Module_3

Team Members:

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Project Title:

Limitless Replication & its Effect on Cancer Pathogenesis

Project Goal:

This project seeks to identify and compare patterns between presence/absence of the CDKN2A and HTRA1 genes and development of glioblastoma and melanoma.

Disease Background:

- Cancer hallmark focus: Limitless Replicative Potential
- Overview of hallmark: Disruptions in cell-to-cell signaling are not the sole reason for macroscopic tumor development due to other regulation factors causing cell attrition. Limitless replicative potential is a phenotype that pops up during the process of tumor progression. This means they "breach the mortality barrier" at some point. It is uncertain whether "circumvention of cellular senescnece" plays a role.
- Genes associated with hallmark to be studied (describe the role of each gene, signaling pathway, or gene set you are going to investigate): The ALT mechanism drives this potential. Typically, telomeres shorten, leading to cell death. However, ALT maintains telomeres, which means replication can occur for longer. Cell imortalization can occur by expressing the telomerase enzyme in cells. Mice carrying the CDKN2A gene (producing p16INK4A protein) exhibit tumors with higher telomerase activity (espeicially when exposed to carcinogens) compared to those without the gene. They are generally considered tumor-prone compared to mice without the gene.

- The HTRA1 plays a role in regulating cell growth. According to the article linked below, its absence can contribute to delayed senesence, has siimlar consequences to the telomere maintenence characteristic of limitless replicative potential.

https://pubmed.ncbi.nlm.nih.gov/29863874/#:~:text=Recent%20evidence%20suggests%20that%

Cancer type: Glioblastoma

- Prevalence & incidence Glioblastoma is the most prevalent cancer of the brain/central nervous system, making up 47.7% of all cases. It occurs in 3.21 out of 100,000 people. Individuals are typically diagnosed around the age of 64, and the disease is more common in men. The survival rate is low at 40% within the first year following receiving diagnosis. The rate drops to 17% in the second year. https://www.aans.org/patients/conditions-treatments/glioblastoma-multiforme/
- Risk factors (genetic, lifestyle) & Societal determinants Age, radiation exposure, and genetics are all potential risk factors for the development of glioblastoma. While age is a risk factor, the disease can affect all ages. Ionizing radion is specifically listed; it is often used to treat cancer. Syndromes such as Lynch syndrome or Li-Fraumeni Syndrome increase risk, as well as DNA changes passed between parents and children. https://www.mayoclinic.org/diseases-conditions/glioblastoma/symptoms-causes/syc-20569077 A preliminary study showed that low income populations had different survival rates, but more research is needed. https://www.clinicaltrials.gov/study/NCT03900689
- Standard of care treatments (& reimbursement) Glioblastoma is treated by surgery followed by radiation for 6 weeks followed by chemotherapy over a 6-month period. During surgery, the surgeon removes as much of the tumor as possible and will sometimes implant devices that release chemotherapy. https://www.hopkinsmedicine.org/health/conditions-and-diseases/glioblastoma-multiforme-gbm-advancing-treatment-for-a-dangerous-brain-tumor#:~:text=The%20standard%20of%20treatment%20for,three%2C%20four%20years%20o
- Biological mechanisms (anatomy, organ physiology, cell & molecular physiology) Glioblastoma tumors are made up of abnormal astrocytic cells but also include other types of cells and dead cells. The tumors invade neighboring regions of the brain or the ventribular system, but typically not outside of these regions. They are classified as Astrocytoma IDH-wildtype tumors with microvascular proliferation, necrosis, EGFR amplification, TERT promoter mutation, or combined gain of chromosome 7/loss of chromosome 10 copy number changes. They are common in the frontal lobe, but can also appear in temporal, parietal, and occipital lobes. They are not common in the cerebellum. https://www.abta.org/tumor_types/glioblastoma-gbm/

Cancer type: Melanoma

- Prevalence & incidence It is estimated that in 2022, about 1.5 million people in the United States were living with melanoma. New cases appear in 21.9 per 100,000 men and women yearly, with the death rate being 2 out of 100,000. 2.2% of people (both men and women) will be diagnosed at somepoint in their life based on 2018-2021 data. An estimated 104,960 new cases have been diagnosed in 2025. https://seer.cancer.gov/statfacts/html/melan.html

- Risk factors (genetic, lifestyle) & Societal determinants UV exposure, moles, having lighter skin/hair/eye color, family/personal history, having a weak immune system, old

age, being male, and having a genetic condition xeroderma pigmentosum are all risk factors. UV exposure is noted as a major risk factor, coming from the sun or tanning beds/sun lamps. This is because UV rays damage the DNA inside skin cells. Moles are benign pigmented tumors. One type of mole that is at small risk for developing into a melanoma are dysplastic nevi. https://www.cancer.org/cancer/types/melanoma-skin-cancer/causes-risks-prevention/risk-factors.html

- Standard of care treatments (& reimbursement) Treatment varies based on stage of development. Wide excision surgery is typically used, and care follwing it depends on the stage of development. For example, for stage 0, samples from surgery are sent to a lab to see if cancerous cells are present, and care continues from there. For later stages, action is taken to protect the lymph nodes/see if cancer spread to them. https://www.cancer.org/cancer/types/melanoma-skin-cancer/treating/by-stage.html

- Biological mechanisms (anatomy, organ physiology, cell & molecular physiology) Cutaneous melanoma results from the transformation and uncontrolled proliferation of melanocytes. These are found in the deepest layer of the epidermis (stratum basale), which is the most superficial layer of the skin. Sunlight exposure starts a process called active melanogenesis, which helps protect the skin becaused mealnin absorbs UV light. https://www.sciencedirect.com/science/article/pii/S2352304222001027

Methods:

The ML method we are implementing is classification, specifically binary classification, which is a supervised learning model that assesses data points and their features to be able to predict labels for a new data set. Specifically, it utilizes the relationship between the features and classes of training data sets to find out which features corresspond to each class. Once it learns/it is trained, it can be used to predict the class of data points in a new dataset based on its features. A model will decide if a datapoint with certain features fits into a class based on the relationships it learned in the training dataset.

https://www.ibm.com/think/topics/classification-machine-learning

```python
In [2]: import pandas as pd
        import gzip
        import re
        import numpy as np
        from pathlib import Path
        from sklearn.decomposition import PCA
        import matplotlib.pyplot as plt

        # ----------------------------
        # Output folder
        # ----------------------------
        OUT = Path(r"C:\Users\leahb\OneDrive\BME2315\Module3")
        OUT.mkdir(parents=True, exist_ok=True)
```

```python
(OUT / "raw").mkdir(parents=True, exist_ok=True)

# -----------------------------
# Random seed
# -----------------------------
SEED = 42
np.random.seed(SEED)

# -----------------------------
# URLs
# -----------------------------
CLIN_URL = "https://ftp.ncbi.nlm.nih.gov/geo/series/GSE62nnn/GSE62944/suppl/GSE6294
CTYPE_URL = "https://ftp.ncbi.nlm.nih.gov/geo/series/GSE62nnn/GSE62944/suppl/GSE629
TPM_URL = "https://ftp.ncbi.nlm.nih.gov/geo/samples/GSM1536nnn/GSM1536837/suppl/GSM
GTF_URL = "https://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_19/gen

# -----------------------------
# Helper functions
# -----------------------------
def fetch(url, dest):
    if not dest.exists():
        print(f"Downloading {url}")
        import urllib.request as ureq
        ureq.urlretrieve(url, dest)
    else:
        print(f"Found {dest.name}")

def read_gz_table(path, sep="\t", index_col=None):
    return pd.read_csv(path, sep=sep, index_col=index_col, compression='gzip')

# -----------------------------
# Download files
# -----------------------------
clin_gz = OUT / "raw" / Path(CLIN_URL).name
ctype_gz = OUT / "raw" / Path(CTYPE_URL).name
tpm_gz = OUT / "raw" / Path(TPM_URL).name
gtf_gz = OUT / "raw" / Path(GTF_URL).name

for url, dest in [(CLIN_URL, clin_gz), (CTYPE_URL, ctype_gz), (TPM_URL, tpm_gz), (G
    fetch(url, dest)

# -----------------------------
# Load clinical and cancer-type info
# -----------------------------
clin = read_gz_table(clin_gz, sep="\t").transpose()
clin.index.name = "sample"

ctype = read_gz_table(ctype_gz, sep="\t")
ctype.columns = ["sample", "cancer_type"] + list(ctype.columns[2:])
ctype = ctype.set_index("sample")

# -----------------------------
# Load protein-coding genes
# -----------------------------
pc_genes = []
with gzip.open(gtf_gz, "rt") as fh:
```

```python
    for line in fh:
        if line.startswith("#"):
            continue
        parts = line.rstrip("\n").split("\t")
        if len(parts) < 9 or parts[2] != "gene":
            continue
        attrs = parts[8]
        m_name = re.search(r'gene_name "([^"]+)"', attrs)
        m_type = re.search(r'gene_type "([^"]+)"', attrs)
        if m_name and m_type and m_type.group(1) == "protein_coding":
            pc_genes.append(m_name.group(1))
print(f"Protein-coding genes: {len(pc_genes)}")

# ------------------------------
# Load TPM in chunks (memory-efficient)
# ------------------------------
chunks = []
for chunk in pd.read_csv(tpm_gz, sep="\t", compression='gzip', index_col=0, chunksi
    chunk = chunk.loc[chunk.index.intersection(pc_genes)]
    chunks.append(chunk)
expr = pd.concat(chunks)
print("Expression shape after protein-coding filter:", expr.shape)

# ------------------------------
# Filter low-expression genes and small libraries
# ------------------------------
keep_gene = (expr >= 1.0).sum(axis=1) >= int(np.ceil(0.10 * expr.shape[1]))
expr = expr.loc[keep_gene]

lib = expr.sum(axis=0)
expr = expr.loc[:, lib >= 1e4]

# ------------------------------
# Align samples safely
# ------------------------------
common_samples = expr.columns.intersection(clin.index).intersection(ctype.index)
expr = expr.loc[:, common_samples]
clin = clin.loc[common_samples]
ctype = ctype.loc[common_samples]

X = np.log2(expr + 1.0)
print("Filtered and aligned expression shape:", X.shape)

# ------------------------------
# Stratified subsampling (optional)
# ------------------------------
TARGET, MIN_N, MAX_N = 80, 50, 100
counts = ctype["cancer_type"].value_counts()
types_order = counts.index.tolist()
take = []

rng = np.random.default_rng(SEED)
for ct in types_order:
    cols = np.where(ctype["cancer_type"].values == ct)[0]
    n_avail = len(cols)
    if n_avail < MIN_N:
```

```python
        continue
    k = min(MAX_N, max(MIN_N, TARGET, 0), n_avail)
    pick = rng.choice(cols, size=k, replace=False)
    take.extend(list(ctype.index[pick]))

# Ensure specific cancer types included
must_keep = ctype[ctype["cancer_type"].isin(["GBM", "SKCM"])].index.tolist()
take = list(set(take).union(must_keep))

X = X.loc[:, take]
clin = clin.loc[take]
ctype = ctype.loc[take]
print("After subsampling:", X.shape)

# ------------------------------
# Pick top variance genes
# ------------------------------
def pick_top_var(df, max_genes):
    v = df.var(axis=1)
    keep = v.sort_values(ascending=False).head(max_genes).index
    return df.loc[keep]

g0 = 3000 if X.shape[1] > 900 else 5000
X = pick_top_var(X, g0)
print("Top variance genes shape:", X.shape)

# ------------------------------
# PCA for GBM and SKCM
# ------------------------------
merged_meta = clin.copy()
merged_meta.insert(1, "cancer_type", ctype["cancer_type"].values)

genes_of_interest = ["CDKN2A", "HTRA1"]
X_t = X.T
merged = merged_meta.join(X_t)

# Compute presence/absence
for g in genes_of_interest:
    threshold = merged[g].median()
    merged[f"{g}_presence"] = (merged[g] > threshold).astype(int)
    merged[f"group_{g}"] = merged.apply(lambda row: f"{row['cancer_type']}_{'Presen

# Filter for GBM and SKCM only
merged_gbm_skcm = merged[merged['cancer_type'].isin(['GBM', 'SKCM'])]

X_gbm_skcm = X.loc[:, X.columns.isin(merged_gbm_skcm.index)]
X_pca = X_gbm_skcm.T

pca = PCA(n_components=2)
principal_components = pca.fit_transform(X_pca)

pca_df = merged_gbm_skcm.loc[X_pca.index, ["cancer_type"]].copy()
pca_df["PC1"] = principal_components[:, 0]
pca_df["PC2"] = principal_components[:, 1]

plt.figure(figsize=(8,6))
```

```python
for ct in ["GBM", "SKCM"]:
    subset = pca_df[pca_df["cancer_type"] == ct]
    plt.scatter(subset["PC1"], subset["PC2"], label=ct, alpha=0.7)

plt.xlabel(f"PC1 ({pca.explained_variance_ratio_[0]*100:.1f}% variance)")
plt.ylabel(f"PC2 ({pca.explained_variance_ratio_[1]*100:.1f}% variance)")
plt.title("PCA of GBM and SKCM samples")
plt.legend()
plt.tight_layout()
plt.show()
```

Found GSE62944_06_01_15_TCGA_24_548_Clinical_Variables_9264_Samples.txt.gz
Found GSE62944_06_01_15_TCGA_24_CancerType_Samples.txt.gz
Found GSM1536837_06_01_15_TCGA_24.tumor_Rsubread_TPM.txt.gz
Found gencode.v19.annotation.gtf.gz

C:\Users\leahb\AppData\Local\Temp\ipykernel_23824\3743562841.py:42: DtypeWarning: Co
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19,9220,9221,9222,9223,9224,9225,9226,9227,9228,9229,9230,9231,9232,9233,9234,9235,9
236,9237,9238,9239,9240,9241,9243,9244,9245,9246,9247,9248,9249,9250,9251,9252,9253,
9254,9255,9256,9257,9258,9259,9260,9261,9262,9263,9264,9266) have mixed types. Speci
fy dtype option on import or set low_memory=False.
  return pd.read_csv(path, sep=sep, index_col=index_col, compression='gzip')
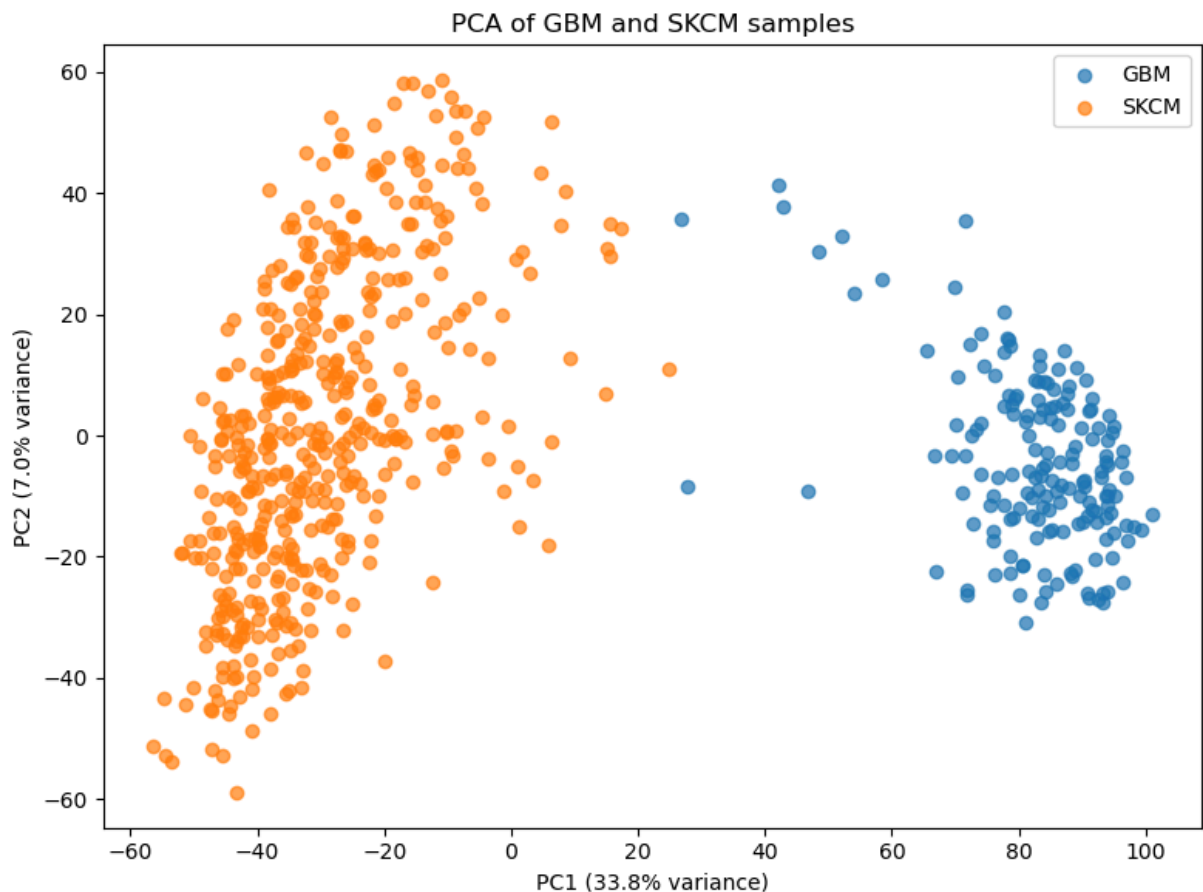
```
Protein-coding genes: 20345
Expression shape after protein-coding filter: (18353, 9264)
Filtered and aligned expression shape: (15716, 9263)
After subsampling: (15716, 2284)
Top variance genes shape: (3000, 2284)
```


PCA of GBM and SKCM samples

Verify and validate your analysis:

- We chose to compute the area under the ROC curve to see how effective the genes are at distinguishing Glioblastoma from Melanoma.

- The closer the area is to 1, the more effective the gene is at distinguishing between Glioblastoma and Melanoma patients. The HTRA1 gene is very effective at distinguishing between the types of cancer as the area under the curve is 0.955, which is very close to one. CDKN2A was much less effective, as the ROC curve indicates a similar result to if it was left to chance.
- This is consistent with research done into the expression of HTRA1 in different types of cancer. A high expression is prevalent in Glioblastoma patients and is linked to cell replication and migration. https://onlinelibrary.wiley.com/doi/10.1111/cns.14605
- Its expression in Melanoma patients is less consistent, with a significant difference between its expression in primary melanoma and metastatic melanoma cells. https://www.nature.com/articles/1205911

In [4]:
```python
# Area under the ROC curve
from sklearn.metrics import roc_auc_score
from sklearn.metrics import roc_curve
from sklearn.linear_model import LogisticRegression

# -----------------------------
# Compute ROC-AUC for each gene of interest (GBM vs SKCM)
# -----------------------------
# Define binary labels: 1 = GBM, 0 = SKCM
y_true = (merged_gbm_skcm["cancer_type"] == "GBM").astype(int)
for g in genes_of_interest:
    x = merged_gbm_skcm[g].values.reshape(-1,1)
    y_score = LogisticRegression(solver="newton-cholesky").fit(x,y_true)
    auc_value = roc_auc_score(y_true, y_score.predict_proba(x)[:, 1])
    print(f"AUC for {g} distinguishing GBM vs SKCM: {auc_value:.3f}")

    # Plot the ROC curve
    fpr, tpr, _ = roc_curve(y_true, y_score.predict_proba(x)[:, 1])
    plt.plot(fpr, tpr, label=f"{g} (AUC={auc_value:.3f})")

plt.plot([0, 1],[0, 1],'k--',label="Chance")
plt.xlabel("False Positive Rate")
plt.ylabel("True Positive Rate")
plt.title("ROC Curves: GBM vs SKCM (gene expression)")
plt.legend()
plt.tight_layout()
plt.show()
```

AUC for CDKN2A distinguishing GBM vs SKCM: 0.515
AUC for HTRA1 distinguishing GBM vs SKCM: 0.955

ROC Curves: GBM vs SKCM (gene expression)
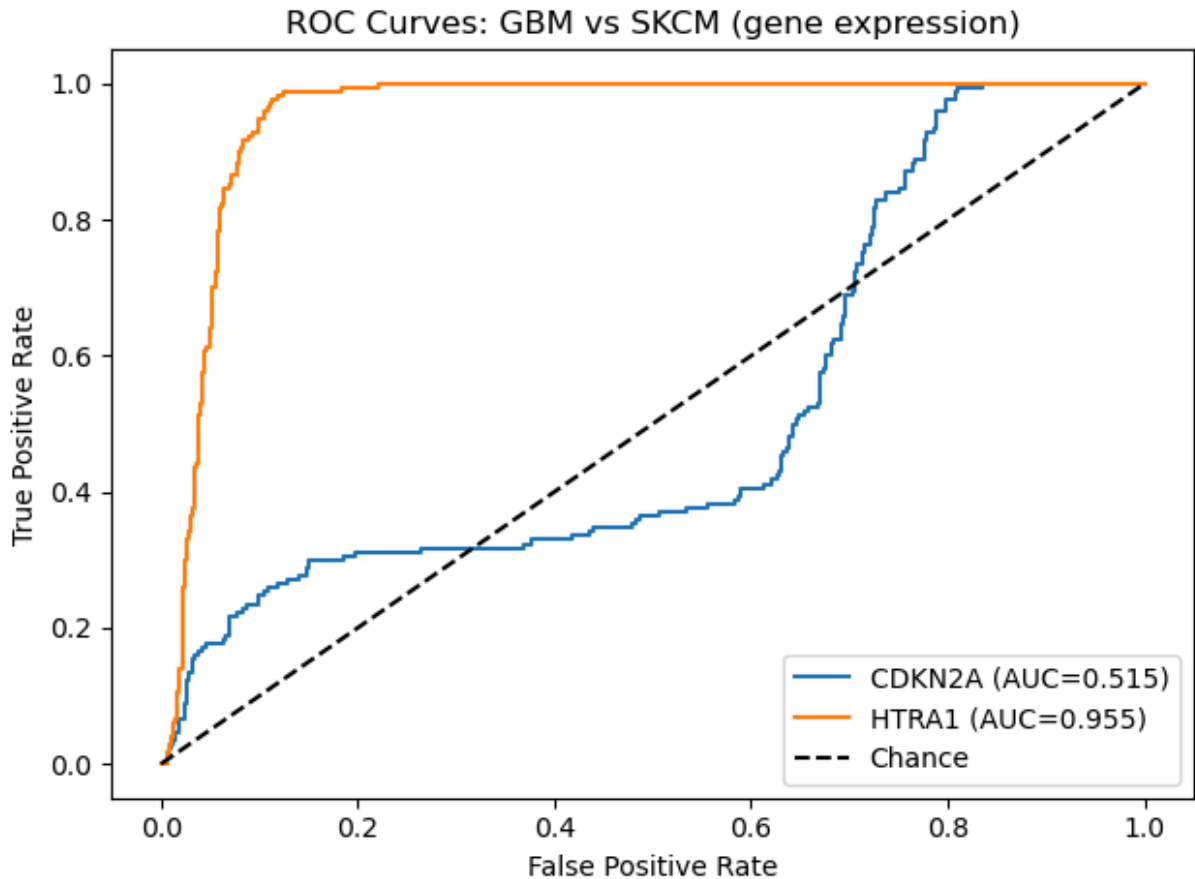
Conclusions and Ethical Implications:

After employing the ML method classification, our plot shows two clusters.

- The blue datapoints = patients with glioblastoma
- orange datapoints = patients melanoma. The plot shows that these are distinct; notably, the clusters are separated wider on the horizontal axis, meaning the difference in gene expression between the clusters (AKA cancer types) is greater than within the clusters. Essentially, patients with different cancer types have more differences in gene expression than those with the same cancer type.

To validate our analysis, we generated an ROC curve. The figure shows that incidence of the HTRA1 gene was much more useful for differentiating cancer type (AUC = 0.955) than the CDKN2A gene (AUC = 0.515).

Going back to our project goal, one pattern we can identify is that patients with the same cancer type have less differences in their gene expression that those with different illnesses. Another is that HTRA1 gene expression better distinguishes glioblastoma and melanoma. The two cancer types we chosen to study in regard to limitless replicative potential because GBM occurs in neurons (replicate less) and melanoma occurs in skin cells (replicate more). Thus a conclusion we can draw is that this gene responsible for regulating cell growth differs in expression level between the two types.

Expanding experiments to test this and training the model will be improved more having more patient data. This would become a large-scale effort, and it is important to maintain high ethical standards when it comes to prserving patient privacy and obtaining the data generally. Patients and families should be aware of what studies like this aim to do, and should not feel pressured to contribute.

Limitations and Future Work:

Some limitations of this dataset include scale and number of genes available to analyze.

Scale

- while the dataset is expansive, training machine learning models is always better with more expansive datasets
- the dataset given is large, but a bigger dataset could be more helpful in making sure models are not fit inaccurately
- a larger dataset can also help unsupervised models make better clusters/groupings

Genes available

- while coding, we ran into problems using one of the CSV files because they were either too big (causing our code to have problems)
- with the smaller CSV file (GSE62944_subsample_topVar_log2TPM.csv.zip), the code worked, but some of the genes we identified relating to our hallmark were not present

In the future, developing a method to clean up the CSV file so that it still includes some key genes weould be useful for further studies for different cancer types guided by different hallmarks. Additionally, future work would benefit from a larger-scale patient dataset obtaining data from a wide range of demographics.

Regarding the direction of future projects expanding on our goal, further experimentation to clarify whether the presence/absence of HTRA1 (or any other gene) specifically increases liklihood of a certain cancer type to develop would be worthwhile. Additionally, exploring whether certain genes were more integral to the development of a specific cancer type would be interesting.

Citations

OpenAI. (2025). ChatGPT (November 13 version) [Large language model]. https://chat.openai.com/

In our project, ChatGPT was used to help us write the code to create/test/train our classification model. It also helped us create key figures such as the PCA plot and ROC curve. It was also used to debug code/troubleshoot.

QUESTIONS FOR YOUR TA:

These are questions we have for our TA.

In [ ]: