Differentiate Colorectal carcinoma and para-carcinoma tissue based on expression profile

Title: Hypoxia-induced cysteine metabolism reprogramming are crucial for the tumorigenesis of colorectal cancer

Gene Expression Omnibus: GSE223119

Data Analyzer: Kai-Wei Chang

Data Owner: Shiyi Yang
Email: octoberfirst@sjtu.edu.cn
Affiliation: Shanghai Jiao Tong University, Shanghai, China

Q1: Main objective of the analysis that specifies whether your model will be focused on prediction or interpretation.

Answer:
This analysis wil be focused on prediction
By determining whether the gene expreisom profiles capable of classifying the sample's origin, future expression profiles of unknown origin may be classified accordingly
In addition, the analysis will compare and contrast the performance of classificaiton tools on low sample, high-dimension data set.
ie. each gene is assumed to be independent feature for this analysis

Q2: Brief description of the data set you chose and a summary of its attributes

Answer:
Metabolic reprogramming is a hallmark of human cancer and cancer-specific metabolism provide opportunities for cancer diagnosis, prognosis, and treatment. However, how metabolic pathways affect the initiation and progression of colorectal cancer remain largely unknown.
This data set includes 40 gene expression profiles of cancer and para-carcinoma tissue (tissue surrounding cancer).
Raw data is composed of gene ID, gene expression on each sample, gene names, and database and gene network category
This analysis attempt to build a model for predicting the tissue origin of unknown gene profiles

In [1]:
```python
#No warning output
def warn(*args, **kwargs):
    pass
import warnings
warnings.warn = warn
```

Q3: Brief summary of data exploration and actions taken for data cleaning and feature engineering.

Answer:
Data Exploration:

- Gene expression has been preprocessed by the data provider, with no missing values
- This data set contain extremely high amount of features (ie. 61,700 genes)

Data Engineering:

- For the project purpose, only gene ID (feature), gene expression, and tissue origin will be considered:
- Removing features that are not expressing or have very low variability among all profiles
- For simplification in this analysis, I randomly selected 3000 features for analysis
- Gene expression may or may not be skewed, and expression levels vary from gene to gene, standardization is applied

Detail analysis see below

In [2]:
```python
#Import Data processing libraries
import numpy as np
import pandas as pd
```

In [3]:
```python
#Load Data, data is preprocessed
rawData=pd.read_csv(r'C:\Users\kai-w\Desktop\03_Supervised Machine Learning Classification\GSE223119_MJ20190424016-gene.tpm.matrix.annot.txt',sep='\t',header=0)
```

In [4]:
```python
#Data overview
rawData.head()
```

Out[4]:

	Unnamed: 0	C10	C11	C12	C13	C14	C15	C18	C1	C20	...	cog	cog_description	KO_id	KO_name	paths	pfam	go	n
0	ENSG00000000003	61.65	37.27	85.58	68.76	32.17	49.63	64.18	10.96	36.86	...	ENOG4111IRY(S:Function unknown)	ENOG4111IRY(Tenomodulin)	K17295	TSPAN6	NaN	PF00335.17(Tetraspannin:Tetraspanin family)	GO:0039532(biological_process:negative regulat...	NP_003261.1(tetraspanin-(isoform a [Homo sapi.
1	ENSG00000000005	0.31	3.39	1.34	2.24	0.25	4.48	1.64	0.15	0.89	...	ENOG410YB96(S:Function unknown)	ENOG410YB96(Tenomodulin)	NaN	NaN	NaN	PF04089.11(BRICHOS:BRICHOS domain)	GO:0005737(cellular_component:cytoplasm); GO:0...	XP_006986474.1(PREDICTED tenomodulin [Peromys.
2	ENSG00000000419	81.96	34.78	56.60	81.13	49.78	121.31	91.79	39.23	41.02	...	COG0463(M:Cell wall/membrane/envelope biogenesis)	COG0463(Glycosyl transferase, family 2)	K00721	DPM1	map00510(N-Glycan biosynthesis)	PF00535.23(Glycos_transf_2:Glycosyl transferas...	GO:0019673(biological_process:GDP-mannose meta...	NP_001303964.1(dolichol phosphate mannosyltran.
3	ENSG00000000457	4.98	1.42	2.84	4.01	4.90	3.86	3.63	4.08	3.95	...	ENOG410XQTG(S:Function unknown)	ENOG410XQTG(S. cerevisiae)	K17542	SCYL3	NaN	PF00069.22(Pkinase:Protein kinase domain); PF0...	GO:0005794(cellular_component:Golgi apparatus)...	XP_003893590.2(protein associating with the ca.
4	ENSG00000000460	7.14	1.99	2.80	9.40	5.90	4.13	9.92	4.05	4.21	...	ENOG4110VTC(S:Function unknown)	ENOG4110VTC(Chromosome 1 open reading frame 112)	NaN	NaN	NaN	PF14868.3(DUF4487:Domain of unknown function (...	NaN	XP_005245374.1(uncharacterizec protein C1orf11.

5 rows × 54 columns

In [5]:
```python
#Data types for each column
rawData.dtypes
```

Out[5]:
```
Unnamed: 0          object
C10                float64
C11                float64
C12                float64
C13                float64
C14                float64
C15                float64
C18                float64
C1                 float64
C20                float64
C22                float64
C23                float64
C24                float64
C25                float64
C3                 float64
C4                 float64
C5                 float64
C6                 float64
C7                 float64
C8                 float64
C9                 float64
P10                float64
P11                float64
P12                float64
P13                float64
P14                float64
P15                float64
P18                float64
P1                 float64
P20                float64
P22                float64
P23                float64
P24                float64
P25                float64
P3                 float64
P4                 float64
P5                 float64
P6                 float64
P7                 float64
P8                 float64
P9                 float64
gene_name           object
length               int64
description         object
cog                 object
cog_description     object
KO_id               object
KO_name             object
paths               object
pfam                object
go                  object
nr                  object
swissprot           object
entrez             float64
dtype: object
```

In [6]:
```python
#Transpose so treat genes as feature, and each profile as independent record
data=rawData.loc[:,rawData.columns.str.match('^C|^P')].rename(index=rawData['Unnamed: 0']).T
data.head()
```

Out[6]:

	ENSG00000000003	ENSG00000000005	ENSG00000000419	ENSG00000000457	ENSG00000000460	ENSG00000000938	ENSG00000000971	ENSG00000001036	ENSG00000001084	ENSG00000001167	...	MSTRG.9662	MSTRG.9664	MSTRG.9666	MSTRG.9667	MSTRG.9925
C10	61.65	0.31	81.96	4.98	7.14	4.40	15.56	38.45	18.87	12.65	...	0.02	0.00	0.0	0.13	0.37
C11	37.27	3.39	34.78	1.42	1.99	3.10	5.15	34.72	11.98	3.12	...	0.10	6.57	0.0	1.17	0.61
C12	85.58	1.34	56.60	2.84	2.80	5.31	8.48	29.41	25.83	11.12	...	0.07	0.00	0.0	0.20	0.17
C13	68.76	2.24	81.13	4.01	9.40	4.46	14.99	45.74	25.93	13.76	...	0.04	0.00	0.0	0.33	0.75
C14	32.17	0.25	49.78	4.90	5.90	2.46	10.40	33.28	17.18	11.91	...	0.27	7.07	0.0	0.36	0.54

5 rows × 61700 columns

In [7]:
```python
#check for presence of empty values
data.isna().values.sum()
```

Out[7]:
```
0
```

In [8]:
```python
#huge feature size, reduce by taking randomly 1000 for the purpose of the project
data.shape
```

Out[8]:
```
(40, 61700)
```

In [9]:
```python
#some features/genes have low variability, remove those
feature_var=pd.DataFrame([[i, data[i].std()] for i in data.columns[:-1]],
                         columns=['feature', 'std']).set_index('feature')
print(feature_var[feature_var['std']>0].sort_values('std'))
```

```
                          std
feature
ENSG00000228856      0.001581
ENSG00000231051      0.001581
ENSG00000156925      0.001581
ENSG00000250231      0.001581
ENSG00000232264      0.001581
...                       ...
ENSG00000211890   6428.959034
ENSG00000240040   6780.842254
ENSG00000212907   7435.193295
ENSG00000198804   8216.179258
ENSG00000228253  18410.117553

[41927 rows x 1 columns]
```

In [10]:
```python
#random select 3000 features/genes that are variable for the project's purpose
features=feature_var[feature_var['std']>0.5].index.to_series().sample(3000).to_list()
data[features].head()
```

Out[10]:

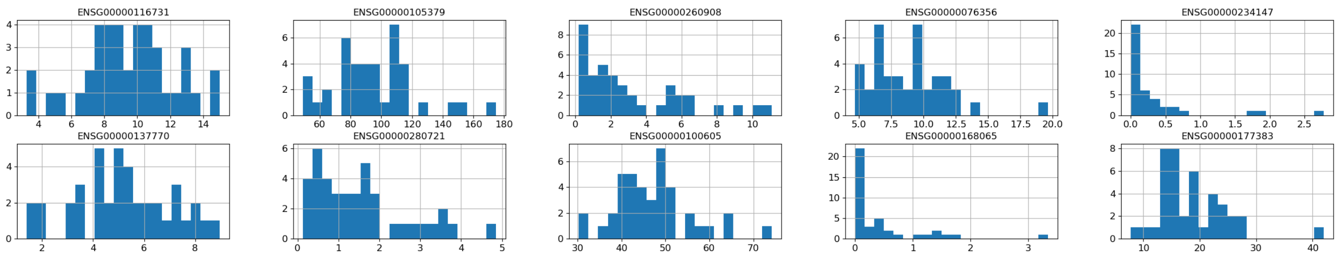
	ENSG00000116731	ENSG00000105379	ENSG00000260908	ENSG00000076356	ENSG00000234147	ENSG00000137770	ENSG00000280721	ENSG00000100605	ENSG00000168065	ENSG00000177383	...	ENSG00000206069	ENSG00000102996	ENSG00000177731	ENSG00000
C10	8.25	95.22	3.32	9.67	0.00	8.97	1.90	32.16	0.40	26.98	...	1.32	25.51	95.05	
C11	5.14	151.07	0.31	4.69	2.78	1.46	3.64	48.93	0.19	19.29	...	4.92	31.44	124.75	
C12	8.53	67.01	0.29	8.34	0.46	5.27	1.54	44.18	3.34	17.14	...	0.85	34.43	75.61	
C13	12.99	48.89	1.45	5.64	0.40	7.68	1.76	42.32	1.24	19.71	...	3.04	30.77	82.29	
C14	14.51	108.77	1.52	8.08	0.00	7.86	0.90	50.16	0.32	27.59	...	3.10	33.86	104.32	

5 rows × 3000 columns

In [11]:
```python
#some features are skwed and some are not, vary dependent on genes
import matplotlib

params = {'axes.titlesize':'12',
          'xtick.labelsize':'12',
          'ytick.labelsize':'12'}
matplotlib.rcParams.update(params)
data[features[0:10]].hist(bins=20,figsize=(30, 5),layout=(2,5))
```

Out[11]:
```
array([[<AxesSubplot:title={'center':'ENSG00000116731'}>,
        <AxesSubplot:title={'center':'ENSG00000105379'}>,
        <AxesSubplot:title={'center':'ENSG00000260908'}>,
        <AxesSubplot:title={'center':'ENSG00000076356'}>,
        <AxesSubplot:title={'center':'ENSG00000234147'}>],
       [<AxesSubplot:title={'center':'ENSG00000137770'}>,
        <AxesSubplot:title={'center':'ENSG00000280721'}>,
        <AxesSubplot:title={'center':'ENSG00000100605'}>,
        <AxesSubplot:title={'center':'ENSG00000168065'}>,
        <AxesSubplot:title={'center':'ENSG00000177383'}>]], dtype=object)
```



Q4: Summary of training at least three linear regression models which should be variations that cover using a simple linear regression as a baseline, adding polynomial effects, and using a regularization regression. Preferably, all use the same training and test splits, or the same cross-validation method.

Answer:

Linear Regression, Polynomial Regression, Linear Regression with LESSO regularization

Given only few data record, bagging and boosting may be less suit for this analysis

Based on the findings by the research group, they found the transporter genes of cystine and cysteine are all upregulated in colorectal cancer by tumor microenvironment induced ROS through transcription factor ATF4. Given such fact, whether gene expression pattern may be used to predict ATF4 expression pattern is of interest.

Preprocessing

In [12]:
```
#Load preprocessing libraries
from sklearn.preprocessing import StandardScaler
from sklearn.preprocessing import PolynomialFeatures
```

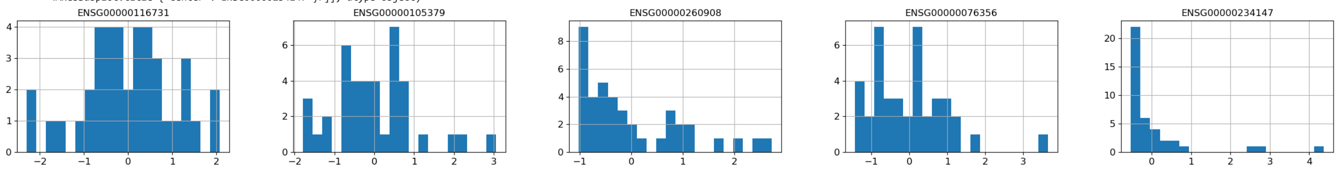
In [13]:
```
#identify ID that map to ATF4
ATF4_id=[rawData[rawData.gene_name=='ATF4'].iloc[0,0]]
```

In [14]:
```
#subset of data for standardize and binarize labels
X=pd.DataFrame(StandardScaler().fit_transform(data[features]),columns=features)
Y=pd.DataFrame(StandardScaler().fit_transform(data[ATF4_id]),columns=['ATF4'])
pf = PolynomialFeatures(degree=2, include_bias=False)
X_pf = pf.fit_transform(data[features])
print(X.iloc[:5,:4])
print(X_pf)
print(Y.head())
```

```
   ENSG00000116731  ENSG00000105379  ENSG00000260908  ENSG00000076356
0        -0.447179        -0.006876         0.055265         0.264150
1        -1.609993         2.144600        -0.977947        -1.429403
2        -0.342488        -1.093593        -0.984812        -0.188144
3         1.325085        -1.791619        -0.586631        -1.106336
4         1.893406         0.515102        -0.562603        -0.276563
[[8.2500000e+00 9.5220000e+01 3.3200000e+00 ... 5.2707600e+03
  0.0000000e+00 0.0000000e+00]
 [5.1400000e+00 1.5107000e+02 3.1000000e-01 ... 2.3299929e+03
  0.0000000e+00 0.0000000e+00]
 [8.5300000e+00 6.7010000e+01 2.9000000e-01 ... 3.0349081e+03
  0.0000000e+00 0.0000000e+00]
 ...
 [8.9500000e+00 9.1450000e+01 2.9100000e+00 ... 4.7692836e+03
  4.6270200e+01 4.4890000e-01]
 [1.0340000e+01 8.0200000e+01 9.3500000e+00 ... 5.0651689e+03
  0.0000000e+00 0.0000000e+00]
 [3.5400000e+00 1.1419000e+02 1.5100000e+00 ... 1.0909809e+03
  9.9090000e+00 9.0000000e-02]]
       ATF4
0 -0.110166
1  0.197281
2 -0.185572
3  0.536964
4  1.441271
```

In [15]:
```
#data distribution after standard scaling, some skewness presist
X.iloc[:,:5].hist(bins=20,figsize=(30, 3),layout=(1,5))
```

Out[15]:
```
array([[<AxesSubplot:title={'center':'ENSG00000116731'}>,
        <AxesSubplot:title={'center':'ENSG00000105379'}>,
        <AxesSubplot:title={'center':'ENSG00000260908'}>,
        <AxesSubplot:title={'center':'ENSG00000076356'}>,
        <AxesSubplot:title={'center':'ENSG00000234147'}>]], dtype=object)
```



In [16]:
```
#train test split
from sklearn.model_selection import train_test_split

#Load libraries for classifier and reports
from sklearn.linear_model import LinearRegression
from sklearn.linear_model import Lasso

#Load report libraries
from sklearn.metrics import r2_score

import time
```

In [17]:
```
#train-test split, double check for splitted data set
X_train, X_test, Y_train, Y_test = train_test_split(X, Y, test_size=0.4, random_state=5)
print("X_train: {}, Y_train: {}, X_test: {}, Y_test: {},".format(X_train.shape,Y_train.shape,X_test.shape,Y_test.shape) )
```

```
X_train: (24, 3000), Y_train: (24, 1), X_test: (16, 3000), Y_test: (16, 1),
```

Linear Regression

In [23]:
```
#Linear regresison model
linR=LinearRegression()

#train
linR_time = time.time()
linR.fit(X_train,Y_train)
linR_time_train = time.time() - linR_time
#test
linR_time = time.time()
Y_pred_linR = linR.predict(X_test)
linR_time_pred = time.time() - linR_time
linR.coef_
```

Out[23]:
```
array([[-4.73740017e-04,  5.03606954e-04, -3.72638149e-04, ...,
        -4.22145505e-26,  8.06827903e-06,  1.20522737e-03]])
```

In [29]:
```
#coefficients imply each feature takes little effects to the predictability of ATF4 expression
print("Number of coefficients greater than 0.01: ",(abs(linR.coef_) > 0.01).sum() )
print("Number of coefficients greater than 0.001: ",(abs(linR.coef_) > 0.001).sum() )
```

```
Number of coefficients greater than 0.01:  0
Number of coefficients greater than 0.001:  1221
```

LASSO

In [19]:
```
#LASSO regularization
lso = Lasso(alpha=0.01) #try some levels of regularzation

#train
lso_time = time.time()
lso.fit(X_train,Y_train)
lso_time_train = time.time() - lso_time
#test
lso_time = time.time()
Y_pred_lso = lso.predict(X_test)
lso_time_pred = time.time() - lso_time
```

```
#Identify features of importance
key_features=(lso.coef_ > 0)
print("Number of key features/genes may associate with ATF4 expression: ", key_features.sum())
```

```
Number of key features/genes may associate with ATF4 expression:  14
```

In [20]:
```
#List genes may be of interact with ATF4
geneList=[]
for i in lso.feature_names_in_[key_features]:
    geneList = geneList + [rawData.gene_name[rawData.iloc[:,0].to_list().index(i)]]
geneList
```

Out[20]:
```
['AC011472.3',
 'CATSPERZ',
 'TRIM26',
 'GLRX2',
 'YBX3',
 nan,
 'GTF2E2',
 'TUBA8',
 'UFD1',
 'BST2',
 'ZNF394',
 'NME4',
 'NCS1',
 'AL365203.2']
```

Linear Regression with Polynomial Features

In [21]:
```
#train-test split with polynomial features
X_pf_train, X_pf_test, Y_pf_train, Y_pf_test = train_test_split(X_pf, Y, test_size=0.4, random_state=5)
print("X_train: {}, Y_train: {}, X_test: {}, Y_test: {},".format(X_pf_train.shape,Y_pf_train.shape,X_pf_test.shape,Y_pf_test.shape) )

#train
linR_pf = LinearRegression()
linR_pf_time = time.time()
linR_pf.fit(X_pf_train,Y_pf_train)
linR_pf_time_train = time.time() - linR_pf_time
#test
linR_pf_time = time.time()
Y_pred_linR_pf = linR_pf.predict(X_pf_test)
linR_pf_time_pred = time.time() - linR_pf_time
```

```
X_train: (24, 4504500), Y_train: (24, 1), X_test: (16, 4504500), Y_test: (16, 1),
```

Analysis of each classifiers

In [22]:
```
score_df = pd.DataFrame()

for i, j in enumerate([Y_pred_linR, Y_pred_lso, Y_pred_linR_pf]):
    score_df[i] = [r2_score(Y_test,j)]
score_df = score_df.T
score_df.columns=['R2']
score_df.index=['Linear Regression','Lasso','Polynomial']
score_df['Training time']=[linR_time_train, lso_time_train, linR_pf_time_train]
score_df['Prediction time']=[linR_time_pred, lso_time_pred, linR_pf_time_pred]
score_df
```

Out[22]:

	R2	Training time	Prediction time
Linear Regression	0.725526	0.033981	0.020987
Lasso	0.749997	0.154910	0.020989
Polynomial	0.383518	13.971140	0.216882

Q5: A paragraph explaining which of your regressions you recommend as a final model that best fits your needs in terms of accuracy and explainability.

Answer:

For this data set, Linear Regression and Lasso show similar predictability to ATF4 expression.

Nevertheless, R-square of less than 0.75 suggest the predictability may not be very accurate in these tested models

On the other hand, polynomial features have dramatic reduced accuracy and high training and prediction time, therefore not recommanded.

Q6: Summary Key Findings and Insights, which walks your reader through the main drivers of your model and insights from your data derived from your linear regression model.

Answer:

Comparing Linear regression and Lasso outcomes. Linear regression suggested each genes may take minor effects to ATF4 expression, therefore suggested a robust system in gene network, and that contradict with data provider's finding

On the other hand, Lasso regularization narrow down the effective terms to several genes, which may imply regulatory network for further analsis. Also these genes makes more sense in a gene regulatory model.

Q7: Suggestions for next steps in analyzing this data, which may include suggesting revisiting this model adding specific data features to achieve a better explanation or a better prediction.

Answer:

Several genes are involved in regulatory networks involvein ATF4, whether such predictability applicable for other genes of importance is of question. Additionally, the predictability is not effective, further analysis over the gene expresion may be required for better predictability based on gene expresion profiles.

In [ ]: