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IDENTIFYING AND PREDICTING KEY CONNECTED GENES IN ORAL ADENOID CYSTIC CARCINOMA AND COLON CANCER CELLS TREATED WITH ALPHA-TOCOPHEROL

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ABSTRACT

Introduction: Adenoid cystic carcinoma (ACC) is a rare malignant neoplasm primarily found in salivary glands, accounting for about 1% of all head and neck cancers. Alpha-tocopherol, a powerful antioxidant, has been linked to cancer cell behaviors, and its relationship with oral ACC and colon cancer cells treated with alpha-tocopherol could reveal overlapping therapeutic targets and strategies. **Objective:** To identify highly

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connected genes in ACC and colon cancer (CC) using a multi-step approach involving bioinformatics analysis, machine learning, and immune infiltration analysis. **Methods:** Gene expression data from the NCBI GEO dataset were used to analyze ACC and normal salivary gland tissue. The preprocessing stage involved data normalization and identification of differentially expressed genes (DEGs) using statistical frameworks like limma in R or DESeq2. Cytoscape was used to visualize gene interaction networks, and Cytohubba identified top hub genes. Feature selection methods and the Random Forest algorithm were applied for classification models, validated through cross-validation. Immune cell infiltration in tumor samples was analyzed using tools like CIBERSORT, xCell, or TIMER. **Results:** Hub genes such as CXCL13, TEKT3, and KCNA4 were identified, playing key roles in cellular signaling, immune responses, and tumor progression. **Conclusions:** The study reveals crucial hub genes in ACC and colon cancer biology, emphasizing the need for ongoing exploration and validation for therapeutic advancements in oncology.

KEYWORDS: Adenoid cystic carcinoma; colon cancer; hub genes; tocopherol.

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IDENTIFICACIÓN DE GENES CENTRALES EN EL CARCINOMA ADENOIDE QUÍSTICO Y CÉLULAS DE CÁNCER DE COLON TRATADAS CON ALFA-TOCOFEROL MEDIANTE ANÁLISIS BIOINFORMÁTICO Y APRENDIZAJE AUTOMÁTICO

RESUMEN

Introducción: El carcinoma adenoide quístico (ACC) es una neoplasia maligna rara que se encuentra principalmente en las glándulas salivales, representando aproximadamente el 1% de todos los cánceres de cabeza y cuello. El alfa-tocoferol, un potente antioxidante, se ha relacionado con comportamientos de células cancerosas, y su relación con el ACC oral y las células de cáncer de colon tratadas con alfa-tocoferol podría revelar objetivos y estrategias terapéuticas comunes. **Objetivo:** Identificar genes altamente conectados en el ACC y el cáncer de colon (CC) mediante un enfoque que combina análisis bioinformático, aprendizaje automático y análisis de infiltración inmune. **Métodos:** Se utilizaron datos de expresión génica del conjunto de datos NCBI GEO para analizar tejido de ACC y glándulas salivales normales. La etapa de preprocesamiento incluyó normalización de datos e identificación de genes diferencialmente expresados (DEGs) utilizando paquetes estadísticos como limma en R o DESeq2. Se empleó Cytoscape para visualizar redes de interacción génica y Cytohubba para identificar genes centrales. Se aplicaron métodos de selección de características y el algoritmo Random Forest para modelos de clasificación,

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validados mediante validación cruzada. También se analizó la infiltración de tipos de células inmunes en muestras tumorales utilizando herramientas como CIBERSORT, xCell o TIMER. **Resultados:** Se identificaron genes centrales como CXCL13, TEKT3 y KCNA4, que desempeñan roles clave en la señalización celular, respuestas inmunes y progresión tumoral. **Conclusiones:** El estudio revela genes centrales cruciales en la biología del ACC y el cáncer de colon, destacando la necesidad de exploración continua y validación para avances terapéuticos en oncología.

PALABRAS CLAVE: Carcinoma adenoide quístico; Cáncer de colon; Genes centrales; Tocoferol.

INTRODUCTION

Adenoid cystic carcinoma (ACC) is a rare malignant neoplasm primarily found in salivary glands. Still, it can also occur in various sites in the head and neck region, including the oral cavity. It accounts for approximately 1% of all head and neck cancers and is most commonly diagnosed in middle-aged adults, with a slight female predominance (1). ACC presents with painless masses or swelling, causing dysphagia, odynophagia, and nerve-related symptoms. Histologically, ACC is characterized by unique growth patterns and cellular architecture, including cribriform patterns, tubular structures, and solid areas. ACC is a rare cancer that can occur between 10 and 96 years of

age, with a peak in the 6th decade of life. Treatment involves surgical resection followed by adjuvant radiation, with disease-specific survival reaching 89% at 5 years (2,3). However, ACC is known for its late distant metastasis and poor long-term prognosis. The path of ACC metastasis is mainly hematogenous, with the lung being the most commonly involved organ. Factors such as large tumor size, positive margins, and large nerve involvement can predispose to metastasis in ACC (4,5). ACC is a rare malignancy arising from secretory glands, often in the salivary glands. Its growth is slow and distant, with colon metastasis being rare (6). Treatment involves surgery, radiation therapy, and chemotherapy. ACC has a high potential for recurrence. Colon cancer, also known as colorectal cancer, starts in colon cells and can be treated with surgery, chemotherapy, radiation therapy, and targeted therapies. ACC has a unique histology consisting of epithelial and myoepithelial cells, with three growth patterns: cribriform, tubular, and solid. Metastatic ACC in the colon should be differentiated from primary colonic adenocarcinoma using immunohistochemical studies. ACCs show genetic heterogeneity, with MYB-NFIB translocation and PI3K alterations being common.

Immunohistochemistry plays a vital role in diagnosing ACC, employing markers such as cytokeratins and vimentin (7). The primary treatment for ACC is

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surgical excision, which can be difficult due to the tumor's infiltrative characteristics. Radiation therapy is frequently advised after surgery, particularly for patients at high risk of recurrence. Conversely, chemotherapy has typically proven ineffective for ACC because of its resistance to standard chemotherapy drugs (8). Patient prognosis varies based on factors like the tumor's site, stage at diagnosis, and histopathological characteristics (9–13). While some patients may achieve long-term survival, ACC is recognized for late recurrences, which can occur many years after initial treatment. A multidisciplinary strategy that includes surgery and adjuvant therapy is essential for improving patient outcomes (3)

A previous study using bioinformatics highlighted two prominent genes (FN1 and SPP1) associated with mucoepidermoid carcinoma (MECa) and two others (EGF and ERBB2) linked to adenoid cystic carcinoma (AdCC). These genes are significant in salivary gland neoplasms' pathogenesis, progression, and prognosis. They should be explored as therapeutic targets for tumor growth and metastasis, offering insights for new biomarker development (1). Adenoid cystic carcinoma is a rare malignancy affecting the salivary glands, characterized by slow growth but a strong tendency to invade nerves and metastasize. Investigating the genetic framework of ACC, especially regarding treatments like alpha-tocopherol (vitamin E), could unveil potential therapeutic

avenues and deepen our understanding of the disease. Key genes associated with ACC include MYB, KLF4, EGFR, CCND1, PTEN, and ACTB. MYB plays a crucial role in tumor development and is essential for cellular proliferation and differentiation. Alterations in KLF4 expression have been implicated in various cancers, including ACC, and adjusting its expression may affect tumor dynamics. Targeting EGFR could potentially hinder growth and invasion in ACC. The overexpression of Cyclin D1 in numerous cancers, including ACC, indicates it might serve as a promising therapeutic target (14,15).

Colon cancer (5,7), or colorectal cancer, is a prevalent malignancy that originates from the inner lining of the colon, ranking as a major cause of cancer-related fatalities. It commonly arises from adenomas, precancerous growths that can evolve into malignant tumors. Risk factors, including age, family history, dietary habits, lifestyle choices, and medical conditions, contribute to its development. Symptoms encompass alterations in bowel habits, the presence of blood in stool or rectal bleeding, abdominal pain, weight loss, and fatigue. Diagnosis generally involves colonoscopy, imaging studies, and biopsy procedures. Treatment may include surgical tumor removal, chemotherapy for more advanced stages, radiation therapy specifically for rectal cancer, and an increasing focus on targeted therapies in colon cancer care (16–19).

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Alpha-tocopherol (20), a potent antioxidant, is associated with various behaviors of cancer cells, such as inducing apoptosis, inhibiting cell proliferation, and enhancing immune responses. The effects of this compound on adenoid cystic carcinoma (ACC) cells may be influenced by gene-environment interactions, biomarker potential, and resistance mechanisms. These genes could serve as biomarkers for treatment effectiveness, implying that combined therapies might produce better results than single-agent treatments. Grasping the significance of these genes is vital for identifying therapeutic targets, understanding tumor biology, and advancing personalized medicine. Future studies will be crucial in converting these complex interactions into viable treatment strategies. Examining gene functions in ACC can lead to innovative therapeutic approaches and improved patient management. A prior study reported that early supplementation with α-Tocopherol significantly reduced the risk of esophageal cancer caused by N-nitrosomethylbenzylamine (NMBA) in a rat model of ESCC. Furthermore, it inhibited cell proliferation and encouraged cell cycle arrest in a Het-1A cell model. α-Tocopherol enhanced the expression of PPARγ and its downstream tumor suppressor PTEN, which were triggered by PPARγ knockdown. However, the impact of α-Tocopherol on Akt inhibition was not evident in established tumors or cancerous cell lines. This study implies that α-Tocopherol

could act as a PPARγ agonist for preventing esophageal cancer.

The relationship between oral ACC (21,22) and colon cancer cells treated with alpha-tocopherol (vitamin E) is an intriguing area of study that spotlights the potential for shared pathways and therapeutic strategies in different types of cancer. Exploring these interrelations can yield insights into the molecular mechanisms of cancer progression and the impact of dietary or pharmacological interventions on tumor behavior. The study explores the relationship between oral ACC and colon cancer cells treated with alpha-tocopherol. Both cancers share common signaling pathways, such as growth factors, inflammatory responses, and cellular apoptosis. Alpha-tocopherol may modulate these pathways as an antioxidant, potentially influencing cellular senescence, apoptosis, and metastatic potential. Alpha-tocopherol has shown potential for antiproliferative effects on various cancers, possibly inhibiting cell cycle progression and promoting apoptosis. Research suggests that alpha-tocopherol treatment in colon cancer may reduce inflammation and promote apoptosis, which could apply to ACC, given the tumors' shared characteristics of slow growth and tendency for perineural invasion. The tumor microenvironment significantly influences cancer behavior, and understanding how vitamin E affects the signaling cascades in diverse microenvironments of oral ACC and

colon cancer may reveal overlapping therapeutic targets and strategies for effective intervention. Both cancers can manipulate immune responses in their favor, and the immunomodulatory effects of alpha-tocopherol could provide critical insights into enhancing antitumor immunity in both types. Nutrition is a critical factor in cancer therapy, and the role of alpha-tocopherol in modulating the inflammatory environment and inhibiting tumor growth emphasizes the importance of dietary components in cancer management. Exploring the potential of dietary interventions, such as alpha-tocopherol supplementation, on tumor behavior can foster a holistic approach to cancer care, integrating lifestyle choices with conventional treatments (13,23–25).

Predicting relationships between ACC and colon cancer about alpha-tocopherol treatment could enhance therapeutic strategies, biomarker development, immunotherapy insights, understanding resistance mechanisms, and translational research. Identifying and predicting interactomic hub genes (26,27) is essential for effective treatment in personalized cancer care. In summary, exploring the relationship between oral adenoid cystic cancer and colon cancer cells under alpha-tocopherol treatment presents a valuable opportunity to advance our understanding of cancer biology and refine therapeutic strategies. By integrating insights into the interaction of these cancers with dietary antioxidants, such as alpha-tocopherol, we can foster innovative treatment methods and

improve patient outcomes in oncology. Thus, this study aims to predict and identify hub genes related to adenoid cystic carcinoma and alpha-tocopherol-treated colon cancer cells.

Materials and Methods

1. Dataset Overview

Dataset To identify highly connected genes in ACC and colon cancer (CC), a multi-step approach involving bioinformatics analysis, machine learning, and immune infiltration analysis can be employed. Below is a detailed methodology outlining these steps:

2. Data Acquisition and Preprocessing

2.1 Gene Expression Data

Using the NCBI GEO dataset (28), GSE153002 investigates global gene expression in ACC (30) and normal salivary gland tissue. Additionally, top differential gene analysis was performed. Using the NCBI GEO GSE14476, eight samples were analyzed: four for alpha-tocopherol and four for gamma-tocopherol. Differential gene expression analysis was performed in colon cancer cells.

The gene expression analysis preprocessing phase entails normalizing data to ensure consistency and make comparisons easier. This normalization can be performed using various techniques, such as Reads Per Kilobase of transcript per Million mapped reads

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(RPKM), Transcripts Per Million (TPM), or quantile normalization. A log transformation may also be applied to stabilize the variance across samples, enabling more accurate data analysis. The subsequent important step involves identifying differentially expressed genes (DEGs), necessitating strong statistical analysis. Researchers can effectively pinpoint DEGs that exhibit significant differences between tumor and normal samples in ACC and CC by employing statistical testing frameworks like the limma package in R or DESeq2. A significance threshold is often established to ensure the results are trustworthy, commonly defined by adjusted p-values lower than 0.05 and a fold change exceeding 2.

3. Network Analysis

Cytoscape (29) It is an open-source software platform for visualizing and analyzing gene interaction networks. It helps identify highly connected nodes using metrics like Degree, Betweenness Centrality, and Clustering Coefficient. Cytohubba, with its maximum clique centrality method, is used to identify top hub genes from differential gene expression, providing critical biological insights. Integrating differential gene expression data into network analysis frameworks like Cytoscape and CytoHubba involves constructing a list of gene identifiers and associated expression metrics. Then, interaction data is compiled, including known gene-gene, protein-protein, or regulatory interactions. The data is then loaded into Cytoscape

and visualized using various layout algorithms.

Centrality metrics identify highly connected nodes (hubs) within the constructed gene interaction network. Degree centrality measures the number of direct connections a node (gene) has within the network. In contrast, betweenness centrality evaluates the number of times a node acts as a bridge along the shortest path between two other nodes. The clustering coefficient measures the degree to which nodes in a network tend to cluster together, suggesting localized communication. The CytoHubba plugin within Cytoscape is employed to refine the identification of essential hub genes further. The plugin provides various methods for hub analysis, including the Maximum Clique Centrality function, which evaluates the maximum cliques of a network and recognizes nodes integral in connecting them. The output from CytoHubba enumerates the top hub genes based on their Maximum Clique Centrality scores, representing nodes with critical connectivity and regulatory roles.

4. Machine Learning

4.1 Feature Selection Methods

Feature selection is essential in data analysis and machine learning, enhancing model performance by removing irrelevant or redundant features. There are three primary categories of feature selection methods: filter, wrapper, and

embedded. Filter methods function independently of any machine learning algorithm, concentrating on the inherent properties of the data. Examples include the ANOVA F-value, mutual information, and the chi-squared test. Wrapper methods engage a specific machine learning algorithm and evaluate the performance of subsets of features. Techniques like Recursive Feature Elimination (RFE) and Sequential Forward Selection (SFS) eliminate the least important features based on model performance metrics. Embedded methods merge the qualities of filter and wrapper methods by integrating feature selection into the model training process. Examples include LASSO regression, Elastic Net regularization, and Random Forest importance scores. Choosing an appropriate feature selection method is crucial for constructing an effective machine learning model, affecting the efficiency and accuracy of predictions.

4.2 Classification Models

This analysis uses the Random Forest algorithm, an ensemble learning technique that generates multiple decision trees during training. The parameters for this analysis include averaging 500 trees, which reduces variance, and try = sqrt(p), which specifies the number of variables randomly sampled as candidates at each split in the trees. This choice helps reduce overfitting and enhances model accuracy.

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4.2.1 Validation

The model's robustness will be ensured through a 5-fold cross-validation approach, repeated 10 times. The dataset is divided into five parts, with the model trained in four parts and validated in the fifth part. This process increases the reliability of results by providing a comprehensive assessment of the model's performance across multiple data subsamples.

4.2.2 Performance Metrics:

The Random Forest model's effectiveness is evaluated using several performance metrics. These include the Area Under the ROC Curve (AUC), which measures the likelihood of a randomly chosen positive instance ranking higher than a randomly chosen negative instance. The model's accuracy is measured by the ratio of correctly predicted instances to the total instances in the dataset. Precision measures the accuracy of positive predictions, while recall measures the model's ability to capture positive instances. The F1 score combines precision and recall into a single score, which is useful for datasets with imbalanced classes. The Matthews Correlation Coefficient (MCC) is a more informative metric for binary classification problems, considering all four categories of confusion matrix outcomes.

5. Immune Infiltration Analysis

5.1 Estimation of Immune Cell Types:

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To analyze the infiltration of various immune cell types within tumor samples, it is essential to utilize advanced computational tools such as CIBERSORT, xCell, or TIMER. These tools assess the gene expression profiles obtained from the tumor samples, allowing researchers to quantify and estimate the levels of different immune cells in the tumor microenvironment. By leveraging these sophisticated platforms, researchers can gain insights into the immune landscape of tumors, which is crucial for understanding tumor biology and the immune response.

5.2 Correlation Analysis:

The next step involves performing a correlation analysis after estimating the immune cell infiltration levels. This can be done by examining the expression levels of genes that are highly interconnected within the tumor. Researchers can investigate potential associations and relationships between tumor biology and immune response by correlating these gene expression levels with the immune cell infiltration scores. Applying appropriate statistical tests, such as the Spearman correlation coefficient, is important to assess these relationships rigorously. Additionally, visualizing the results can greatly enhance comprehension; tools like heatmaps and scatter plots can effectively illustrate the correlations, making it easier to identify significant patterns or trends in the data.

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Results

Identifying hub genes in adenocarcinoma and colon cancer through the analysis of differential gene expression and interaction networks represents a significant step toward understanding the molecular mechanisms of these diseases. Our results delineate a complex landscape of gene interactions, with specific hub genes emerging as pivotal players in the disease processes. The study reveals significant changes in gene expression in adenocarcinoma and colon cancer, suggesting potential biomarkers for future research. The circular interactome network graph demonstrates a highly interconnected network of key genes, with top-ranked genes like CXCL13, TEKT3, and KCNA4 serving as significant hubs. These genes may mediate cellular signaling, immune responses, and tumor progression. The analysis also reveals correlations between hub genes and immune cell proportions, with strong positive correlations with Macrophages-M1, moderate positive correlations with CD4_T_cells, and a notable negative correlation with B_cells. However, the results warrant cautious interpretation due to low Mean Squared Error values and negative R^2 scores, indicating suboptimal model complexity, choice of features, or potential overfitting. Further refinement of the analytical approach is necessary to enhance predictive accuracy.

The volcano plot displays the results of differential expression analysis in ACC,

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highlighting genes with significant upregulation (red) and downregulation (blue). The x-axis represents the log2 fold change, and the y-axis shows the -log10(p-value). Most genes are not significantly differentially expressed, with red and blue points indicating genes that meet the significance threshold (adjusted p-value < 0.05 and |log2 fold change| > 2). The plot reveals a subset of genes with significant expression changes, providing insights into potential biomarkers or therapeutic targets in ACC (Figure 1a).

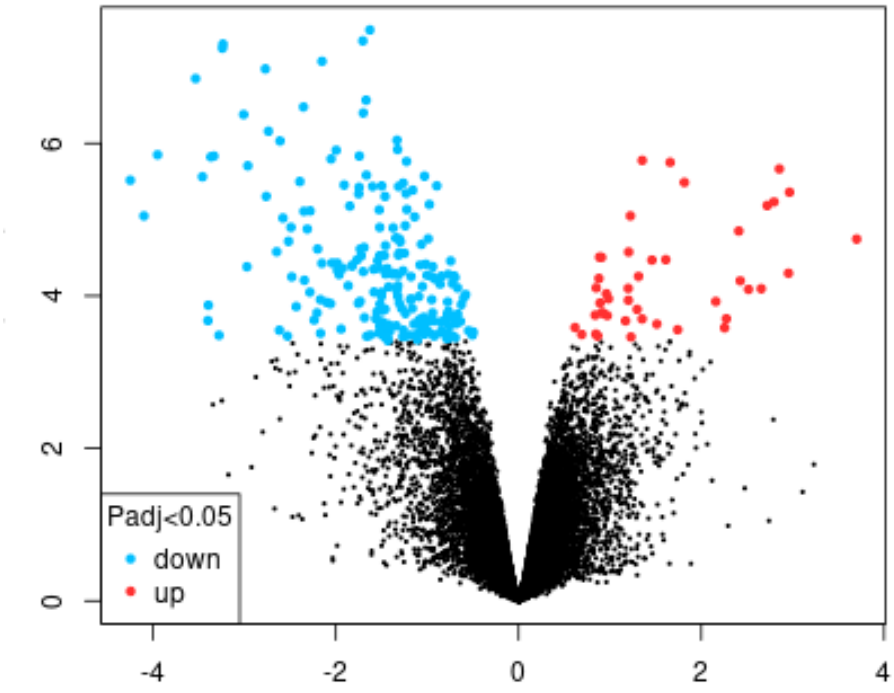

Figure 1. Differential Expression Analysis in Adenoid Cystic Carcinoma (ACC) and Colon Cancer (CC). **a.** Volcano Plot of Differential Expression in ACC

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The volcano plot illustrates the differential expression analysis in CC, with upregulated (red) and downregulated (blue) genes. The x-axis represents the log2 fold change, and the y-axis shows the -log10(p-value). Genes meeting the significance threshold (adjusted p-value < 0.05 and |log2 fold change| > 2) are highlighted, while the majority of genes show no significant change. This analysis identifies key genes with significant expression changes in CC, offering potential insights into the molecular mechanisms underlying the disease (Figure 1b).

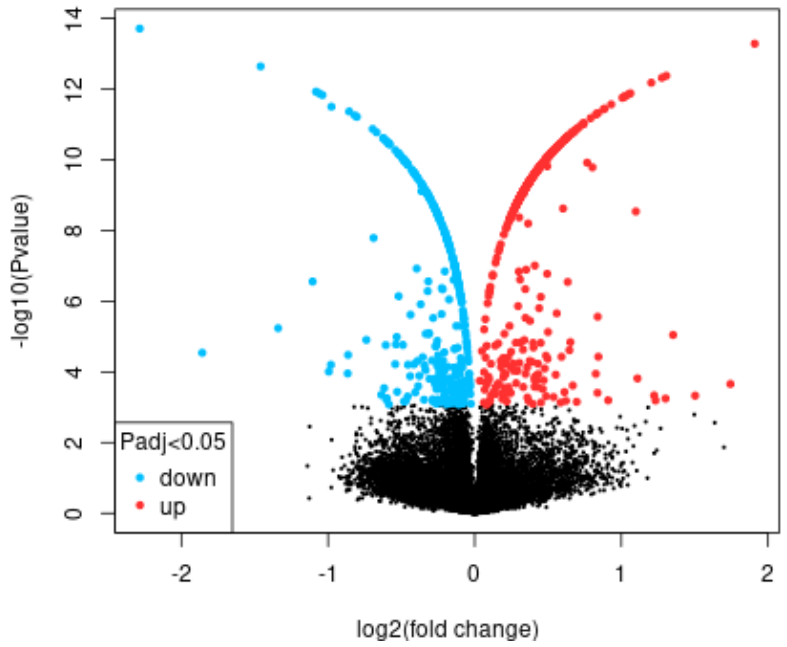

Figure 1. Differential Expression Analysis in Adenoid Cystic Carcinoma (ACC) and Colon Cancer (CC). **b.** Volcano Plot of Differential Expression in CC.

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The circular interactome network graph depicts gene interactions, with nodes representing genes and edges representing interactions between them. The network is highly interconnected, providing a global view of gene-gene relationships. The layout emphasizes the complexity and connectivity of the interactome, highlighting potential regulatory hubs and functional modules. This visualization offers a comprehensive overview of the gene interaction landscape, revealing key genes and pathways that may play critical roles in the disease (Figure 2a).

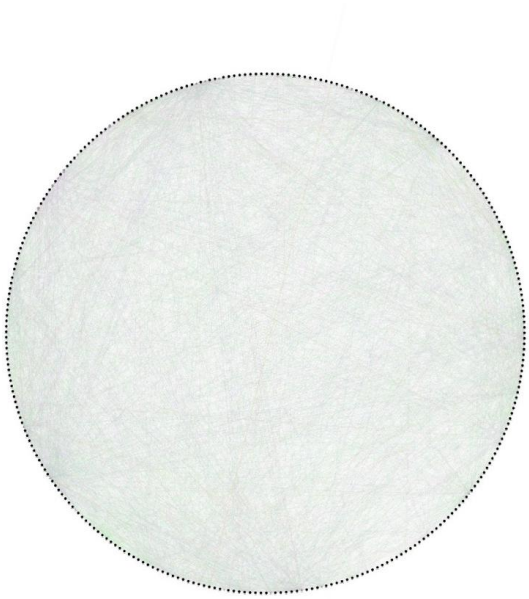

Figure 2. Gene Interaction Networks in Adenoid Cystic Carcinoma (ACC) and Colon Cancer (CC). **a.** Circular Interactome Network of Gene Interactions

The interactomic network graph displays the top 50 genes ranked by Maximum Clique Centrality (MCC) scores. Hub genes are highlighted in red and orange, indicating their central role in the network. The densely connected structure focuses on these hub genes, which are likely to have significant regulatory or functional importance. This network highlights critical hub genes with high connectivity, suggesting their potential as biomarkers or therapeutic targets (Figure 2b).

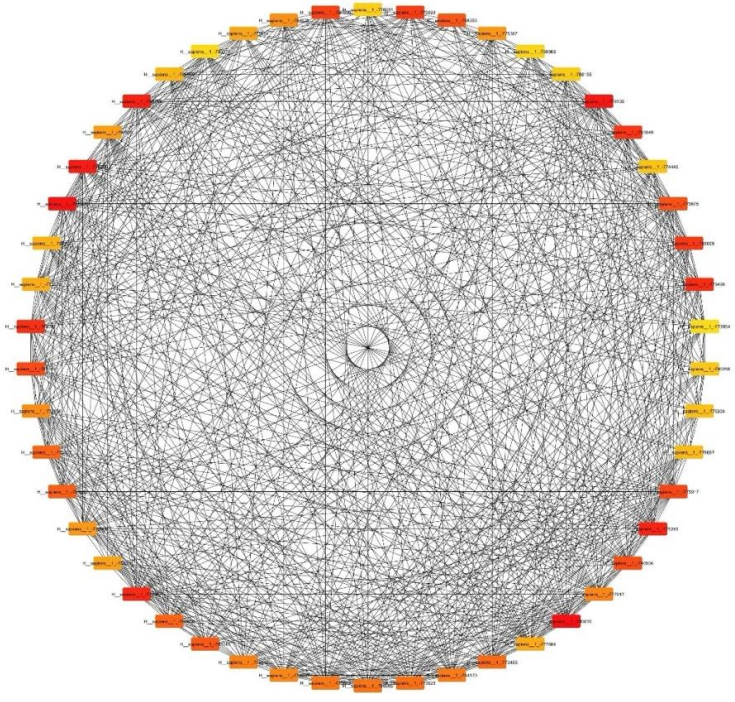

Figure 2. Gene Interaction Networks in Adenoid Cystic Carcinoma (ACC) and Colon Cancer (CC). **b.** Interactomic Network of Top 50 Hub Genes Ranked by MCC Scores

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The network graph illustrates relationships between genes, with top hub genes highlighted in blue and green. Most genes cluster in a central region, indicating strong interconnectivity, while some genes are isolated, suggesting fewer interactions. Prominently highlighted hub genes, such as CXCL13, TEKT3, and KCNA4, are identified as key players in the network due to their high connectivity. This visualization emphasizes the central role of hub genes in the network, which may have critical regulatory or functional importance in the disease context (Figure 3a).

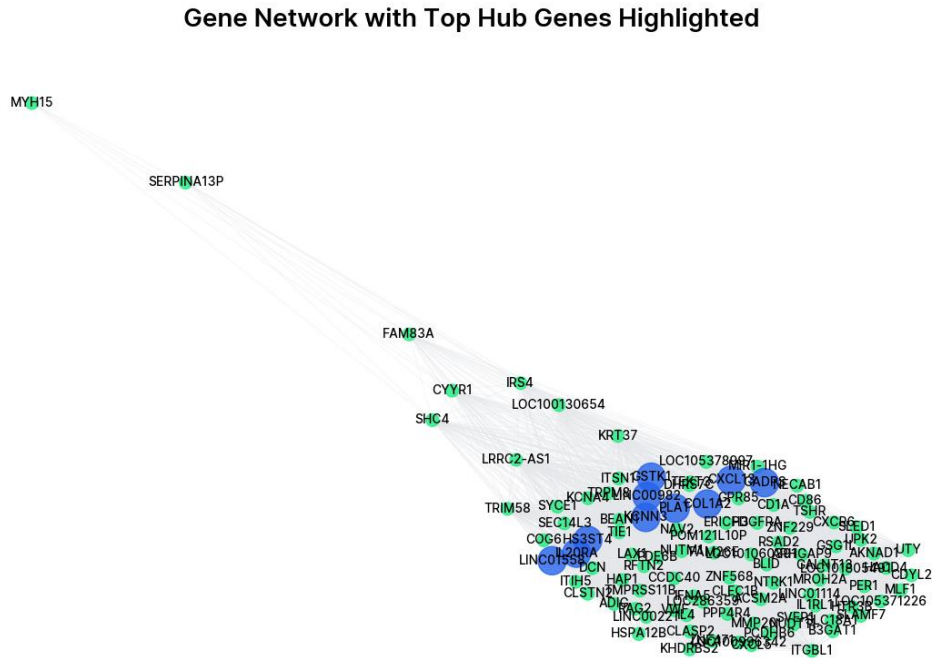

Figure 3.

Network Analysis and Immune Cell Distribution. **a.** Network Graph Highlighting Top Hub Genes.

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The boxplot displays the distribution of proportions for different immune cell types across samples. The median proportions are relatively low for all cell types. B_cells, CD8_T_cells, and CD4_T_cells exhibit similar distributions, suggesting comparable infiltration levels in the tumor microenvironment. This analysis provides insights into the immune landscape of the samples, highlighting the relative abundance and variability of immune cell types, which may influence tumor biology and immune response (Figure 3b).

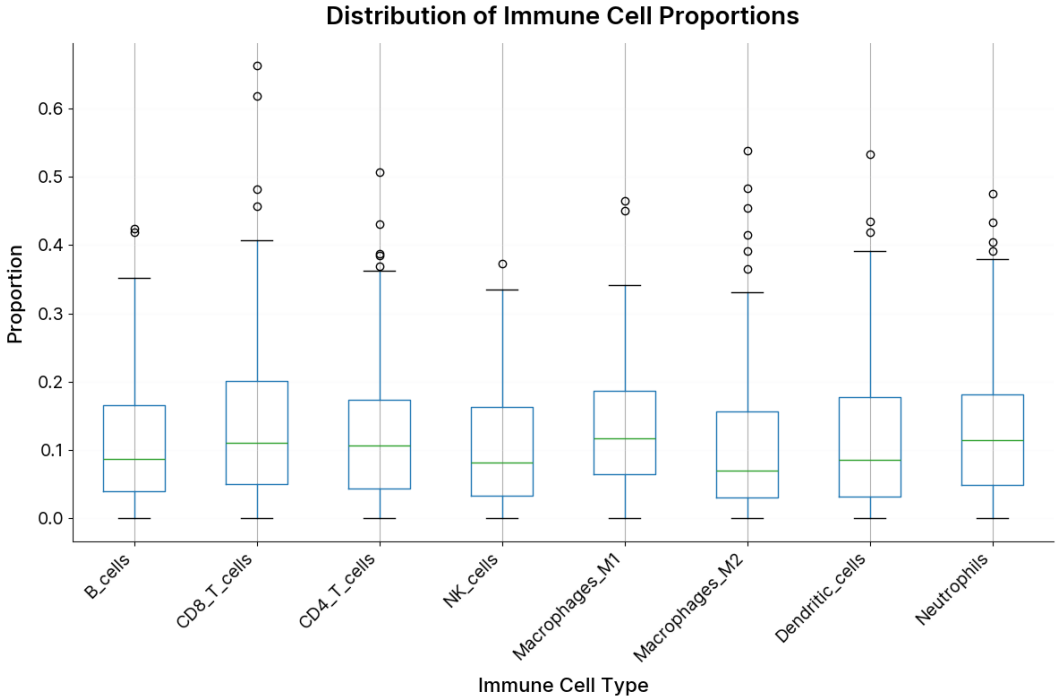

Figure 3. Network Analysis and Immune Cell Distribution. **b.** Boxplot of Immune Cell Type Distribution Across Samples

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The heatmap displays the correlation coefficients between hub genes and immune cell proportions (Figure 4), with the color scale representing the strength and direction of correlations: red indicates positive correlation, blue indicates negative correlation, and white indicates no correlation. Key observations include strong positive correlations between certain hub genes and Macrophages-M1, moderate positive correlations with CD4_T_cells, and negative correlations with B_cells. This heatmap provides a comprehensive view of the relationships between hub genes and immune cell infiltration, highlighting potential interactions that may influence tumor biology and immune response.

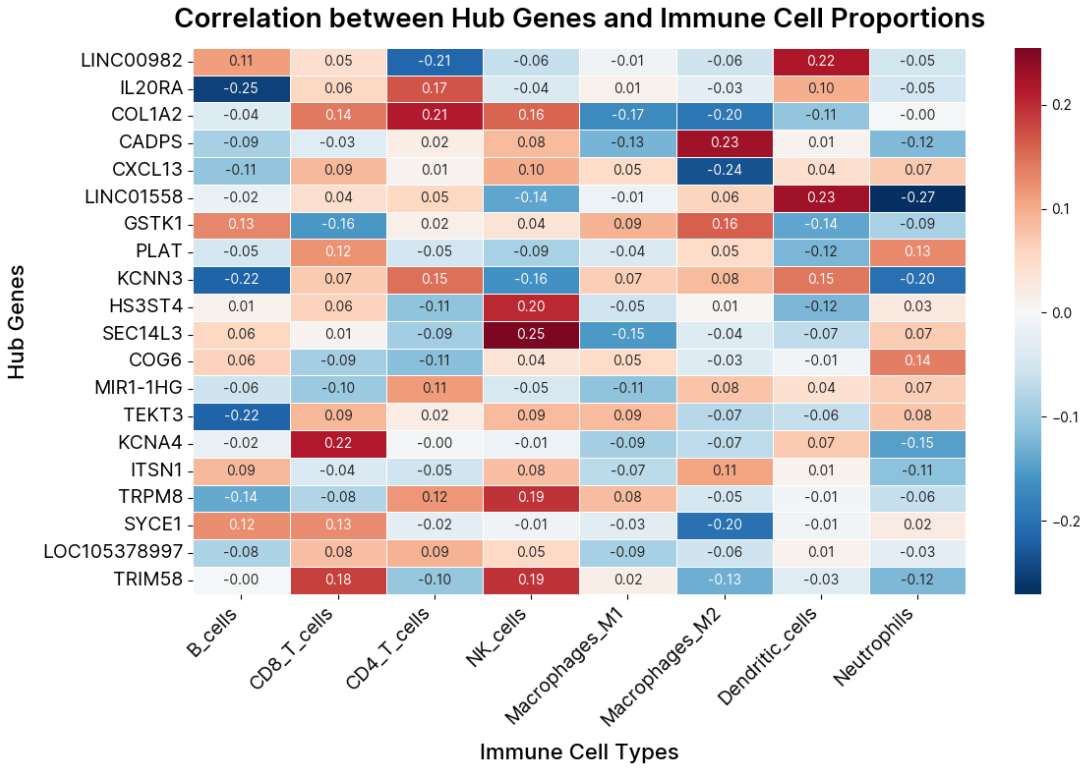

Figure 4. Correlation Heatmap Between Hub Genes and Immune Cell Proportions.

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The regression analysis in Figure 5 highlights the associations between key genes and the outcome, as well as the performance of the LASSO and Random Forest models. While the low MSE values suggest that the models' predictions are close to the true outcomes, the negative R^2 scores indicate that neither model effectively explains the variability in the target variable. This discrepancy may stem from inappropriate model assumptions, dataset characteristics, or the need for alternative modeling approaches. Despite these limitations, the identification of top genes such as CXCL13, TEKT3, and KCNA4 provides valuable insights for further research and potential clinical applications.

The LASSO regression model identifies TEKT3, KCNA4, and KCNN3 as having the largest positive coefficients, indicating a strong positive association with the outcome. In contrast, GSTK1 and TRIP58 exhibit negative coefficients, suggesting a negative association. The model's performance metrics include a Mean Squared Error (MSE) of 0.0154 and an R^2 Score of -0.4045. The low MSE indicates that the model's predictions are relatively close to the true outcomes. However, the negative R^2 score suggests that the model performs worse than a simple mean-based prediction, indicating it cannot effectively explain the variability of the target variable. This may be due to inappropriate model selection, incorrect assumptions, or dataset

characteristics, warranting further investigation (Figure 5a).

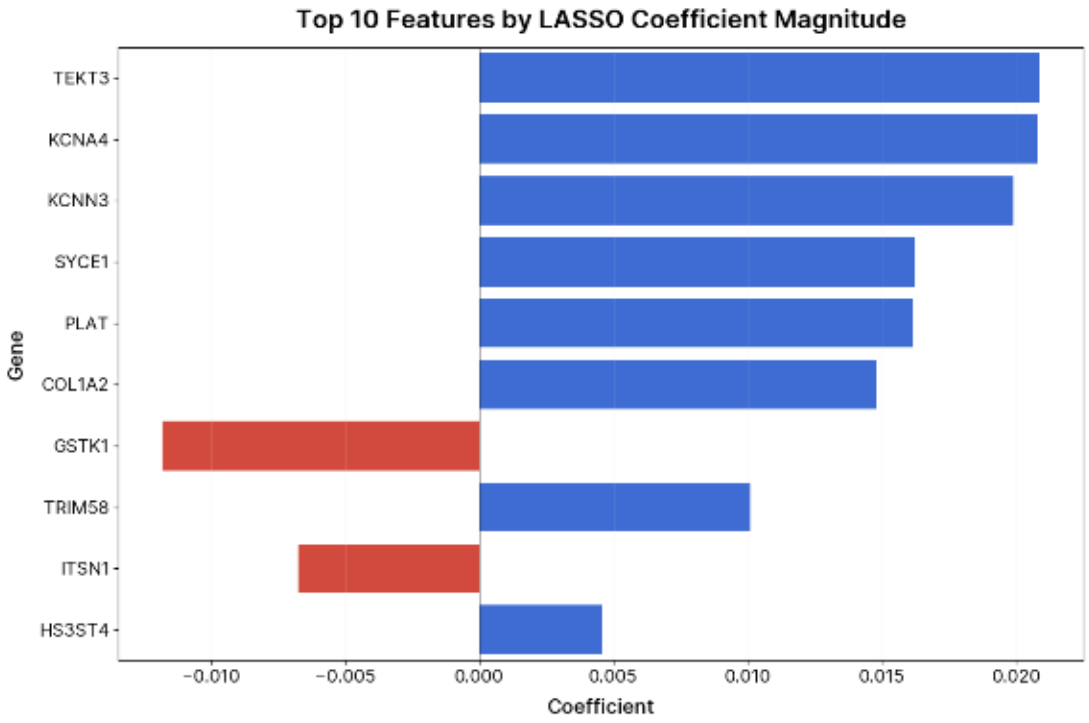

Figure 5. Regression Analysis of Gene Associations. **a.** LASSO Regression Model Coefficients.

The Random Forest regressor highlights the top 10 important features, focusing on gene-related data. The top five genes are CXCL13, COL1A2, HS3ST4, KCNA4, and SYCE1, followed by IL20RA, TEKT3, SEC14L3, LINC00982, and PLAT. The model's performance metrics include a Mean Squared Error (MSE) of 0.0146 and an R^2 Score of -0.3317. Similar to the LASSO model, the negative R^2 score indicates poor model performance, suggesting it cannot

adequately explain the target variable's variability. The importance scores range from 0.00 to 0.12, with CXCL13 having the highest importance and PLAT the lowest among the top 10. This analysis provides valuable insights for guiding further research and potential clinical applications (Figure 5b).

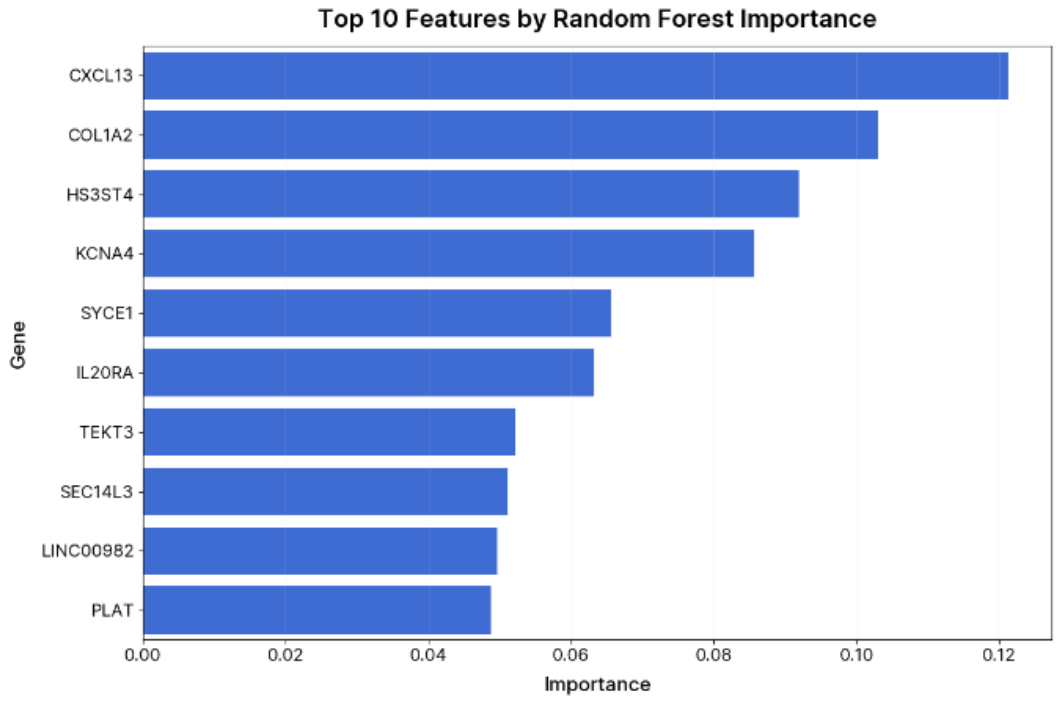

Figure 5. Regression Analysis of Gene Associations. **b.** Random Forest Regressor Feature Importance

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Discussion

ACC is a rare malignant neoplasm that typically arises in salivary glands but can also occur in various tissues, including the head, neck, breast, and lung. Its characteristics include a slow growth pattern, rich perineural invasion, and a propensity for late recurrences (4,5,30). While both ACC and CC are types of malignancies, they originate from different tissues and exhibit distinct biological behaviors, risk factors, and treatment approaches. Although there is no direct link between the two, they may be explored within the broader context of cancer biology, genetics, and treatment strategies. One previous study indicates that δ-T is the most effective form of tocopherol inhibiting prostate cancer cell growth by inducing cell cycle arrest and apoptosis. Its primary inhibited target was the phosphorylation of AKT on T308. δ-T attenuated the EGF/IGF-induced activation of AKT, which was further supported by the expression of dominant active PIK3 and AKT in the prostate cancer cell line DU145. The data also suggests that δ-T interferes with EGF-induced EGFR internalization, inhibiting AKT's receptor tyrosine kinase-dependent activation (20,31).

ACC is marked by genetic changes that may affect its growth and advancement. Notable genes include CXCL13, COL1A2, HS3ST4, KCNA4, and SYCE1. CXCL13, a chemokine, recruits B cells and plays a vital role in immune response and inflammation. Elevated CXCL13 levels may shape the tumor

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microenvironment by drawing immune cells, which could enhance immune evasion or foster a tumor-friendly environment. COL1A2 (9,10,19) encodes a part of type I collagen, essential for tissue structure (17). Variations in COL1A2 expression may influence the tumor stroma and ECM remodeling, impacting tumor cell behavior related to growth and invasiveness. HS3ST4 plays a role in producing heparan sulfate, a glycosaminoglycan key to cell signaling and ECM interactions. KCNA4 encodes a potassium channel that manages membrane potential and cell excitability. Abnormal KCNA4 expression in cancer can affect cell growth, death, and spread. SYCE1 is crucial for meiosis, the formation of the synaptonemal complex, and the maintenance of genome stability during gamete production. IL20RA (Interleukin 20 Receptor Alpha) is part of the receptor complex for interleukin-20 and is involved in the immune response. Disruption of IL20RA may influence the inflammatory response within ACC's tumor microenvironment. SEC14L3 relates to lipid metabolism and may affect signaling pathways. LINC00982 is a long, non-coding RNA associated with gene regulation that influences chromatin remodeling and expression. Understanding these genetic links is vital for improving ACC management and treatment (1,32).

TEKT3, KCNA4, and KCNN3 play roles in various biological processes and have implications in multiple cancers, including ACC. TEKT3 belongs to the

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tektin protein family, which is crucial for the structural organization of cilia and flagella. Changes in ciliary function are linked to tumor progression and metastasis (26,33); thus, dysregulation of TEKT3 could affect cell signaling pathways governed by primary cilia. KCNA4 encodes a voltage-gated potassium channel that regulates excitability in several cell types, including neurons and muscle cells (14,15). Altered expression or functioning of KCNA4 has been noted in various cancers, affecting cell growth, apoptosis, and invasion. KCNN3 encodes a calcium-activated potassium channel responsive to increased intracellular calcium levels, aiding in membrane potential regulation and impacting cell signaling, gene expression, and cellular response. Insights into these genes' roles in specific cancers may reveal valuable therapeutic targets and strategies to enhance patient outcomes.

Hub genes discovered through validation studies might play crucial roles in tumor biology. Additional research is required to uncover how these genes influence immune responses and identify potential therapeutic targets for immunotherapy in adenocarcinoma and colon cancer. Understanding the mechanisms behind these correlations while integrating multi-omics data could provide new perspectives on tumor-immune microenvironment dynamics, advancing our knowledge of therapy resistance and tumor progression. The hub gene identification study has limitations, such

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as dependence on bioinformatics predictions, possible inaccuracies from models like LASSO and Random Forest, and experimental validation (34–36). Sample size and dataset heterogeneity could influence the reliability of associations. Future research should aim to clarify mechanistic links between adenocarcinoma and colon cancer and identify relationships using computational analyses.

Conclusion

Investigating differential gene expression and interaction networks has revealed important hub genes vital in adenocarcinoma and colon cancer biology. Although this study has provided valuable insights for future research, the results highlight the importance of ongoing exploration and validation to maximize these findings for therapeutic progress in oncology.

Conflict of Interest

The authors have no conflicts of interest to declare

Author contribution

Conceptualization: Carlos M. Ardila. Methodology: Carlos M. Ardila. Software: Pradeep Kumar Yadalam. Formal analysis: Carlos M. Ardila. Investigation, Carlos M. Ardila. Data curation: Carlos M. Ardila. Writing-original draft preparation, Carlos M. Ardila; writing-review and editing, Carlos M. Ardila.

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