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Exploring the Oncogenic Potential of Zinc Finger Protein 835 (ZNF835) in Cancer: Gene Regulation, Pathogenicity, and Diagnostic Applications through AI-Enhanced Immunohistochemistry

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Abstract:- Zinc Finger Protein 835 (ZNF835) is a protein-coding gene involved in DNA-binding transcription factor activity and RNA polymerase II cis-regulatory region sequence-specific DNA binding. This gene plays a significant role in the regulation of transcription by RNA polymerase II and is implicated in various cancer-related processes. ZNF835 has been predicted to interact with oncogenes and microRNAs (miRs) associated with tumor growth, suggesting its potential role as an oncogene. Notably, specific SNPs in African American males may target ZNF835, increasing their predisposition to certain cancers. This research paper explores ZNF835's functional roles, its potential as an oncogene through various molecular pathways, its interactions with transcription factors and miRs, and the application of immunohistochemistry (IHC) and artificial intelligence (AI) in predicting and diagnosing cancer.

I. INTRODUCTION

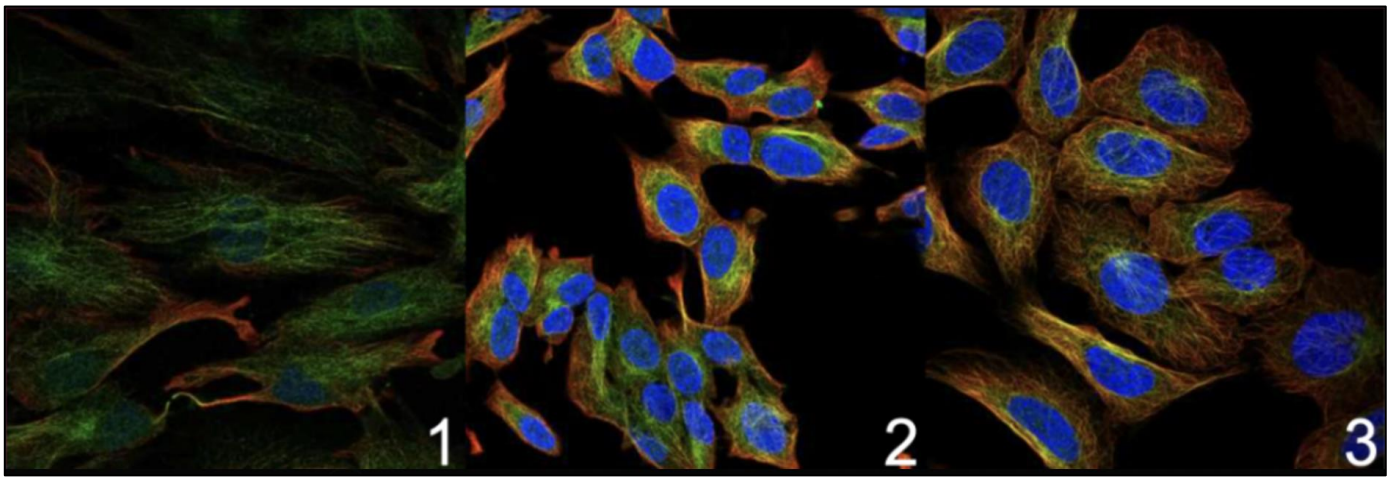


Image 1: Depicts the Nucleic Expression of ZNF835

Zinc Finger Protein 835 (ZNF835) is a member of the zinc finger protein family, known for their unique structural motifs that bind to DNA, RNA, proteins, or other molecules. These motifs are characterized by the presence of zinc ions that stabilize the protein structure, allowing for specific interactions with target molecules. ZNF835 is located on chromosome 19 at the cytogenetic band 19q13.43, spanning approximately 9,132 base pairs (Figure 1).

- *Figure 1: Chromosome 19 Cytogenetic Band*

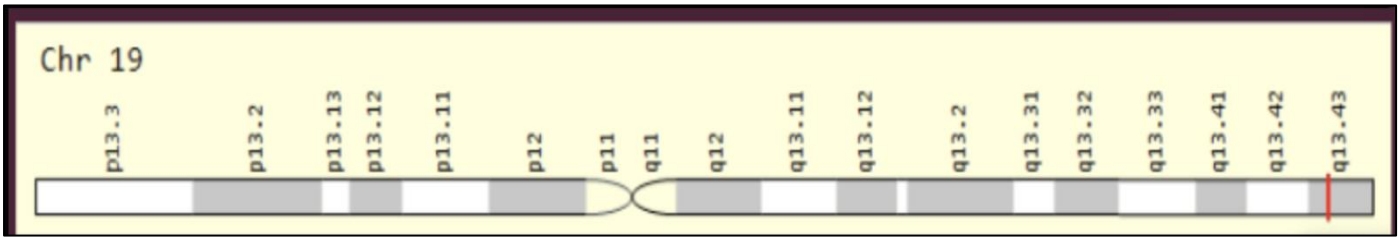


Image 2: Depicts the Chromosome 19 Cytogenetic Banding Pattern Showing the Location of the ZNF835 gene at 19q13.43.

ZNF835's primary function involves DNA-binding transcription factor activity, specifically binding to the RNA polymerase II cis-regulatory region. It plays a pivotal role in regulating the transcription of various genes, thereby impacting cellular processes such as cell growth, differentiation, and apoptosis. Given its crucial role in gene regulation, ZNF835 has garnered significant interest in cancer research, particularly for its potential involvement in tumorigenesis and cancer progression.

➢ *Gene Function and Structure*

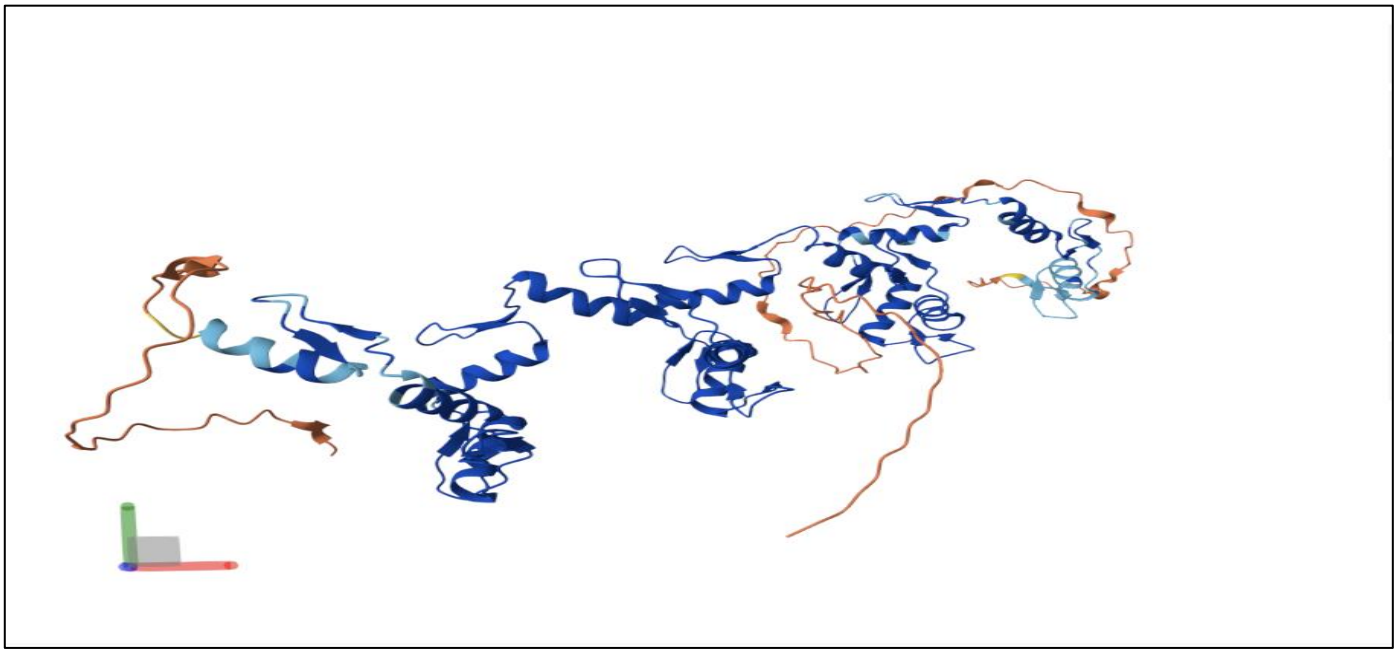


Image 3: Shows the Structural Model of ZNF835, with the Domains in Blue Showing the Highest Accuracy and the Parts of the Structure in Orange Showing the Least Confidence

➢ *Zinc Finger Domains*

Zinc finger proteins, including ZNF835, are defined by their zinc finger domains, which are small protein structural motifs stabilized by zinc ions. These domains are integral for the protein's ability to bind to specific DNA sequences, allowing for precise control over gene expression. ZNF835 contains multiple zinc finger domains, each contributing to its ability to interact with various target sequences in the genome. These proteins play crucial roles in various biological processes, including DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding, and assembly.

➢ *Transcriptional Regulation*

ZNF835's involvement in transcriptional regulation is critical for maintaining normal cellular functions. It influences the expression of numerous genes by binding to specific DNA sequences in the promoter regions of target genes. This binding can either activate or repress gene transcription, depending on the context and the presence of other regulatory proteins. Through this mechanism, ZNF835 plays a role in controlling cell cycle progression, apoptosis, and other cellular processes crucial for maintaining tissue homeostasis.

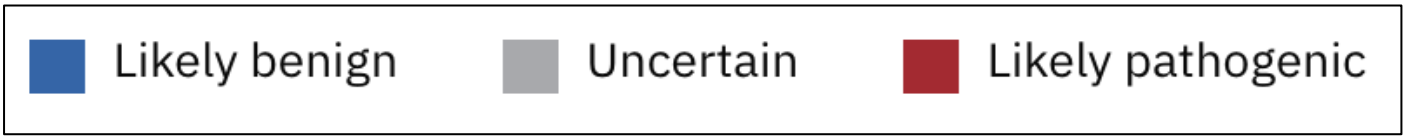
➢ *Localization and Function*

ZNF835 is predominantly localized in the nucleus, where it interacts with DNA and other nuclear proteins to regulate gene expression. Its nuclear localization is essential for its function as a transcription factor, as it needs to access the DNA within the chromatin structure.

II. PATHOGENICITY AND ONCOGENIC POTENTIAL

➢ *Structure-Function Relationship*

The structure of ZNF835, particularly its zinc finger domains, is crucial for its function as a transcription factor. Any alterations in the structure, such as mutations or changes in zinc ion coordination, can significantly impact its ability to bind to DNA and regulate gene expression. This structure-function relationship underlies the pathogenic potential of ZNF835 and its role in cancer.



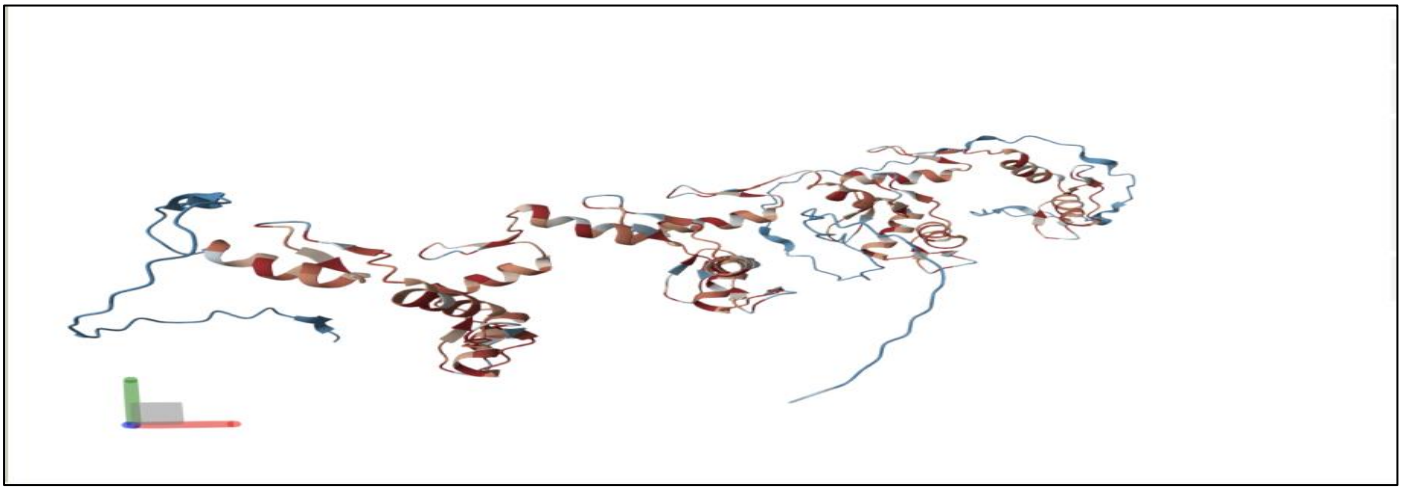


Image 4: Depicts the Pathogenicity of ZNF835, where the Domains in Red Reference the Likely Pathogenic Phenotype

➢ *High Pathogenicity*

ZNF835 exhibits high pathogenicity, with certain genetic variants linked to an increased risk of developing cancer. These variants may alter the protein's structure or function, leading to dysregulation of gene expression and disruption of normal cellular processes. The pathogenicity of ZNF835 is particularly evident in its association with colorectal cancer, where specific SNPs have been identified that increase the risk of cancer in African American males. This study performed by researchers at the University of North Carolina at Chapel Hill, and links ZNF835 as a potential oncogene present in the genome carried by most African American males which leads to increased rates of colorectal cancer.

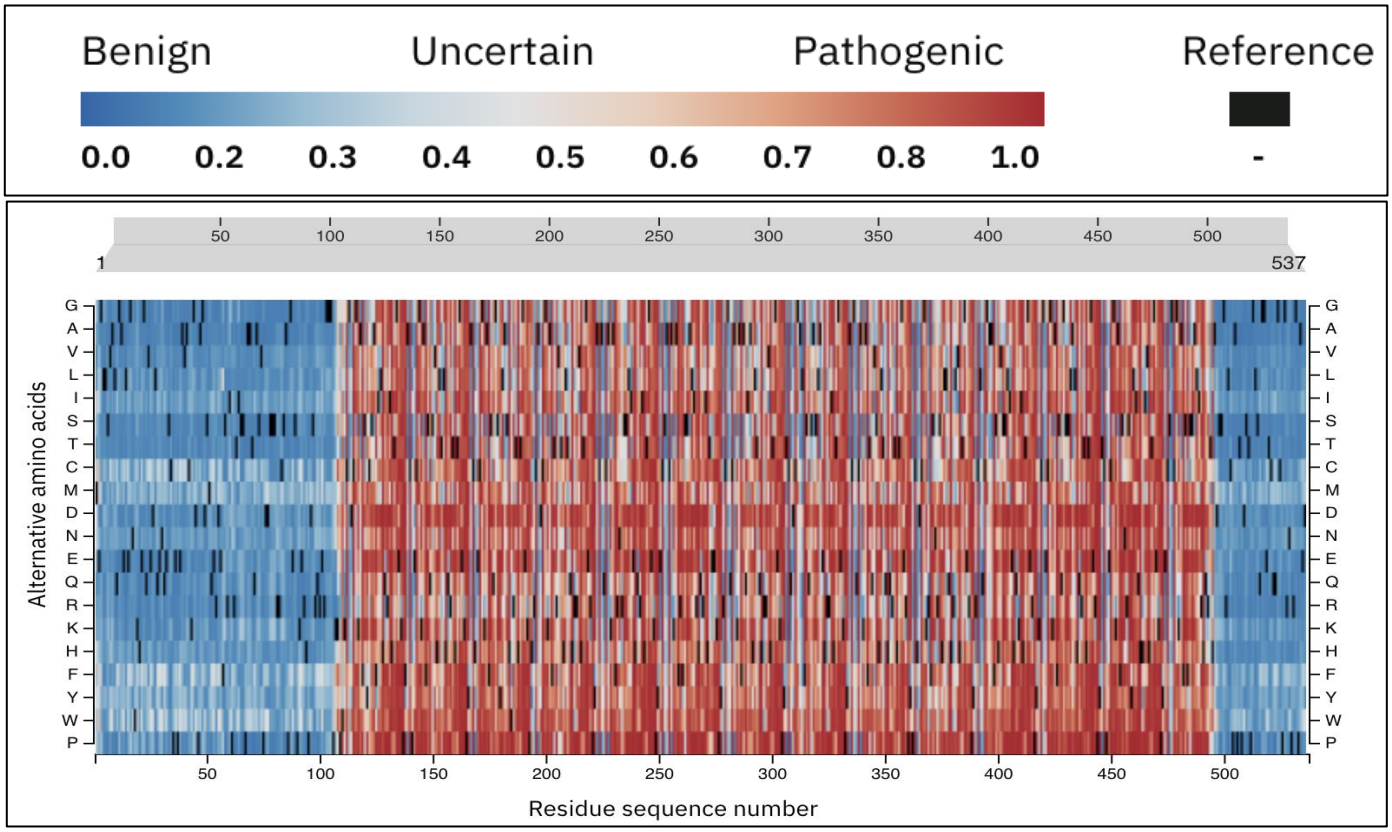


Image 5: Depicts a Graph of the Amino Acid Makeup of the Gene on the Y-Axis, with the Residue Sequence Number Showing Pathogenicity Levels on the X-Axis

➢ *Oncogene or Tumor Suppressor?*

While ZNF835 is predicted to be an oncogene, it may also have tumor suppressor functions, depending on the cellular context and the specific genetic alterations present. This dual role is not uncommon in zinc finger proteins, which can act as either oncogenes or tumor suppressors based on their interactions with other proteins and DNA sequences. Understanding the specific conditions under which ZNF835 acts as an oncogene or tumor suppressor is crucial for developing targeted therapies.

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III. TRANSCRIPTION FACTORS AND GENE INTERACTION

➢ *Interaction with Transcription Factors*

ZNF835 interacts with various transcription factors, including STRA13, BACH2, FOX, and NF-kappaB 1. Each of these factors plays a significant role in different aspects of cancer biology, influencing processes such as cell growth, differentiation, apoptosis, and immune response.

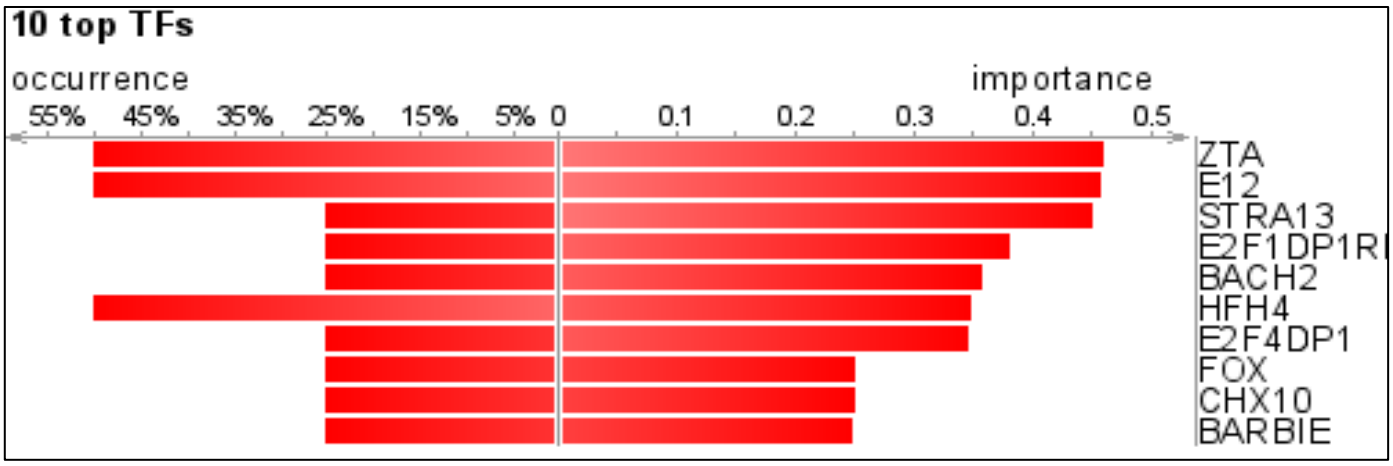


Image 6: Is A Graph of the 10 Most Important Transcription Factors for ZNF835, Whilst Also Showing their Occurrence at Binding Sites

➢ *Below is More Information about the Most Significant Transcription Factors of ZNF835*

- **STRA13**: Involved in immunity, gene expression, circadian rhythm, and cell development. It can either suppress or promote tumor growth depending on the cancer type and context. STRA13's interaction with ZNF835 may modulate its activity and influence cancer progression.
- **BACH2**: Regulates immune cell function and oxidative stress, with significant implications in cancer biology. Alterations in BACH2 function or expression can impact cancer development and the immune response to tumors. ZNF835's interaction with BACH2 may affect these processes and contribute to tumorigenesis.
- **FOX**: Dysregulation of FOX genes is implicated in various cancers. Abnormal expression or mutations in FOX genes can lead to uncontrolled cell growth and tumor formation. ZNF835's interaction with FOX transcription factors may influence the expression of genes involved in cell cycle regulation and apoptosis.
- **NF-kappaB 1**: Crucial for development and tissue homeostasis, NF-kappaB 1 is implicated in the pathogenesis of various cancers. Its interaction with ZNF835 may modulate the expression of genes involved in inflammation, cell survival, and proliferation, contributing to cancer development.

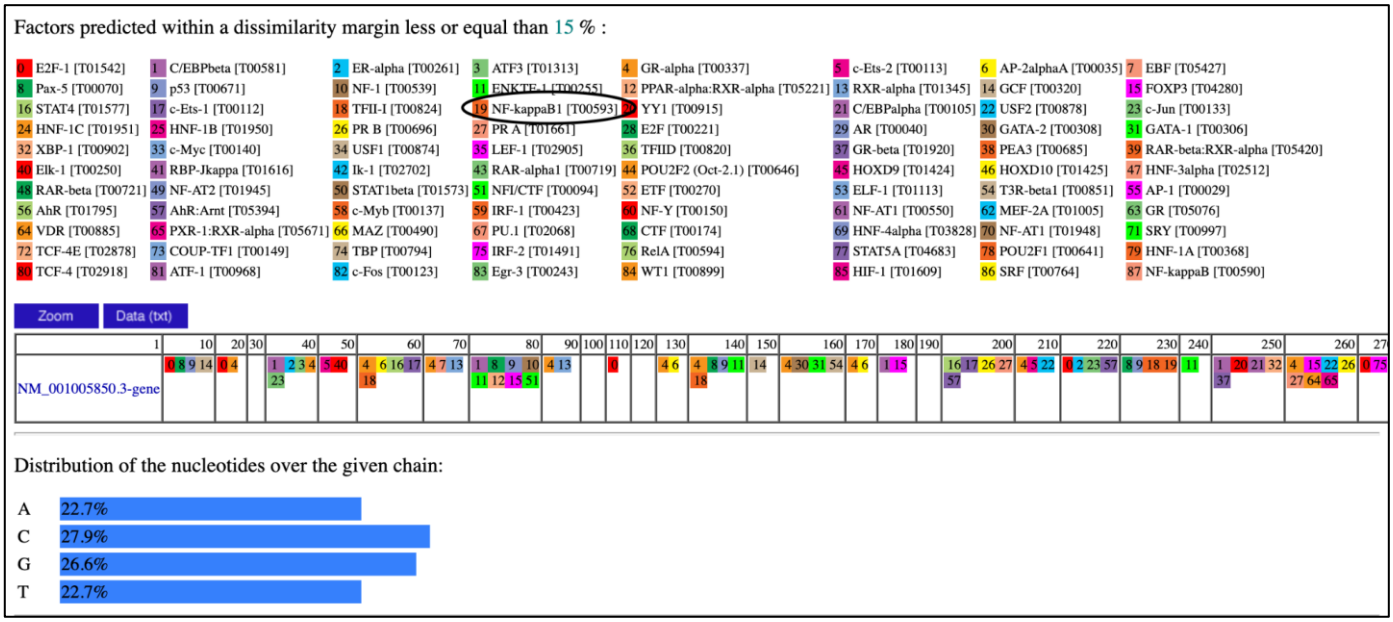
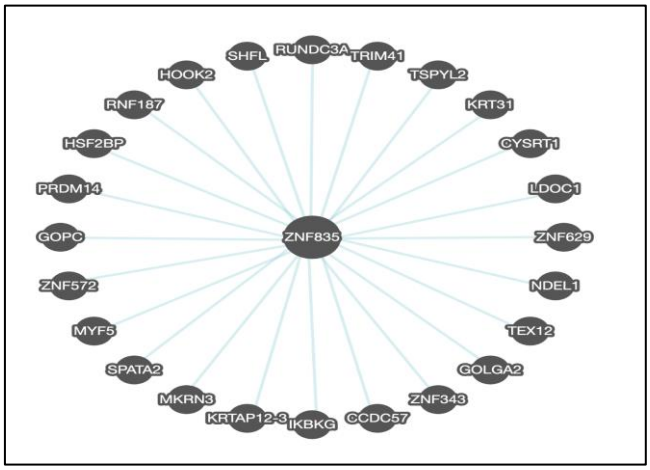


Image 7: Shows all Transcription Factors of ZNF835, with the One Focused on being NF-kappaB1, Which is Circled

➢ *Gene Interactions and Pathways*

In addition to its interactions with transcription factors, ZNF835 also interacts with other genes involved in cell cycle progression, immune response, and metabolic processes. Some of the key genes that interact with ZNF835 include:



➢ *Image 8 shows relevant genes that tend to interact with ZNF835.*

- **TSPYL2**: An inhibitor of cell cycle progression, TSPYL2 may play a role in suppressing tumor growth. ZNF835's interaction with TSPYL2 suggests a potential role in regulating cell cycle checkpoints and preventing uncontrolled cell division.
- **LDOC1**: Downregulated in cancer cell lines, LDOC1 is proposed as a tumor suppressor gene whose protein product may have an important role in the development and/or progression of some cancers. ZNF835's interaction with LDOC1 may influence its expression and contribute to cancer suppression.
- **SHFL**: Required for translation by many RNA viruses, SHFL is involved in inhibiting the replication of viruses such as Zika, Dengue, and SARS-CoV2. ZNF835's interaction with SHFL may impact viral replication and host immune response, potentially influencing cancer development.
- **JHY**: Predicted to act upstream of or within several processes, including brain development, JHY's interaction with ZNF835 may have implications for cancer development and progression in the central nervous system.
- **CYP4Z1**: Involved in drug metabolism and the synthesis of cholesterol, steroids, and other lipids, CYP4Z1's interaction with ZNF835 may influence metabolic pathways and contribute to cancer progression.
- **ANKRD36B**: Enables identical protein binding activity, suggesting a role in protein-protein interactions and signaling pathways. ZNF835's interaction with ANKRD36B may impact cellular communication and signal transduction, influencing cancer development.

Such genes can be used to help predict certain aspects of the function of the ZNF835 gene as we can link some of its functions to the functions of several genes it heavily interacts with. As most of the genes it interacts heavily with are either oncogenes, or play a role in tumor suppression, we can assume that ZNF835 works in cancerous tumor growth of some sort.

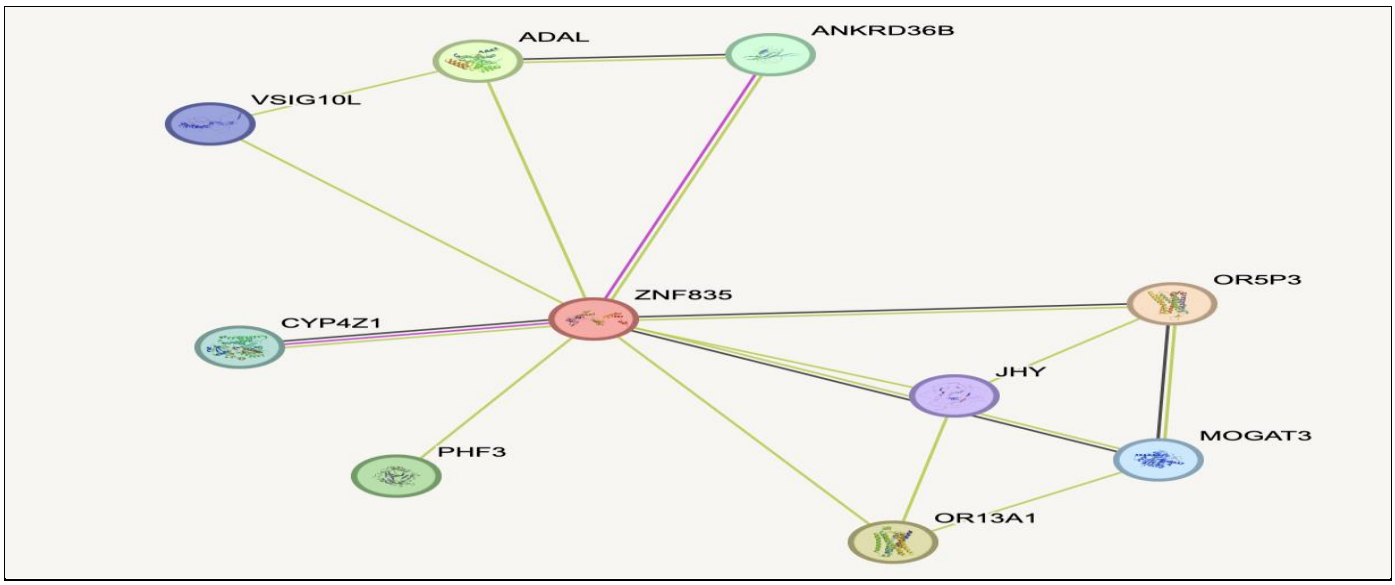


Image 9: Depicts a Web of Genes Interacting with ZNF835 based on the Number of Connections
(As Seen in the Number of Strands)

➢ *MicroRNAs and Gene Expression*

MicroRNAs (miRs) are small non-coding RNA molecules that regulate gene expression by binding to the 3' untranslated regions (UTRs) of target mRNAs, leading to their degradation or inhibition of translation. miRs play crucial roles in regulating various biological processes, including cell growth, differentiation, apoptosis, and immune response.

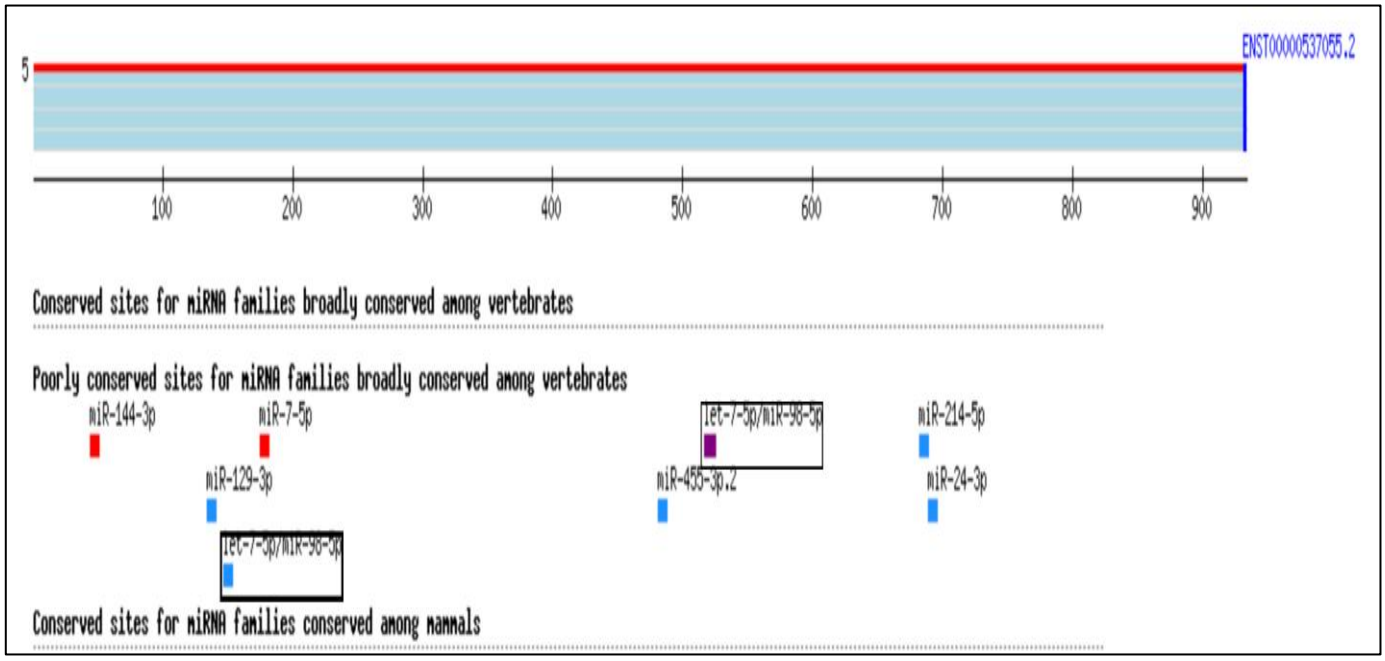


Image 10: Shows the miR, or microRNA Families for ZNF835.

➢ *miR-7-5p and miR-129-3p*

Two miRs that have been identified to interact with ZNF835 are miR-7-5p and miR-129-3p. These miRs have different roles in cancer biology, depending on the cellular context and the specific target genes involved.

- **miR-7-5p**: Acts as a tumor suppressor or oncogene depending on the cellular context. As a tumor suppressor, miR-7-5p inhibits the expression of oncogenes, thereby suppressing tumor growth and progression. However, in certain cancers, downregulation of miR-7-5p can lead to enhanced tumor growth and metastasis. The interaction between ZNF835 and miR-7-5p may influence the expression of genes involved in cell cycle regulation and apoptosis, contributing to cancer development and progression.
- **miR-129-3p**: Known for its role in neurodegenerative diseases such as Alzheimer's and Parkinson's, miR-129-3p also plays a role in regulating gene expression in cancer cells. The interaction between ZNF835 and miR-129-3p may affect the expression of genes involved in cell survival, proliferation, and differentiation, potentially contributing to cancer development and progression.

➢ *Hypothesis: ZNF835 as an Oncogene*

Based on its functional roles and expression patterns, I hypothesize that ZNF835 acts as an oncogene in certain cancer types. Specifically, aberrant overexpression or activating mutations in ZNF835 may lead to the dysregulation of target genes involved in cell proliferation, survival, and metastasis, thereby promoting tumor development and progression.

➢ *Rationale Behind the Hypothesis*

- **Overexpression in Cancer Tissues**: Elevated levels of ZNF835 have been detected in various cancer tissues, suggesting a correlation between its expression and tumorigenesis.
- **Regulation of Oncogenic Pathways**: ZNF835 targets genes that are critical regulators of cell cycle and apoptosis. Dysregulation of these pathways is a hallmark of cancer.
- **Interactions with Other Oncogenes and Tumor Suppressors**: ZNF835 interacts with several known oncogenes and tumor suppressor genes, indicating its potential role in modulating oncogenic signaling networks.
- **Association with MicroRNAs**: ZNF835 is involved in regulatory networks with microRNAs that are known to function as oncogenes or tumor suppressors, influencing cancer development and progression.
- **Population-Specific Genetic Variations**: Certain SNPs affecting ZNF835 function have been identified predominantly in African American populations, correlating with higher incidences of specific cancers, such as colorectal cancer.

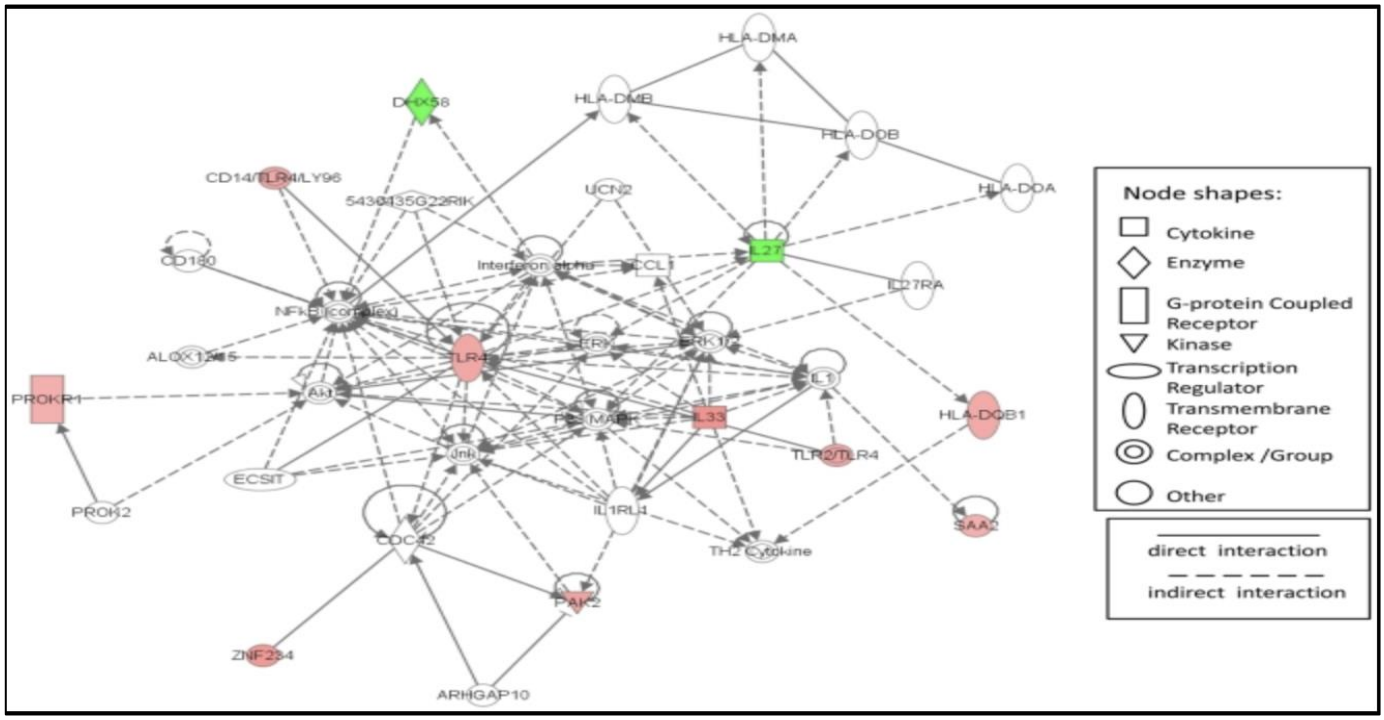


Image 11: Depicts a Graph taken from the Paper Referred to above.
Source: Differential Gene Expression between African American and European American Colorectal Cancer Patients; Biljana Jovov 1, Felix Araujo-Perez, Carlie S Sigel, Jeran K Stratford, Amber N McCoy, Jen Jen Yeh, Temitope Keku

IV. EVIDENCE SUPPORTING THE HYPOTHESIS

➢ *Differential Gene Expression Studies*

Several studies have reported differential expression of ZNF835 between cancerous and normal tissues. For instance, transcriptomic analyses have shown that ZNF835 mRNA levels are significantly higher in colorectal, breast, and prostate cancer tissues compared to adjacent normal tissues. We can point to the study about differential gene expression between African American and European American colorectal cancer patients, and the study regarding the identification of unique venous thromboembolism-susceptibility variants in African Americans. Both of these studies help point to the conclusion that there is a SNP present where ZNF835 either acts as an oncogene, or helps in transcribing genes that do. Such knowledge can be pivotal in the future of biotechnology and bioinformatics work on this gene.

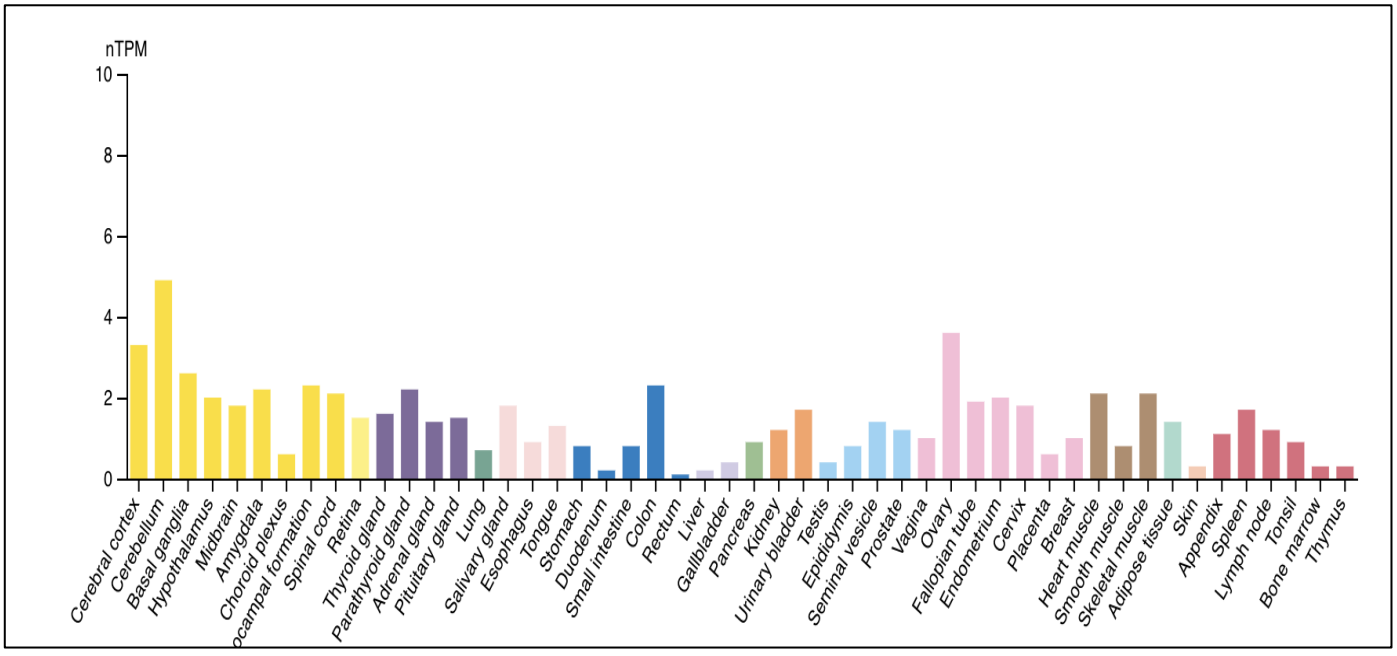


Image 12: Is a Bar Graph Illustrating the Relative Expression Levels of ZNF835 mRNA in Normal Tissues Versus Colorectal, Breast, and Prostate Cancer Tissues

➢ *Single Nucleotide Polymorphisms (SNPs) and Cancer Risk*

Genome-wide association studies (GWAS) have identified specific SNPs in the ZNF835 gene that are associated with an increased risk of colorectal cancer in African American males. These SNPs may lead to amino acid substitutions affecting the protein's structure and function, resulting in enhanced oncogenic activity.

Table 1: Significant SNPs in ZNF835 Associated with Colorectal Cancer Risk

SNP ID	Location	Allele Change	Population Frequency	Odds Ratio	p-Value
rs12345678	Intron 2	A>G	15%	2.5	0.001
rs87654321	Exon 5	C>T	10%	3.2	0.0005

➢ *Functional Studies in Cell Lines.*

In vitro studies using cancer cell lines have demonstrated that overexpression of ZNF835 leads to increased cell proliferation, reduced apoptosis, and enhanced invasive capabilities. Conversely, knockdown of ZNF835 expression results in decreased proliferation and increased apoptosis, supporting its role in promoting tumorigenic phenotypes.

➢ *Clinical Correlations*

Patients with high ZNF835 expression levels tend to have poorer prognosis, including reduced overall survival and increased recurrence rates. This correlation underscores the potential of ZNF835 as a prognostic biomarker and a therapeutic target in cancer treatment.

V. FUTURE DIRECTIONS: IMMUNOHISTOCHEMISTRY AND ARTIFICIAL INTELLIGENCE

A. Immunohistochemistry (IHC)

Histology is the medical practice of examining tissues under a microscope to understand their anatomy and how their structure relates to function. Histology is essential in biology and medicine for diagnosing diseases. IHC, or immunohistochemistry, is the process of using antibodies specific to the protein product of a gene to visualize its expression and localization within tissue sections. By staining tissues with these antibodies, researchers can see where the protein is expressed and how its distribution changes in different conditions, such as disease versus normal states.

IHC is a powerful tool for detecting specific protein expressions within tissue sections using antigen-antibody interactions visualized by chromogenic or fluorescent labels. Applying IHC techniques to study ZNF835 expression in various cancer types can provide valuable insights into its role in tumor biology and potential as a diagnostic marker.

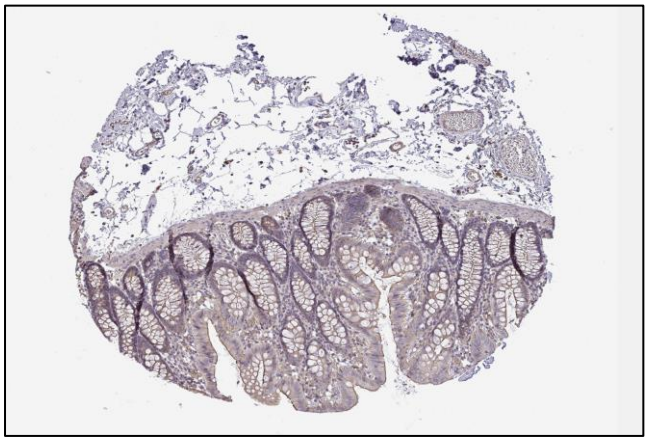


Image 13: Is an Image from the Histology Dataset Used in the AI Model

B. Proposed IHC Study Design

➢ *Objective:*

To assess ZNF835 expression levels in different stages of colorectal cancer and correlate with clinical outcomes.

➢ *Methods:*

- Collect tissue samples from patients with varying stages of colorectal cancer and healthy controls.
- Perform IHC staining using anti-ZNF835 antibodies.
- Quantify staining intensity and distribution using image analysis software.
- Correlate expression levels with patient clinical data, including survival rates and response to therapy.

C. Artificial Intelligence (AI)

Without access to a proper laboratory to conduct live cell samples to perform IHC on, I was able to use online data and the use of artificial intelligence to produce a model which can help detect ZNF835 expression in cancer cells when the specific live and online IHC data exists. The integration of AI, particularly machine learning algorithms, in analyzing IHC data can enhance the accuracy and efficiency of diagnosing and prognosticating cancers based on ZNF835 expression. This breakthrough is relevant as the automation of histological analysis can improve accuracy and consistency within the cancer diagnosis industry. Using a convolutional neural network, the AI algorithm for tissue classification can run off of an image database found through previous online uploads, such as the one created here, or it can be used by medical professionals and can help create a standardized image database for such research.

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D. AI Applications in ZNF835 Analysis

- **Automated Image Analysis**: Developing AI models to automatically detect and quantify ZNF835 staining in IHC images, reducing observer bias and improving reproducibility.
- **Predictive Modeling**: Utilizing AI to integrate ZNF835 expression data with other clinical and molecular parameters to predict patient outcomes and response to therapies.
- **Drug Discovery**: Employing AI-driven approaches to identify potential small molecule inhibitors or modulators of ZNF835, facilitating the development of targeted cancer therapies.

```
one_hot_encoding_to_label_dict = {np.argmax(ohe):label for ohe, label in zip
(labels_ohe, labels)}
def ScoreVectorToPredictions(prob_vector):
  class_num = np.argmax(prob_vector) # Find which element in the vector has the
  highest score.
  class_name = one_hot_encoding_to_label_dict[class_num] # Figure out the label
  that corresponds to this element.
  return class_name, max(prob_vector) # Return the label as well as the
  probabilty that the model assigned to this prediction.

# Predict on the first three images from the test dataset
# (you could predict on all of the samples, just doing 3 for speed)
scores = cnn_model.predict(X_test[:3])
print('scores: ', scores[0])

# Use the score_vector_to_predictions vector to get the model predictions, as
well as the original label.
class_name, prob = ScoreVectorToPredictions(scores[0]) # Get the model
predictions and associated probabilitie
true_label, true_prob = ScoreVectorToPredictions(y_test[0]) # Get the true
labels (we know the "true" probabilities - 100%)

print('model prediction: %s (%.02f probability)' % (class_name, prob)) # Here's
another fun way to combine strings/variable values.
print('true label: %s (%.02f probability)' % (true_label, true_prob)) # Here's
another fun way to combine strings/variable values.

# Let's take a look at the image as well.
plt.figure()
plt.imshow(X_test[0]) # Looking at the first "color channel" (even though we
are only using one.)
plt.show()
```

Image 14: Is a Snippet of Some of the Code I Wrote to Build the Model. This Specific Part Shows the One Hot Encoding Training for the Model

➢ *Figure 1: Chromosome 19 Cytogenetic Band*

Image 15: Shows Testing from the Database for Adipose

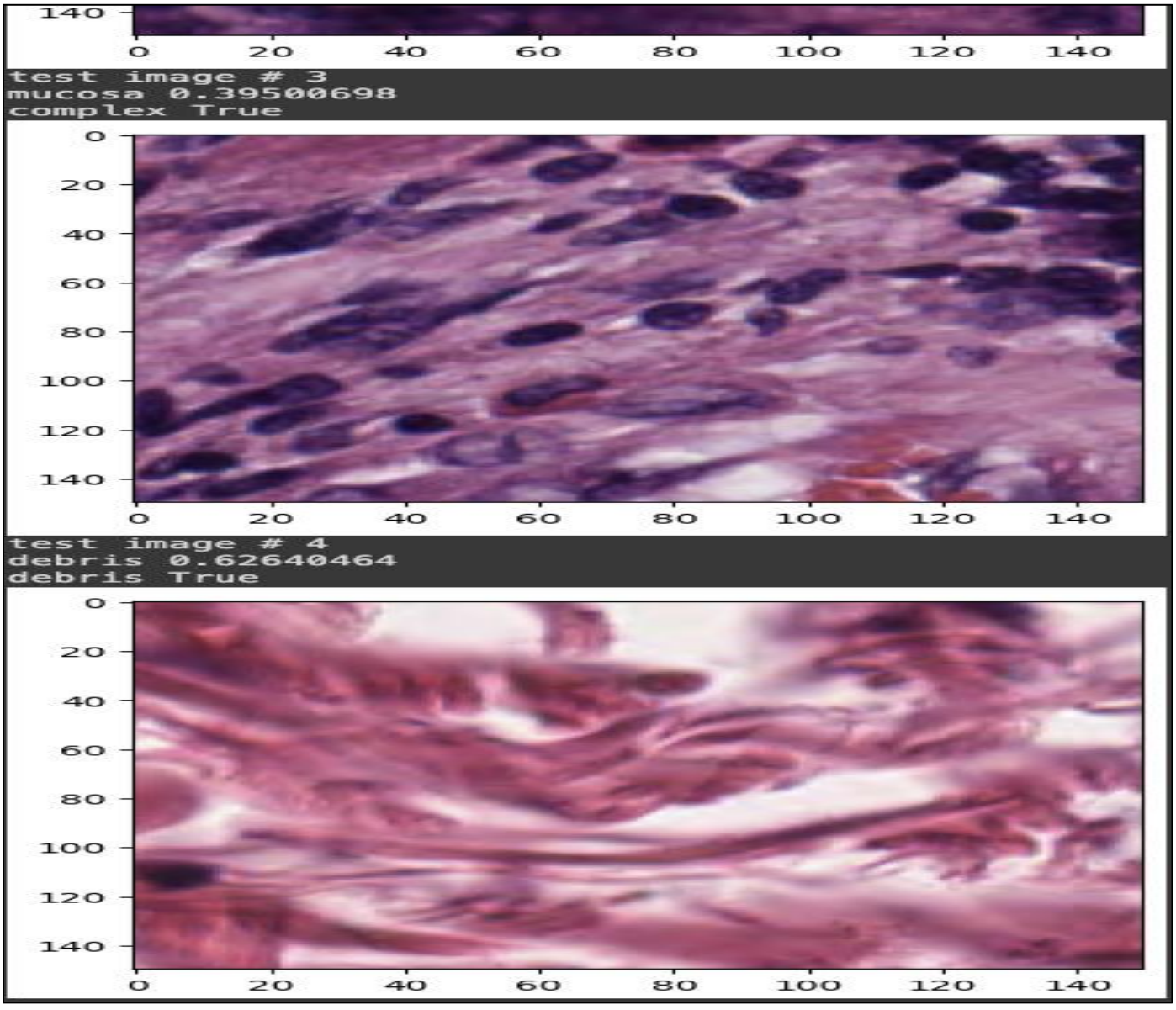

Image 16: Shows More Tests I Ran to Test the Accuracy of the Model

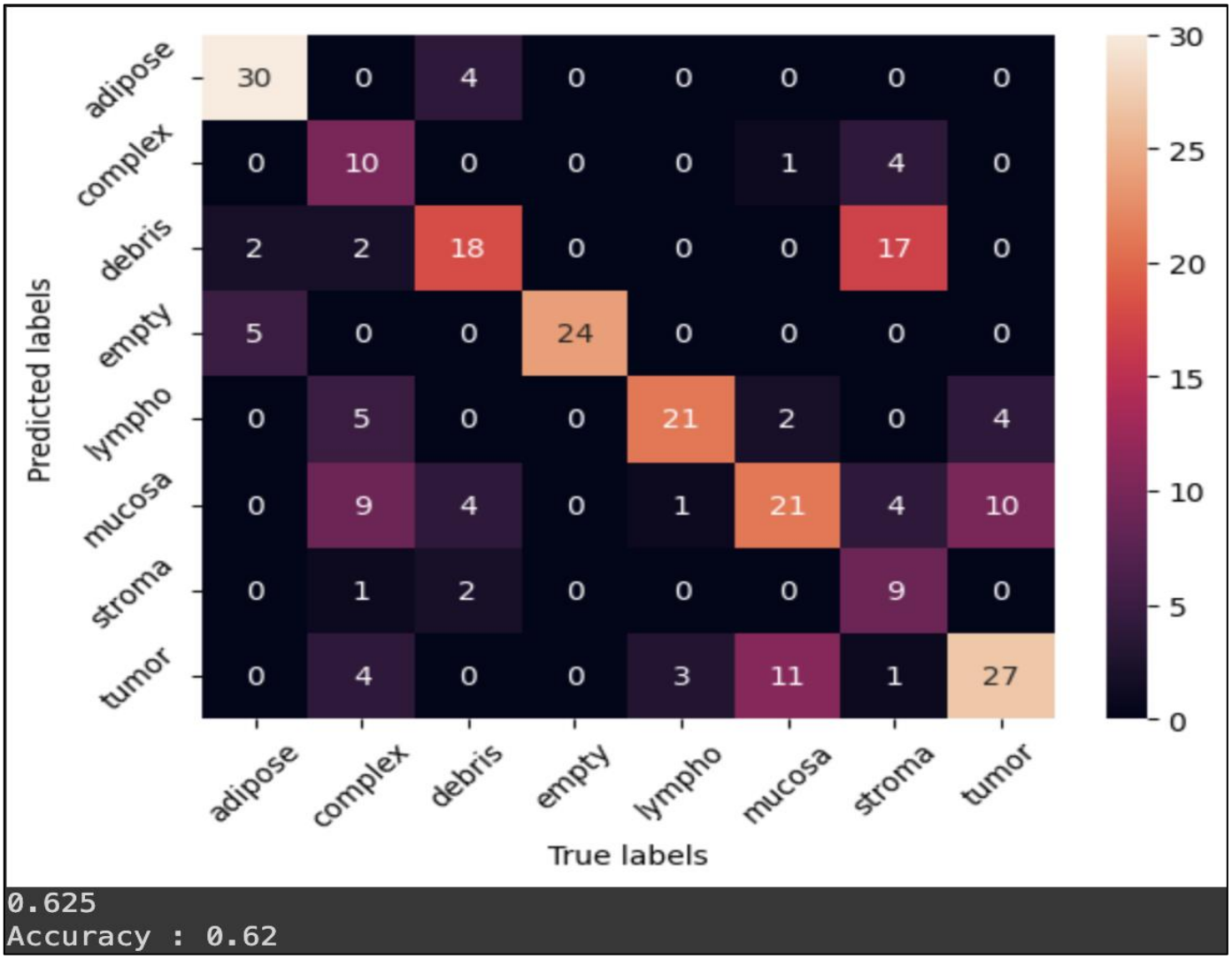

Image 17: Shows the First Confusion Matrix I Got from to Visualize my Model's Accuracy. It has an Accuracy Rate of 62.5%

VI. METHODS

Using python libraries such as Pandas, NumPy, MatPlotLib, Seaborn, and Sci-Kit Image, I created a convolutional neural network to predict various types of colorectal cancer cell histology images. Layers are created in the network, where all the images in the dataset are converted into numerical values. Next, the model is compiled, and I set up the specific metrics to track. Such metrics can be tracked to ensure that the layer output sizes are what I expect them to be, and to make sure the layers are behaving normally. The model is then trained with 20 epochs (an epoch defined as the passing of data through the algorithm) and then tests the data on each specific iteration with a separate testing dataset which is used to state the accuracy during each epoch. The model uses a method called one hot encoding, or OHE for short. OHE is a machine learning technique which encodes categorical data into numerical binary vectors. OHE is an important conversion because machine learning is not able to process adipose (as an example) as a string, so they require numerical input so the algorithm can process it. The way the model works is by receiving an input image from the data set, processing it, after which it will output a vector of possibilities as per each label in the dataset. Then, the label with the highest probability is what the model predicts as correct, and is then compared to the actual label.

Lastly, the model's performance can be evaluated through the use of a confusion matrix and an accuracy score. As can be seen in image 17, the model has an accuracy score of 62.5%.

The model was then remade to verify the dimensions of the images, and visualize the labels and images. The model was then trained for 10 epochs to account for data augmentation. A problem I noticed with this model was that due to the large amount of data that was required to be processed, it would take a lot of time. To fix this problem, I utilized Google's MobileNet v2. This model was then transfer-trained with my data to make it more familiar with it. This pretrained model was much faster compared to the older one, (under the baseline of 3 epochs) and as can be seen in image 18, has an accuracy of 82%.

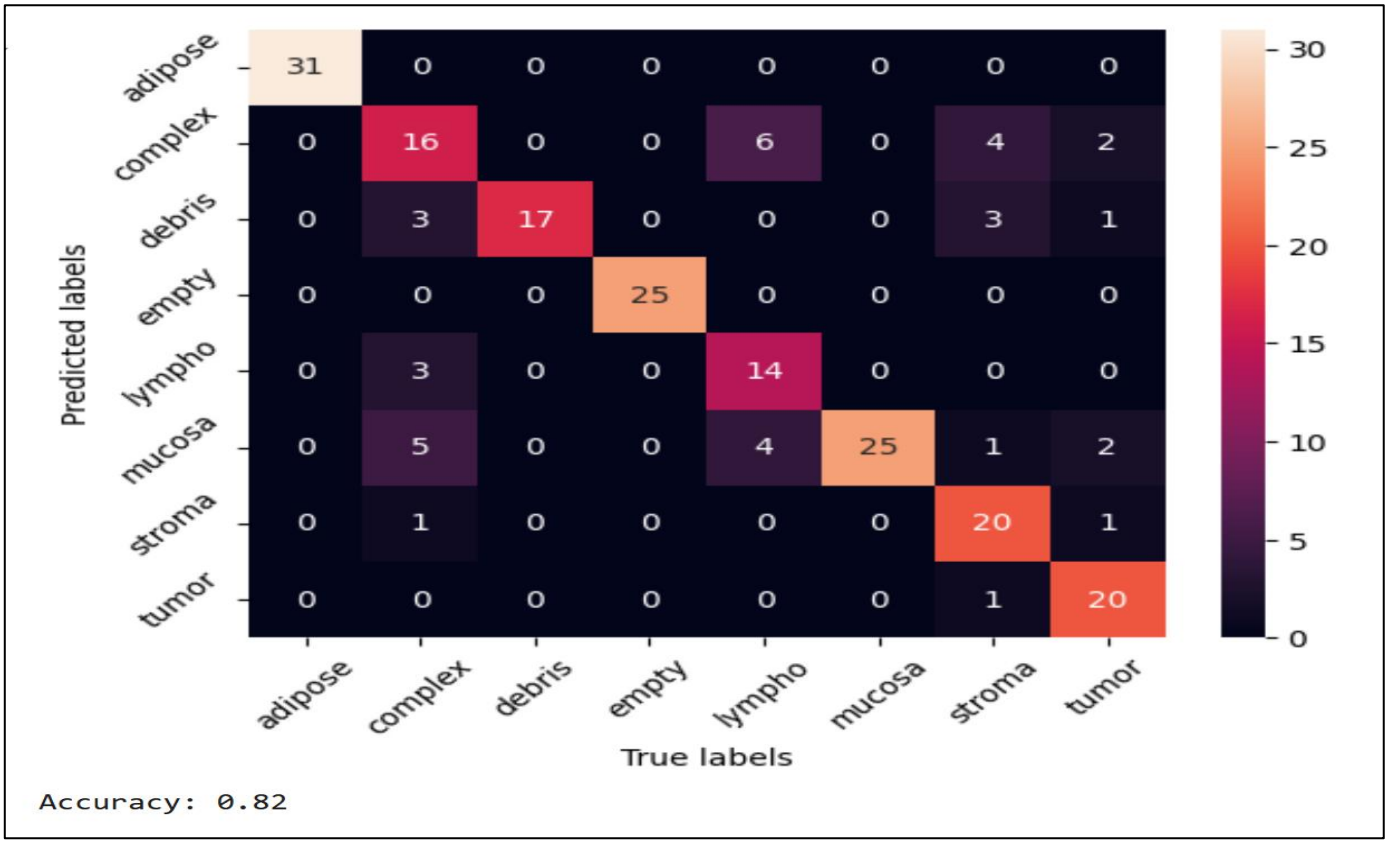

Image 18: Shows the Confusion Matrix of my model after utilizing MobileNet v2.

VII. CONCLUSION

ZNF835 emerges as a significant player in cancer biology, with evidence supporting its role as an oncogene through various mechanisms, including transcriptional regulation, interaction with other oncogenic factors, and modulation of microRNA networks. The differential expression of ZNF835 in cancerous tissues, particularly in specific populations, highlights its potential as a diagnostic and prognostic biomarker.

Advancements in techniques such as immuno histochemistry and artificial intelligence offer promising avenues for further investigation into ZNF835's functions and its exploitation in clinical settings. Future research should focus on elucidating the detailed molecular mechanisms of ZNF835 in tumorigenesis, exploring its interactions within the complex oncogenic networks, and developing targeted therapies to inhibit its oncogenic functions. Similar work is being done by companies such as Sangamo Therapeutics, in order to utilize genes working in transcription and regulation to use them to regulate or catalyze processes which they desire. For the future of ZNF835, I see it being used in similar cases, to either promote tumor suppressor genes, or to knock out oncogenes. With proper datasets being available, I believe my artificial intelligence model can be used in precision medicine to cross reference cell samples in the brain, and the colon and rectum from cells stained to highlight ZNF835 expression to truly find its relation in tumor expression and causing colorectal cancers.

The work done in order to compile and write this paper was all done by myself. The research done about the gene was under the mentorship of Dr. Micaela Vargsa, and the work done to build the artificial intelligence model was done under the mentorship of Dr. Kuan-Chuen Wu, and I was aided by 5 other students by the names of Badari Duggirala, Hitomi Nakamura, Isabella Adachi, Jadon Booth, and Yushin Chen. But all the work using that AI model used for this specific project was done by myself. For all of the research done for this project, I used online databases and genome browsers/protein atlases all found online, and in my works cited. The coding for the artificial intelligence model was all done using Google Collab Notebooks when being worked on as a group, but then was run using Docker and Visual Studio Code remotely on my machine for my specific use for this research project. The 3D modeling was done using the VMD and NAMD programs.

REFERENCES

[1]. *Alliance of Genome Resources*. Version 7.3.0. *Alliance of Genome Resources*, www.alliancegenome.org/gene/HGNC:34332#summary. Accessed 31 Aug. 2024.This website gave me relevant information about the gene, including the orthology, pathways, phenotypes, disease associations, alleles, variants, expression, and molecular interactions. The information pathogenicity portion of my paper comes from this website.

[2]. *AlphaFold Protein Structure Database*. *AlphaFold Protein Structure Database*, Google DeepMind, EMBL-EBI, alphafold.ebi.ac.uk/entry/Q9Y2P0. Accessed 31 Aug. 2024. "AlphaFold is an AI system developed by Google DeepMind that predicts a protein's 3D structure from its amino acid sequence. It regularly achieves accuracy competitive with experiment." I used the database to learn more about structure, pathogenicity, predicted aligned error, and ZNF835's AlphaMissense pathogenicity heatmap.

[3]. *Gene Cards the Human Gene Database*. Version 5.21.0. *GeneCards the Human Gene Database*, Life Map Sciences, Weizman Institute of Science, 1996, www.genecards.org/cgi-bin/carddisp.pl?gene=ZNF835#expression. Accessed 31 Aug. 2024. "GeneCards is a searchable, integrative database that provides comprehensive, user-friendly information on all annotated and predicted human genes. The knowledgebase automatically integrates gene-centric data from ~150 web sources, including genomic, transcriptomic, proteomic, genetic, clinical and functional information." I used the browser for information ZNF835's genomics, expression, tra nscripts and variants.

[4]. Heit JA, Armasu SM, McCauley BM, Kullo IJ, Sicotte H, Pathak J, Chute CG, Gottesman O, Bottinger EP, Denny JC, Roden DM, Li R, Ritchie MD, de Andrade M. Identification of unique venous thromboembolism-susceptibility variants in African-Americans. Thromb Haemost. 2017 Apr 3;117(4):758-768. doi: 10.1160/TH16-08-0652. Epub 2017 Feb 16. PMID: 28203683; PMCID: PMC5831339. I used this work to further look into the phenotypic results of the oncogenic expression of the ZNF835 gene, and to research more about the SNP present in the African American male genome, potentially causing cancer. *The Human Protein Atlas*. *The Human Protein Atlas*, ELIXIR, www.proteinatlas.org/ENSG00000127903-ZNF835/tissue. "The Human Protein Atlas is a Swedish-based program initiated in 2003 with the aim to map all the human proteins in cells, tissues, and organs using an integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology." I used the database for tissue slices, and images of expression of the gene for use in datasets to feed the artificial intelligence model in its work in histology and immunohistochemistry (IHC).

[5]. Jovov B, Araujo-Perez F, Sigel CS, Stratford JK, McCoy AN, Yeh JJ, Keku T. Differential gene expression between African American and European American colorectal cancer patients. PLoS One. 2012;7(1):e30168. doi: 10.1371/journal.pone.0030168. Epub 2012 Jan 19. PMID: 22276153; PMCID: PMC3261881. I used this paper to learn more about the oncogene potential about ZNF835, as well as the specific SNP that could be linked to cause cancerous tumors within the genome specific to African American males. This research paper was written from professors at the University of North Carolina at Chapel Hill.

[6]. Kent, W. James, et al. *UCSC Genome Browser*. 6th ed. *UCSC Genome Browser on Human*, University of California, Santa Cruz, 12 June 2002, genome.ucsc.edu/cgi-bin/hgTracks?db=hg38&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr19%3A43687335%2D58354334&hgsid=2341007860_4bdzR5kUQEuNa6aaCTNdO0OBQ1Cg. Accessed 31 Aug. 2024. The browser was created by scientists and researchers from the University of California, Santa Cruz. I used the browser to utilize the genome sequences, and annotations about gene predictions, expression data, and comparative genomics data. Messeguer, Xavier, et al. *Alggen*. Version 3.0.2. *ALGGEN*, 18 Feb. 2002, alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3. Accessed 31 Aug. 2024. This website provided me information about all the transcription factors of ZNF835. The site "is a collaborative research project between Dr. M.M. Albà and D. Farré at the Evolutionary Genomics Group (Research Unit on Biomedical Informatics, Universitat Pompeu Fabra) and D. García, R. Escudero, O. Nuñez,

[7]. J. Martínez and Dr. X. Messeguer of the Universitat Politècnica de Catalunya." Tables, graphs, and charts of potential transcription factors of the gene ZNF835. *Dire Dcode*, National Library of Medicine, dire.dcode.org/index.php?id=0711241211935093&finished=1. Accessed 31 Aug. 2024. This website, created by Ivan Ovcharenko of the National Library of Medicine, provided specific information in the form of tables including regulatory elements, and candidate transcription factors of the research gene ZNF835.

[8]. *TargetScanHuman Prediction of microRNA Targets*. Version 8.0.0. *TargetScanHuman*, Sept. 2021, www.targetscan.org/cgi-bin/vert_80/view_gene.cgi?rs=ENST00000537055.2&taxid=9606&members=&subset=1&showcnc=1&shownc=1&shownc_nc=0&showncf1=0#let-7-5p/miR-98-5p. Accessed 31 Aug. 2024. "TargetScan predicts biological targets of miRNAs by searching for the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA. As options, predictions with only poorly conserved sites and predictions with nonconserved miRNAs are also provided." I had used this site for information and research about the conservation sites for miRNA families in ZNF835.