Differential expression & coexpression analysis of clear cell renal carcinoma vs normal cells

Dataset downloaded from The Cancer Genome Atlas

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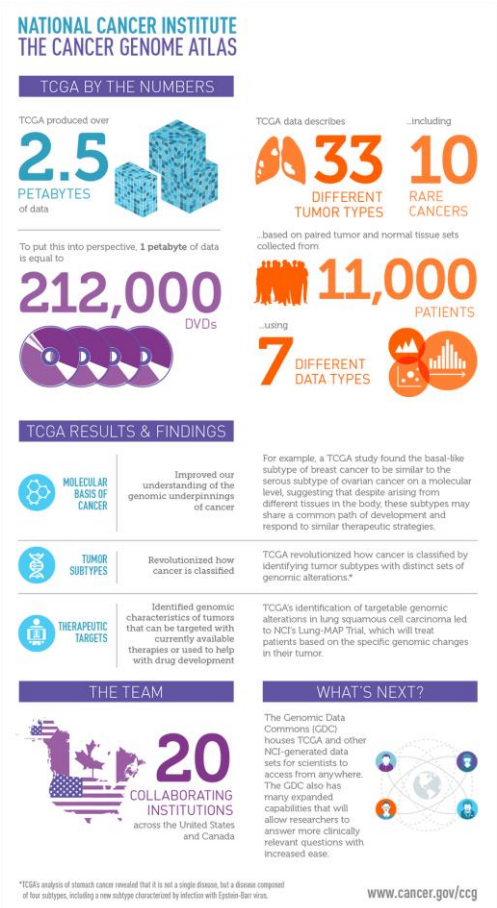
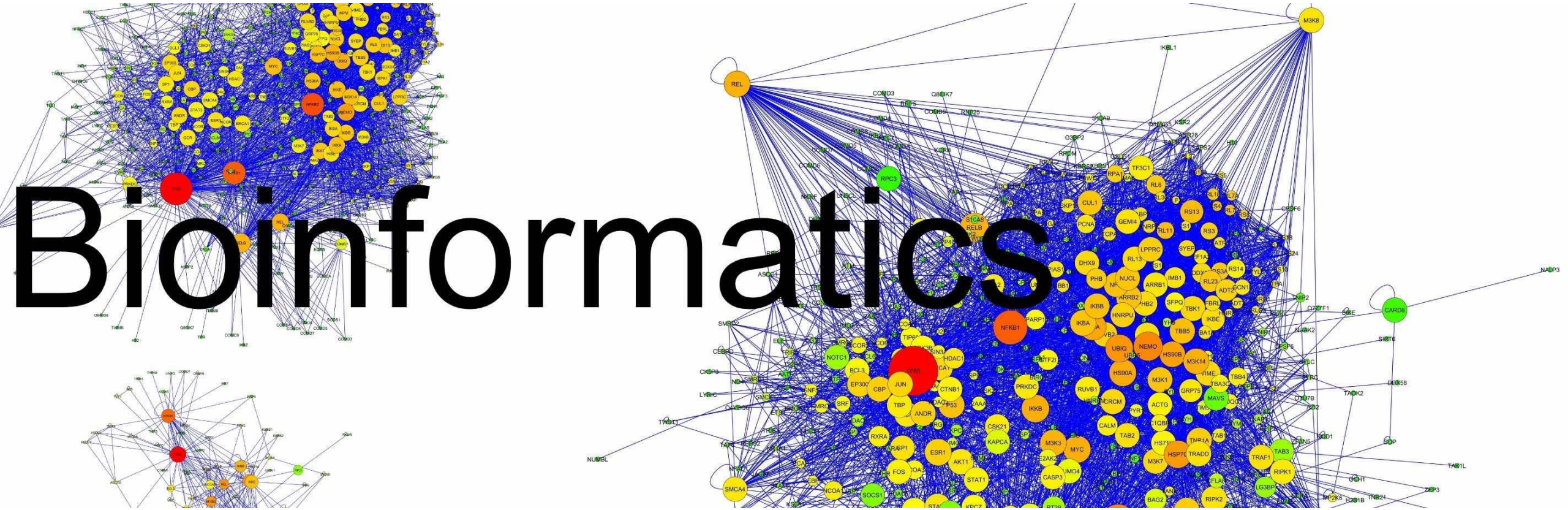
Springboard – Data Analysis with Python

The Cancer Genome Atlas (TCGA)



Clear cell renal carcinoma

- Clear cell renal carcinoma is a form of kidney cancer which primarily afflicts men in their sixties and seventies
- It is called ‘clear cell’ because the cells generally have a clear cytoplasm surrounded by a distinct cell membrane containing round and uniform nuclei
- In general, renal cell carcinomas are a kidney cancer that originates in the lining of the proximal convoluted tubule, a part of the network of small tubes in the kidney responsible for transporting urine
- Initial treatment involves partial or complete removal of the affected kidney, and absent metastasis, the five year survival rate post-surgery is 65 – 90%
- The greatest risk factors for RCC are lifestyle related e.g. obesity, smoking, and hypertension


Bioinformatics

Problem identification and target audience

- Novice bioinformaticians need accessible resources to learn programmatic techniques for genomic analysis
- There are many different approaches for solving common genomic problems
- In this analysis, we want to identify differences in gene expression between cancer and normal cells, in order to identify the genes involved in cancer formation and metastasis
- Identification of those genes allows us to develop strategies to inhibit the deleterious functions of oncogenes and oncoproteins

Workflow

- Find and import dataset
- Data wrangling
- Filtering
- Exploratory data analysis
- Correlation analysis
- Differential expression analysis
- Unsupervised learning
- Hypothesis testing
- Interpretation of results

Find and import data

- The data, gene expression values for cancer (clear cell renal carcinoma) and normal cells, was downloaded from TCGA as a csv file
- First, the working directory was changed to reflect the path where the dataset is stored
- Then, the dataset was assigned to a filename object
- Then, the dataset was imported into a Jupyter Notebook using the pandas read_csv command
- Finally, the columns containing the gene names was set as the index
- The dataset can be found at:
  - https://tcga.xenahubs.net/download/TCGA.KIRC.sampleMap/HiSeqV2.gz

Out[22]:

sample	TCGA-BP-4162-01	TCGA-CJ-5677-11	TCGA-DV-5566-01	TCGA-BP-5191-01	TCGA-BP-5200-01	TCGA-BP-4347-01	TCGA-BP-4770-01	TCGA-B0-5696-11	TCGA-BP-4762-01	TCGA-BP-4158-01	...	TCGA-B0-5104-01	TCGA-A3-3313-01	TCGA-B2-5633-01	TCGA-CJ-4872-01	TCGA-CJ-5684-01	TCGA CJ 4886 0
ARHGEF10L	10.5030	10.8969	10.7612	10.2063	10.0616	10.0193	8.4364	11.1427	9.9150	10.7621	...	10.5586	9.5556	10.2652	9.4981	10.2751	9.952
HIF3A	5.5283	6.4943	5.7842	5.0063	5.3326	7.6102	6.0422	5.9789	3.7802	7.2039	...	6.2061	4.8253	5.1680	8.6195	6.2080	6.311
RNF17	3.8036	0.0000	0.0000	0.0000	0.0000	0.3386	0.0000	0.0000	0.0000	0.0000	...	0.0000	0.0000	0.0000	0.0000	0.0000	0.000
RNF10	11.4379	12.2130	11.5478	12.3439	11.5149	11.4065	12.1963	12.3946	11.3734	11.3251	...	11.7338	11.7709	11.4968	11.9561	11.7518	11.569
RNF11	11.4180	11.8248	11.3190	10.3413	11.2923	10.9971	11.8936	11.7217	12.2329	11.2993	...	11.7629	10.2373	11.4637	11.4391	11.3992	11.493

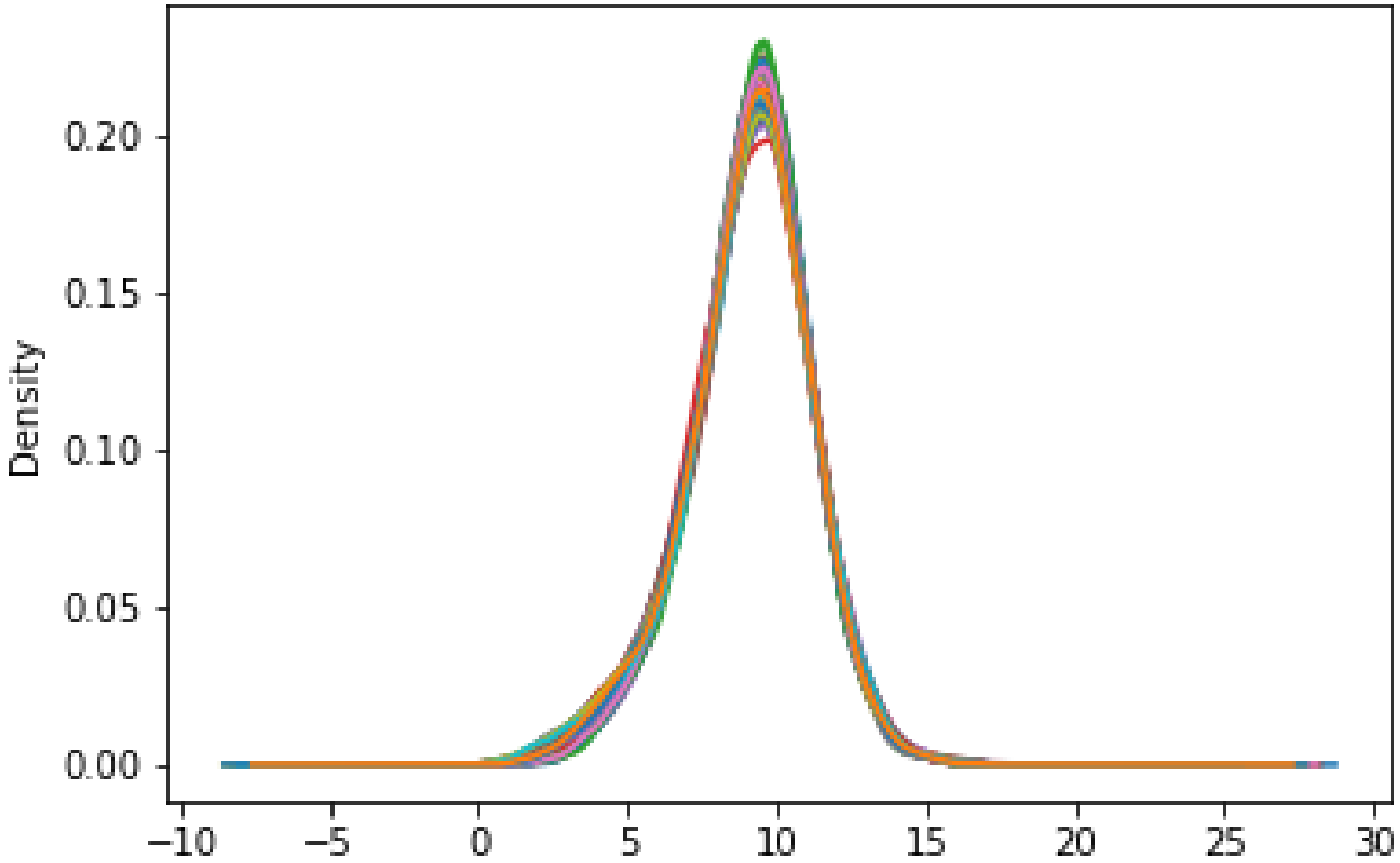
5 rows × 606 columns

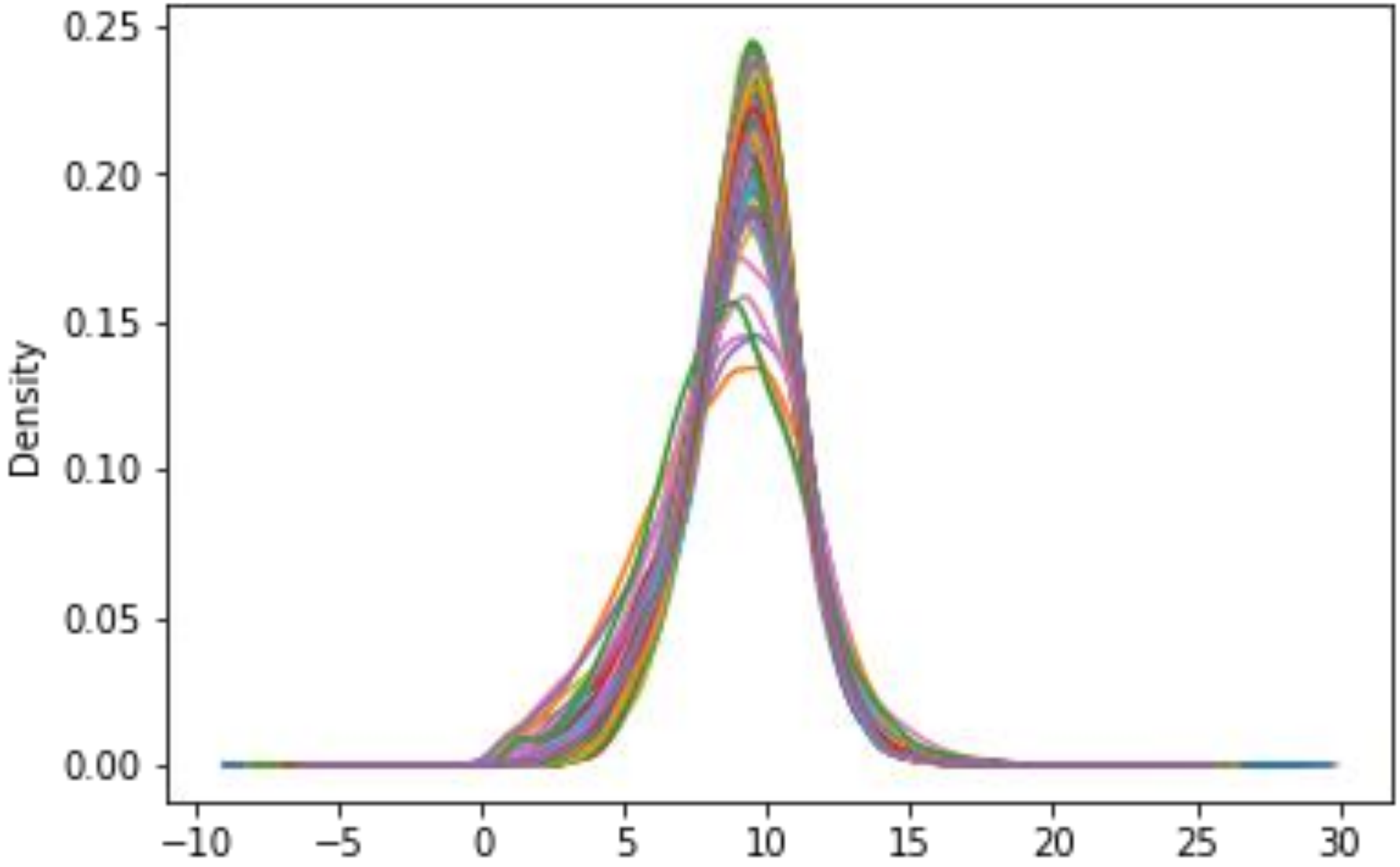
Data Preparation

Data wrangling and filtering

- All rows with zero values were dropped.
- The dataset was split into two Pandas dataframes:
  - The first dataframe contains normal cell gene expression values
  - The second dataframe contains cancer cell gene expression values
- The original dataset has been normalized and log2 transformed.

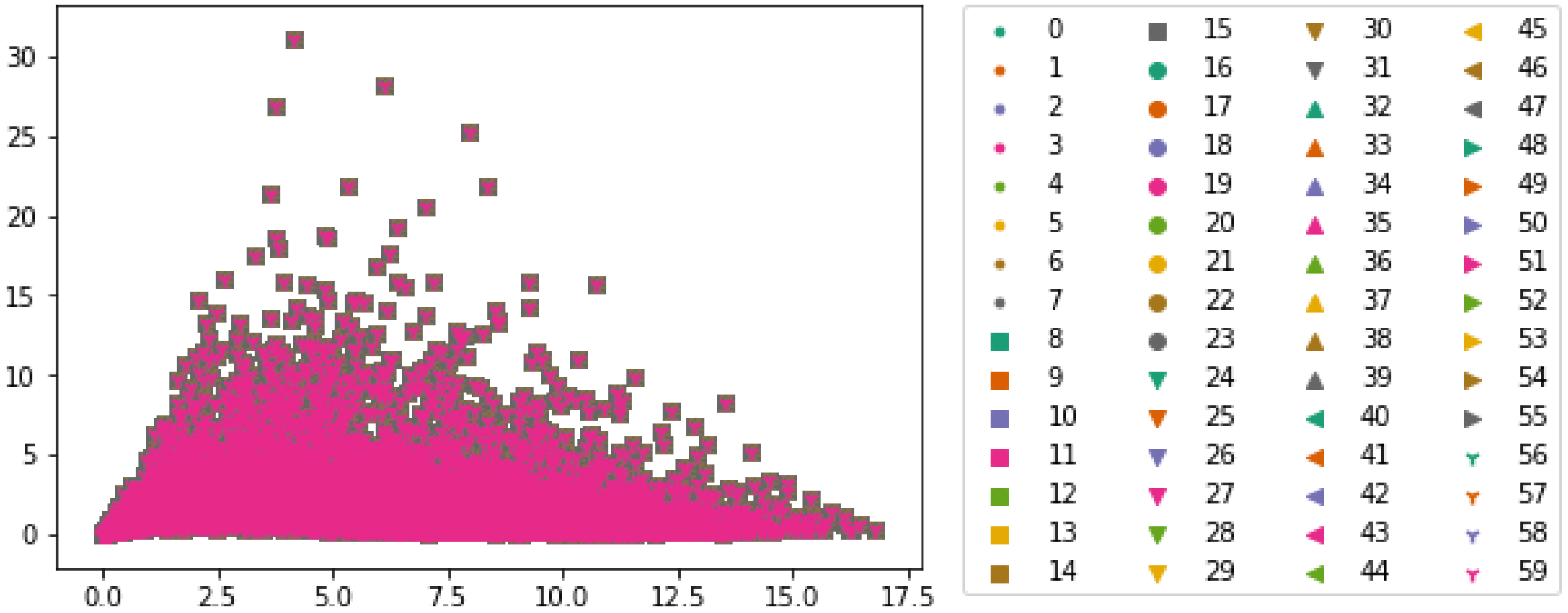
Exploratory Data Analysis


Density
0.20
0.15
0.10
0.05
0.00
−10
−5
0
5
10
15
20
25
30


0.25
0.20
0.15
0.10
0.05
0.00
Density
−10
−5
0
5
10
15
20
25
30

Confirm the shape of the distribution

- The two plots shown above are kde (density) plots
- We can use kde plots to check the normality of our data
- The first plot plots normal cell expression values
- The second plot plots cancer cell expression values
- As shown, the data appears to be normally distributed


30
25
20
15
10
5
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
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29
30
31
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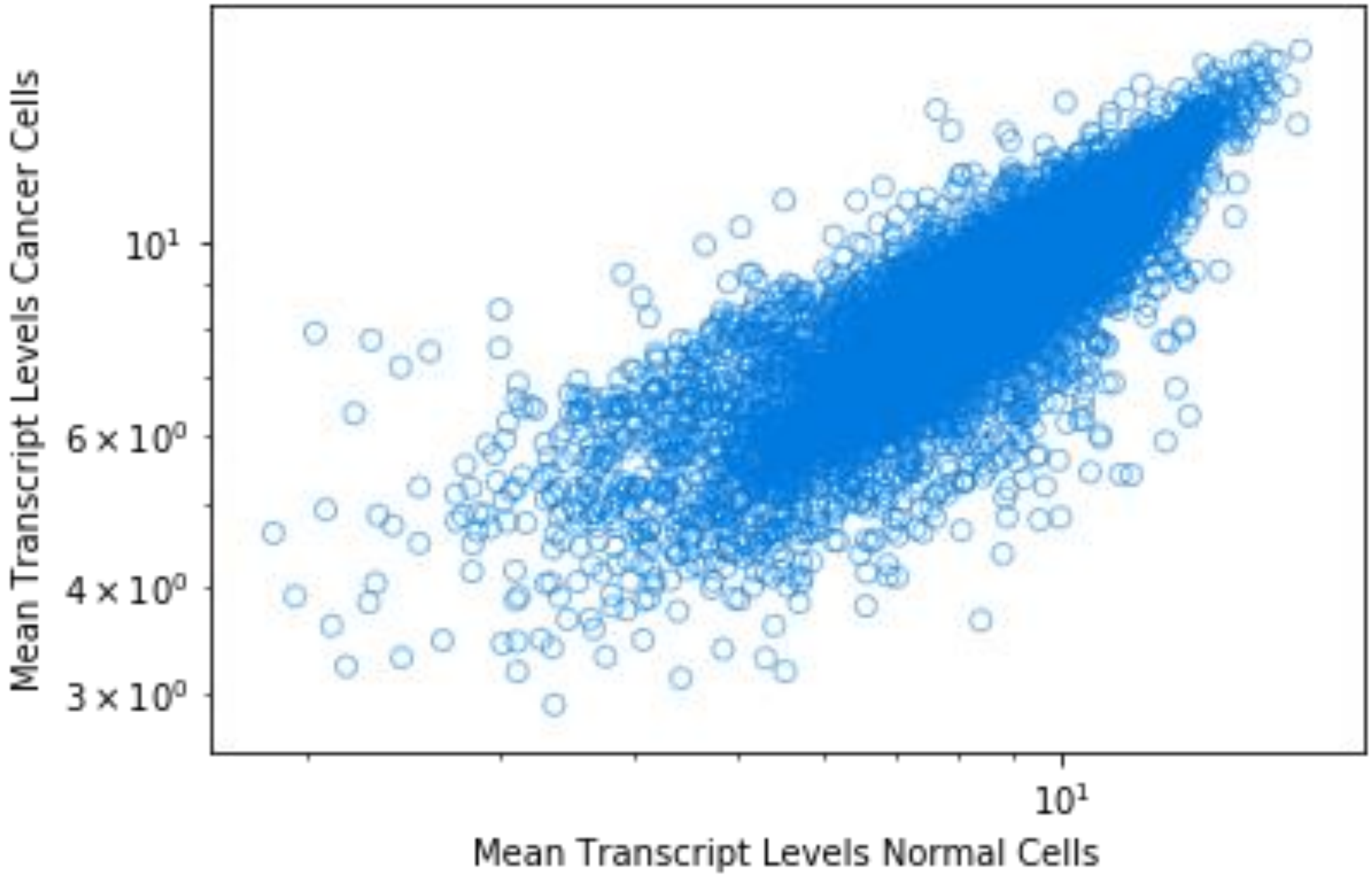

Mean Transcript Levels Cancer Cells
10^1
6×10^0
4×10^0
3×10^0
10^1
Mean Transcript Levels Normal Cells

Confirm that true biological differences exist between cancer and normal cell gene expression

- The first scatter plot (above) plots the mean against the variance of the entire dataset
- The second scatter plot plots mean expression levels for cancer genes against normal genes
- These plots suggest real biological differences between cancer and normal gene expression levels

Differential expression analysis

The t-test method was used for this analysis

- There are a number of different methods which can be used for differential expression analysis
- They include the t-test method and other methods such as empirical Bayes
- In this case, because the number of samples is large and the data is normally distributed, the t-test method was appropriate
- The two images below show the t-test formula and the top 25 differentially expressed genes

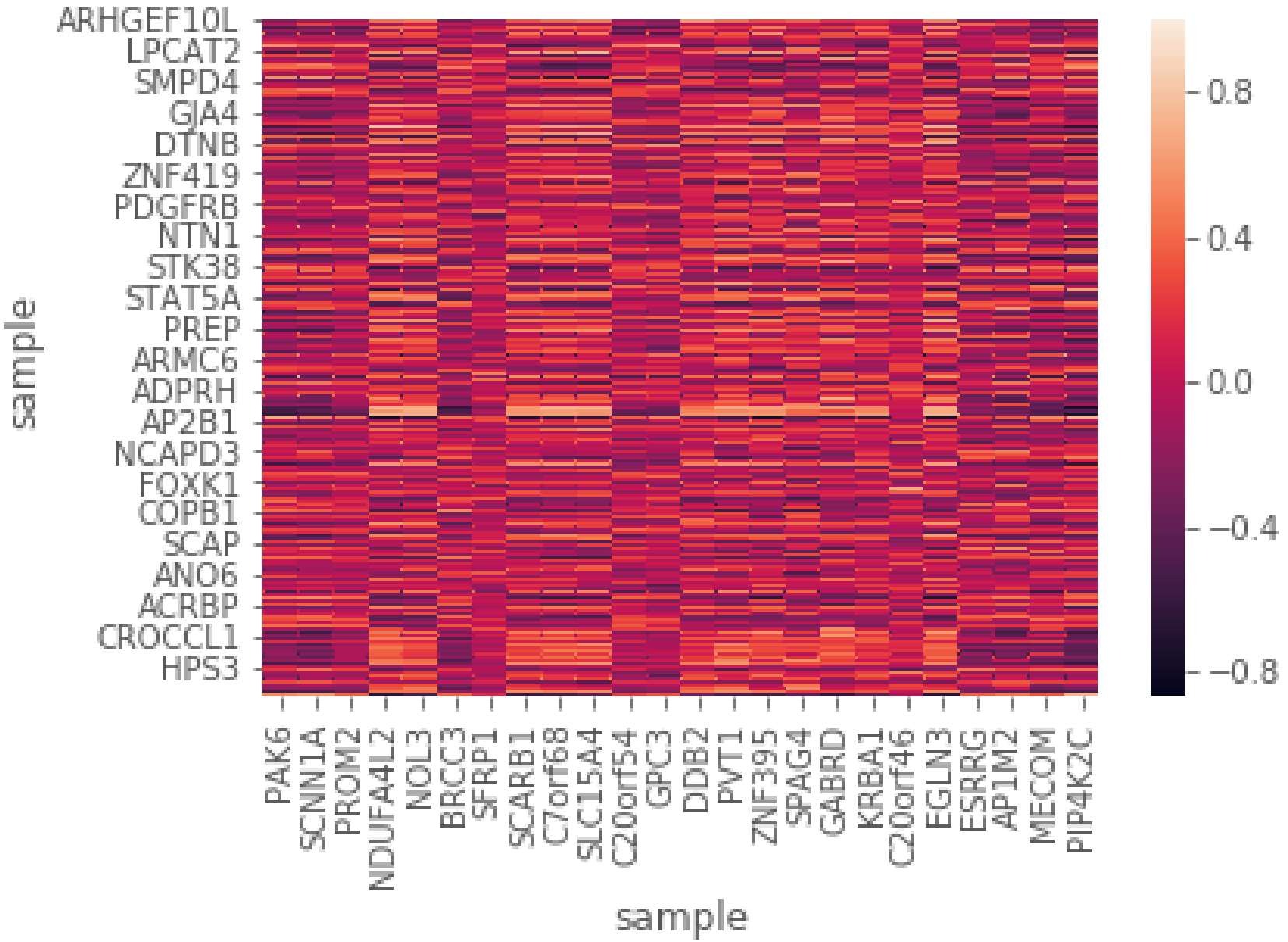
$$t = \frac{\bar{x}_T - \bar{x}_C}{\sqrt{\frac{var_T}{n_T} + \frac{var_C}{n_C}}}$$

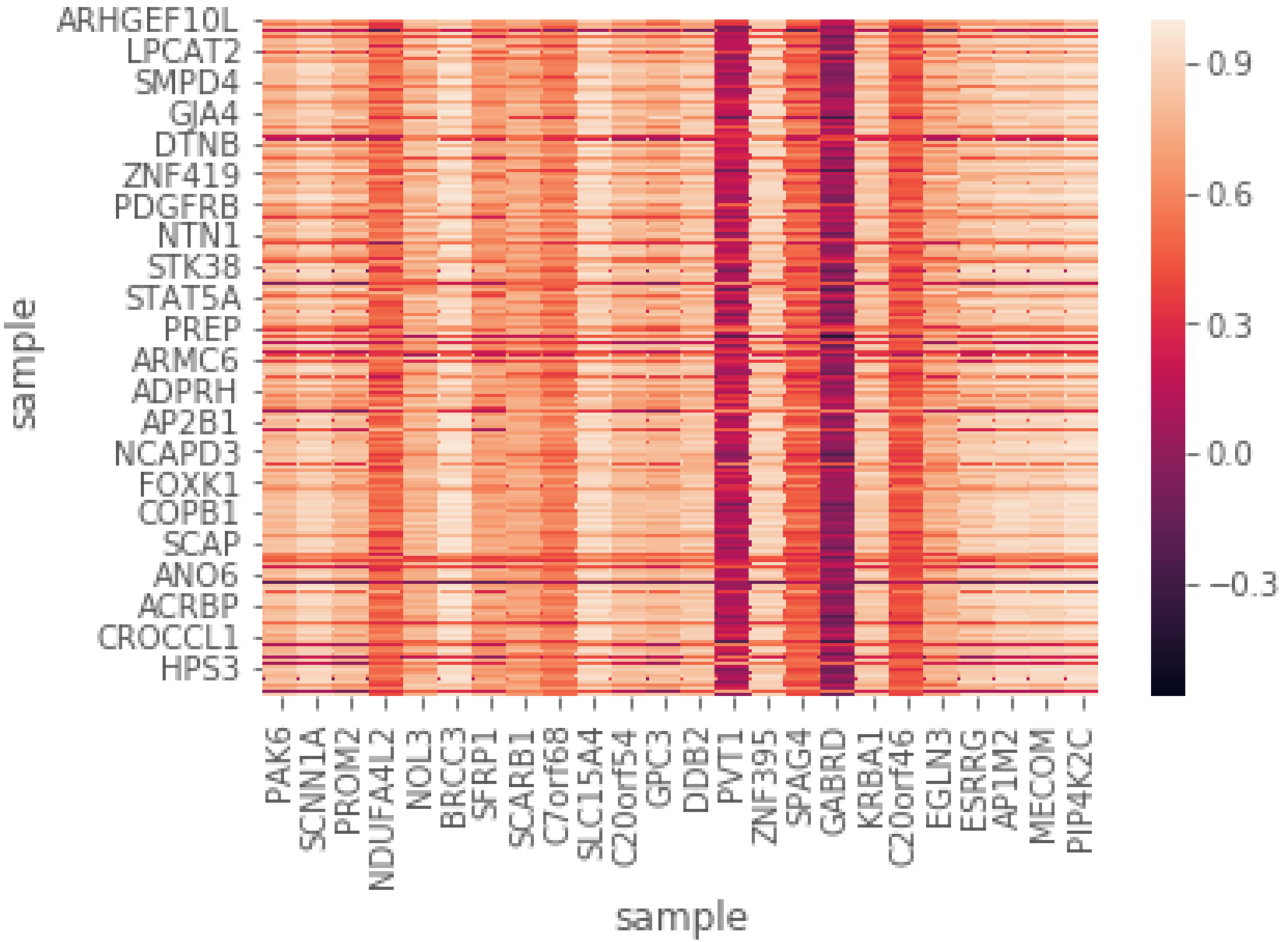
In [59]:
```
results = diffexp.iloc[1:25]
results
```

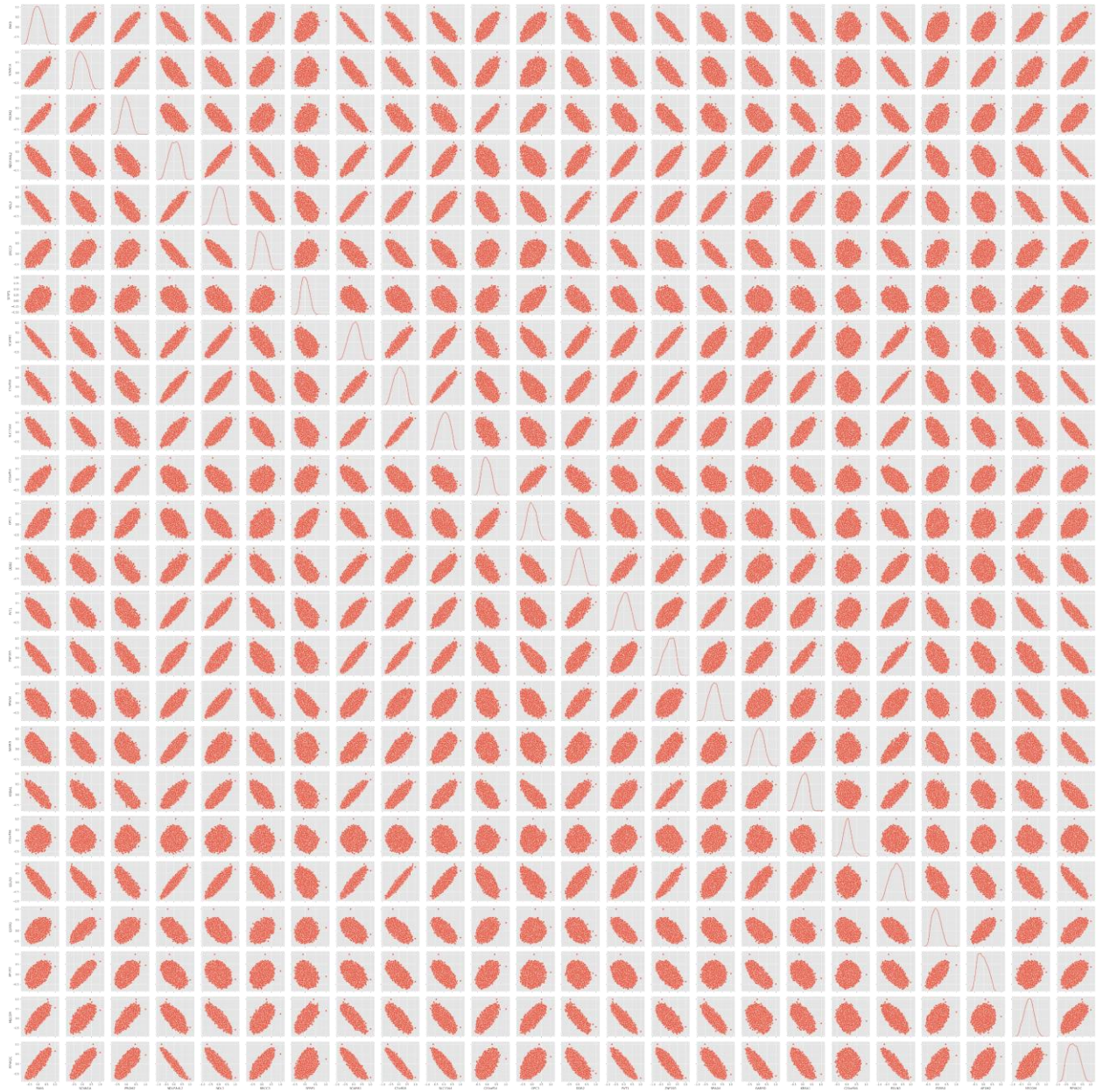
Out[59]:
```
sample
PAK6         45.037406
SCNN1A       41.761799
PROM2        41.630070
NDUFA4L2     41.419890
NOL3         41.102853
BRCC3        39.666073
SFRP1        39.578176
SCARB1       39.336044
C7orf68      39.328517
SLC15A4      38.738762
C20orf54     37.866042
GPC3         37.633499
DDB2         37.573465
PVT1         37.449893
ZNF395       36.747181
SPAG4        36.731714
GABRD        36.592500
KRBA1        36.530676
C20orf46     36.410145
EGLN3        36.152210
ESRRG        36.070852
AP1M2        36.035638
MECOM        35.674698
PIP4K2C      35.659240
Name: ttest, dtype: float64
```

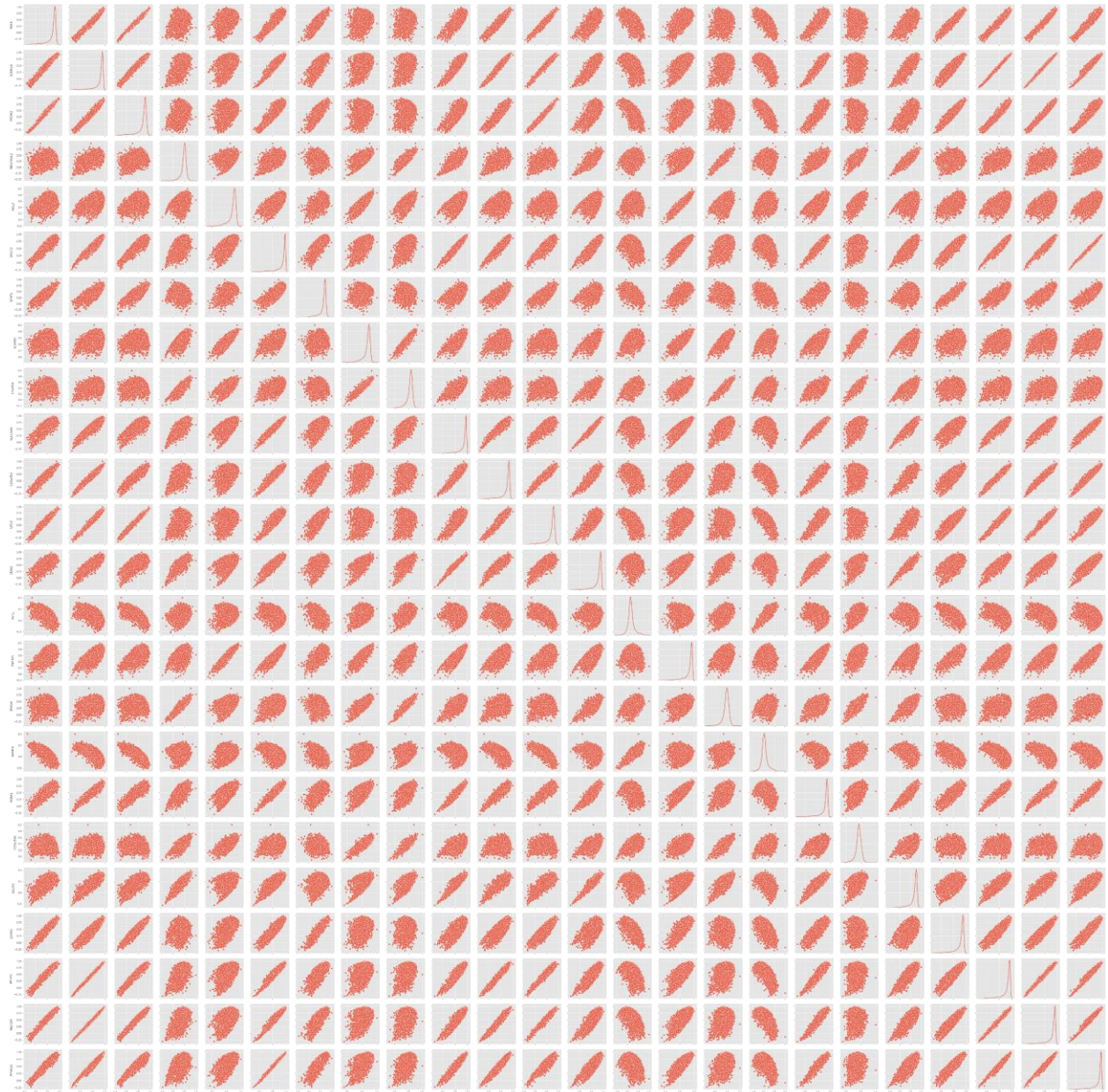
Correlation analysis

- Correlation analysis was done using the Pandas corr() method
- Heatmaps were generated showing correlation with our differentially expressed genes
- We notice a large number of negatively correlated genes in the cancer expression dataframe
- Conversely, we see a large number of positively correlated genes in the normal cell expression dataframe
- This is not surprising, since oncogenes produce deleterious proteins which often inhibit important cellular functions such as cell cycle control and apoptosis
- Pairs plots were also generated to further demonstrate the trends which appear in the heatmaps


ARHGEF10L
LPCAT2
SMPD4
GJA4
DTNB
ZNF419
PDGFRB
NTN1
STK38
STAT5A
PREP
ARMC6
ADPRH
AP2B1
NCAPD3
FOXK1
COPB1
SCAP
ANO6
ACRBP
CROCCL1
HPS3
sample
PAK6
SCNN1A
PROM2
NDUFA4L2
NOL3
BRCC3
SFRP1
SCARB1
C7orf68
SLC15A4
C20orf54
GPC3
DDB2
PVT1
ZNF395
SPAG4
GABRD
KRBA1
C20orf46
EGLN3
ESRRG
AP1M2
MECOM
PIP4K2C
sample
0.8
0.4
0.0
−0.4
−0.8


ARHGEF10L
LPCAT2
SMPD4
GJA4
DTNB
ZNF419
PDGFRB
NTN1
STK38
STAT5A
PREP
ARMC6
ADPRH
AP2B1
NCAPD3
FOXK1
COPB1
SCAP
ANO6
ACRBP
CROCCL1
HPS3
sample
PAK6
SCNN1A
PROM2
NDUFA4L2
NOL3
BRCC3
SFRP1
SCARB1
C7orf68
SLC15A4
C20orf54
GPC3
DDB2
PVT1
ZNF395
SPAG4
GABRD
KRBA1
C20orf46
EGLN3
ESRRG
AP1M2
MECOM
PIP4K2C
sample
0.9
0.6
0.3
0.0
−0.3





Top correlated genes

- Next, a dataframe was created containing correlated values for the top 25 differentially expressed genes
- In order to perform the analysis, the number of replicates in the cancer gene expression dataframe was reduced to 74
- Then, a correlation analysis was done which identified genes with expression values closely correlated with the top 25 differentially expressed genes
- This gene set was then used in hierarchical clustering

Create dataframes with correlated values for differentially expressed genes

In [66]:
```
diff_exp1 = correlations1[['PAK6', 'SCNN1A', 'PROM2', 'NDUFA4L2', 'NOL3', 'BRCC3', 'SFRP1', 'SCARB1', 'C7orf68', 'SLC15A4', 'C20o
```

In [67]:
```
diff_exp2 = correlations2[['PAK6', 'SCNN1A', 'PROM2', 'NDUFA4L2', 'NOL3', 'BRCC3', 'SFRP1', 'SCARB1', 'C7orf68', 'SLC15A4', 'C20o
```

Create object with only highly correlated genes

In [123]:
```
top_corr1 = diff_exp1[(diff_exp1 > abs(0.90)).any(1)]
top_corr1 = diff_exp1[(diff_exp1 < abs(1.0)).any(1)]
```

In [69]:
```
top_corr1.shape
```

Out[69]:
```
(8973, 24)
```

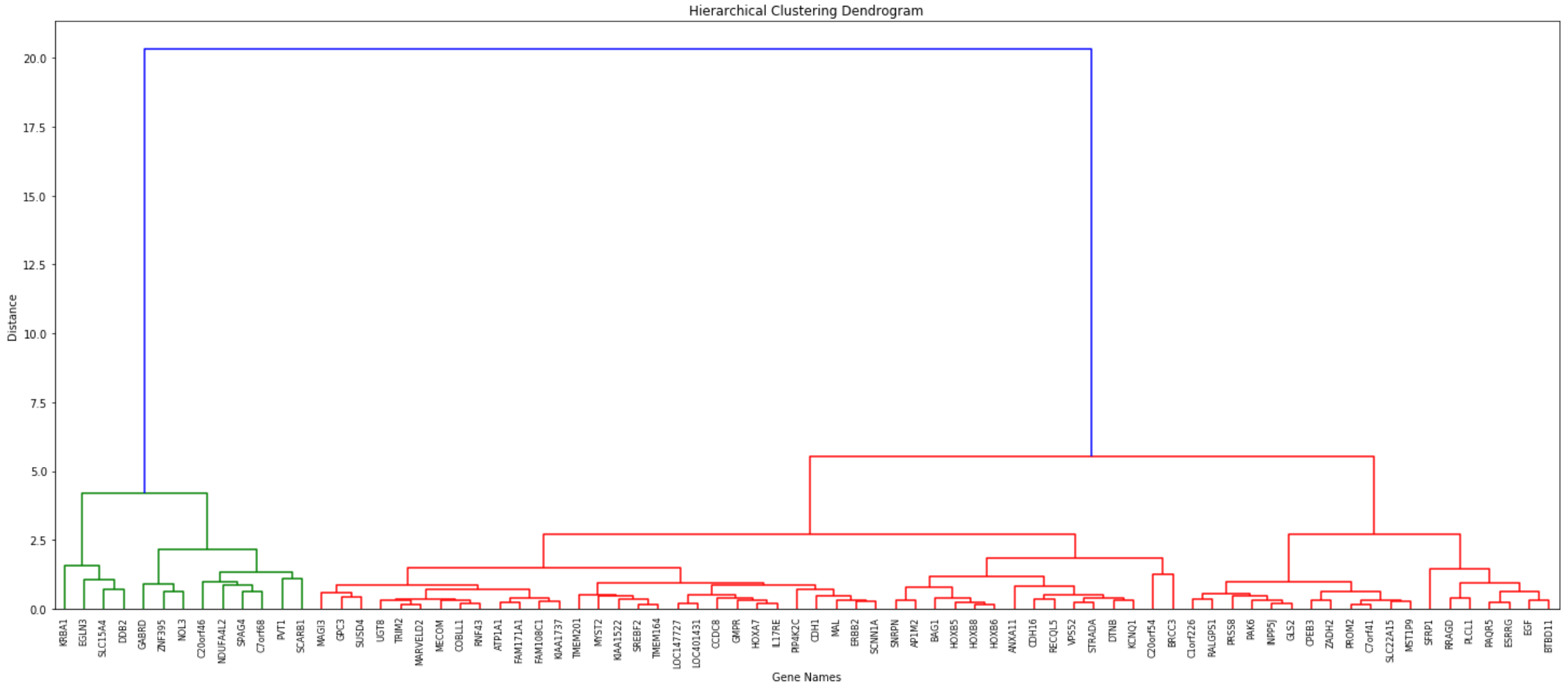
In [124]:
```
top_corr2 = diff_exp2[(diff_exp2 > abs(0.90)).any(1)]
top_corr2 = diff_exp2[(diff_exp2 < abs(1.0)).any(1)]
```

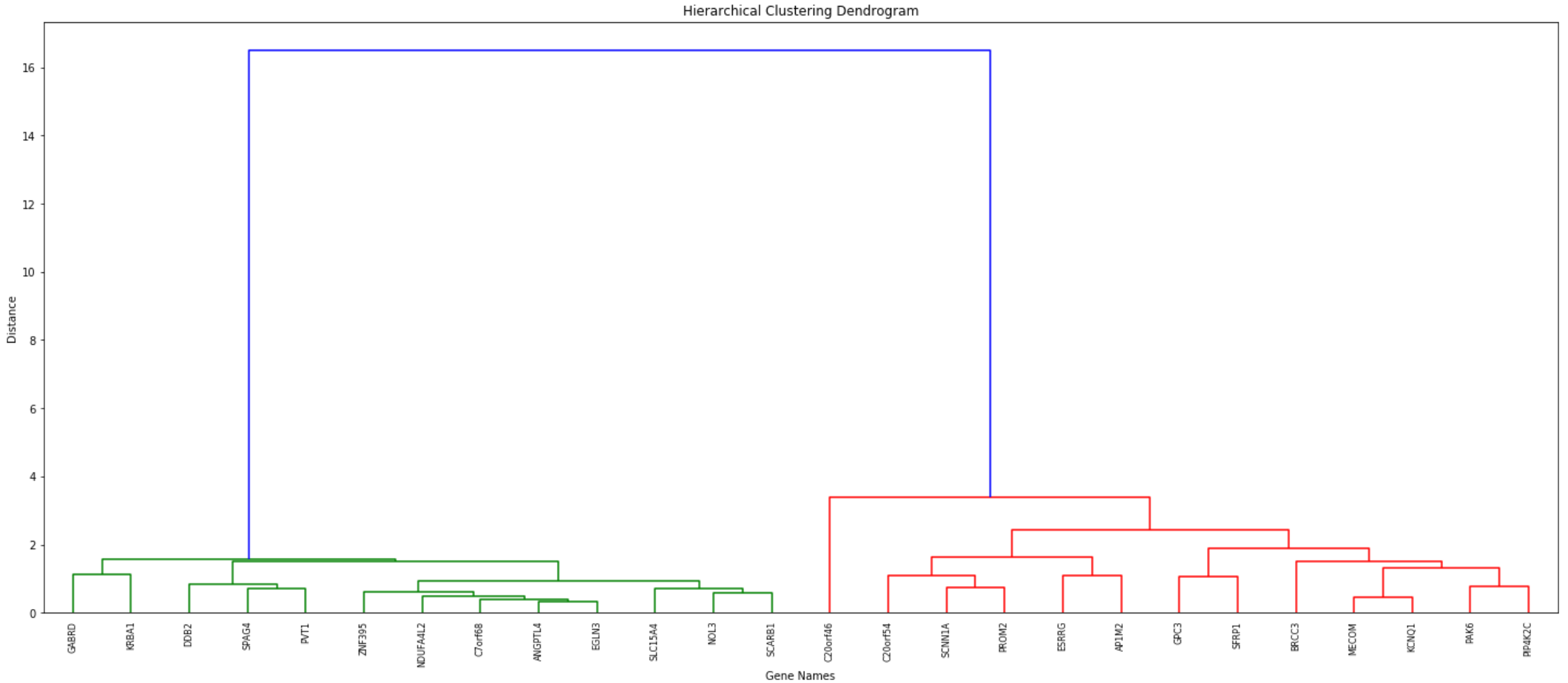
In [126]:
```
top_corr2.shape
```

Out[126]:
```
(12580, 24)
```

Unsupervised learning

- As detailed above, hierarchical clustering was done on genes closely correlated with the top 25 differentially expressed genes
- This operation was done to identify a gene candidate with a unique expression profile
- The gene ANGPTL4 was identified
- A survey of the literature was done to elucidate on the role ANGPTL4 plays in tumorigenesis


Hierarchical Clustering Dendrogram
Distance
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
KRBA1
EGLN3
SLC15A4
DDB2
GABRD
ZNF395
NOL3
C20orf46
NDUFA4L2
SPAG4
C7orf68
PVT1
SCARB1
MAGI3
GPC3
SUSD4
UGT8
TRIM2
MARVELD2
MECOM
COBLL1
RNF43
ATP1A1
FAM171A1
FAM108C1
KIAA1737
TMEM201
MYST2
KIAA1522
SREBF2
TMEM164
LOC147727
LOC401431
CCDC8
GMPR
HOXA7
IL17RE
PIP4K2C
CDH1
MAL
ERBB2
SCNN1A
SNRPN
AP1M2
BAG1
HOXB5
HOXB8
HOXB6
ANXA11
CDH16
RECQL5
VPS52
STRADA
DTNB
KCNQ1
C20orf54
BRCC3
C1orf226
RALGPS1
PRSS8
PAK6
INPP5J
GLS2
CPEB3
ZADH2
PROM2
C7orf41
SLC22A15
MST1P9
SFRP1
RRAGD
PLCL1
PAQR5
ESRRG
EGF
BTBD11
Gene Names


Hierarchical Clustering Dendrogram
Distance
0
2
4
6
8
10
12
14
16
GABRD
KRBA1
DDB2
SPAG4
PVT1
ZNF395
NDUFA4L2
C7orf68
ANGPTL4
EGLN3
SLC15A4
NOL3
SCARB1
C20orf46
C20orf54
SCNN1A
PROM2
ESRRG
AP1M2
GPC3
SFRP1
BRCC3
MECOM
KCNQ1
PAK6
PIP4K2C
Gene Names

NULL Hypothisis: ANGPTL4 is not overexpressed in clear cell renal carcinoma tumor cells relative to expression levels in normal cells

Alternate Hypothesis: ANGPTL4 is overexpressed in clear cell renal carcinoma tumor cells relative to expression levels in normal cells

In [14]:
```
df1.loc['ANGPTL4'].mean(axis=0)
```

Out[14]:
```
7.894029166666665
```

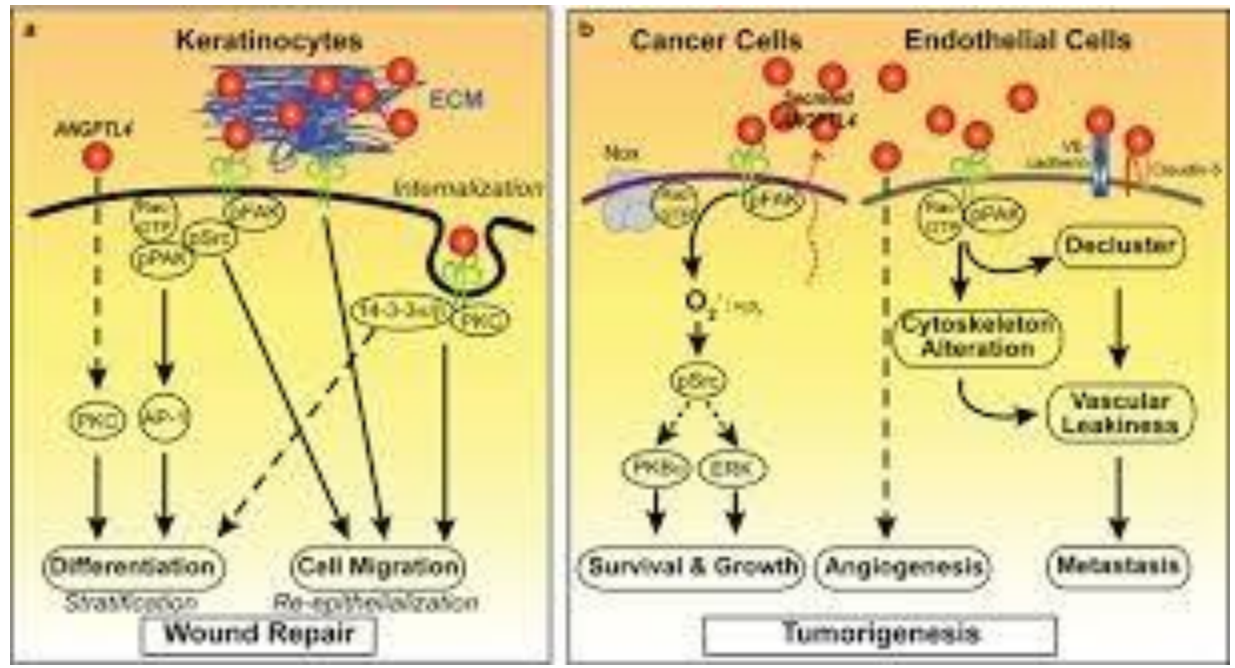
In [15]:
```
df2.loc['ANGPTL4'].mean(axis=0)
```

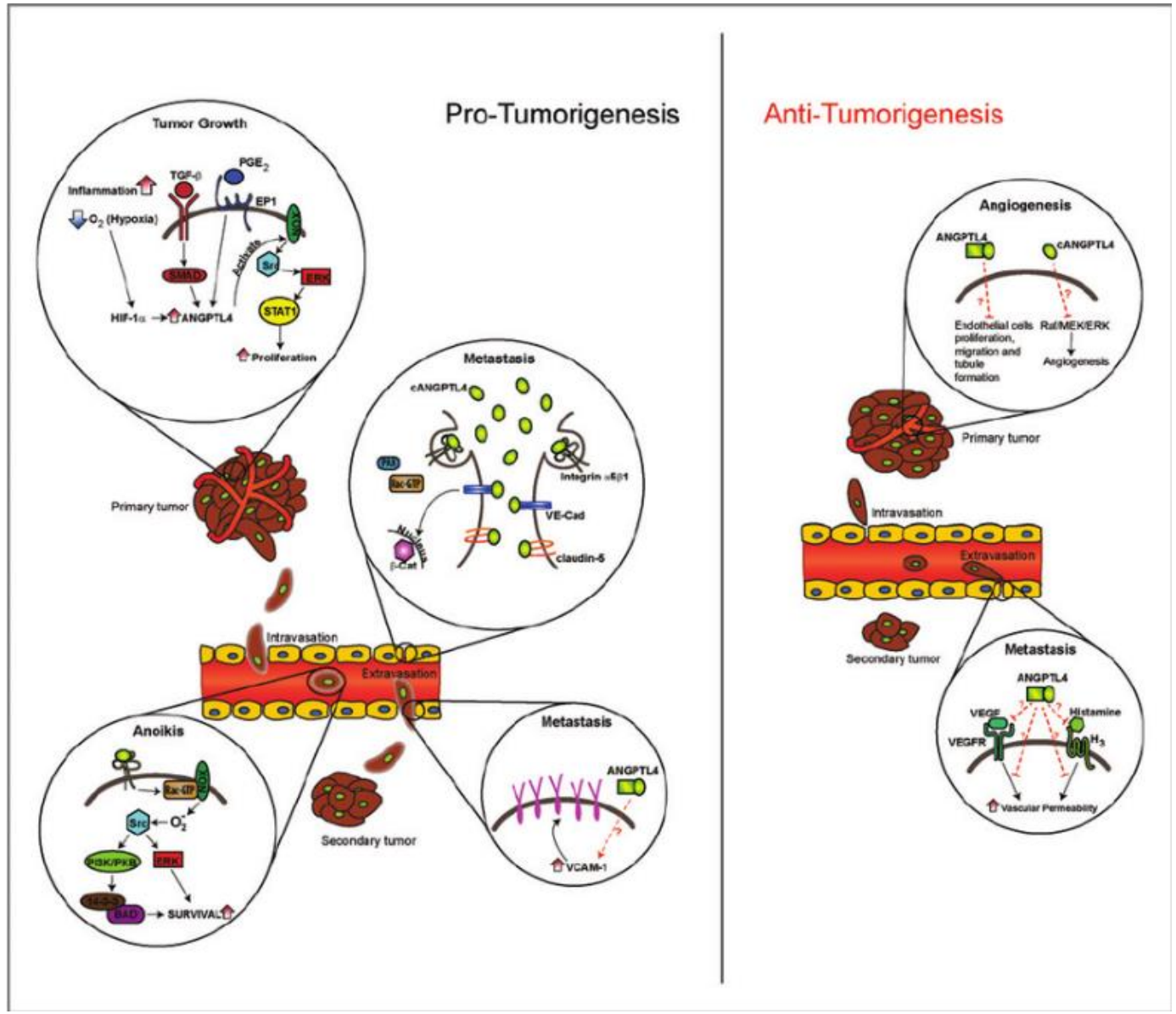
Out[15]:
```
13.540759626168226
```

In [16]:
```
from scipy import stats
stats.ttest_ind(df1.loc['ANGPTL4'], df2.loc['ANGPTL4'])
```

Out[16]:
```
Ttest_indResult(statistic=-23.8749431619333, pvalue=2.8860803388857175e-89)
```

- Our p-value is < 0.05, providing strong evidence against the NULL hypothesis
- Therefore, we can reject the NULL hypothesis
- This shows that ANGPTL4 is overexpressed in clear cell renal carcinoma tumor cells compared to expression levels in normal cells


a
Keratinocytes
ECM
ANGPTL4
Internalization
Rac
GTP
pPAK
pSrc
pPAK
14-3-3α/β
PKC
PKC
AP-1
Differentiation
Stratification
Cell Migration
Re-epithelialization
Wound Repair
b
Cancer Cells
Endothelial Cells
Secreted ANGPTL4
Nox
pPAK
Rac
GTP
pPAK
VE-cadherin
Claudin-5
Decluster
Cytoskeleton Alteration
Vascular Leakiness
pSrc
PKBα
ERK
Survival & Growth
Angiogenesis
Metastasis
Tumorigenesis


Pro-Tumorigenesis
Tumor Growth
Inflammation
TGF-β
PGE2
EP1
O2 (Hypoxia)
Arachidonic acid
Src
ERK
SMAD
HIF-1α
ANGPTL4
STAT1
Proliferation
Primary tumor
Metastasis
cANGPTL4
Integrin α6β1
VE-Cad
claudin-5
β-Cat
Intravasation
Extravasation
Anoikis
Rac-GTP
PI3K/PKB
FAK
BAD
SURVIVAL
Secondary tumor
Metastasis
ANGPTL4
VCAM-1
Anti-Tumorigenesis
Angiogenesis
ANGPTL4
cANGPTL4
Endothelial cells proliferation, migration and tubule formation
Raf/MEK/ERK
Angiogenesis
Primary tumor
Intravasation
Extravasation
Secondary tumor
Metastasis
ANGPTL4
VEGF
VEGFR
Histamine
H3
Vascular Permeability

Conclusion

- As shown, our p-value is < 0.05, therefore we reject the NULL hypothesis, and we can assert that ANGPTL4 is overexpressed in clear cell renal carcinoma cells
- It is important to note, although there has been extensive research elucidating on the role ANGPTL4 plays in tumorigenesis and important biological functions such as glucose homeostasis, we were able to identify ANGPTL4 with no prior knowledge
- This project demonstrates basic techniques for gene expression analysis, and shows how open ended data analysis can spearhead biological research and hypothesis development

References

- The Cancer Genome Atlas: About TCGA, retrieved July 31, 2018, available at, https://cancergenome.nih.gov/abouttcga
- Clear Cell Renal Cell Carcinoma, Wikipedia, last edited Nov. 28, 2017, available at, https://en.wikipedia.org/wiki/Clear_cell_renal_cell_carcinoma
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- Jie Tan, Ming & Teo, Ziqiang & Sng, Ming Keat & Zhu, Pengcheng & Tan, Nguan Soon. (2012). Emerging Roles of Angiopoietin-like 4 in Human Cancer. Molecular cancer research : MCR. 10. 677-88. 10.1158/1541-7786.MCR-11-0519
- Z. Pengcheng et al., A decade of research, Bioscience Reports (Dec. 22, 2011)