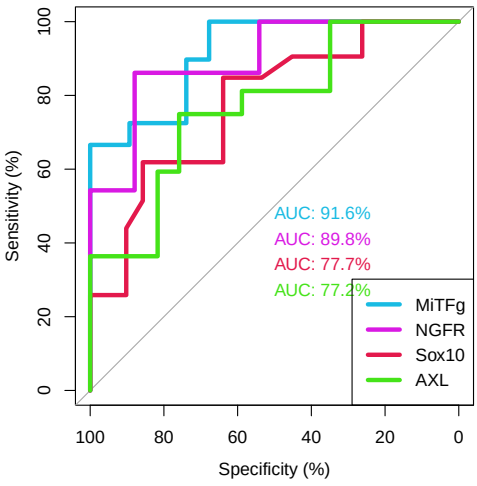
AP-1	Error	Condition	#Observations
Phospho_ATF2	17.2	Neural crest-like	1340
Phospho_p38	19.5	Transitory	2065
Phospho_Fra1	24.7	Melanocytic	793
NF_kappaB	7.4	Melanocytic	793

What the results tell us:

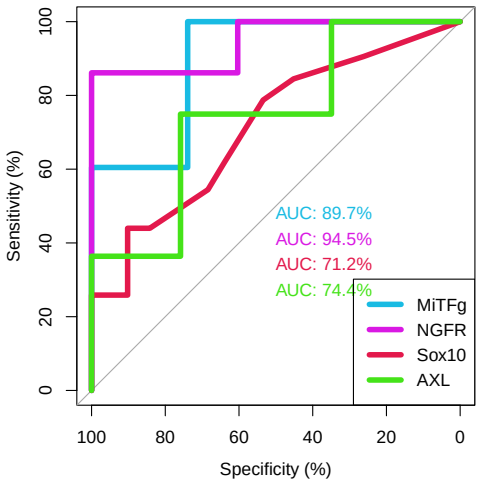
- Initial Condition for each Drug
- High accuracy becomes Melanocytic States
- Lot of # Transistory States found on Drug Vem + Tram

Prediction (Classification Trees) : ROC curves (Sensitivity vs. Specificity)

NF_kappaB's ROC curves



Phospho_p38's ROC curves



Research Question:

- Do protein levels in experimental condition 'X' change over time 't'?

Experimental condition:

- Time point = 0.5h Drug = 0

AP-1	Condition
Phospho_ATF2	Undifferentiated
Phospho_p38	Melanocytic
NF_kappaB	Melanocytic

- Time point = 0.5h Drug = 1

AP-1	Condition
Phospho_p38	Transitory
Phospho_Fra1	Melanocytic
NF_kappaB	Melanocytic

Process (Two Hypothesis Testing)

Methods:

- Categorize the 4 genes as high or low.
- Identify the Cellular Phenotype using given information of genes.
- Calculate the test static: the mean difference between two time periods.
- Perform the two sample hypothesis test.

Hypothesis:

$$H_0 : M_{0.5} = M_{15}$$

$$H_A : M_{0.5} \neq M_{15}$$

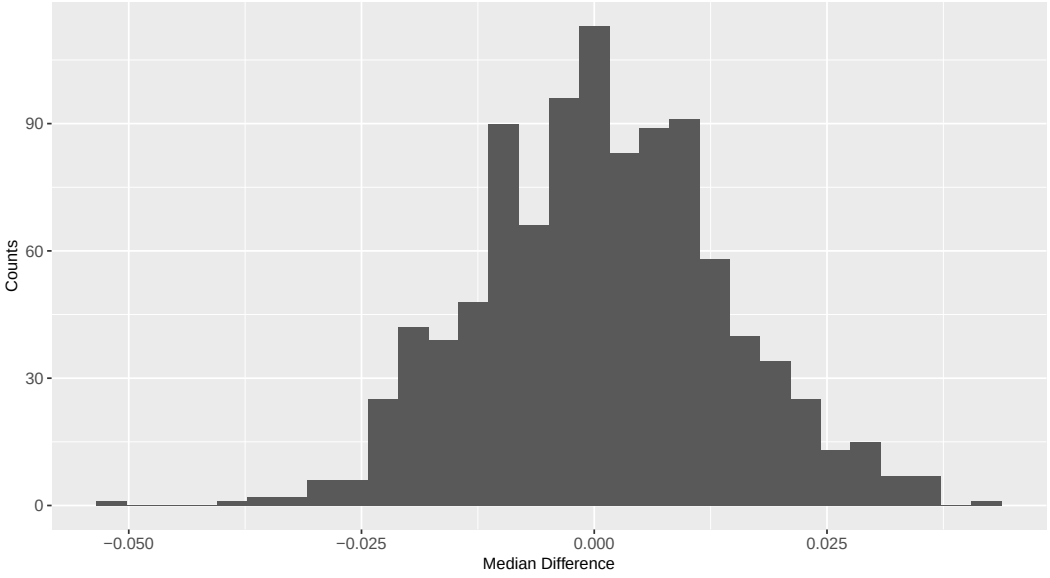
`Significance value alpha=0.01`

Results

p-value	Reject/Not
$P_1 = 0$	Reject
$P_2 = 0$	Reject
$P_3 = 0$	Reject
$P_4 = 0$	Reject
$P_5 = 0$	Reject

- Assume Null Hypothesis is true
- No single value that is as or more extreme than the test statistic
- Reject all the null hypothesis

Prediction: Drug0 - Difference between 0.5 and 15h of NF_kappaB



Overall Results

Linear regression

Decision Trees

- Identified some phenotype at 0.5 hour time point
- Initial Melanocytic condition found in 2 main TF
- Detected as high sensitivity and specificity

Two Hypothesis Test

- Detected that there's difference between 0.5 and 15 hours
- Rejected the Null Hypothesis testing: $\alpha = 0.01$
- It may have some effect from drugs

Limitation

Example of limitation

- Bias and over fitting
- Type I and type II error
- Confounding variables - multicollinearity

Conclusion with Future Perspective

Conclusions

-
-
-

How this results will help?

1.
2.
3.

Reference

[1] AP-1 transcription factor network explains diverse patterns of cellular plasticity in melanoma Natacha Comandante-Lou, Douglas G. Baumann, Mohammad Fallahi-Sichani bioRxiv 2021.12.06.471514; doi: https://doi.org/10.1101/2021.12.06.471514

[2] Tables for the Classification Trees: https://docs.google.com/spreadsheets/d/13_1a_-0V5JHSz73iriPjz50Bo9Y6YCpHou2AdM0XbMA/edit#gid=0